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Characterization of Bioaerosols and their Relation with OC, EC and Carbonyl VOCs at a Busy Roadside Restaurants-Cluster in New Delhi

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ABSTRACT

Measurement of biological particles (bioaerosols) in ambient air is of great importance as it is directly linked with the health issues. However, data on the bioaerosols characterization are scarce. Here we report on the concentration and characterization of bioaerosols (including bacterial and fungal aerosols) as well as determination of organic and elemental carbon (OC and EC) in total suspended particulate matter (TSPM) at a busy roadside restaurants-cluster site in New Delhi. In addition, 14 carbonyl volatile organic compounds (carbonyl VOCs) were also measured and their relationship with bioaerosols and OC/EC is assessed. The culturable airborne bacterial and fungal concentrations (CAB and CAF) at restaurant area varied significantly in different seasons ranging from 1.7×10^4–9.8×10^4 (averaged $6.3 \times 10^4 \pm 2.6 \times 10^4$ cfu m^{-3}) and 3.5×10^2–9.5×10^3 ($3.9 \times 10^3 \pm 3.1 \times 10^3$ cfu m^{-3}) cfu m^{-3}, respectively. Major concentration peaks of TSPM, OC, EC as well as bacterial and fungal aerosols were found in winter and spring seasons. These peaks can be attributed to the low atmospheric boundary layer (ABL) height and favourable meteorological conditions for microbial growth in winter and spring seasons in New Delhi. Good correlations ($R^2 > 0.5$) were observed between CAB, CAF, TSPM and OC. On the other hand, CAB and CAF were not found to be correlated with carbonyl compounds ($R^2 < 0.2$) indicative of their diverse sources. The bacterial identification was done by 16s rDNA sequencing and the identified strains were *Bacillus sp., Bacillus firmus, Bacillus licheniformis, Bacillus cereus, Bacillus pumilus, Acinetobacter sp.* and *Acinetobactor radioresistens* gene. Predominant fungal genera identified were *Aspergillus*, *Cladosporium*, *Alternaria* and *Fusarium*, which are known for adverse health effects causing numerous allergic and pathogenic inflammations.

***Keywords*:** Bioaerosol; Bacteria; Fungi; Carbonyls; Organic and elemental carbon.

INTRODUCTION

Airborne biological particles or bioaerosols are present everywhere in the atmosphere. A significant portion of atmospheric carbonaceous aerosols is comprised of biological particles such as bacteria, fungi, viruses, pollens and their by-products. Chow *et al.* (2015) attributed that the sum of fungal spores, pollen grains, and plant detritus contributed for an average of 11–15% of PM_{10} and 24–33% organic carbon (OC) mass in an agricultural town in California. Similarly, Bauer *et al.* (2008) found that at a suburban site, fungal spores comprised 6% and 14% of the OC mass in spring and summer, respectively. Womiloju *et al.* (2003) reported that fungi and pollen accounted for 12–22% of total OC mass in $PM_{2.5}$. Bioaerosols usually vary in size from a few nanometres to hundreds of micrometer in diameter. They can either exist as individual entities or form aggregates of biological structures and also attached to soil dust particles, water droplets and chemical constituents of aerosols (Szymczak and Gorny, 2010). They can have negative effect on agricultural and public health by acting as pathogenic, allergic, toxic and carcinogenic agents. In addition, they can affect atmospheric systems by serving as cloud condensation and atmospheric ice nuclei and thus can affect hydrological processes and radiation transfer in the atmosphere (Cox and Wathes, 1995).

In many Asian countries, cluster of road side restaurants are very common near public places such as markets, offices, factories, workshops, construction sites, etc. Rapid urbanization process and unprecedented industrial and economic development in metropolitans has caused the over population growth, leading to increase in the number of such restaurants and food stalls. Large concentrations of

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chemical and microbial components of particulate matter are aerosolized in such areas originating largely from cooking related activities as well as heavy traffic, commercial/ industrial activities of nearby area other than crowd itself.

Several studies have revealed that apart from vehicular emissions, cooking as one of the most important particle generating activities in the urban atmosphere (Abdullahi *et al.*, 2013). Watson *et al.* (1998) found that about 15% of fine organic aerosol was generated by meat cooking activities during the Northern Front Range Air Quality Study (NFRAQS) in 1997 in Denver, Colorado. Similarly, during an air quality campaign in Beijing in 2008, it was found that ~24% of total organic mass was attributed to cooking related particulates (Huang *et al.*, 2010).

Cooking process generates two kind of emissions, one is from the combustion of fuel used for cooking which includes biomass (coal/wood), liquid petroleum gas (LPG), kerosene, etc. and another from cooking itself. Cooking fumes contains various allergic, mutagenic and carcinogenic compounds (Vainiotalo and Matveinen, 1993). Majumdar *et al.* (2015) reported high carbonyl levels resulted from primary emission from uncontrolled open burning of biomass and coal associated with cooking activity at a residential/commercial area in Kolkata, India. Various reactions take place during the high temperature treatment of food such as degradation of sugars and fats, pyrolysis of proteins and amino acids (Svendsen *et al.*, 2002). These cooking processes lead to production of aldehydes, such as formaldehyde, acetaldehyde, acrolein, butanal, hexanal, etc. (Vainiotalo and Matveinen, 1993). Formaldehyde and acetaldehyde were among the predominant light gas-phase organic compounds released from the charbroiling processes in the urban atmosphere (Schauer *et al.*, 1999a). In addition, meat charbroiling was also recognized as one of the sources for the high molecular weight aldehydes (Schauer *et al.*, 1999a). Recently, during laboratory experiments performed by Klein *et al.* (2016) to characterize the cooking emissions, it was found that emission from charbroiling, deep frying and shallow frying were dominated by aldehydes of varying composition depending upon the type of oil used and food being cooked. Feng *et al.* (2005) also reported that besides vehicular exhaust, cooking was another major source of carbonyl compounds in the ambient air of Guangzhou city, China.

Furthermore, bioaerosol concentrations are also usually found higher near restaurant areas where it could be originating from occupants, microbial growth on food-storage, leftover food and organic waste (Yusup *et al.*, 2014). The most influential factors affecting the ambient air quality of restaurant areas are the different style of cooking methods, fuel type used, the type of food being cooked, temperature, extraction/ventilation equipment used, number of occupants and the hygiene maintained. The food being cooked is usually exposed to air, and thus, could be contaminated due to poor air quality.

