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ATMOSPHERIC BACTERIAL PROFILE AND SUSPENDED PARTICULATE MATTER OF LAHORE CITY

JAVED I. QAZI, MUDASSAR YASIN AND SHAGUFTA SHAHJAHAN

Microbiology Laboratory, Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore(JIQ, MY). Environmental Protection Agency, Lahore, Pakistan(SS).

ABSTRACT: Air of ten different localities of Lahore was sampled with the help of Hi-volume sampler for 15 minute by using Whatman glass microfiber filter papers. A piece of 4×4cm² of each filter was then suspended in sterile water and processed for enumeration of bacteria on nutrient, EMB and mannitol salt agar media. Plates of these media were also exposed directly to the atmospheres of sampling areas. It was found that air of the city harboured high to considerable profiles of viable bacteria corresponding to the amount and size distribution of suspended particulate matter (SPM). The agar plates directly exposed to the atmosphere indicated that the number of colony forming units (CFU) of bacteria including coliforms and *Staphylococcus aureus* in general, followed the higher and lower values of concentration and size of SPM respectively. Atmosphere of Ravi Chowk depicted more than 600 value of CFU, while the lowest figure, 35 appeared on the plate exposed in the Quaid-e-Azam Campus of the University of the Punjab. Surprisingly, these areas also expressed highest and lowest counts for the *S. aureus* i.e. 385 and 3 CFU. Concentrations of SPM μg/m³ for the Ravi Chowk and Quaid-e-Azam campus areas measured 240.53 and 3.16, respectively. Amongst the coliform, CFU values of *E. coli* represented the highest figures and ranged from 8 to 125 for the different study areas. CFU obtained for water immersed SPM reflected a range of 134×10□ to 101×10¹□/ 4×4cm² of the air filter on nutrient agar for different study areas. The CFU values on EMB and mannitol salt agar media ranged from 300×10□ to 45×10□ and nothing to 182×10□, respectively for different urban locations. These results are suggestive that besides other toxicological effects of the atmospheric SPM of an industrial city with heavy traffic load, they should also be considered seriously in the spreading of various types of bacteria including pathogens. The study indicates importance of size and mass distribution of airborne particles and correlates these attributes to microbial types and densities to be disseminated.

Key Words: Biopollutants in air, Size and mass of atmospheric particles, Aeromicrobiology, Coliforms in air, *Staphylococcus aureus* in air.

INTRODUCTION

Atmospheric pollution is an indispensable consequence of big citie's life characterized by heavy traffic and industrial set up(Wittoroff *et al.*, 1994; Sacre *et al.*, 1995). Dust and soot particulate matter is a major component of the atmosphere of such cities (Schwartz *et al.*, 1993; Anderson *et al.*, 1996). This form of pollution is directly concerned with acute human health problems, ranging from skin irritation to cardiac and respiratory diseases (Dockery and Pop, 1994; Burnett *et al.*, 1995; Schwartz, 1996; Poloniecki *et al.*,1997; Hwang and Chan, 2002) and cancer (Netteshei *et al.*, 1975; Repace and Lowrey, 1985; Bhatia *et al.*, 1998). A number of cases

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of asthma attacks have been associated with air pollutions (Rossi *et al.,* 1993; Castellsague *et al.,* 1995). The air borne particles may contain heavy and transition metals. The chemical composition of particles has been shown to determine their health effects (Tepper *et al.,* 1994; Dusseldorp *et al.,* 1995; Campen *et al.,* 2001). Besides aforesaid health problems even mortalities have been associated with air pollution in different cities (Schwartz, 1994; Wordley *et al.,* 1997).

Various studies have shown that adverse health effects and mortalities are associated with number and mass of ambient ultrafine particles (Oberdoster *et al.,* 1995; Peters *et al.,* 1997). Another very important aspect of suspended particulate matter (SPM) is that it carries and disseminates microbes which can contaminate / spoil food and cause infections, especially respiratory diseases (Radmore *et al.,* 1998; Reiman and Uitti, 2000; Chang *et al.,* 2001, Dutkiewicz *et al.,* 2001; Whyte *et al.,* 2001). Practically such attempts addressing microbial status and SPM have not been even initiated for second big city of Pakistan. Therefore, this effort was made to survey the microbial air pollution in connection with amount and size distribution of SPM in air of 10 areas of Lahore. Viable counts of coliforms, *Staphylococcus aureus* and on nutrient agar are reported here. This information is valuable for public health authorities. Monitoring programs may be designed on these lines to assess the aerial SPM pollution generated by motor vehicles and industrial activities and its impact on bacterial dispersion.

