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Endotoxin: Indicator of Indoor Air Quality – A Case Study

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Abstract – Endotoxins may play an important role in the development of various symptoms in occupational environments where exposure to bacteria is prevalent.

The standard analytical method for endotoxin is the Limulus amebocyte lysate (LAL) assay. The Gel-Clot method is one of the simplest, least expensive and has good sensitivity technique of LAL assay. In this study volumetric sampling technique has been applied for enumeration and quantification of airborne viable counts Endotoxin from indoor and outdoor air of school. An Endotoxin (Pyrogen)-free filter cassette with PTFE membrane filter has been used for air sampling. 240L air volume has been sampled for sampling of Endotoxin at the rate of 4LPM. Extraction of Endotoxins from the collected air samples has been done in Endotoxin-free water to avoid the evolving of false positive results during assay. Assay results indicate that Endotoxin level (EU/m3) has been higher in indoor air than outdoor air. It is an indicator inside source of Endotoxins.

Key Words – Chemical Markers, Airborne Dust, Endotoxins, Indoor Air Quality (IAQ)

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INTRODUCTION

Exposure to biological agents (including microorganisms) is associated with a wide range of major public health issues, such as infectious diseases, acute toxic effects and allergies (Douwes et al 2003). Deterioration in indoor air quality (IAQ) is attributed to the occurrence of microbial population. In hospital premises many healthy people including the vulnerable group of weak, elderly, and infirm people, may be exposed to such contaminants and thus may be very sensitive to biological hazards. In particular, hospitalized patients could be at significantly increased risk of bio-aerosol exposure (Augustowska et al 2006).

Endotoxins is major component of the outer membrane of both pathogenic and non-pathogenic Gram-negative bacteria (GNB) and known as more or less ubiquitous in the environment and are present in normal indoor environment as constituents of house dust (Park et al 2001). Endotoxins are found to be associated with the Sick Building Syndrome (SBS) symptoms, to acute chronic lung diseases asthma and asthma-like symptoms (Jacobs 1997, Caillaud 2009). Respiratory inflammation, its dysfunctions and failure of lung function etc are consequences of exposure of high concentrations of airborne endotoxin in the occupational environment (Farokhi *et. a.l* 2018)

Air sampling, for detection and quantification of airborne culturable bacteria and non-culturable its cellular component - *Endotoxins* was carried out simultaneously indoor and outdoor in a hospital premise.

METHODOLOGY

Two sampling points indoors and two outdoors were selected for detection of airborne bacteria and endotoxins. A new volumetric approach developed by Agarwal and Jhamb (1998) was used for simultaneous quantification of airborne gram-negative bacteria and Endotoxins. An indigenous sampler APM 823 (fabricated by Envirotech Pvt Ltd., New Delhi) was used for the collection of samples using poly-tetra-flouro-ethylene (PTFE) membranes (47 mm diameter) at an adjustable flow rate.

Culturable form of total bacteria was isolated on Petri plates containing nutrient agar medium and incubated for 24hrs. at 37°C temperature, while collected air filters extracted with non-pyrogenic water to prepare a suspension of trapped particles for extraction of Endotoxins. Endotoxins were analyzed using Gel Clot- Limulus Amoebocyte Lysate (LAL) assay method using Endotoxin's kit (Charles River Laboratories Inc. USA). All reagents were prepared and re-constituted as per the instructions provided by the manufactures. Control standard Endotoxins (CSE) was used for positive

control, while pyrogen free water (LAL reagent water) was used as negative control.

Principal of Gel Clot method

Endotoxins produces opacity and gelatin in LAL when equal amount of LAL reagent and specimen incubated for 60 minutes at 37°C indicates. A positive response on the gel clot test indicates that there is an number of Endotoxins in the sample. A positive test is defined as the formation of firm gel capable of maintaining its integrity when the test tube is inverted at 180°. Endotoxin's level is calculated and expressed as number of EU (Endotoxins unit) present in per cubic meter of air.