Occupational workers, customers/clients and downwind population are exposed to the cooking related health risks as well as to PM laden airborne microorganisms. Despite the above mentioned facts, very few reports are available on the air quality of such food premises, especially from Asia including Indian subcontinent region. To the best of our knowledge, this study provides a first report on the concentration and characterization of the bioaerosols (CAB and CAF) as well as determination of organic and elemental carbon (OC and EC) in total suspended particulate matter (TSPM) at a busy road side restaurants-cluster site in New Delhi, India. Apart from microbial and chemical constituents studied in airborne particulate matter, total 14 carbonyl compounds were also measured in the gaseous phase (cooking is recognised as one of the major sources of carbonyl compounds as discussed above). Relationship between the ambient air pollutants and bioaerosols were also evaluated in order to gain insight to their sources.

MATERIALS AND METHODS

Site Description

New Delhi city (location: 28°12'N–28°53'N, 77°50'E–77°23'E, 218 m above sea level) experiences a monsoon influenced subtropical climate with high difference between summer and winter temperature. Apart from temperature swings, the city experiences a very dense foggy weather and low atmospheric boundary layer (ABL) height conditions in winter. During spring and summer seasons, dust storm events which originate from the Thar desert (situated at the South-West of New Delhi) or sometimes from Middle East (far West of New Delhi) also affect the climate of Delhi (Kumar *et al.*, 2016).

Aerosol samplings were carried out at a cluster of restaurant site (nearby area of PVR, Naraina) in New Delhi. As shown in Fig. 1 the sampling site was located near (at a distance of ~10 m) a busy two-lane road and an industrial area (Naraina Industrial Area phase-1) comprised of cosmetics, pharmaceuticals, printing, dyeing and motor workshops. Approximately 55 various eateries were situated within the 100 m radius of the sampling site including open-and semi-open air canteen type restaurants (18), established restaurants with closed kitchens (4), confectioneries (3), temporary food stalls (14), open-air tea stalls (11), fast food outlets (3) and bakeries (2). The processes used for cooking were mostly pan/wok-frying, deep frying, roasting, grilling, baking, boiling and broiling. Liquid petroleum gas (LPG), kerosene and solid fuels (coal, wood) were the predominant fuels used for cooking at the sampling site. Crude biomass fuels such as raw/semi wood, dried cow-dung and coconut shells were also seen at some temporary stalls.

Air Sampling

Ambient aerosol samples were collected using a low-volume handy sampler (APM 821, Envirotech Instruments Pvt. Ltd., India) operated at flow rate of 2 L min^{-1}. The instrument was placed at a height of ~1.5–2 m above the ground level for sampling to simulate exposure in the human breathing zone. Samplings were carried out for the duration of 3–4 hours during the period of 7:00–14:00 hours (local time). Before each run, the sampler was carefully wiped with 70% ethanol and allowed to dry. The samples were collected sequentially in winter, spring and summer seasons (i.e., from January to June) of the two consecutive years, 2014 and

Fig. 1. Location of the sampling site.

2015. Meteorological parameters, i.e., temperature and relative humidity were taken from weather monitoring station of Indian Agricultural Research Institute (IARI), which is situated within 5 km proximity of the sampling site. Description of the samples collected and corresponding meteorological conditions are summarized in Table 1. A total of 36 samples were collected for bioaerosols, TSPM and OC/EC (2 samples were collected on each sampling day). However, samples for May 13th, 19th and 21st, 2015 could not be analysed for bioaerosols.

Quartz filters (Whatman QM-A) of 25 mm diameter (prebaked at 550°C for 6 hours) were used for aerosol sampling. Before and after sampling the filters were kept in desiccators for 24 hours at room temperature to equilibrate at a constant relative humidity. A parallel sampler was deployed during each sampling event to collect aerosol particles on quartz filters for gravimetric mass determination and initial and final weights of these quartz filters were taken using a microbalance (Sartorius, ME 5-F; Germany). The balance was conditioned for 12 hours and auto calibrated before each weighing session, according to manufacturer's instructions. Quartz filters have been reported to be optimum for retrieval of trapped micro-organisms (Kenny *et al.*, 1998; Stagg *et al.*, 2010). In addition to this, filtration samplers allow sampling without the loss of collection efficiency compared to that of impinging sampling. However, filtration can cause dehydration stress in the captured microorganisms but it depends on the sampling duration and can be controlled by reducing the sampling time (Mandal *et al.*, 2011). Moreover, the advantage of use of quartz filters as sampling media is that a combined study of microbial and chemical constituents (especially OC, EC) in aerosols could be performed. After each sampling event, quartz filter samples were brought to the laboratory in sterile containers maintaining the temperature around 4°C using dry ice packs and immediately processed for viable count analysis. New para air monitoring for airborne carbonyl VOCs (formaldehyde and 13 other compounds, Table 3) was done during spring (March–April, 2015) and summer (May, 2015) seasons for the duration of 3 hours between 10:00–14:00 hours. A low-flow air sampler (Poltech Instruments Pvt. Ltd., India) with the flow rate of 80 mL min^{-1} was used for collection of carbonyl compounds using Supelco make L$_P$DNPH cartridges. A total of 20 samples were collected to measure carbonyl VOC's. Ozone scrubber was used with the sampler to restrict further reactions of carbonyls (Wang *et al.*, 2010).

Sample Analysis

Carbonaceous Aerosols

Organic carbon and elemental carbon (OC and EC) were analyzed using a thermal-optical technique based Carbon analyzer (Atmoslytic Inc., USA), following the Interagency Monitoring of Protected Visual Environments (IMPROVE) thermal/optical reflectance (TOR) protocol (Chow *et al.*,

Table 1. Sample/sampling details.

S. No.	Sampling Date (dd/mm/yyyy)	Sampling Instruments	No. of Samples	Average Temperature during sampling hours (°C)	Average Relative Humidity during sampling hours (%)
1.	02/01/2014	Handy sampler	2	9.1	85.6
2.	28/01/2014	Handy sampler	2	15.9	78.9
3.	04/03/2014	Handy sampler	2	18.2	72.3
4.	26/03/2014	Handy sampler	2	22.3	72.9
5.	25/05/2014	Handy sampler	2	31.6	52.7
6.	04/06/2014	Handy sampler	2	33.7	39.5
7.	11/02/2015	Handy sampler	2	13.2	64.4
8.	12/02/2015	Handy sampler	2	15.4	73.4
9.	02/03/2015	Low volume sampler	2	16.2	87.0
10.	26/03/2015	Low volume sampler	2	28.7	52.3
11.	15/04/2015	Handy and low volume sampler	4	27.0	57.1
12.	28/04/2015	Handy and low volume sampler	2	30.8	54.7
13.	06/05/2015	Handy and low volume sampler	4	34.2	38.6
14.	08/05/2015	Handy and low volume sampler	4	36.3	41.7
15.	11/05/2015	Handy and low volume sampler	2	37.9	44.1
16.	12/05/2015	Handy and low volume sampler	2	35.3	45.4
17.	13/05/2015	Handy and low volume sampler	2	33.5	73.4
18.	19/05/2015	Handy and low volume sampler	4	37.3	41.0
19.	20/05/2015	Handy and low volume sampler	4	34.2	42.1
20.	21/05/2015	Handy and low volume sampler	4	34.1	39.2

2004) and assuming carbonate carbon in the sample to be negligible. Typically, a punch of the filter (0.5 cm^2) was placed in a quartz boat inside the thermal desorption chamber of the analyzer, and then stepwise heating was applied as described in Mandal *et al.* (2013).

