An official publication of the International Society for Energy, Environment and Sustainability (ISEES)

Journal of Energy and Environmental Sustainability

Journal homepage : www.jees.in

Particulate matter pollution: Characterization of bioaerosols in South Indian cities

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ARTICLE INFO

Received : 20 December 2019
Revised : 15 April 2020
Accepted : 20 April 2020

Keywords:
Urban air pollution, bioaerosols, airborne bacteria, protein, endotoxin

ABSTRACT

In the present study, we aimed to report biocomponents associated with airborne particulate matter (PM) collected from South Indian cities. PM-10 samples were collected from ambient air quality monitoring sites from Hyderabad (n=48) and Chennai city (n=44). We are reporting bioaerosols such as proteins, endotoxin, bacterial and fungal DNA concentration from these cities. Measurements have shown that average protein concentration was approximately 5.2% and 4.8% of the average PM mass concentration in the above cities. Our study showed that total bacterial DNA constitutes only a small fraction (0.1-0.01%) of total DNA associated with PM.However, the average fungal DNA concentration was approximately 8.6% and 3.7% of the total DNA concentration. We also found that, airborne endotoxin is significantly correlated with PM mass concentrations ($p<0.001$).In both cities, endotoxin was also found to be correlated with trace elements such as calcium ($p<0.001$). This may indicate that endotoxin might have originated from resuspended dust. Average carbohydrate concentration accounts for 2-4.3% of average PM mass concentration. Taking together, our study shows that biogenic particles are significant part of ambient PM.Also, our study emphasizes that components of biological origin could play a significant role in ambient PM induced adverse health effects and calls for thorough investigations into airborne biological components.

1. Introduction

Epidemiological studies have identified an association between ambient particulate matter (PM) exposure and increased rates of morbidity and mortality (Dockery et al., 1993; WHO, 2016). Studies have shown associations between PM exposure and lung inflammatory and cardiovascular diseases (Tanaka et al., 2013; Wu et al., 2018). Also, it is important to note that several studies have shown evidence for the association of PM exposure with anti-inflammatory usages (Dusseldorp et al., 1995;von Klot et al., 2002). Studies have also shown that, airborne biological particles constitute major portion of atmospheric organic matter and is largely remain unidentified and uncharacterized (Jaenicke 2005; Bowers et al., 2011). In many European sites, airborne biological particles contribute about 25% to the total particulate mass (Fröhlich-Nowoisky et al., 2016). PM consists of several inflammatory inducing biocomponents such as endotoxin, beta-glucan and other pathogen associated molecular patterns (Gangamma 2012; Gangamma 2014). These components are likely to play important role in innate immunity activation. Many studies have shown that the biological components associated with coarse fraction of the ambient PM significantly influence the PM induced proinflammatory response in vitro (Monn and Becker 1999). Microbial components present in the PM were found to be an important component responsible in eliciting the cytokine response (Gangamma, 2014). Therefore, it is important to characterize biocomponents of ambient PM.However, in India, there is a scarcity of studies on biocomponents associated with PM and health effects.

Bacteria in the ambient air environment are originated from natural as well as anthropogenic sources. Sources of bacterial aerosols include agricultural activities, water bodies, animals, soil, waste dumping ground, plants and wastewater treatment plants (Gangamma 2014; Gangamma et al., 2011). Airborne bacteria is an important component of bioaerosols in natural and urban environments (Jaenicke 2005; Elbert et al., 2007; Jaenicke et al., 2007; Gangamma et al., 2011; Gangamma 2014). Endotoxin (lipopolysaccharide, LPS) is a cell wall component of Gram-negative bacteria. Studies have shown the capability of inhaled endotoxins to cause respiratory disease or to have an influence on lung function (Rylander, et al., 1983; Schwartz et al., 1995; White, 2002). Fungi can survive in harsh environmental conditions. Viable forms of fungi can be found in many harsh environments (Elbert et al., 2007). Therefore, fungi are expected to survive in a harsh air environment. Studies have found that fungi are an important fraction of airborne bioparticles (Bauer et al., 2007). In general, the aerodynamic diameter of fungi ranges from 2-20µm (Ingold et al., 2001). Fungal components are identified by innate immune receptors such as mannose receptors, beta-glucan receptors, and toll-like receptors (TLRs). Therefore, these components of fungi may induce a significant level of proinflammatory responses (Levitz, 2004). However, there is a relative scarcity of studies to determine the fraction of fungal content in the biocomponents associated with PM.

