BIOAEROSOLS IN A RESEARCH LABORATORY

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ABSTRACT

Laboratory procedures can generate aerosolized particles that are respirable and therefore potentially hazardous to the laboratory workers including researchers. Bioaerosols in the indoor environment of a postgraduate research laboratory in the Department of Microbiology, Ahmadu Bello University, Samaru-Zaria were investigated within a period of 16 weeks in the year 2007. The highest bacterial count: 8.3×10^3 cfu/ml and lowest: 0.95×10^2 cfu/ml were obtained in 2nd and 4th week while the highest: 4.0×10^3 cfu/ml and lowest: 0.41×10^3 fungal counts were obtained in 16th week and 4th week of sampling respectively. The correlation is significant between the bacterial and fungal mean concentration ($p<0.05$) using t-test (two tailed), Kendall's and Spearman's rho correlation analysis. Some of the representatives which constituted bacteria and fungi isolated in this study include *Staphylococcus spp*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus* spp, *Klebsiella* spp, *Aspergillus niger*, *Mold* and *Fusarium*. These organisms could significantly deteriorate indoor air quality therefore, safe microbiological principles and practices must be highly esteemed.
Keywords: Aerosol, Bacteria, Fungi, Indoor, Laboratory,

INTRODUCTION

The prescence of microoganisms in the atmosphere has been known for a long time and are primarily associated with aerosols (Fahlgren *et al*,2010).Exposure of these bioaerosols have been observed in a variety of working environments and as well related to occupational health symptoms (Timm *et al*,2009). Air serves as a mode of transport for the dispersal of bioaerosols from one location to another and the composition and concentration vary with the source and the dispersal in the air until deposition (Jones and Harrison,2004). Environmental factors such as temperature, wind velocity, and relative humidity have been reported to significantly influence the survival and transport of airborne microorganisms, affecting their ability to colonize on surfaces after deposition (Rosas *et al*,1993). Harsh environmental conditions tend to decrease the number of viable airborne organisms. However, fungal spores, enteric viruses, and amoeba cysts are somewhat resistant to the environmental stresses but bacteria and algae are more susceptible except bacterial endospores known to be associated with those of *Bacillus spp* which are quite resistant (Gregory,1973). However, microbial amplification occurs under favourable conditions rendering the indoor environment a source of bioaerosol exposure resulting in deterioration of indoor air quality (IAQ) including offensive odors,deterioration of research materials, building materials, and adverse human effects (stezenbach 2004). Inhalation, ingestion,and dermal contact are routes of human exposure to bioaerosols but inhalation serves as the

predominant route resulting in potential risk of infection to laboratory staff and researchers who continuosly come in contact with pathogenic organisms (Bridson,1995). The objective of this study is to determine the concentration and composition of bacterial and fungal aerosols present in the indoor environment of the research laboratory in question. This might be needed in medical evaluations, remedial procedures, assessment of health hazards and useful proactive indoor air monitoring (Brian *et al*,2002).

MATERIALS AND METHOD

This study was undertaken in and focused on the main postgraduate laboratory of the department of microbiology, Ahmadu Bello University (ABU) Samaru-Zaria. It is located on the first floor among offices, having a big size refrigerator, an incubator, hot air oven, autoclave, fume chamber and other equipments as shown in Fig.1 below.

Fig.1: A cross section of the laboratory

Air samples (100cm2) were collected using an air sampler placed at a height representative of the normal human breathing zone (1.5m above floor level) and impacted onto a prepared nutrient agar (NA) in a petri dish already fixed in the air sampler for bacteria isolation. Consequently, the same quantity of air was collected and impacted onto prepared potato dextrose agar (PDA) plate impregnated with 0.5g/l of the antibiotic chloramphenicol in the air sampler (Nevalainen *et al*,1992) for fungal isolation. The NA plate was incubated at 37^0C for 24 hours and the PDA plate was incubated at room temperature and observed for five days. The colonies were enumerated and the different isolates were characterized based on their cultural, microscopical, and biochemical properties (Collee *et al,* 1989, Cheesebrough, 2001, Bergey's manual 1983) whereas the fungal isolates were identified by examining their morphology and microscopically the spores and hyphae (Sutton 1998, Burnett and Hunter

1972).The immediate outdoor air of the laboratory was sampled inorder to establish the relationship with the indoor environment.Workbench surfaces were also sampled and compared with those of the air.These procedures were repeated for a period of 8weeks in dry season and another 8weeks within the wet season. Correlation analyses were carried out to describe relationship between the mean concentration using T-test, Kendall's and Spearman's rho.

