Airborne Bacterial Evaluation of Indoor and Outdoor Environments of AU School in Visakhapatnam

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***Abstract*—The issue of healthy schools is a global concern as the schools are one of the critical social infrastructures in a society, and children are particularly at risk of lung damage and infection caused by poor air quality. Bio aerosols such as bacterial and fungal cells and their spores are along with non-biological particles, airborne particulate matter have been related to long term health issues of human beings as well as flora, and fauna. To identify the different risks and to establish exposure thresholds, microbiology of air samples from a series of indoor environments must be characterized, i.e. the different microorganisms (bacteria and fungi) must be identified and quantified. In this paper, the concept of air hygiene; measuring and assessing the level of bioaerosols in both the indoor & outdoor environments of select schools is proposed to serve as a guide to indicate overall air quality, associated with airborne infections. Current bio aerosol sampling in schools is carried out using Andersons' 6- stage sampler to indicate the environmental sources of bioaerosols.**

Keywords*— *bioaerosol, bacteria, outdoor environments, sampling, impactors, biochemical tests

I. INTRODUCTION

Aerosols are liquid or solid particles suspended in a gaseous medium with size ranges from 0.001 to 100 µm. Bio aerosols consists of aerosols containing microorganisms (bacteria, fungi, viruses) or organic compounds derived from microorganisms (endotoxins, metabolites, toxins and other microbial fragments), gases, vapours, particulate matter, etc that are in the air capable of eliciting diseases that may be infectious, allergic, or toxigenic with the conditions being acute or chronic.

The majority of bacteria naturally present do not cause adverse health effects. Some bacteria are even essential to both the human body and the environment. Health risks appear when the concentrations of some species become abnormally high. Some bacteria are recognized as the agents responsible for infectious diseases. The health risk related to the presence of Legionella pneumophila bacteria, namely legionnaire's disease, is well documented in previous research studies Bioaerosols are either injected into the atmosphere by chance (e. g., wind, rain and bursting bubbles) or processes governed by natural selection. Though bio aerosols is an important area of research, it has not been very well attempted in India. Considering the importance of bioaerosols, the studies have been carried out in schools to assess the bacterial levels in indoor & outdoor environments which are the first of its kind reported from Visakhapatnam. The study was aimed to find out the concentration of culturable bacteria in air along with their Gram characterization. An attempt has also been made to correlate the concentration of bacteria with meteorological parameters.

II. SOURCES OF BIO AEROSOLS IN OUTDOOR ENVIRONMENTS

Numerous activities serve as the origin of bioaerosols in indoor & outdoor environments. Outdoor sources include commercial activities, open drain systems, construction activities, poor sanitary conditions prevailing, improper solid waste disposal, increased traffic, etc. Indoor sources include dust arising from old wooden furniture's, poor cleaning activities, lack of ventilation, dusty walls & corridors etc. Although no regulations regarding microbial contamination or bioaerosol concentrations are mandated for residential, office, or class room environments, it is generally accepted that outdoor sources of bio aerosols may be significant when differences are noted between indoor and outdoor concentrations and/or populations.

III. MATERIAL AND METHODS

The objective of bioaerosol sampling is the efficient removal and collection of biological particles from the air in a manner, which does not affect the ability to detect the organisms, (e.g., alteration in culturability or biological integrity). This ability is dependent on the physical and biological characteristics of the organisms and on the physical features of the sampling instrument. Although the number of methods available for the collection of micro-organisms, there are in fact a limited number of approaches suitable for

collecting micro-organisms from bioaerosols. The methods available for monitoring bioaerosols in the vicinity of different school locations are discussed here. The principle concepts for the collection of airborne micro-organisms are impaction, impingement, filtration, cyclone scrubbing, electrostatic precipitation and sedimentation.

Impaction method uses inertial forces to collect particles or micro-organisms in the air. The air is drawn through the impaction sampler and forced to change direction. This causes the particles with too high inertia to become impacted onto a solid surface. Impingement technique uses the same approach as impaction except that the particles are collected in a liquid rather than onto a solid medium. In the process of Filtration collects micro-organisms by drawing the air through a porous material, usually a membrane filter. The collection efficiency of this process depends on the physical properties of the particle and the filter, and the flow rate of the air. In Cyclone scrubbing technique, the air is forced into a centrifugal motion and particles with a high enough inertia are forced onto the wall of the sampler. Electrostatic precipitation process allows the airborne particles (including micro-organisms) to electrically charged, on entering the sampler, causing them to drift and be deposited onto a suitable collection substrate. Electrostatic precipitation is reported to be a less severe collection strategy than impaction or impingement due to the significantly lower velocities encountered by the particles. Sedimentation method (also described as gravimetric sampling) is the simplest sampling strategy available, with micro-organisms collected on agar plates following their passive deposition from air. Although this approach is reported to be suitable for studies seeking to identify the presence of particular micro-organisms, it cannot give a value for the number of micro-organisms present per unit volume of air as the volume of air sampled is not determined (it is a non-volumetric method). Sedimentation methods are therefore not suitable for monitoring bio aerosols.

