Environmental Science & Technology

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# Characterization and Proinflammatory Response of Airborne Biological Particles from Wastewater Treatment Plants

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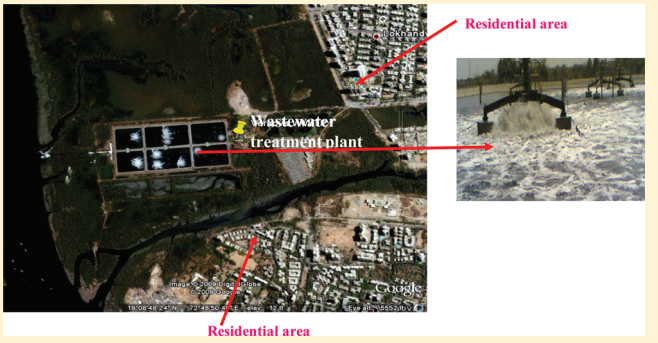
Supporting Information

**ABSTRACT:** Wastewater contains a variety of microorganisms, and unit operations in the plants could release these biological components into the air environment. These airborne biological particles could have adverse health effects on plant workers and the downwind population. This study provides a first report on the concentration and characterization of the airborne biological particles in six wastewater treatment plants in Mumbai, India. The study indicates that 49% and 27% of the samples exceed, respectively, the exposure limit for airborne endotoxin and bacteria in occupational settings. Endotoxin was identified as the single most important component of the particulate matter responsible for induction of proinflammatory indicator (tumor necrosis factor-α) in in vitro assay. Identification of several clinically important bacterial species in the samples suggests that the workers at the treatment plant are exposed to opportunistic and infectious bacteria. Principal component analysis was used to identify the groups among the bacterial species which serves as the signature for transport study. Analysis also shows that the component related to spore-forming bacteria is present in all samples.






## INTRODUCTION

Wastewater contains a variety of potentially pathogenic microorganisms and their toxic components. Several unit operations such as surface aeration, mechanical sludge removal, and screening in treatment plants may cause aerosolization of these biological components into the air environment.[1,2] Depending on the physical properties of these aerosolized biological particles and meteorological conditions, they are likely to be carried by the air currents and may reach significant distances.[3−5] This in turn could expose the workers working in the immediate vicinity to these biological components and may also affect the downwind population. Bacteria are an abundant and important class of the microorganisms present in wastewater treatment plants (WWTPs). Studies have shown that exposure to airborne bacteria and its components, such as endotoxin, has a significant effect on human health.[6,7]

Several studies have characterized the airborne bacteria in WWTPs. The important bacterial species reported in the literature includes species belonging to the genus *Klebsiella, Serratia, Enterobacter, Citrobacter, and Pseudomonas*. The occurrence of these airborne bacterial species is found to vary from plant to plant due to the characteristics of wastewater, operational characteristics, and meteorological parameters.[8−10] Many of the bacterial species found in WWTPs have been linked with short-term as well as long-term heath effects in the workers.[6,11] Exposure to airborne bacteria causes respiratory tract infections, hypersensitivity, and allergies among workers. A significant health hazard is also posed by microbial toxins, of which endotoxin is considered the most important in WWTPs.[12,13]

Endotoxin is a cell wall component of Gram-negative bacteria (GNB) which plays an important role in bacterial pathogenicity.[14] Airborne endotoxin is found to be an important factor associated with respiratory diseases in various occupational settings.[15,16] The most common symptoms reported among WWTPs workers include respiratory disorder, gastrointestinal tract infections, joint pain, fatigue, and headache.[17,18] Endotoxins are potent activators of several immune cells such as macrophages and epithelial cell line both in vitro and in vivo. Several studies have shown that endotoxin explains a major part of the total pro-inflammatory cytokine production from human immune cells activated by the ambient particulate matter.[19,20] These studies indicate that even trace levels of endotoxin must be considered in evaluating the immune responses of airborne particulates. Even though health symptoms and endotoxin concentration are correlated in WWTPs, the role of endotoxin associated with WWTP particulate matter (PM) in the inflammatory responses is not well studied.

**Received:** October 29, 2010
**Accepted:** March 3, 2011
**Revised:** February 28, 2011
**Published:** March 22, 2011

In spite of their importance, the biological particles in WWTPs has not been well studied in India. There is a scarcity of data on the characteristics of biological particles near WWTPs as well as similar sources in India. Particularly, the location of some of these plants in Mumbai also makes these studies relevant. Many of the WWTPs in the Mumbai peninsula are situated near highly populated areas of the city, and under favorable meteorological conditions, the airborne microorganisms generated at the plants could be transported to the nearby populated areas. Moreover, Mumbai is located in a subtropical region, and its warm and humid climate provides favorable conditions for survival of bacteria and bacterial components in the ambient environment. The present study characterizes the airborne bacteria and elicits the influence of endotoxin on inflammatory induction in in vitro assay across the six WWTPs in Mumbai. This study is part of an endeavor to address the airborne biological particles and determine their health effects at these locations. The results of this study would provide preliminary information for assessing the health effects and transport of these biological particles from WWTPs in Mumbai.