Carbonyl VOCs

Sampling and analysis of carbonyl compounds were carried out following EPA compendium method TO 11 (EPA, 1999). L_PDNPH cartridges (Supelco, USA) were extracted following solid phase extraction method using 1.5 mL of acetonitrile. Formaldehyde and 13 other carbonyl compounds (Table 3) were quantified using reverse phase high performance liquid chromatography (HPLC) with ultraviolet (UV) absorption detector at 360 nm (Dionex, ICS-3000; California). Acetonitrile-water gradient was used as eluent and tetrahydrofuran was used as modifier. The target compounds were quantified using an external calibration curve plotted using TO 11/IP6A carbonyl-DNPH mix standard (Supelco, USA) (Majumdar *et al.*, 2015).

Quantification of Culturable Airborne Bacteria and Fungi (CAB and CAF)

Microbial extraction and quantification was done following the protocols described by Stagg *et al.* (2010) and Bowers *et al.* (2009). Quartz filter aliquots were extracted by shaking (refrigerated/incubator shaker MAXQ 6000-8CE, Thermo scientific) at 200 rpm for 2 hours at 5 ± 2°C in 10 mL of 50 mM Tris buffer (Himedia, India). At such temperature the microbial particles can be expected to remain static without leading to multiplication. Instead of applying vortexing or ultrasonic agitation, we opted shaking for microbial extraction, which is reported not to cause injury to cells (Wang *et al.*, 2001). The extraction efficiency of microbial particles off the filters was not quantified in our study. However, Bowers *et al.* (2009) found shaking time of 2 hours at 200 rpm was sufficient for extracting most of the particles. Anti-clumping agent (GIBCO, Life Technologies) was also added while extraction to avoid clumping of microbial particles. Ringers solution, added with anti-clumping agent was used to prepare the dilution series from the initial extraction suspension. Bacterial colonies were plated on Nutrient agar (NA) media amended with 200 mg L^{-1} cyclohexamide (SRL, India) to inhibit fungal growth, which has been previously reported not to affect bacterial counts (Lighthart and Shaffer, 1994; Tong and Lighthart, 2000). The NA plates were incubated at 37°C, and the observation was recorded up to 48 hours. While fungal colonies were plated on Malt extract agar (MEA) (Himedia, India) with 100 mg L^{-1} streptomycin (SRL, India) added to inhibit bacterial growth (Malý *et al.*, 2000) and incubated at 25°C up to 5 days. Emerging colonies on agar plates were counted and, using the known volume of air sampled, number concentration was calculated as colony forming units per cubic meter of air (cfu m^{-3}) as follows:

$$\text{Concentration (cfu m}^{-3}) = [(\text{Number of colonies}/10^{-n})(V_1/V_2)]/F \times T \quad (1)$$

where n is the dilution factor, V_1 is the volume of extraction suspension applied to each filter aliquot (10 mL), V_2 is the volume of diluted suspension inoculated on each agar plate (0.1 mL), F is flow rate of the sampler (m^3 min^{-1}) and T is sampling duration (min).

Laboratory blanks (LB) and field blanks (FB) were also prepared for each sample set. Laboratory blanks were

unexposed, fresh media samples, while field blanks were the samples handled the same way as field samples, including loading the quartz filters into the sampler, labeling, extraction, inoculation and incubation except that no air was drawn through the sampler. All results were FB corrected. Microbial concentration observed in field blanks was found to be less than 6% of the minimum concentration measured for real samples.

Microbial Identification

Bacterial Identification

The bacterial colonies appeared on NA plates were selected on the basis of difference in their size, shape, colour, and surface properties. The streak plating was performed in order to isolate pure colonies and, to keep the culture viable, sub culturing was done at an interval of 15 days and preserved at ~4°C in a refrigerator. Total genomic DNA from the bacteria was isolated by N- cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method (Wilson, 2001). Quantity of the isolated DNA was checked in UV-VIS spectrophotometer (Vivaspec Biophotometer, Germany). 16s rDNA sequencing analysis was done at Triyat Scientific Co., Nagpur, India. PCR amplification of 16s region was carried out following the protocol described previously (Fang *et al.*, 2014) using 27F (AGA GTT TGA TCC TGG CTC AG) and 1522R (AAG GAG GTG ATC CAG CCG CA) universal primers set. The amplified products were purified and then subjected to automated DNA sequencing using Applied Biosystems genetic analyzer following the manufacturer's instruction. The nucleotide sequences obtained were compared with the sequences available in the GenBank database using the NCBI BLAST program and the sequence similarity to the closest phylogenetic relative was used as an indication of identity.

Fungal Identification

The individual fungal colonies were selected and isolated based on their appearance, colour and distribution of fungal growth and subsequently pure cultures were obtained by quadrant streaking method. The pure cultures were maintained on MEA plates at ~4°C. Following the isolation and purification, the colonies were stained with lacto phenol cotton blue and subjected to genus selection by their morphological and microscopic examination using taxonomic keys available in the literature (Owen *et al.*, 1992; St-Germin *et al.*, 1996; Klich, 2002). Further Identification of fungal strains was carried out at Indian Type Culture Collection (ITCC), Indian Agriculture Research Institute (IARI), New Delhi, India (http://www.iari.res.in/files/ITCC_catalogue_1936-2012-10102013.pdf).

Statistical Analysis

The arithmetic mean and standard deviations were calculated using Microsoft office Excel. The CAB, CAF, TSPM, OC, EC, carbonyl concentrations and meteorological parameters (temperature and relative humidity) were subjected to statistical linear regression analysis to examine the correlation among them, and p values less than 0.05 were considered to be statistically significant. Here, correlation coefficients (R^2) point to the degree to which different parameters vary with each other, either because they belong to the same particles, to different particles of same origin, or influenced by the same meteorology (Chow *et al.*, 2015).

