COMPARATIVE MICROBIAL ASSAY IN RELATION TO SEASONAL VARIATION IN SELECTED CLINICAL LABORATORIES IN ZARIA

Shiaka, G.P., Yakubu, S.E. and Olonitola, O.S
Department of Microbiology
Ahmadu Bello University, Zaria, Nigeria
email: petergs10@yahoo.com

ABSTRACT

Microbial contaminants of the indoor environment of two clinical microbiology laboratories of a private (Lab A) and a government (Lab B) owned hospitals in Zaria were investigated within the dry season: January and March, and wet season: July and September in the year 2007. The highest and lowest bacterial concentration were obtained in the 4th week $16.0x10^3$cfu/ml in lab A and 0.09×10^3cfu/ml in the 1st week of sampling in wet season in the same laboratory. The fungal count was highest in Lab B: $4.0x10^3$cfu/ml and lowest: $0.09x10^3$cfu/ml in the fifth week of sampling in dry season. The bacterial mean concentration was highest in Lab B: 7.70×10^3cfu/ml in dry season and lowest in wet season in lab A: 2.61 $x10^3$cfu/ml. The fungal mean concentration in Lab A was the highest while the lowest was found in Lab B: $1.06x10^3$cfu/ml. there was a significant correlation between fungal counts in Lab A's dry and wet season at 0.05 using pearson correlation (2-tailed) and also between fungal counts in wet season of lab B. However, no significant correlation in bacterial counts.The level of microbial concentration in this study is moderate but can be potentially allergic or infectious. This is a further justification for safety practices in clinical laboratories irrespective of seasons.

Keywords: Airborne, Bacteria, Concentration, Fungi and Laboratory.

INTRODUCTION

Biocontaminants occur in the indoor environments and it is widely accepted as an important indoor air pollutant by the indoor air quality researchers. Major indoor air biocontaminants include bacteria, fungi, dust mites, viruses, and protozoa (Chao *et a*l,2001).They posses the capability to grow and multiply on variety of building materials and surfaces under favourable conditions which could result in the deterioration of indoor air quality most especially when they become airborne and are present in high concentrations. (Chao *et al*, 2001). More so, when these organisms are manipulated they could be intrinsically released to the indoor environment causing laboratory associated infections (Bardana, 2003). Some could be transported into the indoor environment via airborne transport of spores by means of ventilation (Li *et al*, 2007). Bacteria and fungi are ubiquitous in the atmosphere and many can remain viable despite the challenges associated with their survival, including ultra violet (UV) exposure, low moisture levels, and extreme oligotrophic conditions (Jones and Harrison, 2004). Many airborne bacteria and fungi have been reported to cause human diseases (Salem and Gardner, 1994) particularly in immunocompromised individuals or in those that are sensitive to allergenic and toxigenic biological material (Burge and Rogers, 2000),

resulting in medical symptoms which may attract notice including eye and sinus irritation, sore throat, headache, fatigue and dizziness (Stetzenbach *et al,* 2004). The possible routes include respiratory tract inhalation, alimentary tract ingestion, skin and conjunctivae (Bridson, 1995). Weather parameters such as humidity, sunlight, temperatures, wind and the organisms characteristics tend to govern the ultimate fate of airborne microorganisms (Zanneti, 1993). Fungi and bacteria predominate in various environments hence, are often considered in indoor air quality investigation essential components (Li *et a*l, 2007). Their detection and quantification in indoor environments could be necessary in medical evaluations, determination of remediation procedures, assessment of health hazards and proactive indoor air quality monitoring (Brian *et al*, 2002). However, monitoring is hampered by lack of methods that provide precise, accurate, and representative exposure estimate for biocontaminant. Nevertheless, the development of molecular methods for the identification of bacteria in environmental samples gives us the ability to survey all, or nearly all of the bacteria present in a given volume of air without introducing a cultivation bias (peccia and Hermandez, 2006). This research investigated the bacterial and fungal load present in a private and government owned hospital laboratories within a period of 8 weeks in dry season and 8 weeks in wet season.