## MATERIAL AND METHODS

**Sampling of Airborne Bacteria and Endotoxin.** The monitoring of airborne biological particles was carried out at six municipal WWTPs of the Municipal Corporation of Greater Mumbai (MCGM), Mumbai, India. The monitoring of bioaerosols in these sites was carried out during May−June 2009. The location of these sampling sites is depicted in Figure S1, Supporting Information. The details of the location of each site and sampling location inside each WWTPs are briefly given in the Supporting Information.

A biosampler (SKC Inc., PA, USA) was used for sampling of airborne endotoxins and bacteria. Before each sampling, the samplers were made endotoxin free by baking them at 270 °C for 30 min.[21] The endotoxin-free biosampler was filled with 20 mL of endotoxin-free water (Lonza, India), and air was sampled at a flow rate of 12.5 LPM over a period of 60−90 min. The exact sampling time was decided based on approximate estimates of aerosol concentration at the various sampling sites. Some loss of sampling medium due to evaporation was observed during sampling. Hence, after 30 min of sampling, the sampling medium was added aseptically. To ensure contamination-free sampling, field blanks were included for each set of samples. The samples were distributed into aliquots and stored at −20 °C for analysis of endotoxin.[22] All samples were analyzed for the presence of culturable bacteria within 24 h of their collection.

**Concentration of Airborne Bacteria and Endotoxin.** In the laboratory, each sample was plated in triplicate onto tryptic soya agar (TSA) plates for determining the total culturable bacteria. The agar medium was prepared using 40 g/L TSA (Himedia, India) and supplemented with 0.5 g/L cycloheximide (S.D.fine Chemicals, India). A 100−250 $\mu$L aliquot was used for plating, and uniform spreading was achieved with the help of Plate Master (Himedia, India). The plates were incubated at 37 ± 1 °C for 2−5 days. The concentration of airborne bacteria was reported as CFU/m$^3$.

The samples stored at −20 °C were analyzed for airborne endotoxin using the kinetic quantitative chromogenic limulus ambeocyte lysate (KQCL) assay method (Lonza, India). The results were expressed in terms of endotoxin unit (EU) and have a potency of 13 (EU/ng). The assay was carried out according to manufacturer recommendations. All standards, samples, field blanks, and endotoxin-free water was analyzed in duplicate.

**Characterization of Airborne Bacteria.** The individual colonies observed on TSA plates were selected and isolated based on their morphology, including size, shape, color, and surface properties. A total of 174 distinct colonies of bacteria were isolated from different plates. Samples were also plated on MacConkey and Eosin methylene blue (EMB) agar to isolate most of the bacterial species present in the samples. However, the number of colonies observed on these media was not used for determining the concentration of airborne bacteria.

The bacterial colonies were identified up to species level using the Biolog Manual System-1(Biolog, Inc., Hayward, California). The classification and identification protocol was followed according to the manufacturer. The steps used for these classifications are outlined in Figure S2, Supporting Information. The screening protocol is described in the Supporting Information. Significant efforts were made to test the Biolog Manual identification system in the laboratory. Known bacterial species were use to test the ability of the identification system. Some of the bacteria were also cross checked by growing them on selective growth media. A few of the bacterial colonies were also checked with the 16s RNA technique. Sequencing was carried out at Macrogen, Korea. Further analysis of results for identification of species was performed using the NCBI BLAST analysis system. The results confirm the biolog identification system.

**Whole Blood Assay and Proinflammatory Response Induced by Airborne Endotoxin.** Twenty five randomly selected samples were subjected to the whole blood assay (WBA) for measuring the pro-inflammatory cytokine such as tumor necrosis factor (TNF-α). Venous blood, collected in a EDTA-coated vacutainer (BD bioscience, India) from a healthy donor, was used within 2 h of withdrawal. The fresh WBA was conducted by adding 100 $\mu$L of blood into a pyrogen-free tube containing 100 $\mu$L of samples and 350 $\mu$L of 0.9% saline. The mixture was incubated at 37 °C for 18 h. Cell viability was assessed at regular intervals during an experiment using trypan blue exclusion assay. The negative saline controls were included in the experiments. Measurement of TNF-α was carried out as per the manufacturer's instruction (Invitrogen, India). A brief protocol for the assay is explained in the Supporting Information. All samples and controls were analyzed in duplicate. Similar experiments were repeated for standard endotoxin (Sigma, India) samples at different concentrations with the same lot of whole blood.