RESULTS AND DISCUSSIONS

TSPM and Associated OC, EC

Total suspended particulate matter (TSPM) and associated organic and elemental carbon (OC and EC) concentrations across the seasons ranged from 208.3–679.2, 66.2–259.5 and 7.4–89.2 μg m^{-3}, respectively (Table 2, total 36 samples). Fig. 2 depicts the average concentrations measured on each sampling day. Higher concentrations of TSPM, OC and EC could be attributed to the mixed contribution of emissions from food premises, high traffic flows and industrial emissions at the sampling site. Results of this study are found to be comparable with the previous findings of Srivastava *et al.* (2007) (annual TSPM range: 100–384 μg m^{-3}) and Shandilya *et al.* (2007) (average 687.7 ± 117.4 μg m^{-3}) in New Delhi. TSPM, OC and EC concentrations varied in order of winter > spring > summer (Fig. 3). The higher concentrations found in winters could be attributed to the low atmospheric boundary layer height conditions and slow dispersion rate in Delhi. Mandal *et al.* (2013) also observed the higher concentrations of PM_{10}, OC and EC (453.45 ± 62.92, 159.40 ± 25.93 and 47.14 ± 11.42 μg m^{-3}, respectively) in December 2011 at Naraina Industrial Area, Delhi. The TSPM and OC were found to be moderately correlated (R^2 = 0.64) and as Fig. 3 shows the average contribution of total carbonaceous aerosols to TSPM was estimated to be 58 ± 9%. The total carbonaceous aerosol concentration was calculated as the sum of organic matter (OM = 1.6 × OC)

Table 2. Summary of TSPM, OC, EC and bioaerosols (CAB and CAF) measured at restaurants-cluster site, New Delhi.

Parameter	Winter (January–February, 2014–15)		Spring (March–April, 2014–15)		Summer (May–June, 2014–15)	
	Average ± Std. dev.	Range	Average ± Std. dev.	Range	Average ± Std. dev.	Range
TSPM (μg m^{-3})	592.4 ± 78.9	482.6–679.2	509.6 ± 71.9	418.7–595.8	414.2 ± 97.4	208.3–495.9
OC (μg m^{-3})	195.2 ± 40.9	147.4–259.6	171.4 ± 44.8	134.9–241.9	131.3 ± 31.9	66.2–169.6
EC (μg m^{-3})	49.5 ± 17.5	30.3–77.9	43.2 ± 12.2	25.3–60.3	37.5 ± 21.9	7.4–89.2
CAB (cfu m^{-3})	$7.4 \times 10^4 \pm 5.9 \times 10^3$	6.4×10^4–8.0×10^4	$7.8 \times 10^4 \pm 2.1 \times 10^4$	2.9×10^4–9.8×10^4	$4.6 \times 10^4 \pm 1.8 \times 10^4$	1.7×10^4–6.5×10^4
CAF (cfu m^{-3})	$4.5 \times 10^3 \pm 2.6 \times 10^3$	1.5×10^3–8.3×10^3	$6.1 \times 10^3 \pm 2.2 \times 10^3$	2.6×10^3–9.5×10^3	$1.3 \times 10^3 \pm 8.4 \times 10^2$	3.5×10^2–2.3×10^3

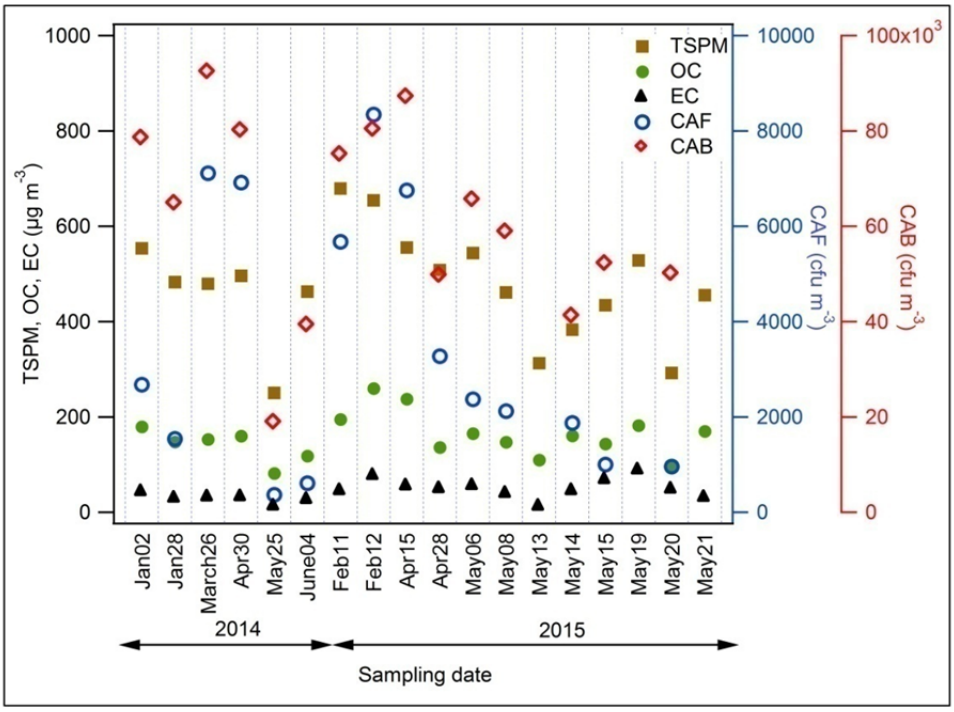

Fig. 2. TSPM, OC, EC and bioaerosols (CAB and CAF) concentrations on sampling days at restaurants-cluster site, New Delhi.

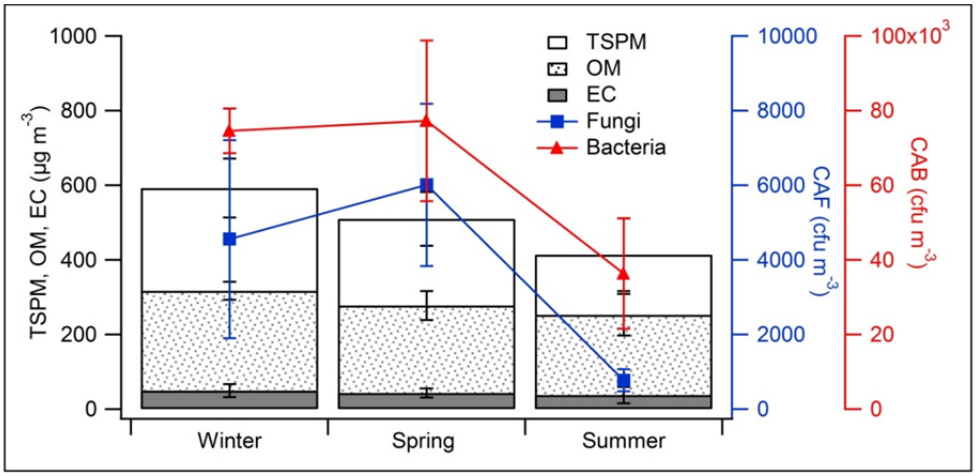

Fig. 3. Seasonal variations in the TSPM, OM, EC, CAB and CAF concentrations at restaurants-cluster site, New Delhi.

and elemental carbon (Cao *et al*., 2005). Mandal *et al.* (2013) also reported the annual average contribution of total carbonaceous aerosol in PM_{10} as 62% at Naraina industrial area of Delhi.