MATERIALS AND METHODS

Ten air samples of different areas of Lahore were taken with the assistance of the EPA air sampling facility on dates indicated in table 1. The bacteriological media's composition and identification of coliforms were made as described by Merck (1996/97). Whatman glass microfiber filters (20.3×25.4cm) were kept in polythene bags and exposed over night to formalin gas in a closed glass chamber. They were removed out of the glass chamber and polythene envelopes were closed. These presterile filters were fitted in Hi volume air sampler after arrival at the sampling point and allowed to suck in the air for 15 minutes. The air filters were removed from the sampling facility, put in the polythene bags and the flaps of the envelopes were closed. Nutrient and EMB agar plates were exposed directly in air for half, while mannitol salt agar plate for three minutes at the same time, at each study area. These were brought to laboratory and incubated at 37°c for 24 hours and then observed for microbial growth and enumeration of colony forming units (CFU). The air filters were cut into pieces in the laboratory. For microscopic observations of the suspended particulate matter (SPM), 2.9×2.2 cm pieces from half portion of each filter sheet were cut and particulate matter collected on them was tapped by finger jerks on clean slides. Each slide was tapped by three such pieces of filter sheets to achieve maximum matter on the slide. Then SPM on one set of slides was embedded in safranin plus glycerine in 1:1 ratio followed by sealing with cover slips and cutex. Microscopic appearance of SPM and microfibres of the filters was studied from these slides. Photomicrographs of representing areas of the slides and scale of stage micrometer were

taken on a camera fitted microscope. All the photographs were developed at same magnification. The tapped SPM on another set of slides was mounted by Canada balsam and covered by cover slips. These slides following drying were studied for measuring average size (diameter / span range) of particulate matter. For this purpose sizes of ten particles for a given sample were recorded randomly with the help of ocular micrometer at 400X magnification and calibrated as µm by using a stage micrometer. One half of each Whatman glass microfiber filter was kept in desiccator in the presence of excessive KOH and $CaCl_2$ to remove the water contents for more than 24 hours. The desiccated half of each sheet was then weighed to calculate weight of SPM by subtracting the weight of the unexposed filter. Weight of SPM was also calibrated as $\mu g/m^3$ of air. A piece of 4×4cm of each filter was cut in laminar airflow system and immersed in 100ml sterile water on the day of sampling. The glass containers containing the immersed pieces of the filters were shaken for 1 min. On next day the contents in the containers were again shaked and the water was processed for bacterial enumeration. Dilutions of water ranging from 10^2 to 10^{17} were made in a series of test tubes containing 9ml of sterile water by successive transfer of 1ml from a previous dilution and the diluents were stirred for twenty seconds on gyromixer. Initially three dilutions; $1:10^3$ to $1:10^5$ were spreaded evenly onto the surface of each nutrient, EMB and mannitol salt agar plates in an amount of 0.1ml/plate by means of sterile glass spreader. The plates were then incubated at 37°C for 24 hours and colonies appeared were observed, counted and measured by using a colony counter. For samples containing either less than 30 or more than 300 of bacterial colonies the experiment was repeated by using appropriate dilution. CFU obtained on different media, directly exposed to the atmosphere as well as obtained for water suspended SPM of different study areas were correlated to amount and size of SPM of the corresponding samples.

RESULTS

Microscopic observation of the dust and soot particles in the glycerine plus safranin (1:1) embedding showed small fragments of the glass microfiber filters containing SPM for all the samples except one. Interestingly the microfibers of the filters did not take safranin but SPM revealed many shades of the stain. Thus it was possible to visualize different sizes and shapes of the SPM while entrapped within the fibres. Such slides were photomicrographed, by using a camera fitted microscope. As can be seen from Fig. 1.1, SPM of the Thokar Niaz Baig area usually comprised of bigger particles that were of variable dimensions. However, many particles of small and intermediate sizes were also visible. When sample 2 was observed, very few large suspended particles (SP) were visible. However, due to very fine nature of SPM, the microfiber indicated pinkish shade (Fig.1.2). Sample number 3 (Model Town) also appeared similar to sample 2 with relatively lesser concentration of SP as revealed by very light pink shade of the

Table1: ATMOSPHERIC BACTERIAL PICTURES OF VARIOUS SAMPLING AREAS AS VISUALIZED BY EXPOSURE, SUBSEQUENT INCUBATION AND COUNTING COLONY FORMING UNITS ON PETRI PLATES OF DIFFERENT AGAR MEDIA