**Endotoxin Removal Using polymyxinB Sulfate.** In order to elucidate the role of endotoxin on TNF-α production, samples were treated with polymyxin B sulfate-coated agarose beads (10 $\mu$g/mL; Sigma, India) for 30 min to neutralize the endotoxin. The endotoxin concentration was reduced to >99% after pretreatment. The bioactivity of the supernatant was determined using whole blood assay as described above.

**Meteorological Parameters.** Air temperature, humidity, wind speed, and wind directions were recorded throughout the sampling period. The temperature and humidity were recorded every minute using a HOBO data logger (Onset, HOBO U12, USA). A hand-held anemometer and wind vane (Weather Technologies, India) were used to record the wind speed and wind direction.

**Statistical Analysis.** The Lilliefors (a variation of the Kolmogorov−Smirinov test for goodness of fit) nonparametric test was conducted to check if the airborne endotoxin and bacteria data was normally distributed. The variation in the concentration of

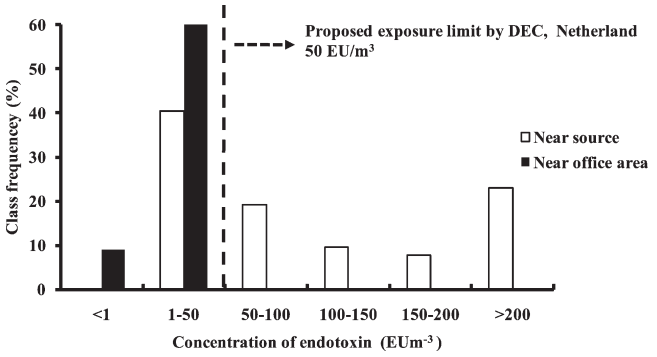



**Figure 1.** Frequency distribution of airborne endotoxin concentrations at six wastewater treatment plants. Concentration of airborne endotoxin of all samples across six wastewater treatment plant was grouped into six groups. The figure shows the exposure limit proposed by DEC, Netherlands (dashed vertical line). The figure also indicates that a significant portion of the samples contains endotoxin concentration greater than 200 EU $m^{-3}$.

airborne endotoxin and bacteria among WWTPs was statistically verified using the Kruskal−Wallis test. The Mann−Whitney-U (MWU)[23] test was used to verify the significant difference in the concentration of airborne endotoxin and bacteria near the source and office area. Principal component, factor analysis (Q mode and R mode) was used for identification of bacterial groups (components) and their contributions in the samples (factor scores). Varimax rotation was used to improve the difference between the principal components. A program in FORTRAN was prepared for the above analysis, and the program was well tested.[24]

## RESULTS AND DISCUSSION

**Endotoxin Is Identified As the Single Major Component Causing Pro-Inflammatory Response.** In the WWTPs, the endotoxin concentration varied over the range of 0.8−741 EU $m^{-3}$. The concentration variation in the plants is due to the operational variability, source water characteristics, and sampling conditions such as wind speed and sampling location. The range and average concentrations of airborne endotoxin observed in the samples are summarized in Table S2, Supporting Information. These values are approximately in the range of values reported in the literature.[7,10] The concentration of endotoxin of field blanks was found to be below the detection limit of the assay. The significance of these concentrations can be deduced by comparing with standard exposure limits. About 49% of the overall samples exceeded the exposure limit proposed by the Dutch Expert Committee (DEC) (50 EU $m^{-3} \approx 4.5$ ng $m^{-3}$)[25] for exposure to airborne endotoxin in an occupational environment (Figure 1). The mean concentration near the source is 1 order of magnitude greater than the corresponding concentration in the office area of plants (Figure 1). However, a similar reduction was not observed for bacterial concentration (discussed in a later section). The reduction in endotoxin concentration may not be due to atmospheric dilution alone. Another possibility is that the bigger droplets containing the endotoxin could have evaporated and collected with lesser efficiency by the sampler.[26] The frequency plot in Figure 1 indicates that a significant portion of the samples contains an endotoxin concentration > 200 EU $m^{-3}$. This corresponds to a