The selection of the proper biological sampling apparatus requires consideration of the organism to be collected, as well as other factors including viability, limits of detectability and instrument sensitivity, and physical and economic constraints. A number of inertial impaction samplers have been designed or adapted as viable samplers.The solid-surface sampler projects the particle directly onto the surface of a nutrient growth medium. The medium is then incubated, and the number of colonies is counted. Examples of this type include slit samplers, sieve samplers, and cascade impactors.

The original Andersen microbial sampler is a solid-surface impactor with six stages. Each stage has 200 or 400 precisely drilled holes with the hole diameter becoming progressively smaller from the top to the bottom stage. A petri dish containing an appropriate type of agar is positioned under each stage. As the air flows through the sampler at the standard rate of 28.3 liters/min a particle is deposited by impaction on the agar surface when the momentum imparted to the particle is too great to allow it to follow the airstream to the next stage. The six stages provide a good deal of information on the size distribution of the sampled aerosol cloud, with the top stage collecting basically non respirable particles, those greater than 7 pm, and the lower stages collecting those respirable particles capable of reaching the alveoli. Actual size ranges and efficiencies vary depending on the density of the particle, and some overlapping of stages does occur, but overall results from this sampler have been widely accepted.

IV. Experimental procedure for Anderson sampling Technique

Purpose of sampling is to provide the sampling data required for identification of culturable microorganisms and assessment of possible proliferation and dissemination of bacteria from building reservoirs of indoor and outdoor school environments. The field equipment required are the Andersen N-6 single-stage sampler, sampling media, in 6 plates prepared according to sampler manufacturer's recommendations. Mannitol Salt Agar (MSA), Blood Agar, Pseudomonas, Mac-conkey, EMB Agar and Nutrient Agar are the media used in petri dishes for evolution of bacterial species. Sampling pump capable of meeting sampler manufacturer's flow specification (e.g., 28.3 L/min), with flexible connecting tubing is required. Accessories like Cotton gauze pad, e.g., 4" × 4", Rubbing alcohol, 70% isopropanol and Refrigerant packs, if necessary for keeping samples cool during shipment are also required for carrying out the sampling. The samples should be kept cool but be protected from freezing.

After selecting the sampling site, each sampling pump is calibrated with a representative sample in line. Before each run, each sampler stage is carefully and thoroughly wiped with rubbing alcohol allowed to dry. Make sure air passages are not blocked. Load sampling media into sampler, remove covers from media, and attach sampler to pump with flexible tubing. Special care should be taken to prevent contamination of media during loading and unloading. Do not touch agar surface. Sample at known preset flow for an accurately known time, e.g., 10 min. (In heavily contaminated areas, a shorter sampling time may be necessary.) and Replace covers on sampling media, unload, and pack securely for shipment (plates should be media side up). The collected samples and blanks are to be kept cool (not necessarily ice-cold) and ship as quickly as possible to a laboratory for enumeration and identification. The analysis of the sample is carried out for identification of different bacterial species. Interpretation is subjective and based on total numbers and rank order of taxa in complaint area compared with control areas.

V. Results and Discussion

Sampling site: AU High School was selected for the study. School is located at Chinna Waltair area & are very near (<500m) to the Beach of Visakhapatnam city, which is on the coastline of Bay of Bengal. Sampling was done at the locations of both indoor and outdoor environments of School, i.e., at entrance, Corridor, Assembly hall and in class rooms at morning and afternoon hours (i.e., in between 10am to 11.30 am & 2pm to 4.30 pm). School is sampled twice in a different seasonal periods. i.e., during summer, rainy and winter seasons in the months of April, September and November respectively and their average bacterial concentrations are represented seasonally.

Sampling results for the airborne bacterial concentrations and the average number of colonies for the AU school is presented in Table 1, 2 and 3.In summer, the airborne bacterial concentrations in the selected school ranged from 28. 26cfu/m3 (lowest) in the EMB media to1021.2 cfu/m3 (highest) in Nutrient agar media used. At the outdoor site, the

concentrations ranged from lowest of 60.07cfu/m3 to a highest of 1777.38cfu/m3. The school environment has showed higher outdoor bacterial levels during summer than their indoor bacterial concentrations. In rainy season, the indoor airborne bacterial concentrations ranged from 24.73cfu/m3 to 533.56cfu/m3 where as in the outdoor they ranged from 7.06cfu/m3 to 1053cfu/m3. In winter season the airborne bacterial concentrations ranged from 7.06cfu/m3 to 791.51cfu/m3. Sampling results for the airborne bacterial concentrations and the average number of colonies for the AU school is presented in Table 1, 2 and 3.

