Original Research Article

MICROBIAL ASSESSMENT OF INDOOR AIR QUALITY OF VENTILATION SYSTEMS

H.O. Stanley[1*], B. Onwuna[1], C. J. Ugboma[2]

[1]Department of Microbiology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.
[2]Department of Microbiology, Rivers State University, Nkpolu, Port Harcourt, Rivers State, Nigeria.
***corresponding author: herbert.stanley@uniport.edu.ng**

ABSTRACT

There is a growing interest in indoor air quality for a better quality environment both at home and at work because many people spend at least 80% of their time indoors. The aim of the study was to compare the indoor concentration of airborne bacteria and fungi of air conditioned (AC) buildings and naturally ventilated (NV) buildings using a Supermarket, a Laboratory and an Eatery as a case study. The predominant bacterial isolates were *Staphylococcus*, *Micrococcus* spp., *Escherichia coli*, *Bacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Klebsiella* spp. The fungal isolates belong to the genera *Penicillium*, *Aspergillus*, Mucor, *Trichophyton*, *Fusarium*, *Candida* and *Chaetomium*. The concentrations of airborne bacteria and fungi were determined using settle plate method. In AC buildings the average air concentrations of bacteria (supermarket: 24.2 CFU m^{-3}; laboratory: 29.2 CFU m^{-3}; eatery: 51.0 CFU m^{-3} air) than in NV (respectively: 54.3 CFU m^{-3}; 100.7 CFU m^{-3}; 134.3 CFU m^{-3} air). The average air concentrations for fungal isolates were higher in the eatery due to presence of poorly maintained AC system (supermarket: 7.8 CFU m^{-3}; laboratory: 11.5 CFU m^{-3}; eatery: 56.7 CFU m^{-3} air) than in NV (28.6 CFU m^{-3}; 19.6 CFU m^{-3}; 13.5 CFU m^{-3} air respectively). Findings from this study showed that AC buildings had lower concentrations of bacterial and fungal contamination compared to buildings with natural ventilation.

Key words: Air conditioned buildings, naturally ventilated buildings, bacteria, fungi

1.1 Introduction

Indoor Air Quality (IAQ) refers to the air quality within and around built environments especially as it relates to the health and comfort of the occupants [1]. Indoor air is influenced by the outdoor air such that regular outside air inflow into interiors is recognized as the main source of biological contamination of the indoor environment [2]. Improving IAQ in buildings can greatly improve the wellbeing of occupants.

The purpose of most ventilation systems is to provide thermal comfort and an acceptable IAQ for occupants. With the improvement of standard of living, occupants require more and more comfortable and healthful indoor environment. We spend about 90% of our time indoors and on an average, inhales 14m^3 air per day [3], a consequence of this life style is an increased susceptibility of individuals to indoor air pollutants with a concomitant increase in the incidence of respiratory diseases.

The factors affecting indoor environment mainly include temperature, humidity, exchange rate, air movement, ventilation, particle pollutants, biological pollutants, and gaseous pollutants [4]. Pollutants in a building's air can cause dizziness, headaches, aggravate allergies and asthma, cancer, heart disease, Chronic Obstructive Pulmonary Disease (COPD), as well of infections such as Legionnaire's disease, tuberculosis, flu, pneumonia, rhinitis, bronchitis, pharyngitis, pneumonia, keratitis, conjunctivitis and severe acute respiratory syndrome (SARS) [5-9]. All of these are indications for indoor environment problems related to poorly ventilated systems.

One of the consequences of the worldwide energy crisis in 1970s is the public recognition of the importance of energy saving. Hence more airtight buildings that minimize the loss of energy through the building envelope have been developed ever since. Fresh air is reduced in air-conditioning systems in order to reduce the energy consumption. Meanwhile, synthetic materials and chemical products (e.g., building materials and decorating materials) have widely been used indoors. The combination of low ventilation rates and the presence of numerous synthetic chemicals and wood furnishing, as well as human activities results in elevated concentrations of indoor particle pollutants and volatile organic compounds (VOCs) (e.g benzene, toluene, and formaldehyde). This is deemed to be a major contributing factor to compound hypersensitiveness [10].