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In the present study, we are reporting PM associated biocomponents such as protein, carbohydrates, endotoxin, bacterial and fungal DNA in Hyderabad and Chennai city. We have also studied the relationship between these biocomponents and PM mass concentration. The study shows that such a relationship may indicate that there is a significant contribution of the biogenic particle to PM mass concentration.

2. Methods and materials

2.1. Sampling of airborne particulate matter

Airborne particulate matter samples (PM-10) were collected from Hyderabad and Chennai cities. Chennai is a coastal city (13°4' N, 80°14'E) situated in the southern part of India. The land use pattern is residential cum commercial with a total population of over 7 million. General sources of PM in the city include vehicular emissions, marine aerosols, industrial emissions and re-suspended road dust (CPCB, 2010). All samples were collected from five ambient air quality monitoring sites of TNPCB in Chennai, India (Figure 1A). Adyar (AD) sampling site is located in the southern part of the city. It is a residential and commercial site. Nungambakkam (NB) and Arumbakkam (KL) sampling sites are located on the central and western sides of the city. Ambattur (AB) sampling station located on the north-western side of Chennai city, is surrounded by small and medium scale industries. The site is well known industrial area in the city but also includes unplanned residential areas. This site is likely to receive PM from industry, vehicular emissions and re-suspended road dust. T Nagar (TN) is situated in the central part of the city and commercial-residential site. This site mainly receives PM from vehicular emissions.

Hyderabad is an inland city (13°23' N, 78°29'E) situated in the southern part of India. The land use pattern is residential cum commercial with a total population of over 6.8 million. General sources of PM in the city include vehicular emissions, industrial emissions and re-suspended road dust (CPCB, 2010). All samples were collected from seven ambient air quality monitoring sites of TSPCB in Hyderabad, India (Figure 1B). Sanathnagar (TS) is situated on the north-western side of the city. This site is likely to receive PM from vehicular emissions. Rajendranagar (RJ) is located on the south-west side of the city. This sampling is classified as a residential cum background site. Charminar (CM) is located in the central part of the city. This sampling site is considered as a commercial and traffic area. Uppal is located on the eastern side of the city. Uppal (UP) and Jeedimetla (JM) sites are well known industrial areas in the city. Madhapur (MP) sampling sites are located on the north eastern side of the city. This is a very well known information technology sector of the city and can be considered as a commercial area. Panjakutta (PJ) sampling site receives PM from vehicular emissions.

Particulate matter samples (PM-10) were collected using High volume samplers on quartz fiber filter paper (GE Healthcare, India). The filter papers were de-pyrogenated, conditioned and weighed prior to sampling. A total of forty four and forty eight 8 h average samples were collected from Chennai and Hyderabad. Field blanks were included to ensure contamination free sampling. The PM samples were sealed and transported to the laboratory for further analysis**.**

2.2. Characterization of airborne particulate matter

Airborne particulate matter was extracted into endotoxin free water and stored at -20^{0}C till further analysis. Total protein concentration was measured using BCA assay kit (Thermo Scientific, India). Bovine serum albumin (BSA) is used in preparation of a standard graph to calculate protein concentration of PM samples.Airborne endotoxin was measured using kinetic LAL assay kit (Lonza, India). Endotoxin extracted from *E.coli* is used to develop a standard graph.Airborne carbohydrates were measured using phenol-sulphuric acid method in 96 well plate format (Masuko*et al.*, 2005).Glucose was used to develop a standard graphand carbohydrate concentrations are reported in equivalence to glucose. PM samples were also subjected to acid extractionand analysed for trace elements using ICP-OES (Agilent, India).