RESULTS

The colonies based on growth on agar plates were counted and expressed in colony forming units (CFU) as indicated in Table 1 below:

Table 1: Weekly CFU counts of the air microflora in the laboratory($x10^3$)

WK	Bacteria	Fungi	Total count
1	5.70	2.10	7.80
2	8.30	1.80	10.10
3	5.90	1.30	7.20
4	0.10	0.41	0.51
5	0.95	0.94	1.89
6	0.19	0.98	1.17
7	0.76	0.62	1.38
8	0.95	0.68	1.63
9	0.85	0.64	1.49
10	1.50	0.74	2.24
11	1.60	0.60	2.20
12	1.80	1.10	2.90
13	3.80	1.30	5.10
14	1.50	1.60	3.10
15	2.10	1.90	4.00
16	2.20	4.00	6.20
MC	2.44(65)	1.29(35)	

MC: Mean concentration

The highest bacterial count: 8.3 ×103cfu/ml and lowest: 0.10 ×103cfu/ml were obtained in the 2nd and 4th week of sampling, whereas those of fungi were 4.0 ×103cfu/ml and 0.41 ×103 in the 16th and 4th week as represented in Fig.2.

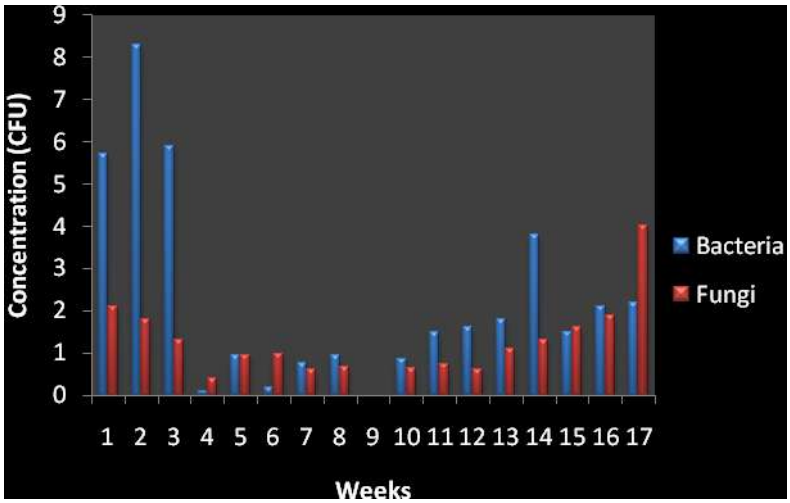

Fig.2: Weekly bacterial and fungal concentration in the laboratory

The total mean concentration were shown in Fig.3 with total bacterial concentration of 65% and that of fungi 35%. The correlation is statistically significant between the bacterial and fungal concentration.

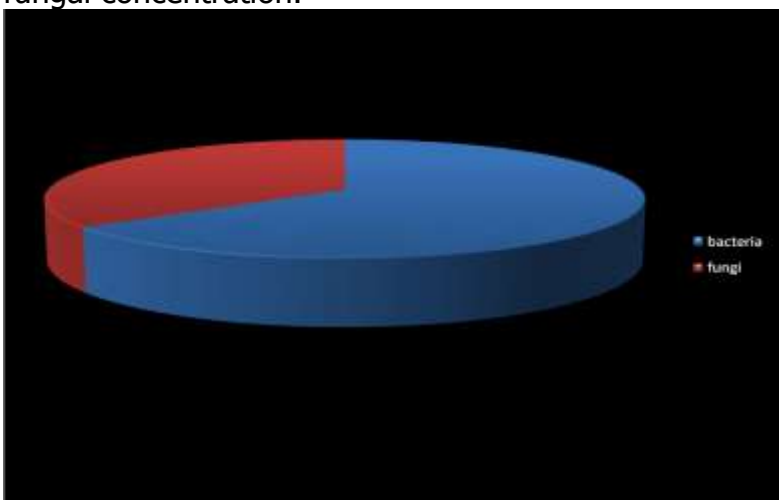

Fig.3: Mean concentration of the bacteria and fungi isolated

Some of the bacterial isolates included *Pseudomonas aeruginosa*, Bacillus *subtilis*, *Staphylococcus aureus* and E*scherichia coli.*The others were *Proteus spp*, *Micrococcus spp*, *Streptococcus spp*, *Klebsiella spp*, and other species of Bacillus which are most frequent. The fungi isolated include A*spergillus niger*, *Fusarium spp*, some Molds and Yeast. Some of the biochemical characterisations carried out are coagulase, urease, methyl red, triple sugar iron (TSI), voges proskaeur. The organisms isolated from the workbench surfaces reflected those in airborne state but more in concentration. Moreso, those of the outdoor environment are

similar to those of indoors but are more diversified and some were unidentified. E.*coli*, *Micrococcus spp*, P.*aeruginosa* and Yeast were not found in the outdoor environment.