TABLE I. AIRBORNE BACTERIAL CONCENTRATION IN SUMMER

Petri Dish No	Media Used	Average number of Colonies		CFU/m^3		Dominant Genera
		Indoor	Outdoor	Indoor	Outdoor	
1	Mannitol Salt Agar	120	190	424.0	671.37	S. aureus S. Epidermis
2	Blood Agar	44	94	155.47	332.15	Staphylococcus Streptococcus
3	Pseudomonas Agar	94	154	332.15	544.16	Pseudomonas sps
4	Mac-conkey Agar	11	30	38.86	106.00	Klebsiella E.coli
5	EMB Agar	08	17	28.26	60.07	E.coli p.aureginosa
6	Nutrient Agar	283	503	1021.2	1777.38	All bacteria

[a.] Sampling Time – 10Min, Flow rate – 28.3 l/min, Temperature-320C ; Relative Humidity-69%

TABLE II. AIRBORNE BACTERIAL CONCENTRATION IN RAINY SEASON

S. No	Media Used	Average number of Colonies		CFU/m3		Dominant Genera
		Indoor	Outdoor	Indoor	Outdoor	
1	Mannitol Salt Agar	37	98	130.74	346.28	S. aureus, S. epidermis
2	Blood Agar	10	06	35.33	21.20	Streptococcus sps
3	Pseudomonas Agar	59	75	208.48	265.01	Pseudomonas sps
4	Mac-conkey Agar	08	02	28.26	7.06	E.coli, Gram neg bacilli
5	EMB Agar	07	05	24.73	17.66	K.pneumonia, P.aeruginosa
6	Nutrient Agar	151	298	533.56	1053.00	All bacterial sps

[b.] Sampling Time – 10Min, Flow rate – 28.3 l/min, Temperature-350C ; Relative Humidity-76%

The recommended maximum limits are: 1000 CFUs/m3 for the total number of bio-aerosol particles set by the National Institute of Occupational Safety and Health (NIOSH); The recommended indoor airborne bacterial levels set by American Conference of Governmental Industrial Hygienists (ACGIH) is (> 500 cfu/m3) and outdoor air level are 1000 CFUs/m3. On an average, we observed relatively higher values of bacteria (CFU/m3) in summer as compared to rainy and winter seasons. It could be due to suitable climatic conditions of tropical region as well as the local activities which can give rise to airborne bacteria. Humans on campus may be significant contributor of bacteria. Apart from these, warm weather of tropics, open waste disposal, cafeteria and vegetation etc. may also contribute high bacterial in concentrated air. Another additional factor of contribution could be the soil dust coming out from construction of buildings in the surroundings of the campus. The presence of suspended soil dust in air supports microbes. During rainy season the higher outdoor air borne bacteria levels may be higher due to open drainage networks present adjacent to the school buildings, geographical location and climatic conditions. The study also showed higher concentration of airborne bacteria during summer & monsoon season than in winter season. On an average, the percentage of Gram +ve bacteria was found higher than Gram –ve bacteria during both the seasons. Among Gram +ve bacteria, mainly cocci dominated over rod shape bacterial species.

TABLE III. AIRBORNE BACTERIAL CONCENTRATION IN WINTER

S. No	Media Used	Average number of Colonies		CFU/m3		Dominant Genera
		Indoor	Outdoor	Indoor	Outdoor	
1	Mannitol Salt Agar	32	24	113.07	84.80	S. aureus
2	Blood Agar	09	17	31.08	60.0	Staphylococcus
3	Pseudomonas Agar	18	37	63.60	130.74	Pseudomonas

4	Mac-conkey Agar	04	15	14.13	53.0	Gram –ve bacilli
5	EMB Agar	02	07	7.06	24.73	Enterobacter, and other gram – ve sps
6	Nutrient Agar	224	180	791.51	636.04	All bacterial sps

[c.] Sampling Time – 10Min, Flow rate – 28.3 l/min, Temp.-310C ; Relative Humidity-68%

CONCLUSION

School is a place where children go to promote their life, may serves as an avenue to contact diseases and diminish the children health. Due to higher concentration of viable counts of bacterial species and lack of standard guidelines of those indoor contaminants in schools with respect to Indian conditions, it is strongly recommended that comprehensive exposure assessment program in schools is required in order to determine whether exposure of indoor air is able to cause risks in children. The present work was among the very few studies evaluating bioaerosols both indoors and outdoors of Andhra University High School located very near to the coast of Bay of Bengal in Visakhapatnam city. The results have shown that the airborne bacterial concentrations for both indoor and outdoor environments of the schools are mostly within the limits. It is advisable that strict measures should be put in place to check the increasing microbial load in the school environment. This is necessary, to set up standards/guidelines and permissible limits for the concentration of microbial community for schools environment. Since, assessment of airborne dust carrying both culturable and nonculturable microbial communities' serve as a better indicator for managing and abating the contemporary health risks and also quality of indoor air. These preliminary results suggested carrying out further comprehensive studies on airborne bacteria at more number of sites and for longer duration. Some bacterial species such as staphylococcus, streptococcus and pseudomonas have been the predominant genera identified.

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