2.3. Measurement of bacterial and fungal DNA of particulate matter samples

For analyzing total DNA of samples, filter samples were extracted using phosphate buffer saline (PBS) and extract was filtered through membrane syringe filter. Further, filter paper was cut into small pieces and DNA was extracted using modified conventional extraction method. The DNA sample was eluted into molecular grade water and stored at -80^{0}C for further analysis.Genomic DNA of *Staphylococcus aureus* was used for preparing standard graph. Purity of extracted DNA was measured using spectrometer (Eppendorf, India). Degenerative primers were synthesized (Sigma, India) for measurement of total bacteria using real time PCR. SYBR Green master mix (Lifetech, India) with Forward primer (5'-CCTACGGGDGGCWGCA-'3) and Reverse primer (5'-GGACTACHVGGGTMTCTAATC3') were used in PCR (Liu et al., 2012). Similarly, fungal DNA concentration of PM samples was measured using real time PCR.The PCR was performed with forward primer ITS1-27F (5'- TACGTCCCTGCCC TTTGTAC-'3) and reverse primer ITS1-217R (5'- TTTCGCTGCGTTCTTCATCG) (Usyk et al., 2017). Genomic DNA from *Phanerochaetechrysosporium* was used for construction of standard graph.

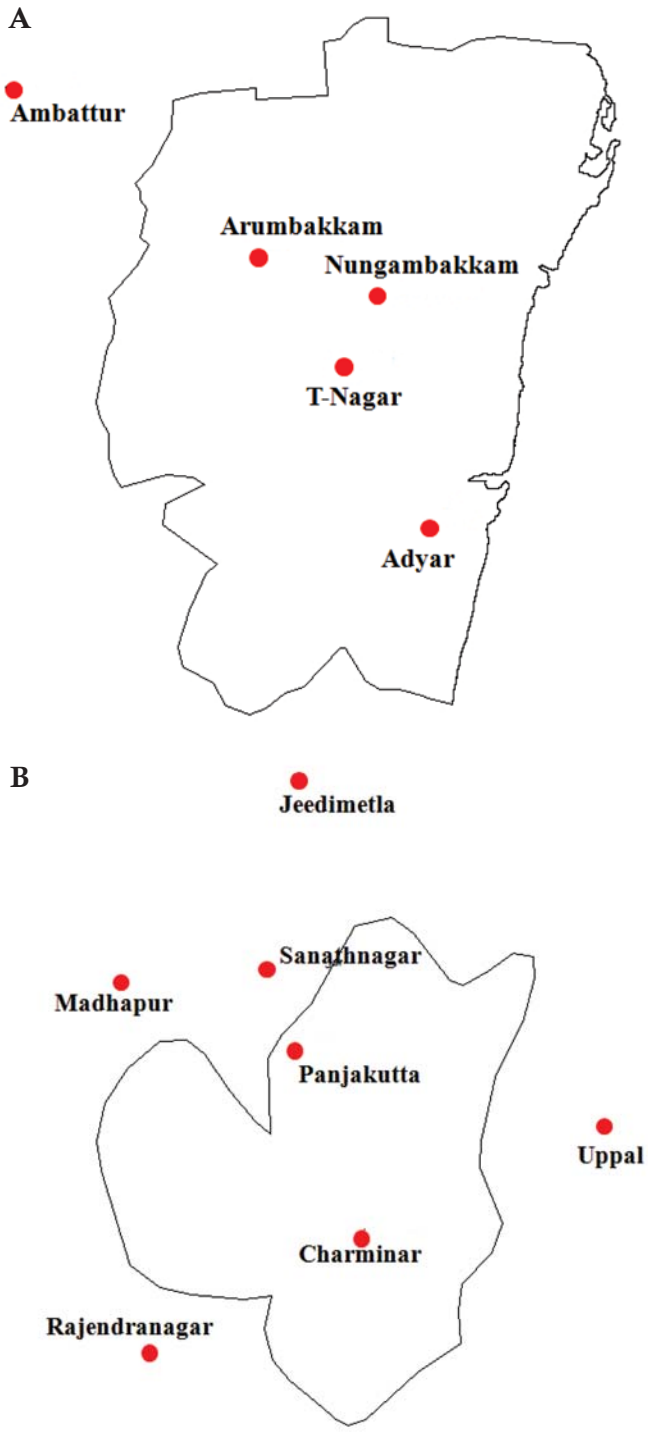

Figure 1.Airborne particulate matter sampling locations. A) Chennai city, B) Hyderabad city