DISCUSSION

In research laboratory, work is usually limited to relatively few organisms already known to the resarchers unlike the diagnostic laboratory. This might be the reason why few organisms were isolated from the indoor aerosols compare to the studies of Shiaka and others in 2007 in which the indoor environment of some hospital laboratories were investigated and published work of Goh *et al*,2000. Bacilli were predominant probably due to the fact that they are generally resistant to many adverse conditions.Aspergillus and Fusarium were the predominated fungi as a result of their high rate of sporulation and moreso, it could be a reflection of the research work involving the detection of *Fusarium spp* in sorghum, uncontrolled opening of fungal plates, and various activities in the laboratory. No gram negative cocci were identified, corresponding to the published work of Normand *et al*, 2009.
The composition and concentration of indoor bioaerosols are known to be subject to seasonal changes (Pangloli *et al*,2008).This might account for the higher levels of fungi obtained between 14-16weeks of sampling. The relative humidity is always very high in the month of September in Zaria, favoring microbial amplification which could become an important source of bioaerosol. Moreso, the low concentrations within week 4 to week 9 as shown in Fig.2 could be associated with the effect of fumigation carried out after the second week of sampling. This suggested that regular fumigation of this laboratory might greatly assist in improving its IAQ.

Not all the indoor fungi are attributed to those of outdoor air (Verhoeff *et al*,1992) as reflected in this study. *Proteus spp*, *pseudomonas aeruginosa*, and yeast were not isolated in the outdoor environment during the period of investigation. *Staphylococcus spp* were fairly predominant probably because their clusters were broken up during the process of sampling (Lundholm,1982). Streptococci are likely discharged into the environment in aerosolized form by sneezing,talking,laughing and might dry up,released to the air, thereby causing infection which could result in furuncles,carbuncles,bacteremia, and some species could adhere on work surfaces and devices resulting to adverse health effect. Klebsiella is known to spread by droplets, a gram negative environmental strain and opportunistic pathogen that can give rise to bacteremia, pneumonia, and other infections. Hands of the researchers could be a main source of transmission (Cheesebrough,2001). Proteus are important nosocomial pathogens which cause UTI, as secondary invaders, they cause septic lesions. P.*aeruginosa* are found in respiratory and urinary tract secretions as opportunistic pathogens. Gram + bacteria were most frequently recovered .This might be due to very warm and dry weather condition that prevailed during the investigation period. The gram – bacteria were fewer probably due to exposure to environmental stress rendering them non-culturable (Heidelberg *et al*,1997). Stewart *et al* in 1995 found that these organisms can be damaged by the impact on agar surface placed in the air sampler although, colonies were formed. Chang *et al*, 2001 and

Olonitola *et al*, 2006 studies performed using Anderson sampler and agar media revealed the presence of culturable gram- bacteria.

In the indoor environment, microbes are determinant of respiratory and general health.this had been established by the association between gram – bacteria endotoxin production and respiratory diseases (Cox, 1989; Flannigan *et al*.,1991).These endotoxins, when inhaled might be very significant because of their effects on humoral and cell mediated immunity, high level might predispose the researchers to illness most especially the immunosuppressed (Etkin,1994). Aspergillus and Fusarium are known to produce potent mycotoxins identified as toxic agents and associated with adverse human health effects (Hossain,*et al* ,2007). The lower level of fungi (34.65%) in this study might be due to hydrophobicity of the spores which facilitate their escape from the media even after they had been captured (Macher *et al*,1995). This was also observed by Thorne *et al*, 1992. No regulations regarding bioaerosol concentrations are currently mandated. However, Rao et al,1996 found out that existing quantitative standards and guidelines for total fungi in indoor air range from <100cfu/m3 to >1000cfum3. WHO,2008 in Development of Guidelines for IAQ stated that hundreds of bacteria and fungi in the prescence of sufficient moisture cause health problems including increased prevalence of respiratory symptoms, allergies and asthma as well as perturbation of the immunological system. Irregular and inadequate cleaning,congestions in the laboratory as shown in fig.1 offered a great opportunity for microbial growth,accumulation, and aerosolization (Pelczar *et al*, 2008). Therefore this study demonstrates the importance of routine sanitization and moreso, efficient and regular use of disinfectants should be highly esteemed. In conclusion, the high concentration of bioaerosols in this study might pose health risks for the researchers including the laboratory workers. However, the low concentrations might potentially exceed the guidelines for internal air quality. These results can assist in medical evaluations,remediation procedures, assessment of health hazards, and improvement in the understanding of the role of these components in IAQ.