Sr. No.	STUDY AREA	DATE/TIME OF SAMPLING	NUTRIENT AGAR ½ min exposure	EMB AGAR ½ min exposure	MANNITOL SALT AGAR 3 min exposure
1.	THOKAR NIAZ BAIG	5.11.01 2:28 to 2:43	11(5a) 36(2-4) 80(<2)	*E. coli=41(1)* *S&S=26(1)* *Ecterobacter=12(5)*	2(6) 8(2) 4(<2)
2.	Q.A. CAMPUS	5.11.01 12:07 to 12:22	8(5.5) 27 (2&<2)	*E. Coli=52* *S&S=19* *Enterobacter=10* Fungi=27	1(5) 2(<2)
3.	MODEL TOWN	5.11.01 1:02 to 1:17	12(5-8) 13(2-5) 47(<2)	*E coli=13* *S&S=6* µi=15	4(6) 11(3-3.5) 6(<2)
4.	CHOWK YATEEM KHANA	14.9.01 12:19 to 12:24	6(8) 34(2-3) 151(<2)	*E.coli=16* *S&S=25* *Enterobacter=1* µi=3	1(7) 19(2-4) 58(<2)
5.	CHOBURJI	29.10.01 2:9 to 2:25	11(6-14) 15(2-6) 120(<2)	*E.coli=92* *S&S=5* *Enterobacter=14*	33(4-6) 99(2-4) 217(<2)
6.	BADAMI BAGH	29.10.01 11:45 to 12:00	9(7-14) 49(2-7) 330(<2)	*E.coli=125* *S&S=40* *Enterobacter=3*	13(5-10) 48(2-4) 71(<2)
7.	RAILWAY STATION	14.09.01 2:08 to 2:23	6(5-10) 15(2-4) 62(<2)	*E.coli=70* *S&S=28* *Enterobacter=6* µi=11 Fungi=1	6(3-5) 19(2-3) 58(<2)
8.	GULBERG	14.09.01 2:25 to 3:10	1(12) 14(2-8) 39(<2)	*E.coli=8* *S&S=7* *Enterobacter=1* µi=6	1(7) 1(4) 11(2&<2)
9.	QANCHI	5.11.01 1:39 to 1:54	9(4) 39(2) 72(<2)	*E.coli=52* *S&S=19* *Enterobacter=10*	1(16) 21(<2)
10.	RAVI CHOWK	29.10.01 11:07 to 11:22	17(7) 275(2-6) 319(<2)	*E.coli=99* *S&S=14* *Enterobacter=8*	18(5-11) 105(2-5) 262(<2)

a: Number of colony forming units. Values within ( ) indicate diameter of bacterial colonies in mm.

S&S=*Shigella & Salmonella;* µi=unidentified

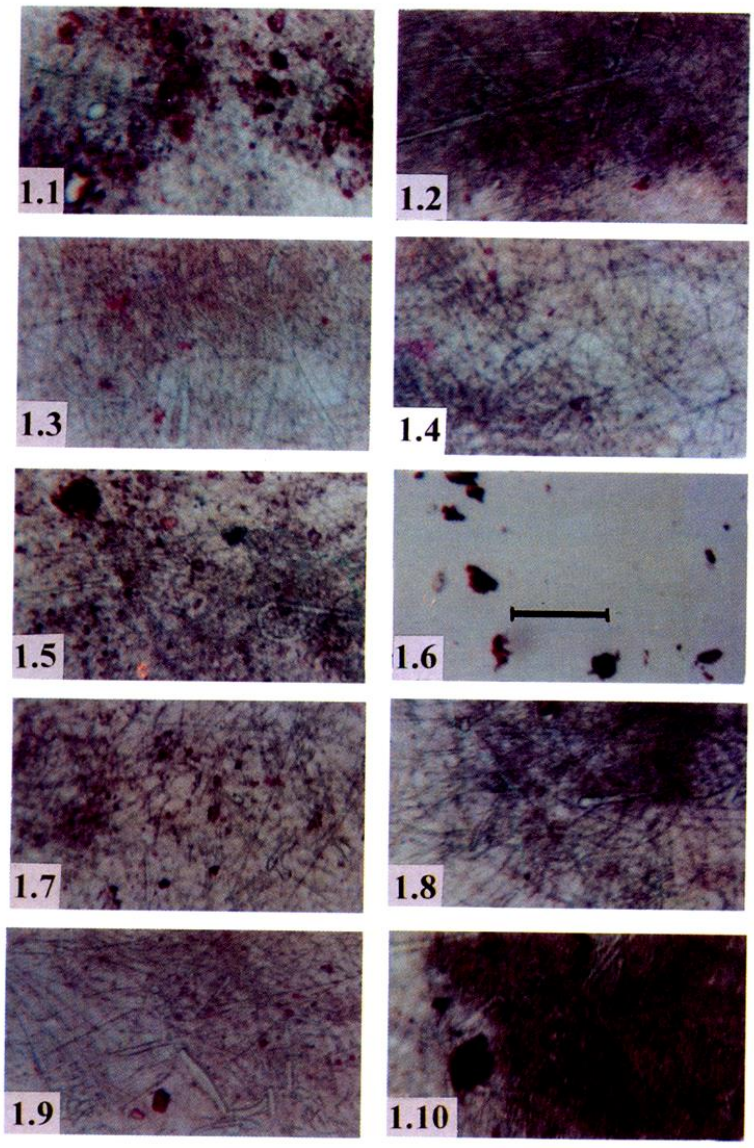

Fig. 1: Microscopic appearance of suspended particulate matter (SPM) entrapped within the Whatman glass, microfiber filters exposed to different areas in the atmosphere of Lahore. Decimated number of each photomicrograph represents the study area. Glycerine plus safranin (1:1) mounted slides. Bar indicates 100μm.