3.0. Results and Discussions

3.1. Significant levels of airborne protein and DNA concentration in South Indian urban cities

We have measured PM associated protein levels using BCA method. There is 48 and 44 number of PM samples from Hyderabad and Chennai respectively.Total protein levels in Hyderabadwere ranged between 1.75 and 20.4μg/m^3 with a standard deviation of 4.6μg/m^3.Average site wise concentrations of protein in the samples were depicted in Figure 2A. In Chennai, total protein levels were ranged between 0.3 and 9.4μg/m^3 with astandard deviation of 1.7μg/m^3 Average site wise concentrations of protein in the samples were depicted in Figure 2B. There is significant($p<0.001$) variation of protein concentration across the sampling sites in Hyderabad city. Similarly, there was variation of protein concentration across sampling sites in Chennai ($p<0.01$). The concentrations of airborne protein in Hyderabad are comparatively high for an urban environmental setting (Menetrez et al., 2007; Chen and Hildemann, 2009).There is significant correlation was observed between protein and PM concentration in Hyderabad($p<0.0001$, Figure 2C). However, there is no correlation between protein and PM concentration in Chennai samples.Total DNA was extracted from PM samples. Concentration of DNA in the extract was measured using Biophotometer (Eppendorf, India). In Hyderabad PM samples, concentration of DNA was found to be varied in the range between 24.7 and 177 ng/m^3. There was a variation in the concentration of DNA across the sampling sites in Hyderabad ($p<0.01$). Highest concentrations were reported from Rajendranagar, Madhapuraand Jeddemetlasampling sites.Similarly, in Chennai samples, concentration of DNA was found to be varied in the range between 54 and 213 ng/m^3. Results show that, there is no variation in the concentration of DNA across sampling sites in Chennai samples. Results are also shows that, there is no correlation between PM and DNA concentration of samples from Chennai and Hyderabad respectively.This is first kind of study reporting DNA concentration of ambient PM.Protein and DNA can be thought as a marker for biological components in the samples. Correlation between protein and PM concentration at Hyderabad samples may indicate that there is a significant contribution of biogenic particle to PM mass concentration.

3.2. Bacterial DNA accounts for only a small fraction of total DNA associated with airborne PM

Bacterial DNA concentration in the PM extract was determined using real time PCR (Life technologies, India). Total bacterial DNA concentration in Hyderabad was ranged between 2.2 and 422pg/m^3 with an average of 96pg/m^3.There was a variation in the concentration of bacterial DNAacross the sampling sites in Hyderabad ($p<0.01$). Highest concentrations were reported from Charminar, Shanthnagar and Jeddemetla sampling sites. In Chennai, total bacterial DNA concentrationswere ranged between 1.15 and 45.2pg/m^3 Bacterial DNA concentration did not shown correlation with protein, PM mass or total DNA concentration. The average bacterial DNA concentration was approximately 0.1% and 0.01% of the total DNA concentration in Hyderabad and Chennai samples respectively.This implies that bacterial DNA is only a small fraction of total DNA in the air samples and presently we do not know about all sources contributing to the total DNA.A complete analysis of contribution of human, plants and microorganisms are needed to apportion the total DNA found in the air samples. This kind of analysis also may reveal the sources of biological components in the samples.

3.3. Significant levels of airborne fungi in South Indian cities

Fungal DNA concentration in the PM extract was determined using real time PCR. The PCR was performed using SYBR Green master mix with forward primer ITS1-27F (5'- TACGTCCCTGCCC TTTGTAC-'3) and reverse primer ITS1-217R (5'- TTTCGCTGCGTTCTTCATCG) (Usyk et al., 2017). The standard graph for measurement of fungi concentration was prepared using *Phanerochaetechrysosporium* culture.The fungi DNA concentration in Hyderabad was found to be ranged between 0.2 and 30.8ng/m^3. There was a variation in the concentration of fungal DNA concentration across the sampling sites in Hyderabad ($p<0.01$). Highest concentrations were reported from Charminar, Rajendranagarand Jeddemetla sampling sites. In Chennai, total fungal DNA concentrations were ranged between 0.28 and 41.65ng/m^3 There was a variation in the concentration of fungal DNA concentration across the sampling sites in Chennai ($p<0.01$). The average fungal DNA concentration was approximately 8.6% and 3.7% of the total DNA concentration in Hyderabad and Chennai samples respectively.This implies that fungal DNA is significantly contributed to total DNA in the air samples and