MATERIALS AND METHODS

Lab A belongs to ST. Luke hospital, Wusasa-Zaria with high level of attendance by patients within Zaria and beyond. It has an area of approximately $30m^2$, uncarpeted, linked to the external environment through a door and two windows, has a ceiling fan as mechanical ventilator. Lab B is sited in Ahmadu Bello University Teaching Hospital Tudun-wada, Zaria. It is larger having an area of approximately $35m^2$, air conditioned, uncarpeted, with one door and six windows. However, there is a permanent opening where an air conditioner was removed.

Sampling Procedures: These involved isolation of airborne bacteria and fungi from the indoor environment of each laboratory after work and almost within the same time twice in a week for a period of 16 weeks. The bacteria were sampled with the aid of a portable microbiology air sampler (MAS-100). It was placed at a height representative of the normal human breathing zone about 1.5m above floor level (Brian *et al*, 2002). $100cm^3$ of air was impacted on a prepared nutrient agar placed in the head of the air sampler and then incubated at 37^0C for 24 hours after which the colonies were enumerated and expressed in colony forming units per cubic meter of air (cfu/m^3 or cfu/ml). Potato Dextrose Agar (PDA) was used for the sampling of fungi instead of nutrient agar. It was impregnated with 0.5g/l of antibiotic chloramphenicol to suppress bacterial growth. The plates were kept at room temperature and observed for 3-5 days for growth.

RESULTS

The bacterial and fungal concentration of each laboratory were represented in Table 1 and Table 2 respectively based on week and season.

Table 1: Weekly Bacterial Counts (x 10^3 cfu/ml)

Period of Observation	Bacteria Colony Count			
	Dry season		Wet season	
	Lab A	Lab B	Lab A	Lab B
Week 1	2.70	9.00	0.09	6.40
Week 2	0.45	14.00	0.72	5.20
Week 3	6.30	4.10	0.99	2.80
Week 4	16.00	11.00	4.50	13.00
Week 5	3.40	12.00	5.20	2.90
Week 6	11.00	3.10	5.80	6.70
Week 7	0.10	3.20	1.80	3.20
Week 8	0.45	5.20	1.80	3.00
TC	40.40	61.60	20.90	43.20
MC	5.05	7.70	2.61	5.40

Lab A: Private owned hospital laboratory
Lab B: Government-owned hospital laboratory
TC: Total colony count
MC: Mean concentration

Table 2: Weekly Fungal Count (x 10^3 cfu/ml)

Period of Observation	Fungal Colony Count			
	Dry Season		Wet Season	
	Lab A	Lab B	Lab A	Lab B
Week 1	3.50	0.40	1.30	0.13
Week 2	3.60	0.14	0.90	1.60
Week 3	3.60	0.27	0.81	1.30
Week 4	3.70	0.80	1.81	0.67
Week 5	0.09	4.00	2.30	1.50
Week 6	1.40	1.90	1.60	2.40
Week 7	1.30	0.67	3.20	3.30
Week 8	0.81	0.27	3.60	3.40
TC	18	8.45	15.51	14.3
MC	2.25	1.06	1.94	1.79

Lab A: Private owned hospital laboratory
Lab B: Government-owned hospital laboratory
TC: Total colony count
MC: Mean concentration

The bacterial counts in Lab A ranged from 0.10x10^3 to 16.0x10^3cfu/ml in dry season and 0.09 x10^3 to 5.80 x10^3cfu/ml in wet season. The bacterial counts in Lab. B ranged from 3.10x10^3

to 14.00 x10^3cfu/ml in dry season and 2.80x10^3 to 13.00x10^3 in wet season as shown in Table 1. The fungal counts in lab A ranged from 0.09 x10^3 to 3.70x10^3cfu/ml in dry season and 0.81x10^3 to 3.60x10^3cfu/ml in wet season. 0.14x10^3 to 4.00 x10^3cfu/ml in dry season and 0.13x10^3cfu/ml in wet season for lab B as shown in Table 2. The bacterial concentration in dry season was highest in the 4th week: 16.00x 10^3cfu/ml in Lab A and lowest in the 7th week of sampling in the same laboratory as indicated in the figures below.