filter (1.3). The atmospheres of Quaid-e-Azam (Q.A.)Campus of the University of the Punjab and Model Town areas which are progressively far from the heavy traffic and industrial localities, are characterized by high and moderate amounts of fine with relatively fewer large-sized SPM, respectively. These microscopic observations were confirmed by lesser values of weight and concentration of SPM for these two study areas (Figs. 2A, 3A). Sample number 4 also revealed less number of larger SP entrapped within the microfibrils (Fig. 1.4). For sample 5 many large, medium and small-sized SP were observed (Fig. 1.5). For sample 6 (Badami Bagh) no fragment of the filter was observed, however, many large and small size particles were observable which indicated that heavier SP might have left the filter easily during the process of slide making (Fig. 1.6). The particles were of varying shapes and sizes. Sample 7 showed moderate amount of medium-sized SP (Fig. 1.7). For sample 8 less large-sized and many fine SP were apparent (Fig. 1.8). This study area i.e., Gulberg is considered a high rank residential area having but scarce heavy traffic. Study area 9 was characterized with large-sized as well as fine SP (Fig. 1.9). Slide for sample 10 had some very large but on the average medium- sized SP (Fig. 1.10). Size (maximum spam) of the atmospheric particulate matter collected from different study areas ranged from about 17 to 46 μm (Fig. 2B).

Nutrient agar plates exposed for half minute to different sampling areas, indicated number of colonies ranging from 35-611. The Q.A. Campus, Model Town and Gulberg areas can be visualized having relatively low atmospheric viable bacterial count. These observations truly followed the microscopic pictures of SPM of these areas. On the other hand Chowk Yateem Khana, Choburjii, Badami Bagh and Ravi Chowk depicted a higher and highest number of bacterial colonies, respectively (Table 1). When the mannitol salt agar plates were analyzed, it appeared that sampling areas number 5 and 10 i.e. Choburjii and Ravi Chowk showed 349 and 385 colonies, respectively. The remaining areas did not show such high profiles of the bacteria in the air. Microbial picture of growth on EMB agar remained in general less than the mannitol salt agar and nutrient agar growths. However, Q.A. Campus, Choburjii, Badami Bagh, Railway Station and Ravi Chowk areas showed more than 100 number of coliform's CFU (Table 1). For bacterial growth on EMB agar it was possible to distinguish *E. coli, Enterobacter, Salmonella* and *Shigella* based upon colonial characteristics according to Merck (1996/97). Atmospheres of Gulberg and Model Town indicated least number of *E. coil* i.e., 8 and 13 CFU, respectively. When the data were presented in the form of graph and correlated with weight of SPM sampled within 15 minutes by Hi Volume air sampler, it appeared that number of CFU on different media, in general, followed the trend exhibited by the values of SPM for different sampling areas (Fig. 2A), except for Thokar Niaz Baig. The high and low profiles of different forms of viable bacteria in the atmosphere thus represented high and low amounts of suspended particles in the air, respectively. Interesting correlation was found when the same data were visualized with reference to the size distribution of particulate matter (Fig. 2B). As can be seen from this figure the sampling areas characterized by low-sized SPM contained, in general, higher number of viable bacteria including coliforms and *Staphylococcus aureus* in the atmosphere. These results clearly

indicated that it is not only the amount of SPM but size of the particles also affect the microbial picture.

Enumeration of CFU of SPM / 16cm^2 of the filters showed highest number on nutrient agar for sample 4. The Yateem Khana Chowk is highly populated and congested area, characterized by heavy traffic. The number of CFU of SPM for the area turned out to be 1×10^{17}. For the remaining sampling areas the lowest count was for sample number 10 (Fig. 3). When the dilutions were spreaded on EMB agar highest CFU count was for sample 7, collected from city railway station. For sample 9 the growth was uncountable even for the dilution 1×10^5. Other sampling areas showed different CFU values, shown in Fig. 3. On mannitol salt agar the highest count was for samples collected from Badami Bagh, an area with high traffic and congested population mainly comprising of passengers coming from and going to other cities. For sample 1,3 and 9, *S. aureus* bacteria were not observed even when the inoculum was taken from first preparation of the samples When the CFU of the diluations were visualized with reference to the concentrations of SPM of respective sampling area a trend opposite to the one observed for directly exposed agar plates, was observed i.e., for higher amounts of SPM the bacterial profiles generally appeared lower (Fig. 3.A). However, correlation of the viable bacterial counts with size distributions of SPM more or less reflected the pattern observed for the CFU values of directly exposed plates (Fig. 3B).

DISCUSSION

The present microbiological survey of atmosphere of the Lahore city indicated that the heavy amount of suspended particulate matter (SPM) harbours different viable bacteria including coliform and *Staphylococcus aureus* species. Appearance of amount of growth on directly exposed nutrient, EMB and mannitol salt agar plates is sufficient to warn the potentially dangerous microbial nature of atmosphere of the city. Apart from the presence of general microbial flora and *S. aureus* in the air, presence of viable coliform bacteria may be considered as an indication of improper management of sewage water. For instance, the highest coliforms CFU/mg of SPM was found in sample representing the Lahore Railway Station. The lowest *E. coli* counts for Gulberg and Model Town areas is easily explained on the basis of high hygienic and sanitary conditions of these two posh areas of the city. Many of the coliforms may not themselves be pathogens, however, they indicate presence of pathogens which travel along the routes of fecal/sewage contaminations. (Gaudy and Gaudy, 1980).