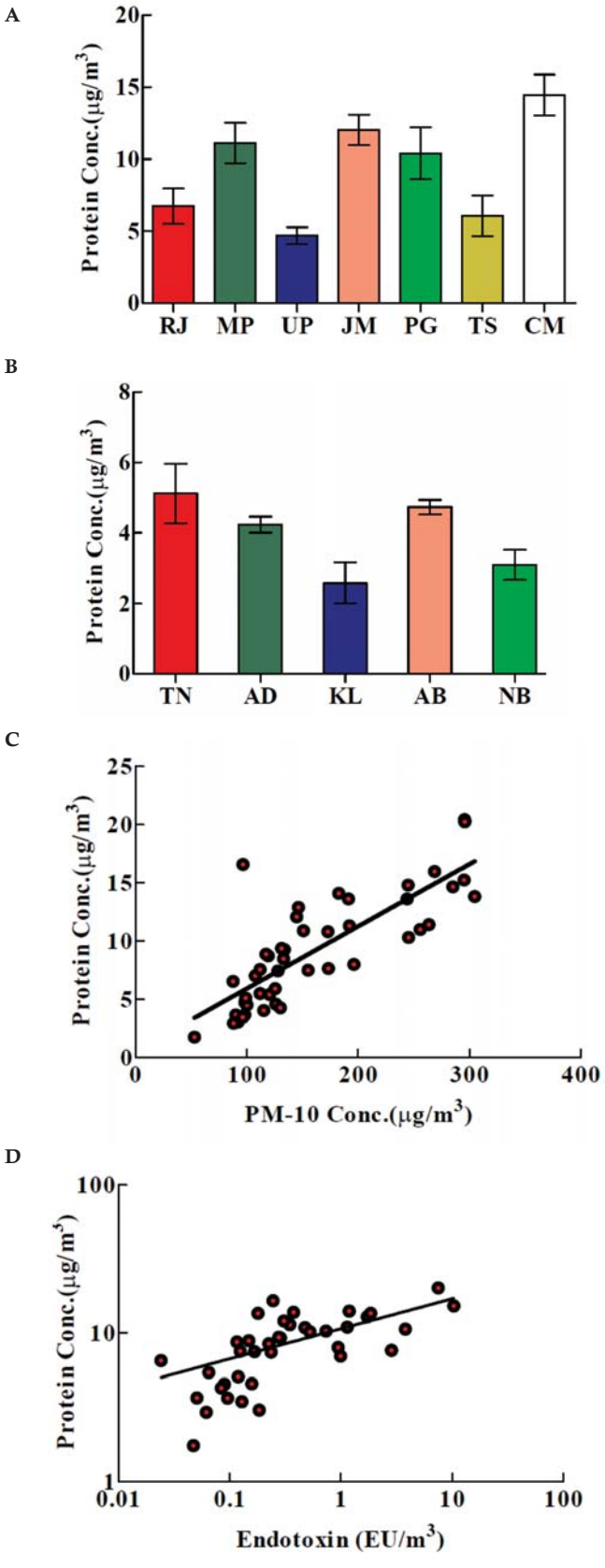

Figure 2. Airborne protein concentration was measured using BCA method. A)Average site wise concentrations of protein in the samples collected from Hyderabad, B) Average site wise protein concentration of from Chennai, C) Relationship between airborne protein and particulate mass concentration, D) Correlation between airborne endotoxin and protein concentration

presently we do not know about all sources contributing to the total DNA.There is significant correlation was observed between protein and fungal DNA concentration in Hyderabad ($p<0.001$).This correlation may indicate that, fungi are source of protein associated with PM. However, fungal DNA concentration did not correlate with PM and total DNA concentration in Hyderabad samples.In Chennai, there is no correlation between fungal DNA, protein and PM concentration was observed.Several studies have been conducted to measure airborne fungi and bacteria at different urban environment in India (Kumar et al., 2013; Agarwal et al., 2016; Lal et al.,2017; Balyan et al.,2017; Sivasakthivel and Nandini, 2017). However, these studies have reported culturable fungi and bacterial aerosols. The current study utilized non-culture based method for reporting the concentration of fungi and bacteria. These studies report significant levels of fungi in ambient PM. Many of airborne fungi and bacteria cannot be cultured on single growth media and this may underestimate concentrations of these bioaerosols. Apart from culturable, non-viable bacterial and fungal aerosols have also play significant role in human health (Gangamma 2014).