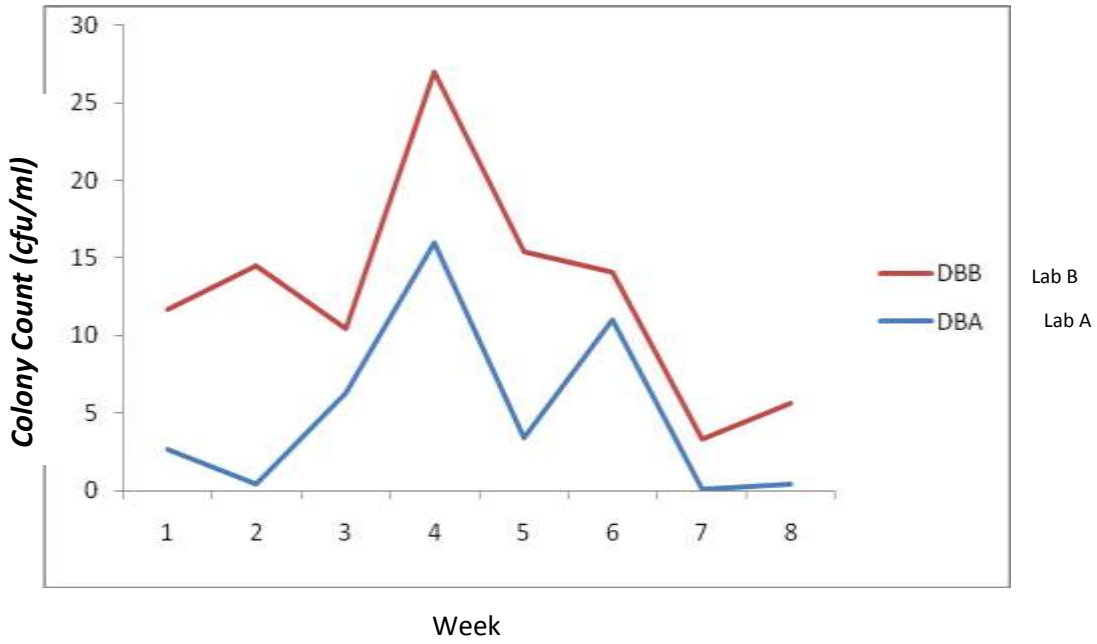

Fig.1: Frequency Curves for Bacterial Colony Counts in Dry Season

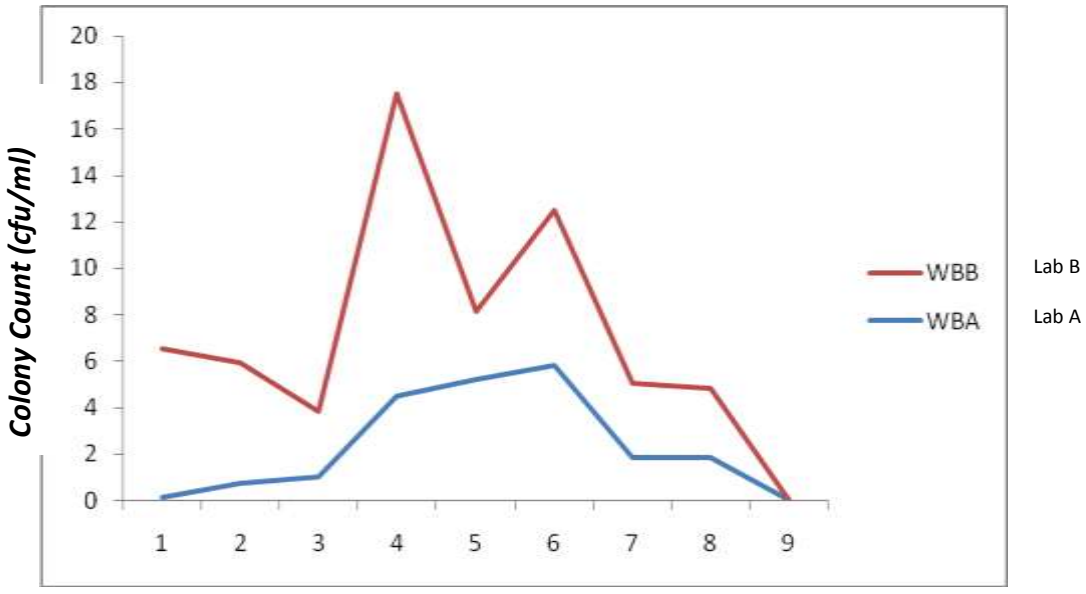

Fig 2: Frequency Curves for Bacterial Counts in Wet Season

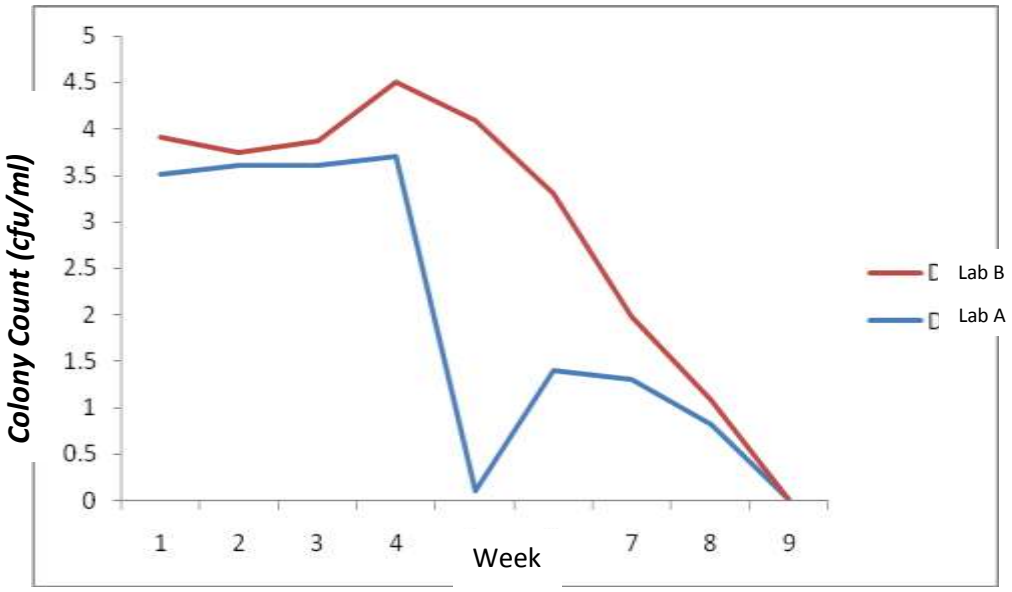

Fig. 3: Frequency Curves for Fungal Counts in Dry Season

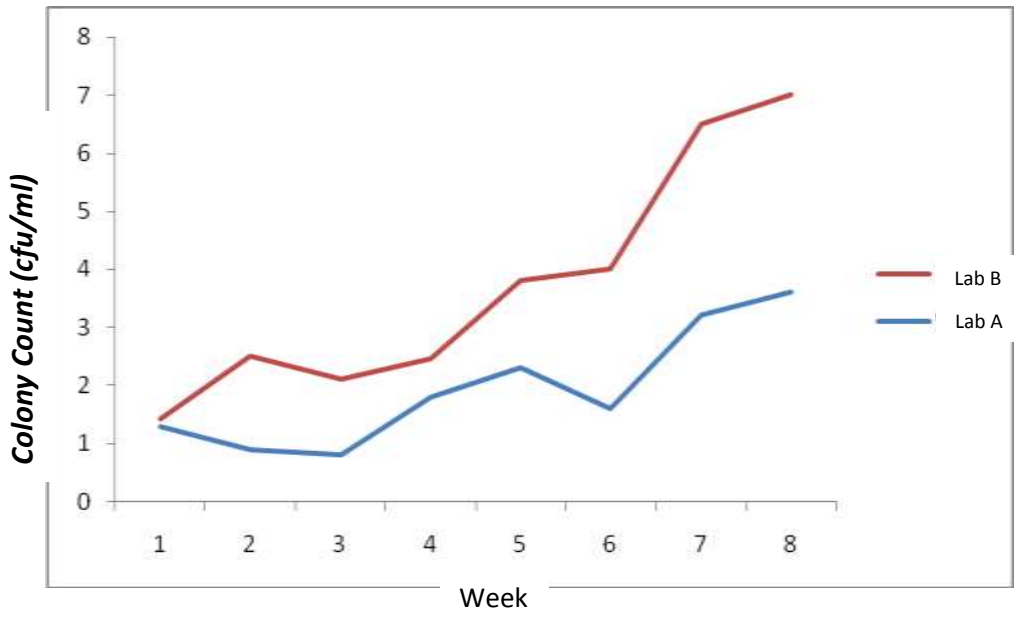

Fig 4: Frequency Curves for Fungal Counts in Wet Season

In fig.2, the highest bacterial concentration occurred in lab B whereas the lowest was in the 1st week in lab A. The fungal concentration was highest in lab B in the 5th week and lowest in lab A in same week for dry season, but in wet season, the highest count was in lab A in the 8th week and lowest in lab B at the 1st week as represented in fig3 and fig.4.