Organic to elemental carbon ratio (OC/EC) provides an idea to identify the possible sources of carbonaceous aerosols. Higher OC/EC ratios are used as an indicator of biomass burning sources while lower ratios are linked with fossil fuel combustions, hence OC/EC ratios could be used to make out different source and transformation characteristics (Kaul *et al.*, 2011). Sandradewi *et al.* (2008) reported an average OC/EC ratio of 7.3 for wood burning and 1.1 from vehicular emissions. Similarly, Watson *et al.* (2001) found an OC/EC ratio of 2.7 for coal combustion, 9.0 for biomass burning and 1.1 for vehicular emission. See and Balasubramanian, 2008 reported that OC/EC ratio from kitchen emission ranged from 4.3–7.7. In our study the OC/EC ratio varied from 1.9–13.2 with an average of 4.5. These high ratios are clearly indicating that the impact of biomass burning and kitchen emissions was more prominent in our samples than vehicular and industrial emissions. A poor correlation coefficient between OC and EC (R^2 = 0.35) concentrations observed in this study again suggested that presence of these species in the ambient air is from different set of source emissions.

Carbonyl VOCs

Formaldehyde and 13 other carbonyl compounds were

measured including acetaldehyde, acetone, propionaldehyde, butanal, crotonaldehyde, benzaldehyde, isovaleraldehyde, valeraldehyde, *o*-tolualdehyde, *m&p*-tolualdehyde, hexanal and 2, 5-dimethylbenzaldehyde (concentration ranges are summarized in Table 3, total 20 samples). Air monitoring for carbonyl compounds was done during spring (March–April, 2015) and summer seasons (May, 2015) between 10:00–14:00 hours. *m&p*-Tolualdehyde was not detected in any sample while 2,5-dimethylbenzaldehyde was found only in four samples.

Out of 14 carbonyls, acetone (21.4 ± 11.8 μg m^{-3}) was the most abundant carbonyl compound followed by acetaldehyde (18.6 ± 11.8 μg m^{-3}) and formaldehyde (10.3 ± 4.5 μg m^{-3}). Nearly 64.8 ± 12.9% of the carbonyls comprised of acetone, formaldehyde and acetaldehyde. Acetone accounted for 29.8 ± 16.4% of total carbonyls, while acetaldehyde and formaldehyde contributed 24.2 ± 7.5% and 10.8 ± 6.8%. The carbonyl levels obtained in this study were comparable to a previous study done in Kolkata during summer season by Dutta *et al.* (2009) (formaldehyde and acetaldehyde levels 14.07–26.12 and 7.60–18.67 μg m^{-3}, respectively). The carbonyl levels also coincide with studies done in other metro cities e.g., average concentrations reported by Feng *et al.* (2005) for formaldehyde, acetaldehyde and acetone in the ambient air were 13.68, 8.33 and 17.76 μg m^{-3}, respectively in Guanzhou, China. Similarly in Brazil, the reported values for formaldehyde, acetaldehyde and acetone were 10.84, 10.43 and 4.14 μg m^{-3}, respectively (Grosjean *et al.*, 2002). On the contrary, ambient average concentrations of formaldehyde, acetaldehyde and acetone (1.68, 0.91 and 1.61 μg m^{-3}, respectively) were found much lower in a coastal/industrial area near the Donana National Park in Spain (Villanueva *et al.*, 2013). The higher acetone concentrations found in current study were in agreement with the results of previous investigators (Christensen *et al.*, 2000; Feng *et al.*, 2005).

Coinciding with the results of the previous study done by Feng *et al.* (2005) on cooking exhaust, in present study the straight chain compounds (C_1–C_6) were comparatively more prominent than aromatic carbonyls (benzaldehyde and *o*-tolualdehyde) and branched chain carbonyl compounds (isovaleraldehyde and 2,5-dimethylbenzaldehyde). Significant concentrations of hexanal (6.6 ± 4.3 μg m^{-3}) were observed in the current study (fourth most abundant carbonyl compound, 8.4 ± 2.3% of total carbonyls), even higher than formaldehyde at times (Table 3). Valeraldehyde and iso-valeraldehyde were also found in considerable levels (2.9 ± 2.0 μg m^{-3}, 4.5 ± 4.2 μg m^{-3}, respectively) in present study. In a very recent study done by Klein *et al.* (2016) reported that hexanal was among the most dominant compounds emitted from heating the cooking oils at high temperatures. Mendonca *et al.* (2015) also observed the significant concentrations of hexanaldehyde along with acetaldehyde and nonanaldehyde, emitted during cooking. Kim *et al.* (2014) reported that valeraldehyde and iso-valeraldehyde along with acetaldehyde are the majorly emitted compounds during fish frying activity. However, major carbonyl compounds (acetone, formaldehyde and acetaldehyde) showed poor correlations with OC and TSPM (Table 4) pointing towards the more complex sources of carbonyls. Formaldehyde, acetaldehyde, propionaldehyde, valeraldehyde, isovaleraldehyde, hexanal and benzaldehyde mostly showed good correlations with each other while these all were poorly correlated with acetone (Table 5).

In our study the average C_1/C_2 and C_2/C_3 ratios were 0.48 (0.14–1.54) and 5.9 (2.0–9.4), respectively. As reported earlier formaldehyde/acetaldehyde (C_1/C_2) ratio typically vary from 1 to 2 in the urban environment to about 10 in rural area (Shepson *et al.*, 1991). Low acetaldehyde/propionaldehyde (C_2/C_3) ratio is indicative of the anthropogenic emissions as it is said that propionaldehyde is originated solely from anthropogenic sources (Andersen *et al.*, 1996). Majumdar *et al.* (2015) also reported the C_1/C_2 ratio as 0.61 in a study done in Kolkata, another metropolitan city of India and, suggested that higher acetaldehyde concentration was due to the strong influence of diesel driven vehicular pollution in Kolkata. In accordance with

Table 3. Summary of carbonyl compounds measured at restaurants-cluster site, New Delhi.