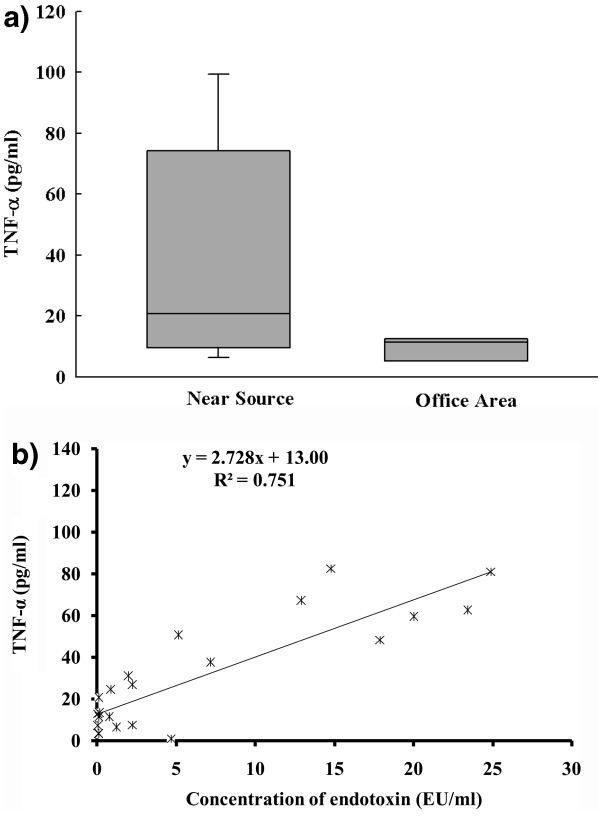



**Figure 2.** Samples collected from WWTPs were used to stimulate the immune cells in the whole blood assay. The TNF-α produced by activated immune cells was measured. The measured TNF-α was used as a measure of the potency of the samples to produce proinflammatory cytokine. (a) Box plot composed of five horizontal lines displaying the 10th, 25th, 75th, and 90th percentiles. Values above the 90th percentile or below the 10th percentile are plotted separately. It compares the proinflammatory potency of samples from near source and office areas of WWTPs. (b) Plot between endotoxin content in the samples and TNF-α produced in WBA stimulation. The straight line represents a linear fit between the data points with a correlation coefficient of 0.75.

lung deposition with a lower bound of 16 and upper bound of 110 ng of endotoxin during 8 h exposure.[27] The concentrations observed in the current study and in vivo/in vitro studies[28,29] indicate that endotoxins are likely to be an important parameter and may have significant health impacts.

The WBA method was used to determine the in vitro pro-inflammatory response (TNF-α) induced by the airborne samples. Figure 2a depicts the TNF-α induced by the samples. As we expected, the samples near the source have a high concentration (approximately 4-times) of TNF-α compared to samples from an office area. Samples have not shown any significant differences among WWTPs or between the treatment plant operations. In addition, TNF-α concentration showed a positive correlation with endotoxin concentration of samples (Figure 2b). This correlation with a single component may have significance because the airborne samples contain a multitude of components present in the wastewater, and many compounds have potential to produce pro-inflammatory response. Therefore, further studies have been conducted to elucidate the biological activity of endotoxin in the sample. We removed endotoxin from the samples by polymyxin pretreatment, and the TNF-α production was measured. The experimental results showed that

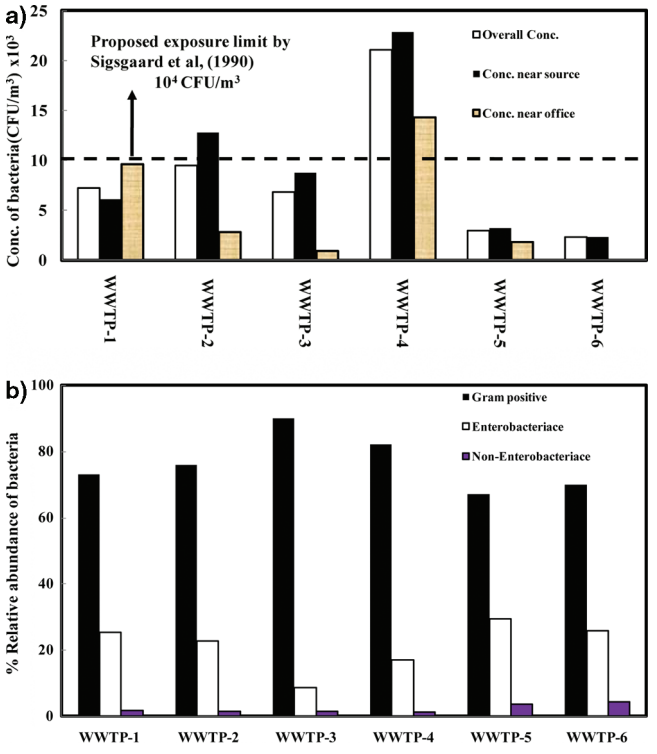



**Figure 3.** Samples collected from WWTPs were plated onto tryptic soya agar (TSA) plates for determining the total culturable bacteria. The distinct colonies were selected based on color size and surface properties of colonies. Several screening tests were conducted to classify the colonies further into different classes. (a) Total culturable bacteria concentration at near source and office areas of WWTPs. Figure also indicates the exposure limit proposed by Sigsgaard et al., 1990 for occupational environments (dashed horizontal line). (b) Concentrations of airborne Gram-positive and -negative bacteria in the WWTPs.

pro-inflammatory production of the samples was completely inhibited by polymyxin. The standard endotoxin (Sigma, India) samples were prepared, and pro-inflammatory response was measured in vitro with the same lot of whole blood. A comparison indicates that the reduction in TNF-α after polymyxin treatment of samples was higher than that expected from endotoxin concentration of the samples. This suggests that endotoxin may be acting as an adjuvant.[20,30,31]