Table 3: Microscopic and Biochemical Characterizations of the Bacterial Isolates

Bacterium	GR	Cit	Ur	Mot	Ind	Gel	MR	Vp	Cat	Nt	Co	Ts1/H_2s
P. aeruginosa	-rods	+	+	+	-	+	-	-	+	+	+	K/AG$^+$
E. coli	-rods	-	+	+	+	-	+	-	+	+	-	A/AG$^-$
S. aureus	+cocci	-	+	-	-	+	-	-	+	+	+	K/AG$^-$
Streptococcus spp	+cocci	+	-	+	-	-	+	-	-			K/A$^-$
Staphylococcus spp	+cocci	-	+	-	-	+	-	-	+	-	-	K/AG$^-$
Proteus	-rods	+	+	+	+		+	-	-	+	-	A/AG$^+$
Micrococcus	+cocci	-	+	-	-	+	-	-	+	-	+	K/A$^\pm$
Bacillus spp	+rod	+	-	-	-	+	-	+	+	-	+	K/A$^+$

GR	-	Gram's Reaction	K/AG	-	Glucose Fermentation with Gas
TSI	-	Triple Sugar Iron	KCN	-	Potassium Cyanide
Cit	-	Citrate	A/AG	-	Triple Sugar Fermentation with Gas
Ur	-	Urease	K/K	-	Non- Fermentation
Mot	-	Motility	K/A	-	Glucose Fermentation
Ind	-	Indole	A/A	-	Triple Sugar Fermentation
Gel	-	Gelatin Liquefaction			
MR	-	Methyl Red			
Cat	-	Catalase			
Co	-	Coagulase			

The bacterial mean concentration was highest in Lab B: $7.56x10^3$cfu/ml in both seasons while the fungal MC was highest in lab A: $1.79x10^3$ cfu/ml and $2.25x10^3$ cfu/ml for dry and wet seasons respectively. The representatives of the isolated bacteria including their microscopic and biochemical characterizations were shown in Table 3. The prominent fungal isolated include Aspergillus spp, *Penicillium spp, yeast, mold,(Barnett and Hunter,1972) among others.* While those of bacteria are *Staphylococcus aureus*, *Streptococcus spp, Proteus* spp,*Bacillus spp*,(Bridson,1995) among others. Generally, the gram negative bacteria were relatively low, bacilli *spp* and asperigillus *spp* occurred most frequently in both seasons.