Parameter (μg m^{-3})	Limit of Detection[#] (μg m^{-3})	Spring (March–April, 2015)		Summer (May, 2015)	
		Average ± Std. dev.	Range	Average ± Std. dev.	Range
Formaldehyde	0.7	9.9 ± 3.6	9.1–15.2	11.5 ± 13.7	1.5–40.3
Acetaldehyde	0.4	15.3 ± 3.0	13.5–19.2	20.3 ± 13.7	12.4–53.5
Propionaldehyde	0.2	4.1 ± 3.1	1.3–8.3	3.9 ± 3.5	1.3–9.7
Acetone	0.4	21.2 ± 6.5	14.3–29.0	23.7 ± 12.5	7.1–43.4
Butanal	0.3	0.4 ± 0.2	BDL–0.9	0.2 ± 0.3	BDL–0.7
Crotonaldehyde	0.4	10.2 ± 8.1	2.5–18.9	6.4 ± 5.5	0.4–15.2
Benzaldehyde	0.4	3.1 ± 1.8	1.5–5.4	2.9 ± 2.0	0.8–7.2
Isovaleraldehyde	0.6	2.9 ± 1.2	1.4–4.2	4.7 ± 4.8	1.0–15.1
Valeraldehyde	0.7	3.4 ± 1.5	1.8–5.2	2.8 ± 2.2	0.8–7.2
o-Tolualdehyde	0.2	0.9 ± 1.01	BDL–2.0	0.5 ± 1.6	0.5–5.1
*m&*ρ -Tolualdehyde	0.8	BDL[*]	BDL[*]	BDL[*]	BDL[*]
Hexanal	0.7	5.9 ± 2.2	4.1–8.8	7.2 ± 4.9	1.9–16.7
2,5-dimethylbenzaldehyde	0.9	0.3 ± 0.5	BDL–1.3	BDL[*]	BDL[*]

[*] BDL-Below Detection Limit.

[#] Limit of Detection is calculated for a sampling volume of 0.019 m^3 corresponding to 4 hours sampling @ 80 mL min^{-1}.

Table 4. Correlation (R^2) matrix of the major parameters measured at restaurants-cluster site in New Delhi.

Parameter	TSPM	OC	EC	CAB	CAF	Formaldehyde	Acetaldehyde	Acetone
TSPM	1	0.645[a]	0.269	0.533[a]	0.509[a]	0.04	0.2	0.047
OC		1	0.349	0.539[a]	0.618[a]	0.036	0.003	0.002
EC			1	0.216	0.121	0.005	0.022	0.014
CAB				1	0.667[a]	0.077	0.038	0.028
CAF					1	0.182	0.752[a]	0.335
Formaldehyde						1	0.712[a]	0.059
Acetaldehyde							1	0.168
Acetone								1

[a] 99% confidence level.

Kolkata and other mega-cities in India, the maximum fleet of heavy- and medium-duty vehicles are diesel driven in Delhi. It suggests that contribution from vehicular emission is also responsible for the observed carbonyl levels in this study. Similar studies done in the urban environment by Grosjean *et al.* (1996) in Los Angeles, USA ($C_1/C_2 = 0.91$ and $C_2/C_3 = 3.84$) and Ho *et al.* (2002), Hongkong, China ($C_1/C_2 = 2.1$ and $C_2/C_3 = 8.38$) again indicate the photochemical formation from anthropogenic hydrocarbons may be an important factor contributing in the production of carbonyl compounds in present study as sampling for carbonyls was done during the peak sun-light hours. As indicated in the site description, the sampling site is situated near an industrial area (Naraina Industrial Area, Phase-1). High acetone concentrations found in our samples and its poor correlation observed with other carbonyl compounds (Table 5), suggest that it has majorly emitted from the solvents use and other industrial uses from those nearby industries.

Hence, to summarize it can be said that the observed carbonyl concentrations in this study have been contributed majorly from the cooking activity, characteristics of the area and the vehicular emission. Photochemical formation as well as industrial emission may also have significant contribution in the level of carbonyl species.

Microbial Quantification

Including India, most of the countries do not have any clear concentration guidelines or regulations for bioaerosols. The World Health Organisation (WHO) issued guidelines for prevention of dampness and associated microbial growth in indoor environments (WHO, 2009) but they do not specify any thresholds for acceptable values of bioaerosols. However, a few European countries such as Germany and Netherlands have given the guideline values for bacterial aerosols as 10,000 cfu m^{-3} for indoor air in different work environments (Mandal *et al.*, 2011 and references therein). Similarly, Swiss guidelines for gram-negative and mesophilic aerobic bacteria in indoor environment are 1000 and 10,000 cfu m^{-3}, respectively. In present study the culturable airborne bacterial and fungal concentrations (CAB and CAF) at restaurant area varied significantly in different seasons ranging from 1.7×10^4–9.8×10^4 (averaged $6.3 \times 10^4 \pm 2.6 \times 10^4$ cfu m^{-3}) and 3.5×10^2–9.5×10^3 ($3.9 \times 10^3 \pm 3.1 \times 10^3$ cfu m^{-3}) cfu m^{-3}, respectively (Table 2, total 30 samples). As shown in Fig. 2 the bioaerosol levels exceeded the suggested exposure limits (described above) on each sampling day. High microbial concentrations found at restaurant area is possibly because it is a heavily crowded public place throughout the year in which different background of people (different age-group, gender, job nature, health, etc.) are present. Large numbers of microbes are emitted during talking/laughing, sneezing, coughing, spitting and peeling of the epidermis. Additionally, microorganisms are also emitted into the air from re-suspension of road/pavement dust due to walking and vehicular transport. Use of various types of organic raw materials, food storage, food spoilage, leftovers, dustbins, lack of sanitization could be other possible causes of high microbial growth at such places. Frequent disposal of restaurant kitchen wastes and food leftovers in the surroundings was also observed in the sampling area. Fang *et al.* (2007) also observed that bacterial concentration were significantly higher at human activity enriched site and highly trafficked site than those at the greener site.