3.4. Concentration of airborne endotoxin in South Indian cities

Endotoxin levels in the ambient PM samples were determined using kinetic LAL assay. Endotoxin concentration in Hyderabad was found to be ranged between 0.02 and 10.4EU/m^3. There was a variation in the concentration of endotoxinacross the sampling sites in Hyderabad ($p<0.01$). High concentrations of endotoxin were observed in Charminar sampling site.There are no ambient standard for endotoxin concentration. However, comparison with occupational exposure limit may give significance of the observed endotoxin concentration. In Hyderabad, none of endotoxin of PM sample exceed exposure limit proposed by the Dutch Expert Committee (50 EUm^{-3}, Spaan et al., 2006). Figure 2D shows that, the concentration of airborne endotoxin is significantly correlated with protein levels of PM samples ($p<0.001$).We also found that, airborne endotoxin is significantly correlated with PM mass concentration ($p<0.001$, Figure 3A). Endotoxin also found to be correlated with trace elements such as calcium ($p<0.001$), magnesium ($p<0.01$) and potassium ($p<0.05$) (Figure 3B, 3C). Calcium and magnesium represents PM sources such as resuspended dust or crust elements. Potassium may also represent sources of PM such as biomass/refuse burning or sea salt.

In Chennai city, endotoxinconcentrations wereranged between 0.5and 65.26 EU/m^3. There was a variation in the concentration of endotoxin across the sampling sites in Chennai ($p<0.001$).Highest concentrations were reported from Nungambakamand TNagar sampling sites.We found that, the concentration of airborne endotoxin is significantly correlated with PM massconcentration ($p<0.001$, Figure 3D). However, airborne endotoxin did not correlate with protein concentration of PM samples.In Chennai, a small percentage (7.5%) of endotoxin samples exceed exposure limit proposed by the Dutch Expert Committee. Endotoxin also found to be correlated with trace elements such as calcium ($p<0.001$) and magnesium ($p<0.001$). But unlike Hyderabad, airborne endotoxin in Chennai did not correlate with potassium. Potassium may also represent sources of PM such as biomass/refuse burning or sea salt. Therefore, airborne endotoxin is not related to biomass/refuse burning in the city.

Endotoxin (lipopolysaccharide, LPS) is a component of the outer membrane of Gram-negative bacteria. Endotoxin is a combination of a polysaccharidepart and a lipid (lipid A) portion. Endotoxin is a potent proinflammatory agent in humans.Animal and epidemiologic studies have clearly shown the capability of inhaled endotoxins to cause respiratory disease, or to have influence on lung function (Rylander, et al., 1983; Schwartz et al.,1995; White, 2002). Therefore measurement of airborne endotoxin assumes importance.In Hyderabad, correlations were found between PM mass, endotoxin and potassium concentration. This indicates that, biomass/refuse burning may have associated with endotoxin. Therefore biomass/refuse burning sources should be further characterized in Hyderabad city.

3.5. Concentration of airborne carbohydrate in South Indian cities

We made an attempt to measure the carbohydrate concentration of PM collected from Hyderabad and Chennai city. Carbohydrate was measured using phenol-sulphuric acid method in microplate format (Masuko*et al.*, 2005).Glucose was used to develop a standard graph for carbohydrate measurements. Therefore, carbohydrate concentrations are reported in equivalence to glucose.Airbornecarbohydrate concentration in Hyderabad city varied between 3.3 to 15.1 μg/m^3 (Figure 4A). There is no variation in the carbohydrate concentration across the sampling sites in the city. Carbohydrates are used as marker for biogenic sources. However, average carbohydrate concentration is accounts for only 4.3 %