**Concentration and Major Classes of Airborne Bacteria.** All samples were plated on TSA in triplicate, and the counts were determined and represented as colony-forming units per $m^3$ of air sampled. The number concentration of bacteria of the samples in terms of a colony-forming unit (CFU) per $m^3$ is summarized in Figure 3a. The range of concentration at the various WWTPs was found to vary from 249 to 41 837 $CFUm^{-3}$. Significant variability of airborne bacterial concentration was observed between the WWTPs ($p \leq 0.05$). The concentration variation among the plants is due to the operational variability, source water characteristics, and meteorological conditions. The concentration at the office area was lower than for the source area in the six WWTPs. (MWU, $p \leq 0.05$). No contamination field blanks by bacteria was observed. Overall, 27% of the samples exceeded the exposure limit proposed by the American Conference of Governmental Industrial Hygienists for 8 h exposure as 10 000 $CFUm^{-3}$.[32]

The bacterial colonies separated from each sample were stored on TSA slants and subjected to several basic screening tests for identifying their general characteristics. The results are summarized in Figure 3b. Gram-positive bacteria (GPB) were found to be predominantly present in all the samples across all WWTPs, with the percentage fractions varying from 67% to 90%. High levels of GNB concentration in WWTPs are reported in the literature.[10] The presence and dominance of different bacterial aerosols are highly dependent on the characteristics of wastewater and the local climatic conditions. In GNB groups of bacteria, ENT group was more predominant (86−94%) in the WWTPs.

**Airborne Bacterial Species Distribution.** The bacterial colonies were identified further to their species level using MicroLog manual system1 (Biolog, Inc., Hayward, California). Twenty four species contained in 13 different genera were identified. These include colonies observed on TSA plates and also additional colonies observed from MacConkey and EMB agar. The later was not included in comparing relative abundance. The genera such as *Bacillus*, *Escherichia*, and *Micrococcus* (63%, 17%, and 8% of total concentration of bacteria) were predominant among all samples. There are eight and five bacterial genus, respectively, in GNB and GPB. *Bacillus* was the most predominant GPB (79%). *Escherichia* and *Pseudomonas* were the predominant genera in GNB (85% and 7%). The genera *Pseudomonas* (85%) and *Pasteurella* (11%) were the dominant NENT bacteria found in the samples. Similarly, the genus *Escherichia* was predominant among ENT (92%).

*Bacilli* spp such as *Bacillus subtils* and *Bacillus pumulis* dominated the total concentration of GPB. These species are spore forming and can survive in harsh environments for a longer period. Analysis shows that the spore-forming bacteria dominates the total culturable bacterial concentration (Figure S3, Supporting Information), and the predominance is more significant at higher concentrations of total bacteria.

Table S1, Supporting Information, shows the frequency of occurrence of bacterial species across WWTPs. One in six indicates that the specific bacterial species was identified only in one treatment plant out of six. Most frequently observed bacteria are *Enterobacter aerogenes, Pseudomonas stutzeri, Bacillus subtils, Corynebacterium nitrilophilus*, and *Micrococcus luteus*. Similar species were also abundant in samples collected from the office area. This table reveals the species similarity between samples in the vicinity of the source and the office area. This table also indicates that the most frequent species found in the office area correspond to the species most frequently found in the samples near the source. *B. subtils, E. coli*, and *M. luteus* were the species that were present in all office area samples. *E. coli* are habitant of human and animals and found lower in concentration in the natural environment. The only bacteria species that is identified exclusively in office area samples was *Brevibacterium otitidis*. The above observations indicate that the office area might be contaminated by the viable bacteria transported from plant sources.

Many of the bacterial species identified from these WWTPs have potential health hazard, and these species are also found in measurements conducted in WWTPs.[8] *Pseudomonas stutzeri* is identified in WWTP samples and clinically important. *P. stutzeri* infections, such as bacteremia/septicemia and eye infection, have been reported by several studies. *Entero cloacae* is another important species isolated from two WWTPs and is considered as a pathogen. All species of *Klebsiella* genus are known pathogens of the respiratory tract.[33,34] *Serratia plymuthica* is identified as a significant opportunistic pathogen to which a variety of

infections, including peritonitis, pneumonia, sepsis, and wound infections, have been attributed.[33] Most of the strains of *E. coli* are commensals of the intestinal tract of human and other animals, indicating contamination by fecal microorganisms. Moreover, the method adopted in this study may not have identified all the bacterial species present in the wastewater facility. Bacterial species such as *Legionella pneumophila* and *Mycobacterium tuberculosis* are identified in WWTPs.[3,35] Overall, the present study suggests that the workers at the treatment plant are exposed to opportunistic and infectious bacteria.