CAB and CAF did not correlate strongly with the meteorological parameters i.e., temperature and relative humidity ($R^2 < 0.5$) in our study. Major microbial concentration peaks found during winter and spring could be associated with high particulate matter concentration and favourable meteorological conditions in Delhi. It is noticeable that unlike TSPM and OM the bacterial and fungal concentrations were comparatively higher in spring than in winter (Fig. 3). This is possibly because apart from high particulate matter levels, the metrological conditions in spring (average maximum temperature in the month of March is ~29°C) are more favourable for microbial growth. On the other hand, comparatively lower concentrations observed in summer could be because of microbial lethal effects of adverse meteorological conditions in New Delhi (average maximum temperature in May is ~40°C) which are more prominent than that of release of microbial flux due to solar ground heating effect in summer. These findings are in contrast to the other studies where the microbial concentrations were higher in summer and warm weather conditions (maximum summer temperature reaches up to 25°C) (Rahkonen *et al.*, 1990; Tong and Lighthart, 2000). However, high winter and low summer microbial concentrations were reported in few studies conducted in Taiwan (Wu *et al.*, 2000; Huang *et al.*, 2002), hence the seasonal variation can be attributed to the geographical, meteorological and other specific conditions of particular study area.

Table 5. Correlation (R^2) matrix of measured carbonyl compounds at restaurants-cluster site in New Delhi.

Parameter	Formaldehyde	Acetaldehyde	Propionaldehyde	Acetone	Crotonaldehyde	Benzaldehyde	Hexanal	Isovaleraldehyde	Valeraldehyde
Formaldehyde	1	0.712[a]	0.469	0.039	0.136	0.884[a]	0.498	0.775[a]	0.332
Acetaldehyde		1	0.593[a]	0.168	0.073	0.561	0.769[a]	0.682[a]	0.523
Propionaldehyde			1	0.172	0.41	0.509	0.836[a]	0.424	0.869[a]
Acetone				1	0.001	0.02	0.203	0.001	0.336
Crotonaldehyde					1	0.247	0.335	0.05	0.34
Benzaldehyde						1	0.455	0.696[a]	0.338
Hexanal							1	0.465	0.784[a]
Isovaleraldehyde								1	0.27
Valeraldehyde									1

[a] 99% confidence level.

Microbial Iidentification

Bacterial Identification

Closest identified phylogenetic relatives (with highest percent similarity) of partial 16S rDNA bacterial sequences obtained are listed in Table 6. The predominant identified strains were *Bacillus sp., Bacillus firmus, Bacillus licheniformis, Bacillus cereus, Bacillus pumilus, Acinetobacter sp.* and *Acinectobactor radioresistens*. Based on our bacterial identification study, the numeration of *Bacillus* was estimated to 69–81% of all identified bacterial genera while *Acinetobacter* comprised 9–14% of all bacterial strain obtained.

Gangamma *et al.* (2011) also reported that *Bacillus* was the prominent genera (63% of the total concentration of bacteria and 79% of the gram-positive bacteria) in the bioaerosol samples of municipal waste water treatment plants of Municipal Corporation of Greater Mumbai, India. Similarly, *Bacillus* was commonly found (35.9%) in the ambient levels of bioaerosols on the Jawaharlal Nehru University (JNU) campus, New Delhi (Srivastava *et al.*, 2012). The majority of bacteria detected at typical open-air restaurant site in Malaysia were gram-positive bacteria (84.3%) (Yusup *et al.*, 2014). Similarly, Gram-positive bacteria were dominant in the indoor air of the restaurants at Hongkong, China, in which *Bacillus* and *Micrococcus* were the most frequently found species (Chan *et al.*, 2009).

The identified *Bacillus* strains (listed in Table 6) are mostly spore forming and can survive in harsh conditions for a longer period. As far as their health effects are concerned, *Bacillus licheniformis* is usually known to cause food poisoning and food spoilage especially in dairy products (Salkinoja-Salonen *et al.*, 1999). *Bacillus cereus*, which was most abundant species in our samples is known for causing food-borne intoxications due to its secretion of emetic toxins and enterotoxins (USFDA, 2007). In addition, *Bacillus cereus* is an opportunistic human pathogen and is linked with periodontal diseases and other infections such as bacteremia, endocarditis, meningitis, pneumsonia, and endophthalmitis in immune-compromised persons (Hoffmaster *et al.*, 2006). *Acinetobactor* is a hardy organism in nature and can survive in wide ranges in temperature, pH and humidity even in dry conditions on particles and dust (Gootz *et al.*, 2008; Manchanda *et al.*, 2010). Similarly, occurrence of *Acinetobactor radioresistens* strain can be attributed to dense crowd present at the sampling site as it is a common bacterial flora of moist skin areas and mucous membrane (Kuo and Chen, 2011).

Fungal Identification

The identified fungal genera were classified as *Aspergillus, Cladosporium, Alternaria, Fusarium, Drechslera Paecilomyces, Penicillium, Mucor, Trichoderma* and *Yeast. Aspergillus, Cladosporium*, *Alternaria* and *Fusarium* were found as most common genera in the samples collected throughout the study period, accounting for almost 78% of all fungal colonies observed on MEA plates (Fig. 4). The significant seasonal variations were observed for *Aspergillus, Cladosporium* and *Alternaria*. *Aspergillus* was the most prominent genera found, especially in winters (51%) followed

Table 6. Phylogenetic relationship of partial 16S rRNA bacterial sequences detected at restaurants-cluster site in New Delhi.

S. No.	Identification (BLAST)	Percentage similarity (%)
1.	*Bacillus sp. (hb38)*	99
2.	*Bacillus sp. (A-BT)*	96
3.	*Bacillus sp. (S11909)*	96
4.	*Bacillus sp. (PM-3)*	96
5.	*Bacillus firmus strain (TY0125)*	99
6.	*Bacillus licheniformis strain (30AA1-3)*	98
7.	*Bacillus licheniformis strain (Sua-BAC012)*	98
8.	*Bacillus cereus strain (WIF15)*	96
9.	*Bacillus cereus strain (SCB001)*	97
10.	*Bacillus cereus strain (DZ102)*	98
11.	*Bacillus cereus strain (30P1-2)*	97
12.	*Bacillus pumilus strain (GC51)*	98
13.	*Acinetobacter sp. (SDT8)*	98
14.	*Acinetobacter sp. (f19)*	99
15.	*Acinetobacter radioresistens gene*	98