According to the Commission of the European Communities [11] mechanical ventilations (heating, ventilation and air conditioning) systems have been shown to cause adverse effects on residents. Seppanen and Fisk [12] reported that there has been an increase in prevalence of sick building syndrome (SBS) between 30% and 60% in the buildings with air-conditioning systems when compared with natural ventilation systems. The aim of air-conditioning systems is to provide occupants with a more comfortable controlled environment. However, such artificial environments may be favourable to microbial pathogens.

It is fair to say that indoor environment problems still exist in many mechanically ventilated buildings, even though some comfortable and healthy air-conditioning systems have been proposed and standards set. In this study, focus will be on microbiological pollutants of air-conditioning and natural ventilation systems in selected public places in Port Harcourt, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Experimental Design

In this study, three sites: medical laboratory, eatery, shopping centre, that use either natural ventilation or HVAC were chosen. These include two supermarkets, two laboratories, and two eateries with different ventilation system. One of each pair of sample sites had air condition and the other used natural ventilation. Samples were collected in duplicates by exposing two plates of each media used for analysis by passive deposition method in the sites.

2.2 Sampling Procedure

The assessment was carried out by exposing Petri dishes containing the appropriate culture media at a convenient place in each of the three study sites, and at approximately one meter above the floor to simulate the breathing zone. Nutrient Agar plates were used for the bacteria, Potato Dextrose Agar plates were used for fungi. The Petri dishes were exposed for 30 minutes to allow time for the microorganisms in the ambient air to settle into the plates which was kept at the centre of each sample collection buildings. Thereafter, the plates were covered immediately wrapped in aluminium foil and transported to the laboratory for incubation. The plates for assessment of bacteria were incubated at 37^0C for 24 hours, while the plates for the assessment of fungi were incubated at room temperature (25^oC) for 5 days.

Microbial assessments were carried out for 3 days, at the time between 12noon when the cooling system must have circulated around the buildings. Number of isolates were enumerated and expressed as Colony Forming Units per cubic meter (CFU m^{-3}) using the equation Omeliansky described in Awad and Mawla [13].

$N = 5a \times 10^4 (bt)^{-1}$

Where:

N= microbial CFU/m^3 of indoor air,

a= number of colonies per Petri dish

b= dish surface cm^2

t= exposure time in minutes

2.3 Identification of Bacterial Isolates

Identification of the isolates was achieved by the observation of colonial characteristics such as size, colour, shape, elevation, consistency and margin. Gram Staining as well as various biochemical tests were used to identify the isolates. The isolates were identified with reference to Bergey and Holt [14] and Cheesbrough [15].

2.4 Identification of Fungal Isolates

A drop of lacto- phenol blue was placed on a clean grease free slide with using a sterile wire loop. Fungi isolates were each teased in the lacto-phenol blue and then covered with a cover slip. Slides were observed under the light microscope with an objective lens of ×40 and the morphological characteristics of the fungi were recorded. The colonial morphologies were noted with reference to Bergey and Holt [14].

2.5 Ethical Consideration

The approval to undertake the study was sought and obtained from my supervisor through the consent of the Head of microbiology department in the University of Port Harcourt. The department also communicated the personnel in the various establishment used as study site.

2.6 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) statistics 20 software was used to determine the statistical significant differences between the concentrations of bacteria and fungi isolated at different sampling sites.