**Similarity Analysis of the Bacterial Species among the Samples.** The droplet mode of transport of bioaerosols, especially live (culturable), have not been assumed to be significant in the past. Only recently have a few studies in multistory buildings, biosolid application, and WWTPs proved that aerosolized transportation of pathogenic bacteria are important.[4,36,37] Transport of viable bacteria from sources is determined by various physical and biological factors. Meteorological conditions and particle characteristics are the physical factors affecting transport of the bacteria in the atmosphere. The ability to survive in the harsh environment is an important factor that governs the transport of the viable biological particle.

The methods adopted in the above investigations include tracking the specific strains of the bacteria and dispersion modeling along with the disease incidence. The transport problem of the biological particles can also be addressed using receptor modeling approaches.[38] A similar technique has been used in different microenvironments to track the pollutants.[39] A combination of receptor modeling techniques and bacterial species identification could be an approachable method for tracking of the biological particles from sources. The factors obtained from principal component analysis (PCA) and their contributions at the source delineated in the present study could be useful for tracking biological particles from these sources.[40,41] Moreover, PCA can help to identify the important species of bacteria (principal components) in all samples. The contribution of these components of all samples will help to elucidate the similarity between sample groups, and analysis may reveal special properties of these bacterial groups (components).

The relationship between the bacterial species present in the samples was analyzed using the R mode (PCA).[24] All species identified were not used for analysis. Species present in only a few samples were removed from PCA. Some of the bacterial species were grouped under the respective family before analysis (Table S2, Supporting Information). The bacterial concentrations were normalized (column normalization) to give equal weightage for all variables. Four factors were identified in the analysis, which combined to explain 78% of data variance was selected for further analysis. The rotated factors identified by R-mode analysis are shown in Table S2, Supporting Information. There are four significant factors, explaining 78% of the data variability. Factor I has three major variables, which are positively related to each other. This factor explains 28% of the data variability, and it indicates the concentration variation of the bacterial group across the samples. *E. coli* and *cedecia* are in enterobacteriaceae and found in sewage, soil, and decaying matter. These bacterial groups commonly utilize simple carbohydrates such as glucose, glucosamine, and dextrin. *Brevibacterum* is an actinobacteria which plays an important role in the decomposition of organic materials and in the carbon cycle. Factor III is signified by the presence of *Micrococcus* bacteria. Even though *Micrococcus* is not a spore former, cells can survive in a harsh environment for extended periods. *Micrococcus* genera has a more general carbon utilization ability. This factor was also found to positively correlate with *cedecia* genera. Factor IV explains 10% of the data variability. This factor includes, in general, acid-metabolizing bacterial groups.

The factor score values give an estimate of the amount of factor contribution in each sample. Factor scores of the office area samples are given in Table S3, Supporting Information. The result shows that most of the samples were significantly associated with factors IV and III. It is corroborated with the fact that the *Bacillus* and *Micrococcus* species of these factors can survive a long time in extreme environments. Sampling was conducted in the typical tropical summer season in Mumbai. The relationship between samples was also analyzed using PCA. Analysis suggests that the samples cannot be separated or differentiated based on species concentration data (Figure S4, Supporting Information). This corroborates with the fact that the bacterial concentration observed in the office area was transported from the plant sources.

In the present study, enumeration and identification of airborne bacteria in WWTP has been carried out using the culture method. It is well known that due to the differences in culturability of microorganisms, the culture method usually underestimates the concentration of airborne bacteria in the environment. However, the culture method is one of the economically viable methods used in the field study of airborne microorganisms. Classification of cultured bacteria from the samples was carried out by rapid biochemical tests. As discussed in previous paragraphs, information could be useful in understanding the transport of biological particles from the WWTPs. However, the present study could not differentiate the bacterial species between plants or unit operations (Figure S4, Supporting Information). This may suggest that there is a need for a better bacterial characterization method that can be used for the transportation study. The nonculture method based on principles of molecular biological and immunological principles has also been used for identification of bacteria.[43] Even though the culture method may provide an economically viable technique, bacterial information obtained from advanced analysis could be useful in tracking bacterial transport as well as for health impact assessment.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Details of sampling locations, sampler description, and analysis methods, and tables and figures that contain additional results. This material is available free of charge via the Internet at http://pubs.acs.org.

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## ■ ACKNOWLEDGMENT

The authors express their sincere thanks to the Division of Earth and Atmospheric Science, Department of Science and Technology, Government of India for providing financial support for carrying out this work (SR/S4/AS:283/2007 & SR/FTP/ES-40/2008). In addition, the authors express their thanks to all officials of the Municipal Corporation of Greater Mumbai (Sewerage commission), Maharashtra for extending facilities and cooperation throughout the sampling program. The first

author would like to thank the Government of India for awarding the Rajiv Gandhi National Fellowship to carry out this research work.

## REFERENCES

(1) Brandi, G.; Sisti, M.; Amagliani, G. Evaluation of the environmental impact of microbial aerosols generated by wastewater treatment plants utilizing different aeration. *J. Appl. Microbiol.* **2000**, *88*, 845–852.