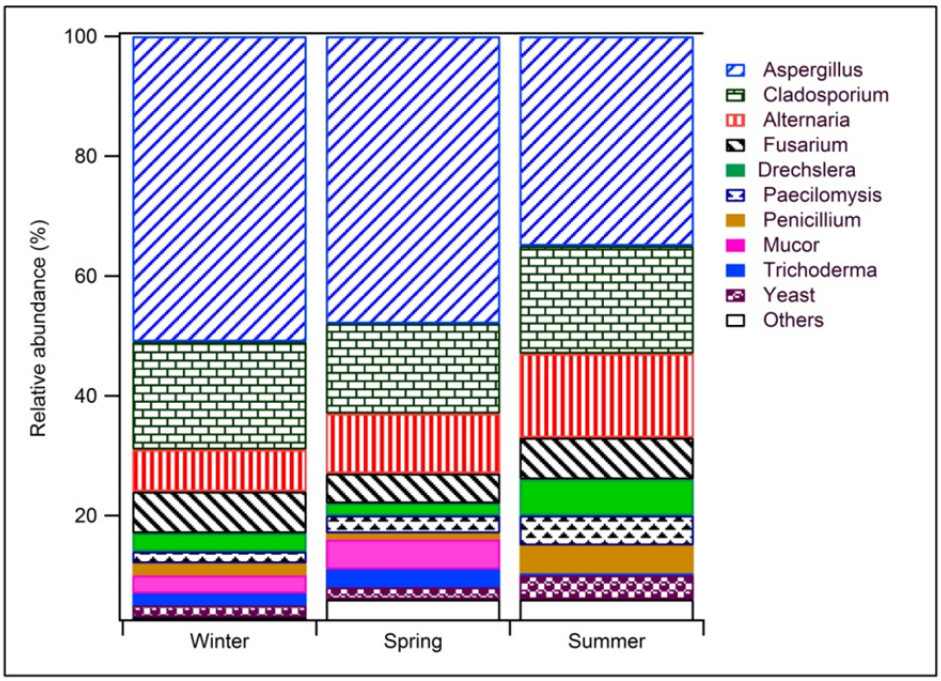

Fig. 4. Relative abundance (%) of fungal genera identified at restaurants-cluster site, New Delhi in winter, spring and summer seasons.

by *Cladosporium* (18%), both are commonly present in food stuffs and soil. *Mucor* and *Trichoderma* were observed only in winter and spring season. No significant differences in the percentage abundances of fungal genera were observed for winter and spring seasons (Fig. 4). Similar to the findings of current study, *Cladosporium, Ustilago, Aspergillus* and *Alternaria* were found as the more prevalent fungi in Delhi's outdoor environment (Gupta *et al.,* 1993). Aerobiological study done in Bangalore city which is located in Southern India showed predominance of *Cladosporium, Alternaria, Aspergillus, Penicillium, Nigrospora, Helminthosporium, Cercospora* and *Curvularia* (Aghase and Vidya, 1997). Important fungal allergens reported worldwide are *Aspergillus, Cladosporium, Alternaria, Penicillium* and *Dechslera* and have been recognized as the major organisms in warm, humid and dry climates (Doory *et al.*, 1984).

Most of the identified fungal spores in present study can be identified as aero-allergens and pathogenic agents of various infections. Members of the *Aspergillus* and *Penicillium* genera are known for causing lung infections as these fungi are able to grow at body temperature (Denning *et al.*, 2014). While, *Cladosporium* and *Alternaria* Species act as allergens, and are associated with severe and fatal episodes of asthma and worse rhinitis, although rarely cause infections (Denning *et al.*, 2014). Overall, it can be stated that people in surrounding area of the restaurant-cluster site

are exposed to opportunistic pathogens and allergens.

It is noticeable that mostly bacterial and fungal spores and hardy species dominated in our sample, which could be possibly due to dehydration stress to other fragile vegetative cells caused by long sampling duration (3–4 hours). Previously, Wang *et al.* (2001) quantified effect of sampling time on the viability of microorganisms. They reported that there was no significant change in the culturability of fungal and bacterial spores for the sampling time ranging from 30 min to 8 hours, which is the usual sampling time for collecting airborne microorganisms with filter samples. However, bacterial vegetative cells lose their viability for the sampling time longer than 10 min.

Correlation between Bioaerosols and Chemical Constituents

CAB and CAF were satisfactorily correlated with TSPM and OC ($R^2 \geq 0.5$, Table 4) indicating that higher concentrations of microbial communities might have contributed to the excessive TSPM and OC concentrations. The fact that large surface area of coarse particles, facilitates the microbial attachment might be the cause of good correlation between TSPM and microbes (however in this study we have not measured fine and coarse particle concentrations). One limitation of this study could be the underestimation of bioaerosols due to the fact that only a fraction of the total bioaerosols collected are culturable. It is reported that less than 1–10% of airborne microbial particles are culturable under specific growth conditions imposed in the laboratory (Tong and Lighthart, 2000; Muratha and Zhang, 2013). However, count-culture method is an easy, convenient and suitable approach for routine analysis because of its low cost and high time resolution. Carbonyl compounds were not found to be correlated with bioaerosols indicative of their diverse sources (Table 4). Interestingly, fungal spores were found to be exceptionally correlated with acetaldehyde (R^2 = 0.75) and acetone (R^2 = 0.34) pointing towards microbial origin of these compounds. It is well known that variety of volatile organic compounds formed in the fungal and bacterial metabolic activities. Scotter *et al.* (2005) reported that acetaldehyde and acetone are among common metabolites of fungi such as *Aspergillus, Fusarium* and *Mucor*, which were prominent species identified in present study.

CONCLUSIONS

Characterisation of aerosols in ambient air samples of a busy roadside restaurants-cluster site in New Delhi during winter, spring and summer of 2014–2015 showed that measured airborne pollutants were likely to be derived from diverse sources such as biomass burning, cooking, vehicular transport and industries. The impact of biomass burning and cooking emissions was found to be more prominent on air quality of the sampling site. The study results showed that biomass burning and cooking exhaust from various restaurants were the most important sources of TSPM, OC and carbonyls, while EC was mostly originated from vehicular exhaust. Carbonyls were likely to be contributed from other sources as well such as vehicular and industrial emissions as well as photochemical formation.

Higher concentrations of airborne microbes were observed in winter and spring rather than summer which is in contrast to the studies done in other countries, suggested that bioaerosol concentration in the atmosphere is a possible variable across climatic zones. *Bacillus* was predominant identified bacterial genera while *Aspergillus, Cladosporium*, *Alternaria* and *Fusarium* were among the most common fungal species identified. These findings provide a better understanding of the airborne microbes present in the ambience of public places and a clue for assessing the seasonal health problem occurs from nose to chest irritation to allergic and pathogenic inflammations resulting from exposure to airborne microbes. The information will be very useful to the policy maker on the issue that bioaerosols measurement could be one of the parameters of National Ambient Air Quality Standards (NAAQS). Continued research in this area is necessary to resolve several questions on the issue.

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