Many studies have correlated amount of SPM with different diseases, health risks and infections. For example, Romieu and Borja-Aburto (1997) proved that particles at high concentration could cause mortality and have related an increase of 100mg/m^3 in 24 hours of average diameter of 10 microns (PM10) ambient levels to a 13% increase in total mortality. Avol *et al.* (2001) concluded that particulate matter, (PM10) had a measurable and potentially important effect on lung function, growth and performance. Similarly,

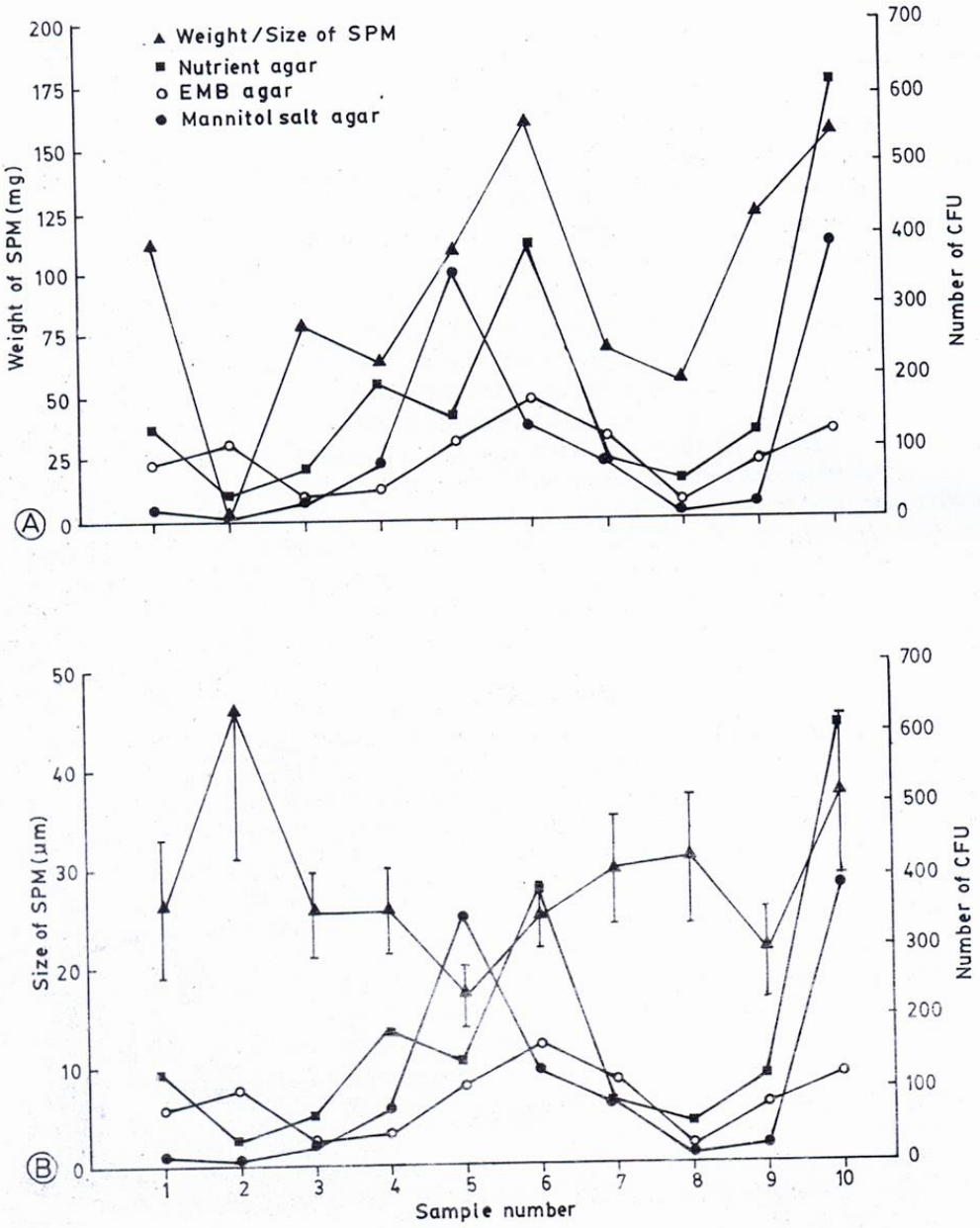

Fig. 2: Colony forming units (CFU) on different agar media plates exposed to the air of different study areas with reference to weight (A) and size (B) of SMP.