(2) Sánchez-Monedero, M. A.; Aguilar, M. I.; Fenoll, R.; Roig, A. Effect of the aeration system on the levels of airborne microorganisms generated at wastewater treatment plants. *Water Res.* **2008**, *42*, 3739–3744.

(3) Blatny, J. M.; Reif, B. A. P.; Skogan, G.; Andreassen, O.; Hoiby, E. A.; Ask, E.; Waagen, V.; Aanonsen, D.; Aaberge, I. S.; Caugant, D. A. Tracking airborne Legionella and Legionella pneumophila at a biological treatment plant. *Environ. Sci. Technol.* **2008**, *42*, 7360–7367.

(4) Nguyen, T. M.; Ilef, D.; Jarraud, S.; Rouil, L.; Campese, C.; Che, D.; Haeghebaert, S.; Ganiayre, F.; Marcel, F.; Etienne, J.; Desenclos, J. C. A community-wide outbreak of legionnaires disease linked to industrial cooling towers — how far can contaminated aerosols spread?. *J. Infect. Dis.* **2006**, *193*, 102–111.

(5) Fracchia, L.; Pietronave, S.; Rinaldi, M.; Martinotti, M. G. Site-related airborne biological hazard and seasonal variations in two wastewater treatment plants. *Water Res.* **2006**, *40*, 1985–1994.

(6) Rylander, R. Health effects among workers in sewage treatment plants. *Occup. Environ. Med.* **1999**, *56*, 354–357.

(7) Douwes, J.; Mannetje, A.; Heederik, D. Work related symptoms in sewage treatment workers. *Ann. Agric. Environ. Med.* **2001**, *8*, 39–45.

(8) Adams, P. A.; Spendlove, L. C. Coliform aerosols emitted by sewage treatment plants. *Science* **1970**, *169*, 1218–1220.

(9) Laitinen, S.; Kangas, J.; Koyimaa, M.; Liesivuori, J.; Martikainen, P. J.; Nevalainen, A.; Sarantila, R.; Husman, K. Workers' exposure to airborne bacteria and endotoxins at industrial wastewater treatment plants. *Am. Ind. Hyg. Assoc. J.* **1994**, *55*, 1055–1060.

(10) Oppliger., A.; Hilfiker, S.; Vuduc, T. Influence of seasons and sampling strategy on assessment of boaerosols in sewage treatment plants in Switzerland. *Ann. Occup. Hyg.* **2005**, *49*, 393–400.

(11) Rinsoz, T.; Duquenne, P.; Greff-Mirguet, G.; Oppliger, A. Application of real time PCR for total bacterial assessment; comparison with epifluoresecence microscopy and culture dependent methods. *Atmos. Environ.* **2008**, *42*, 6767–6774.

(12) Mulloy, K. B. Sewage workers: toxic hazards and health effects. *Occup. Med.* **2001**, *16*, 23–38.

(13) Thorn, J.; Beijer, L.; Jonsson, T.; Rylander, R.; Measurement strategies for the determination of airborne bacterial endotoxin in sewage treatment plants. *Ann. Occup. Hyg.* **2002** a, *46*, 549-554

(14) Rietschel, E. T.; Kirikae, T.; Schade, F. U.; Mamat, U.; Schmidt, G.; Lopnow, H.; Ulmer, A. J.; Zahringer, U.; Seydel, U.; Padova, F. D.; schreier, M.; Brade, H. Bacterial endotoxins: molecular relationships of structure to activity and function. *FASEB J.* **1994**, *8*, 217–225.

(15) Myatt, T. A.; Milton, D. K. Endotoxins. In *Indoor Air Quality Handbook*; Spengler, J. D., Samet, J. M., McCarthy, J. F., Eds.; McGraw-Hill: New York, 2001.

(16) Wouters, I.; Hillhorst, S.; Kleppe, K. M.; Doekes, G.; Douwes, J.; Peretz, C.; Heederik, D. Upper airway inflammation and respiratory symptoms in domestic waste collectors. *Occup. Environ. Med.* **2002**, *59*, 106–112.

(17) Lundholm, M; Rylander, R. Work related symptoms among sewage workers. *Br. J. Ind. Med.* **1983**, *40*, 325–329.

(18) Thorn, J.; Beijer, L.; Rylander, R. Work related symptoms among sewage workers: a nationwide survey in Sweden. *Occup. Environ. Med.* **2002b**, *59*, 562–566.

(19) Long, C. M.; Suh, H. H.; Kobzik, L.; Catalano, P. J.; Ning, Y. Y.; Koutrakis, P. A pilot investigation of the relative toxicity of indoor and outdoor fine particles: in vitro effects of endotoxin and other particulate properties. *Environ. Health Perspect.* **2001**, *109*, 1019–1026.