DISCUSSION

Microorganisms are invariably found in microbiology laboratories and the greatest occupational risk can be associated with the use of pathogenic microorganisms or the handling of contaminated materials (Pikitaro et al, 2008). The high diversity and concentrations of bacteria and fungi obtained in Lab B may be due to the opening where an air conditioner was removed. This exposes the laboratory to the external environment and agrees with the result of Chang *et al*, 2001 in a study of an unenclosed finishing unit of a swine building. However, inappropriate use of disinfectants on work surfaces at the period of study may also be a contributing factor. The influence of weather parameters on airborne bacteria and fungi has been largely demonstrated in environmental reports (Rosas *et al*, 1993). This has reflected in most of the results. For instance, temperature and humidity are usually very high in the 4th and 5th weeks of sampling favoring increase in microbiological

activities. The presence of air conditioners in Lab B reflected this more so, high wind favours sporulation and therefore their spread even from immediate outdoor environment (IEQ, 2004). Fungi produce spores which enable them survive harsh conditions and winds can overturn the top layers of dry soil, dispersing large quantities of mold spores which are drawn into air intakes and buildings (*http://www.imakerews.com*). This could be responsible for the survival of fungi in the indoor environments. Probably, some of these organisms are from immediate outdoor environment of the laboratories in question. Invariably, high seasonal mold spore levels are experienced as obtained in Florida, Louisiana, Texas, New Mexico and southern California in hot climates as reported in IEQ, 2004. This was observed in the study in the sense that molds were prominent during warm and dry climates from late January to March.

Moisture enables reproduction of atypical fungi and bacteria in buildings (Hope and Simon, 2007, Saenz-de *et al*, 2006).This probably accounts for the highest mean counts of bacteria in Lab B in dry season and fungi in Lab A during dry season including high counts in some of the sampling environments despite in the dry season. In addition, bench surfaces in the laboratories were not properly dried during sampling. Bacilli are very resistant to adverse conditions and their spores not easily suppressed by exposure to air (Lansing et al, 2009). This probably accounts for their predominance in both laboratories. Gram negative bacteria were present but few and might be due to environmental stress they were exposed to during sampling (Olonitola *et al*, 2006). Many fungi and gram negative bacteria can produce toxins which may be significant because they are associated with adverse human health effects. The laboratory staff are more prone to these toxins. Such organisms include pseudomonas, Bacilli, *Aspergillus niger*, Rhizopus among others isolated.

CONCLUSION

The presence of high concentration of airborne culturable and potentially allergic or infectious agents could pose health risks for laboratory workers inspite of seasonal variation. However, the low microbial concentrations obtained in some of the weeks might potentially exceed the guidelines for internal air quality (Goh *et al*, 2000), hence the need for routine sanitation and safe microbiological practices in all seasons. In this study, the culturable microbes were mainly gram positive bacteria, few gram negative bacteria, and certain fungi. This can serve as information assisting in medical evaluations, remediation procedures and assessment of health hazards and to improve understanding of the role of these indoor components in indoor air quality investigations especially as it has to do with season since it could influence organisms' survival in the air.

REFERENCES

Bardana, E.J. (2003). Indoor air quality and health. Does fungal contamination play a significant role? *Immunol. Allergy clin. North Am* **1**(2): 291-309.

Barnett, H.H and Hunter, B.B. (1972): Illustrated genera of imperfect fungi (3rd edn) Burgress Pub. Co. Minneapolis Pp.241.

Brian, G. S., Kimberly, H. K., Dana F. W. and George, K.M. (2002): Profile of airborne fungi in Buildings and outdoor in the United States. *Appl. And Envir. Microbiol.* **68**(4): 1743-1756.

Bridson, E.Y. (1995): The Oxoid Manual Pub., Unipath, Ltd Wade Rd, Basingstoke, Hampshire, RG 24 8PW, England.pp50-156.