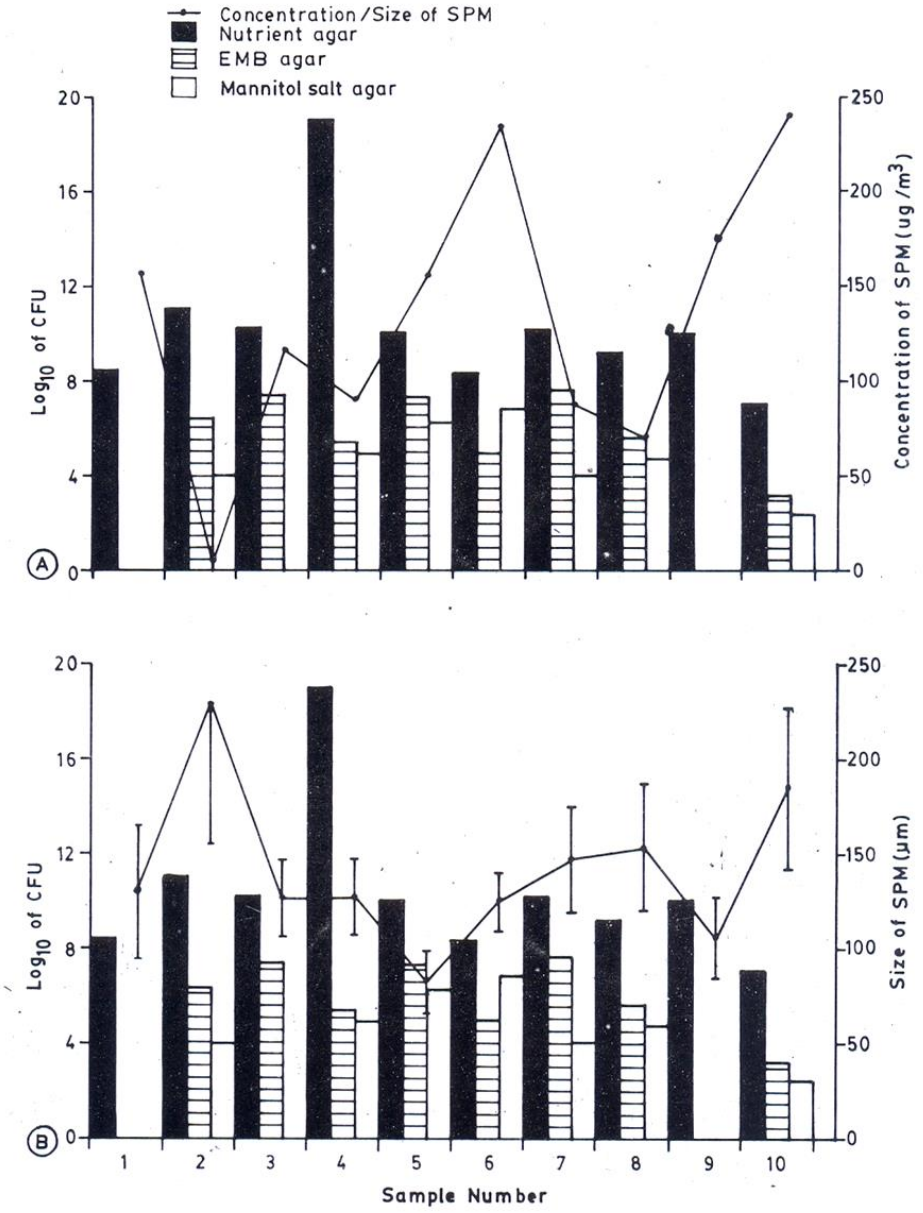

Fig. 3: Colony forming units (CFU) of SPM deposited with 15 minutes / 16 cm^2 of the Whatman glass microfiber filters in different study areas with reference to concentration (A) and size (B) of SPM.

Braga *et al.* (2001) have demonstrated that 10 microgram/m^3 increase in particulate matter, ranging from <10 to 100 microns in size in citie's atmosphere was associated with increase in deaths due to pneumonia. The comparable studies have not been conducted in the study area and the present report is first one to correlate the amount and size of the SPM with certain kind of viable bacteria in the atmosphere. For directly exposed agar plates CFU remained higher for higher amounts of SPM for different areas. Inverse correlations of the concentrations of SPM with CFU values following immersion of SPM in water and its subsequent inoculation, might have occurred due to the water-soluble toxic substances of SPM. Study to assess microbicidal and toxicological nature of the atmosphere's SPM is in progress in our laboratory. The results suggest that soot and dust particles must be managed in addition to managing the poor sewerage. Very high coliform count for railway station area may be considered due, at least in part, to animal crafts still in use in nearby area and human excreta problem. The practice of animal crafts on the roads also results in dissipation of animal manure particles into the atmosphere which may carry germs in addition to coliforms. In conclusion it is indicated that atmosphere is not suitable for human health due to high SPM accompanied by bacterial pollution. Further work is needed to analyze other microbes such as hemolytic bacteria and also chemical nature of SPM in terms of organic, inorganic and biochemical pollutants. Apart from the microbial nature the particles themselves need thorough studies. In this regard high amount of diesel particulate emission due to heavy and outdated traffic of the city is alarming. As diesel particulate emissions have been associated with particulate loadings and health effects (Pepelko and Peerano 1983; Wittoroff *et al.*, 1994). Mauderly (1994) has documented epidemiological evidence from inhaled engine emission. While Bhatia *et al.* (1998) have correlated diesel exhaust exposure with lung cancer.