(20) Ning, Y.; Imrich, A.; Goldsmith, C. A.; Qin, G.; L. Kobzik, L. Alveolar macrophage cytokine production in response to air particles in vitro: role of endotoxin. *J. Toxicol. Environ. Health* **2000**, *59A*, 165–180.

(21) Walters, M.; Milton, D. K.; Larsson, L.; Ford, T. Airborne environmental endotoxin: A cross validation of sampling and analysis techniques. *Appl. Environ. Microbiol.* **1994**, *60*, 996–1005.

(22) Douwes, J.; Versioot, P.; Hollander, A.; Heederik, G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl. Environ. Microbiol.* **1995**, *61*, 1763–1769.

(23) Conover, W. J. *Practical nonparametric statistics*; John Wiley and Sons Inc.: New York, 1971.

(24) Reymend, R.; Joreskog, K. G. *Applied factor analysis in natural sciences*; Cambridge University Press: New York, 1996.

(25) Spaan, S.; Wouters, I. M.; Oosting, I.; Doekes, G.; Heederik, D. Exposure to inhalable dust and endotoxins in agricultural industries. *J. Environ. Monit.* **2006**, *8*, 63–72.

(26) Willeke, K.; Lin, X.; Grinshpun, S. A. Improved aerosol collection by combined impaction and centrifugal motion. *Aerosol Sci. Technol.* **1998**, *28*, 439–456.

(27) Varghese, S. K.; Gangamma, S. Particle deposition in human respiratory system: Deposition of concentrated hygroscopic aerosols. *Inhalation Toxicol.* **2009**, *21*, 619–630.

(28) Vogelzang, P. F.; van der Gulden, J. W.; Folgering, H.; Kolk, J . J.; Heederik, D.; Preller, L.; Tielen, M. J.; van Schayck, C. P. Endotoxin exposure as a major determinant of lung function decline in pig farmers. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, 15–18.

(29) Arbour, N. C.; Lorenz, E.; Schutte, B. C.; Zabner, J.; Kline, J. N.; Jones, M.; Frees, K.; Watt, J. L.; Schwartz, D. A. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat. Genet.* **2000**, *25*, 187–191.

(30) Kindt, J. T.; Goldsby, A. R.; Osborne, A. B.; Kuby, J. *Kuby immunology*: W. H. Freeman: New York, 2006.

(31) Eisenbarth, S. C.; Piggott, D. A.; Huleatt, J. W.; Visintin, I.; Herrick, C. A.; Bottomly, K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* **2002**, *196*, 1645–51.

(32) Sigsgaard, T.; Bach, B.; Malmros, P. Respiratory impairment among workers in a garbage-handling plant. *Am. J. Ind. Med.* **1990**, *17*, 92–93.

(33) Goldman, E.; Green, L. H. *Practical handbook of microbiology*; CRC Press: Boca Raton,2009.

(34) Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schreckenberger, P. C.; Winn, W. C. *Color Atlas and Textbook of Diagnostic Microbiology*; Lippincott, Williams, and Wilkins: Philadelphia,1997.

(35) Pascual, L.; Perez-Luz, S.; Yanez, M. A.; Santamaria, A.; Gibert, K.; Salgot, M.; Apraiz, D.; Catalan, V. Bioaerosol emission from wastewater treatment plants. *Aerobiologia* **2003**, *19*, 261–270.

(36) Reynolds, S. J.; Black, D. W.; Borin, S. S.; Breuer, G.; Burmeister, L. F.; Fuortes, L. J.; Smith, T. F.; Stein, M. A.; Subramanian, P.; Thorne, P. S.; Whitten, P. Indoor Environmental Quality in Six Commercial Office Buildings in the Midwest United States. *Appl. Occup. Environ. Hyg.* **2001**, *16*, 1065–1077.

(37) Tanner, B. D.; Brooks, J. P.; Haas, C. N.; Gerba, C. P.; Pepper, I. L. Bioaerosol emission rate and plume characteristics during land application of liquid class B biosolids. *Environ. Sci. Technol.* **2005**, *39*, 1584–1590.

(38) Friedlander, S. K. Chemical element balances and identification of air pollution sources. *Environ. Sci. Technol.* **1973**, *7*, 235–240.

(39) Mouser, J. P.; Rizzo, M. D.; Rolling, M. F. W.; Van breukelen, M. R. A Multivariate statistical approach to spatial representation of groundwater contamination using hydrochemistry and microbial community profiles. *Environ. Sci. Technol.* **2005**, *39*, 7551–7759.

(40) Thurston, G. D.; Spengler, J. D. A quantitative assessment of source contribution to inhalable particulate matter in metropolitan Bostan. *Atmos. Environ.* **1985**, *19*, 9–25.

(41) Kumar, A. V.; Patil, R. S.; Nambi, K. S. V. Source apportionment of suspended particulate matter at two traffic junctions in Mumbai, India. *Atmos. Environ.* **2001**, *35*, 4245–4251.