RESULTS AND CONCLUSION

Descriptive analysis of two weeks sampling data has been presented in table 1 and 2. Airborne concentration of Endotoxins varied from 0.1 to >1 EU/m^3 (table 1). Total bacterial counts were noticed at higher range at I_2 site (I_2-Out Patients Department) as compare to I_1 lobby (table 2). However, minimum to maximum ranges of bacterial counts and endotoxins were revealed the higher indoor air concentration of two bacterial components. Calculated indoor to outdoor ratio (IO ratio) of indoor-outdoor pollutants was very much close to 1 or more than 1.

Results indicates that –

- Higher indoor concentration of endotoxin and bacteria may be attributed to the inefficient ventilation, cleaning, and level of occupancy. Environmental factors may too significantly influence the airborne concentrations of these agents in hospital environment (Park el al 2013), that cannot be ignored.

- IO ratio pointed out that indoor air was a vital source of culturable and non-culturable bacterial community.

- Likewise, deposition of dust may also bring re-aerosolisation of dust due to moment and various other activities like cleaning, dusting etc. which might have altered the amount of endotoxins and bacteria level of indoor air.

- Total culturable bacterial counts were beyond the upper limits of various standards *viz.* European Commission (> 1000 cfu/m^3 high), National Institute of Occupational Safety Health [NIOSH] (1000 cfu/m^3 for total number of bioaerosol particles), American Conference of Governmental Industrial Hygienists [ACGIH] (<500 cfu/m^3 culturable counts for total bacteria) WHO (for bioaerosol counts at 500 cfu/m^3). In the study, indoor level of viable counts of bacteria were exceeded the concentration level >1000 cfu/m^3.

In a study it has been found that filtration of indoor air may be potential source of indoor air endotoxin (Niu *et. al*. 2020). Hospital indoor air contains a diverse range of microbial population. The importance of the estimation of the quantity and types of airborne microorganisms and that these values can be used as an index for the cleanliness of the environment as well as an index they bear in relation to human health and as source of hospital-acquired infections (Ekhaise et al 2008). Prolonged exposure to airborne endotoxins may be a risk of work related-diseases among occupants (Bouillard 2005). Sampling and testing for Endotoxin in occupational environment has significance to assess the quality of indoor air (Katiyar 2010). It may be one of the important indicators of IAQ complaints and respiratory diseases especially water damaged, old and poorly maintained occupational settings. A combination of mycotoxins and endotoxins air sampling is a useful tool in evaluating microbiological contamination in most water damaged buildings. Since a small fraction (0.1-1%) of the total microflora in an environmental sample can be detected qualitatively and quantitatively by culturing on proper media and microscopic identification.

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Table-1: Indoor and outdoor level of airborne Endotoxins (Eu/m^3).

	I_1	I_2	O_1	O_2
N	16	12	8	12
Mean	0.88	0.95	0.83	0.1238
Range	0.35-1.48	0.12-1.3	0.72-0.89	0.11-0.16
SR	0.08914	0.1153	0.02288	0.004890
One sample t test				
P value	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Wilcoxon Signed Rank Test				
P value	0.0005*	0.0025*	0.0138*	0.0025*

I_1, I_2=Indoor sites, O_1, O_2=Outdoor sites
*=Significant

Table-2: Indoor and outdoor culturable bacterial counts (cfu/m^3).

	I_1	I_2	O_1	O_2
N	14	14	10	10
Mean	1327	4325	1083	2807
Range	804-2200	2708-7916	945-1267	2000-4500
SR	58.24	313.6	30.73	274.2
One sample t test				
P value	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Wilcoxon Signed Rank Test				
P value	< 0.0001*	< 0.0001*	0.0020*	0.0025*

I_1, I_2=Indoor sites, O_1, O_2=Outdoor sites
*=Significant

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