When bacterial profiles (CFU) of different sampling areas were observed in the scenario of respective SPM size distribution, in general an inverse trend was observed for directly exposed media plates as well inoculated with water diluents. This clearly indicates that smaller particles in the atmosphere provide more surface areas for the adsorption, attachment and distribution of microbes in the air. Thence for monitoring atmospheric pollution apart from total load of SPM the size and physiochemical characteristics of the particles must be taken into account, especially when the focus includes aeromicrobiology. It looks pertinent here to indicate that SPM can be examined directly for microorganisms by fluorescence microscopy and fluorescent antibody techniques can be used to examine and enumerate specific microbes (Madigan *et al.*, 1997). Further work will include measurements of surface areas of the SPM and their microscopic topography. This is first report addressing the size of SPM and microbes of the atmosphere of Lahore city. However, many such studies have been conducted in other parts of the world. For instance Burnett *et al.* (1997) have illustrated role of particulate size and hospitalization for cardio respiratory diseases. Similarly Braga *et al.* (2001) have assessed differences in lag structure pattern between matter<10/100 micron meter in diameter and cause specific mortalities due to respiratory and cardiovascular problems.

The sampling of the city air with the help of Hi volume facility, only for fifteen minutes, indicated nature of SPM for different study locations ranging from about 71 to about 240 μm^3 except for sample No.2 having the value of 3.16, with mean diameter range from about 17 to 46 μm. Reported air quality guideline values for PM10 of 70 $\mu g/m^3$ for 24 hours (Tiitanen *et al.*, 1999) and 150 μg (Cicero-Fernandez, *et al.*, 2001) seem much less than the observed values. Thus the atmospheric particulate load of the city Lahore appears more than the safe levels and the amount and size of SPM have bearings on the bacterial profiles and their distribution patterns. Study of pathogenic bacteria and detailed analyses of SPM are required.

REFERENCES

ANDERSON, H.R., PONCE DE LEON, A., BLAND, J.M., BOWER, J.S. AND STRACHAN, D.P., 1996. Air pollution and daily mortality in London: *BMJ* 1987-92., **312**:665-669.

AVOL, E.L., GAUDERMAN, W.J., TAN, S.M., LONDON, S.J. AND PETERS, J.M., 2001. Respiratory effects of relocating to areas of differing air pollution levels. *Am. J. Respir Crit. Care Med.*, **164:**2067-2072.

BHATIA, R., LOPIPERO, P. AND SMITH, A.H., 1998. Diesel exhaust exposure and lung cancer. *Epidemiol.*, **9:**84-91.

BRAGA, A.L., ZANOBETTI, A. AND SCHWARTZ, J., 2001. The lag structure between particulate air pollution and respiratory and cardiovascular deaths in 10 U.S. cities. *J. Occup. Environ. Med.*, **43:**927-933.

BURNETT, R.T., CAKMAK, S., BROOK, J.R. AND KRAWSKI, D., 1997. The role of particulate size and chemistry in the association between summer time ambient air pollution and hospitalization for cardio respiratory diseases. *Environ. Hlth. Propect.*, **105:**614-620.

BURNETT, R.T., DALES, R., KREWSKI, D., VINCENT, R., DANN, T. AND BROOK, J.R., 1995: Association between ambient particulate sulfate and admissions to Ontario hospitals for cardiac and respiratory diseases. *Am. J. Epidemiol.*, **142:**15-22.

CAMPEN, M.J., NOLAN, J. P., SCHLADWEILER, M.C., KOKAVANTI, U.P., EVANSKY, P.A., COSTA, D.L. AND WATKINSON, W.P., 2001. Cardio vascular and thermoregulatory effects of inhaled PM-associated transition metals: a potential interaction between nickel and vanadium sulfate. *Toxicol. Sci.*, **64:**243-252.

CASTELLSAGUE, J., SUNYER, J., SAEZ, M. AND ANTO, J.M., 1995. Short-term association between air pollution and emergency room visits for asthma in Barcelona. *Thorax.*, **50:**1051-1056.

CHANG, C.W., CHUNG, H., HUANG, C.F. AND SU, H.J., 2001. Exposure of workers to air borne microorganisms in open-air swine house. *Appl. Environ. Microbiol.*, **67:**155-161.

CICERO-FERNANDEZ, P., TORRES, V., ROSALES, A., CESAR, H., DORLAND, K., MUNOZ, R., URIBE, R. AND MARTINEZ, A.P., 2001. Evaluation of human exposure to ambient PM10 in the metropolitan area of Mexico city using a GIS-based methodology. *J. Air Waste Manag Assoc.*, **51:**1586-1593.

DOCKERY, D.W. AND POPE, C.A.III. 1994. Acute respiratory effects of particulate air pollution. *Annu. Rev. Publ. Health.*, **15:**107-132.

DUSSELDORP, A., KRUIZE, H., BRUNEKREEF, B., HOFSCHREUDER, P., DE MEER, G. AND VAN, OUDVORST, A.B., 1995. Associations of PM10 and air borne iron with respiratory health of adults living near a steel factory. *Am. J. Respir. Crit. Care Med.*,**152:**1932-1939.