REFERENCES

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

Barnett, H.H. and Hunter, B.B. (1972).Illustrated genera of imperfect fungi (3rd edn) Burgress pub. co. Minneapolis pp. 241.

Bergey's Manual of Systematic Bacterology. (1983). Stanley, J.T., Bryant, M.P., Ptenning.N.and Holt.J. 4th edn. Vol.3.1-100.

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. pp 40-72

Chang, C. W., Chung, H., Huang, C.F. and Su, H.J .(2001). Exposure of workers to airborne microorganisms in open-air swine houses. *Appl. Environ.Microbiol.* **67**: 155-161.

Cheesbrough, M. (2001). District laboratory practice in tropical countries. Part 1 and 2. Cambridge University Press, U.K. 40, West, 20th Street, New York pp. 134-310.

Cox, C.S. (1989). Airborne Bacteria and Viruses. *Sciences Pro. Oxford* **73**:469-500.

Etkin, D.S. (1994). Particulates in the indoor environment characterization and health effects cutter information corp, MA USA. pp 20-25.

Flannigan, B. and Hunter, C.A. (1998). Factors affecting airborne moulds in domestic dwellings in indoor and ambient air quality (Perry, R and Kirk, P.W. eds), pp 4611-8. Selper: London.

Fahlgren, C., Hagstrom,A.,Nilsson,D., and Zweifel,U.L (2010).Annual variations in the diversity,variabilityand origin of airborne bacteria.Appl .and Environmental microbiol.**76**(9): 3015-3025.

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 Biotechnological. **20**: 67-73.

Gregory, P.H (1973). Microbiology of the Atmosphere. 2nd edn. Leonard Hill Aylesbury pp. 7-214

Hossain, M.A., Ahmed, M.S and Ghannoum, M.A (2007). Attributes of *S. Chartarum* and its association with human disease. *J. Allergy Clin. Immunol*. **113**: 200-208

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

Lundholm, I.M.(1982).Comparative of methods for quantitative determinations of airborne bacteria and evaluation of total viable counts. *Appl. Environ.microbiol.***44**:179-183.

Macher, J., Huang, F and Flores, M. (1995). A two year study of mycological indoor air quality in a new apartment *Arch. Envir Health* **46**: 25-29.

Nevalainen, A., Pastuazka .J., Liebhaber. and Willeke .K. (1992). Performance of bioaerosol samplers, collection characteristics and sampler design considerations. *Atmos. Environ*. **26A**: 531-540.

Normand,A.,Vacheyrou,M., Sudre,B., Dick.J.J., and Renaud .P.(2009). Assessment of dust sampling methods for the study of cultivable-microorganism exposure in stables. *Appld. and Environ.Microbiol*.**75**(24):7617-7623.

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.

Pangloli,P.,Dje,Y.,Ahmed,O.,Doane,C.A.,Oliver,S.P., and Draughon,F.A.(2008). Seasonal incidence and molecular characterization of Salmonella from dairy cows, calves, and food borne pathogen.*Dis.***5**:87-96.

Pelczar, M.J., Chan, E.C.S., and Krieg.N.R (2008) Microbiology.5th edn Tata McGraw-Hill pub. company ltd,7 West Patel Nagar, New Delhi 1108 pp99 -114,566-567.

Rao .C.Y.,Proje,H.A. and Cleg.J.C.S.(1996).Review of quality standards and guidelines for fungi in the indoor air. *J of the air and waste mgt assessment.***46**:899-908.

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

Shiaka,G.P.,Yakubu.S.E.,and Olonitola.O.S. (2007). Airborne bacteria and fungi as important components of indoor air quality in selected laboratories in Zaria.pp.39-47.

Stetzenbach, L.D., Amman, H., King, G., Johanning E., and Shaughnessy R.J. (2004). Microorganism mold and indoor air quality. In: American Society for Microbiology (ASM) pub. pp 3-4.

Thorne, P.S., Kiekhaefer, M.S., Whitten, P. and Donham, K.J. (1992). Comparison of bioaerosol sampling methods in barns housing swine. *Appl. Environ. Microbiol.* **58**: 2543-2551.

Timm,M.,Madsen.A.M.,Hansen,J.V.,Moesby,L.,and Hansen,E.W.(2009). Assessment of the total inflammatory potential of bioaerosols by using a granulocyte assay.Appl.Environ.Microbiol.**75** (24):7655-7662.

Verhohoeff, A.P., Van, J.H., Brunekreef, B., Fischerr, P. and Samson R.A. (1992). Presence of viable mold propagules in in-door air in relation to damp and out door air. *Allergy*. **47**(2:1): 83-91.

World Health Organization (2008). Development of WHO guidelines for IAQ : dampness and mould. Regional office for Europe DIC-2100.pp2-6