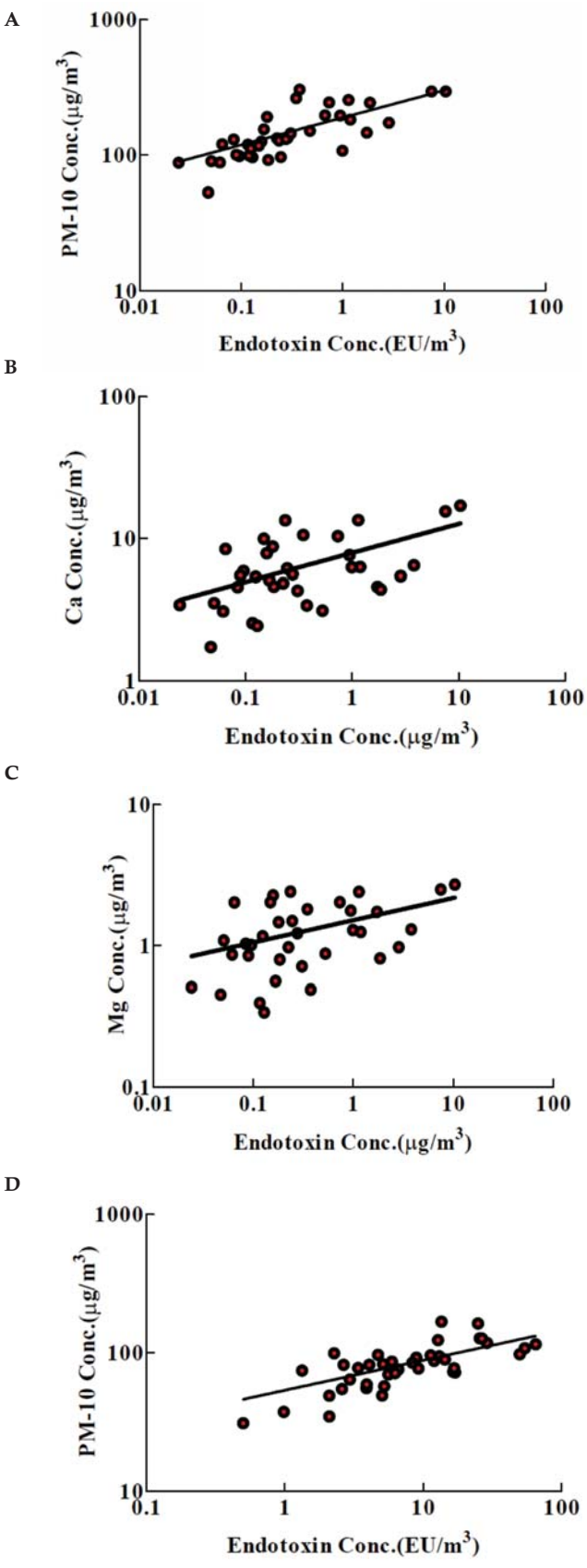

Figure 3. Airborne endotoxin concentration was measured using LAL kinetic assay. A) Relationship between airborne endotoxin and particulate matter mass concentration, B) Correlation of airborne endotoxin with Calcium concentration, C) Correlation of airborne endotoxin with Magnesium concentration, D) Correlation between airborne endotoxin and particulate mass concentration in Chennai city samples