DUTKIEWICZ, J., OLENCHOCK, S., KRYSINSKA-TRACZYK, E., SKORSKA, C. AND SITKOWSKA, J., 2001. Exposure to airborne microorganism in fire board and chipboard factories. *Ann. Agric. Environ. Med.*, **8**:191-199.

GAUDY, A., F. AND GAUDY, E.T., 1980. *Microbiology for Environmental Scientists and Engineers.* McGraw-Hill Book Co.

HWANG, J. S. AND CHAN, C.C., 2002. Effects of air pollution on daily clinic visits for lower respiratory tract illness. *Am. J. Epidemiol.*, **155:**1-10.

MADIGAN, M.T., MARTINKO, J.M. AND PARKER, J., 1997. *Brock Biology of Microorganisms*. Prentice Hall International, Inc. U.S.A.

MAUDERLY, J.L., 1994. Toxicological and epidemiological evidences for health risks from inhaled engine emissions. *Environ. Hlth. Perspec.*,**102:**165-171.

MERCK, 1996/1997. *Microbiology Manual.*

NETTESHEI, P., CREASIA, D.A. AND MITCHELL, T.T., 1975. Carcinogenic and co-carcinogenic effects of inhaled synthetic smog and ferric oxide particles: *J. National Cancer Institute*, **55:**159-168.

OBERDORSTER, G., GELEIN, R.M., FERIN, J. AND WEISS, B., 1995. Association of particulate air pollution and acute mortality: involvement of ultrafine particles. *Inhalation Toxicol.*,**7:**111-124.

PETERS, A., WICHMANN, H.E., TUCH, T., HEINRICH, J. AND HEYDER, J., 1997. Respiratory effects are associated with the number of ultrafine particles, *Am. J. Respir. Crit. Care Med.*,**155:**1376-1383.

POLONIECKI, J.D., ATKINSON, R.W., PONCE DE LEON, A. AND ANDERSON, H.R., 1997: Daily time series for cardio vascular hospital admissions and previous day's air pollution in London, U.K. *Occup, Environ. Med.*, **54:**535-540.

RADMORE, K. HOLZAPFEL. W.H. AND LUCK, H., 1998. Proposed guidelines for maximum acceptable air-borne microorganisms level in dairy processing and packaging plants. *Int. J. Food Microbiol.*, **6:**91-95.

REIMAN, M. AND UITTI, J., 2000. Exposure to microbes, endotoxins and total dust in cigarette and cigar manufacturing: an evaluation of health hazards. *Ann. Occup. Hyg.*, **44**:467-473.

REPACE, J.L. AND LOWREY, A.H., 1985. An indoor air quality standard for ambient tobacco smoke based on carcinogenic risk. *New York State J. Medicine.*, **85:**381-383.

ROMIEU, I. AND BORJA-ABURTO, V.H., 1997. Particulate air pollution and daily mortality: Can results be generalized to Latin American countries? *Salud Publica Mex.,***39**:403-411.

ROSSI, O.V., KINNULA, V.L., TIENARI, J. AND HUHTI, E., 1993: Association of severe asthma attacks with weather, pollen, and air pollutants. *Thorax.*, **48:**244-248.

SACRE, C., CHIRON, M. AND FLORI, J. P., 1995. Development of an exposure index of air pollution caused by motor vehicles for use in epidemiological studies. *Sci. Total Environ.*, **169**:63-69.

SCHWARTZ, J., 1994. What are people dying of on high air pollution days? *Environ Res.*, **64:**26-35.

SCHWARTZ, J., 1996. Air pollution and hospital admissions for respiratory disease. *Epidemiol.*, **7**:20-28.

SCHWARTZ, J., SLATER, D., LARSON, T.V., PIERSON, W.E. AND KOENIG, J.Q., 1993. Particulate air pollution and hospital emergency room visits for asthma in Barcelona. *Thorax.*, **50:**1051-1056.

TEPPER, J.S., LEHMANN, J.R., WINSET, D.W., COSTA, D.L. AND GHIO, A.J., 1994. The role of surface complexed iron in the development of acute lung inflammation and airway hyperrsponsiveness. *Am. J. Respir. Crit. Care Med.*, **149**:A839.

TIITANEN, P., TIMONEN, K.L., RUSKANEN, J.J., MIRME, A. AND PEKKANEN, J., 1999. Fine particulate air pollution, resuspended road dust and respiratory health among symptomatic children. *Eur. Respir. J.,* **13:**266-273.

WHYTE, P., COLLINS, J.D., MCGILL, K., MONAHAN, C.O. AND MAHONY, H., 2001. Distribution and prevalence of air borne microorganisms in three commercial poultry processing plants. *J. Food Port.*, **64:**388-391.

WITTOROFF, D.N., GERTLER, A.W., CHOW, J.C., BARNARD, R.W. AND JONGEDYK, H.A., 1994. The impact of diesel particulate emission on ambient particulate loadings: *Proceed. Air Waste Management Association:* 94W:91.01. Cincinnati, Ohio.

WORDLEY, J., WALTERS, S. AND AYRES, J.G., 1997. Short term variations in hospital admissions and mortality and particulate air pollution. *Occup. Environ. Med.*, **54:**108-116.