Burge, H. and Rogers C. (2000). Outdoor allergens. Environ. Health Perspect. **108**:653- 659.

Chao, H. J., Milton O.K., Schwartz. J, Burge H.A (2001): Dustborne fungi in large office buildings. *Mycopotologica!* **154**:93-106.

Cheesbrough M. (2001). District laboratory Practice in Tropical Countries. Parts 1 and 2 Cambridge Univ. Press; U.K 40. West 20th Street, New York pp. 134-310

Goh, I., Obbard J.P., Viswanbathan, s. and Huang, Y. (2000): Airborne bacteria and fungal spores in the indoor environment; a case study in Singapore, *Acta Biotechnolgical **20**:* 67-73.

Hope, A. P. and Simon, R. A. (2007). Excess dampness and mold growth in homes: an evidence based review of the aeroirritant effect and its potential causes . *Allergy asthma proc.* **28**: 262-270.

http://www.imakenews.com/pure air controls/e-article 000263499.cfm

Jones, A., and Harrison, A. (2004). The effects of meteorological factors on atmospheric bioaerosol concentration: a review: *Total Environ.* **326**:151-180.

Lansing,M.P.,John,P.H.and Donald,A.k.(1999): Microbiol. 4th Edn. McGraw Hill Inc, NY137, 147.

Li, Y., Leung, G, M., Tang, J. W., Yang, X., Choa, C. Y., Lin, J. Z., Nielsen, P. V., Niu, J., Qian, H., Sleigh, A. C., Su, J., Sundell, J., Wong, T. W., Yuen, P. L. (2007). Role of ventilation in airborne transmission of infectious agents in the built environment: A multidisciplinary systematic review. Indoor Air **17**: 1,2-18.

Olonitola, O.S., Yakubu, S.E., Olayinka B.O., Jatau, E.D., (2006). Microbial Contamination of in-Use Disinfectants and Antiseptics in Health facilities Within Zaria, Nigeria. *Nigerian Journal Expt. Appl. Bio.* 7: **2**: 145-151.

Pitkarato,M., Meklin, T., Hyvarinen A., Paulin, L, Auvinen , P., Nevalainen, A.,and Rintala H. (2008). Analysis sequence, analysis of fungal flora in indoor dust by DNA and quatitative PCR, and culture. Inst of Biotech, sequence analysis. Univ. of Heisinki, Finland 233-314.

Peccia, J., and Hernandez, M. (2006). Incorporating PRC-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. Atmos.Eviron.**40**:3941-3961.

Rosas, I. Caldleron, C., Ulloa M. and Lacey, J. (1993): Abundance of airborne Penicillium in relation to urbanisation in Mexico city Appl. *Envr. Microbiol.* **59**: 2648- 2652

Saenz- de-santamaria, M., Postigo I., Gutierrez- Rodriguez, A., Xardonna. G., Guisantes, J., Asturias J., and Martinez, J. (2006). The major allergen of *Alternaria altermata* is exposed in other members of the pleosporaceae family. *Mycoses* **49**:91-95.

Salem, H. and Gardner D, (1994). Health aspects of bioaerosols. En B. Light hart and A. Mohr (ed). Atmospheric microbial aerosols: theory and applications. Chapman of Hall, New York.

Stetzenbach, L .D. (1997): Airborne Microorganism In manual of Environmental Microbiology (C. Hurst, ed) pp619-624. Washington D.C ASM Press.

Stetzenbach L. D., Buttner, M. and Cruz, P. (2004). Detection and enumeration of airborne biocontaminants curr.opin.biotecnol.15:170-174

The IEQ Review: Fungi and bacteria in ventilation system.(2004).Pure air control services 800-422-7873,EXT.802. **1:**1-3**,**

Zanetti, P., Brebbia, C.A., Garcia, J.E. and Millian, G.A. (1993). Environmental Modeling Southermptom Computation Mechanics Pubs. Califonia, USA pp 4-8.