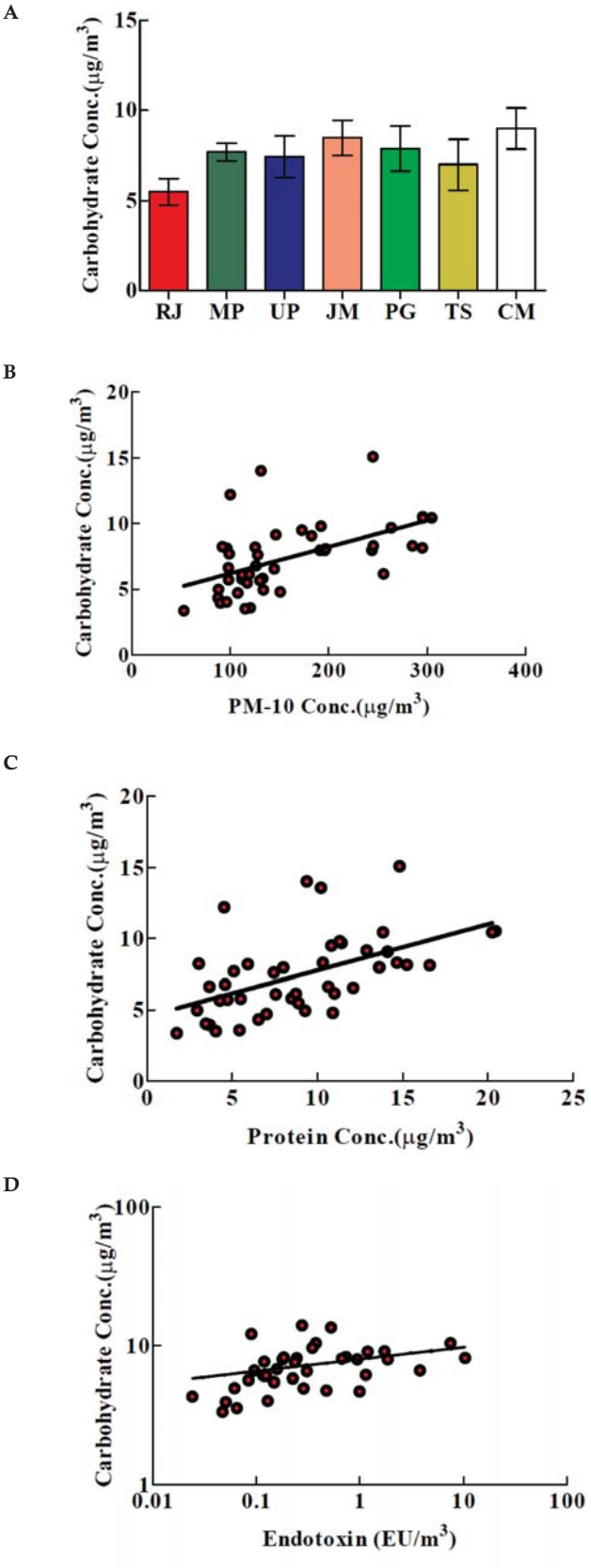

Figure 4. Airborne carbohydrate concentration was measured using phenol-sulfuric acid method. A)Average site wise concentrations of carbohydrates in the samples collected from Hyderabad, B) Relationship between airborne carbohydrate and PM mass concentration. C) Correlation between airborne carbohydrate and protein concentration, D)Correlation between airborne carbohydrate and endotoxin concentration

of PM mass concentration. Further, carbohydrate concentrations show correlation with PM mass concentration ($p<0.001$, Figure 4B), protein ($p<0.001$, Figure 4C) and endotoxin ($p<0.001$, Figure 4D). Trace elemental analysis shows that, carbohydrate is weakly correlated with calcium ($p<0.05$). However, carbohydrate did not correlate with other biocomponents such as total DNA, bacteria and fungi concentration.

In Chennai, airbornecarbohydrate concentration varied between 0.5to 3.4μg/m^3. There is no variation in the carbohydrate concentration across the sampling sites in the city.The average carbohydrate concentration is accounts for only 2 % of PM mass concentration. Further carbohydrate did not show correlation with PM mass concentration and biocomponents of PM. It also did not show correlation with trace elements.Few studies have also reportedsimilar concentrations of carbohydrate from urban environment (Simoneit*et al.*, 2004; Didyk*et al.*, 2000).

Carbohydrates are an important class of soluble organic compounds. Studies have shown that, carbohydrates are significantly contributed to PM (Fu et al., 2008;Jia and Fraser, 2011). Sources of airborne carbohydrates include bioaerosols such as fungi, bacteria and vegetative dust (Jaenicke 2005). Our measurements showed that carbohydrates constitute a significant portion of PM (2-4%). This is higher than the reported carbohydrate concentration in PM (Fu et al.,2012). Carbohydrates are one of the important components of cellular material. Therefore, present study indirectly indicates a significant presence of bioparticles in airborne PM.

4. Conclusions

Our measurement showed that significant levels of protein present PM samples collected from Hyderabad and Chennai city. Correlation between protein and PM concentration at Hyderabad samples may indicate that there is a significant contribution of biogenic particle to PM mass. Measurements showed that total bacterial DNA constitutes only a small fraction of total DNA associated with PM. However, our study results show that fungal DNA is significantly contributed to total biocomponents in air samples. Airborne endotoxin levels were associated with resuspended dust source markers. Endotoxin levels were also correlated with PM mass concentrations and a significant part of PM is resuspended dust (CPCB,2010). Therefore, endotoxin may be mainly originated from resuspended dust. Airborne carbohydrates were account for 2-4% of the PM mass concentration. Therefore, the present study calls for a thorough characterization of biological components of PM and their role in adverse health effects of PM exposure.

Acknowledgements

The authors express their sincere thanks to all officials of Tamil Nadu Pollution Control Board Chennai and Telangana State Pollution Control Board, Hyderabad for extending facilities and cooperation throughout the sampling program.Authors express thanks to Ms. Veekshitha, Ms Sampada and Ms Manisha for their help in carbohydrate analysis. Authors also express thanks to Ms. Rashmi Kurli for her help during sampling program.

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