3.0 RESULTS

The fungal and bacterial contaminants of the various indoor airs were studied by obtaining 18 plate samples from the three sample locations: two supermarkets, two laboratories, and two eateries with different ventilation system. The bacteria isolated from the sample locations are presented in Table 1. The predominant bacteria isolated from the study sites were, *Staphylococcus*, *Micrococcus* spp., *Escherichia coli*, *Bacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Klebsiella* spp. *Staphylococcus* spp. *Micrococcus* spp. and *Escherichia coli* were present in all samples from the air conditioned and naturally ventilated buildings. *Streptococcus* spp., *Enterococcus* spp., *Klebsiella* spp. were not present in the Eatery samples. For all sample sites, *Staphylococcus* spp. was the dominant isolate and *Escherichia coli* the least present.

Table 1: Percentage Occurrence of bacterial isolates from different sample locations

Type of organism	Supermarket		Laboratory		Eatery	
	AC	NV	AC	NV	AC	NV
***Bacillus* spp.**	-	12.2	6.2	4.9	8.5	11.6
***Staphylococcus* spp.**	57	49.8	53.6	48.6	62.4	69.3
***Micrococcus* spp.**	27.1	11.6	15.3	20.4	23.7	16.5
***Klebsiella* spp.**	-	-	6.6	13.1	-	-
***Streptococcus* spp.**	11.8	19.4	9.2	10.8	-	-
Escherichia coli	4.1	3.2	4.4	2.2	5.4	2.6
***Enterococcus* spp.**	-	3.9	4.7	-	-	-
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**

AC = air conditioned buildings; **NV** = naturally ventilated building

The average bacteria count varied significantly in the various sample sites as shown un Table 2. The highest count was recorded in the naturally ventilated samples which are in increasing bacteria population per sample site: Supermarket (54.3 CFU m^{-3} air), Laboratory (95.7 CFU m^{-3} air), Eatery (134.3 CFU m^{-3} air). While the air conditioned sample had lower bacterial population in the order: Supermarket (24.0 CFU m^{-3} air), Laboratory (29.2 CFU m^{-3} air) Eatery (51.0 CFU m^{-3} air).

Table 2: Average bacterial count from different locations

Samples	Supermarket CFU m^{-3} air	Laboratory CFU m^{-3} air	Eatery CFU m^{-3} air
AC	24.0	29.2	51.0
SE	0.8	0.8	4.9
NV	54.3	95.7	134.3
SE	7.9	5.5	35.0

AC = air conditioned buildings; **NV** = naturally ventilated building; SE=standard error

The genera *Aspergillus* and *Penicillium* were found in all AC and NV air sample of the three sites. The genera *Chaetomium* did not occur in the supermarket and naturally ventilated laboratory. *Fusarium* was identified in NV buildings of supermarket and laboratory but not found in the eatery as seen in (Table 3).

The relative frequency of fungi varied according to sample locations as shown in Table 3. The genera that occurred most in the supermarket were in decreasing order, in the AC samples, *Aspergillus* (62.5%), *Penicillium* (25%) and *Trichophyton* (12.5%). As for the NV samples; *Aspergillus* (48.3%), *Penicillium* (31.1%), *Trichophyton* (10.3%) and *Fusarium*(10.3%). In the laboratory the genera in the AC samples were *Aspergillus* (58.3%), *Trychophyton* (25%) and *Penicillium* (16.7%). While in the NV samples the genera were: *Aspergillus* (45%), *Penicillium* (35%), *Trichophyton* (15%) and *Fusarium* (5%). In the eatery, the most frequent fungi genera in the AC were *Penicillium* (71.9%), *Mucor* (19.3%), *Aspergillus* (7%), and *Chaetomium* (1.8%) and in the NV were *Penicillium* (42.9%), *Aspergillus* (28.6%), *Mucor* (14.3%) and *Chaetomium* (14.2%) as shown in Table 3.

Table 3: Percentage Occurrence of fungal isolates from different sample locations

Genera	**Supermarket**		**Laboratory**		**Eatery**	
	AC	NV	AC	NV	AC	NV
***Penicillium* spp.**	25	31.1	25	35	71.9	42.9
***Aspergillus* spp.**	62.5	48.3	58.3	45	7	28.6
***Mucor* spp.**	-	-	-	-	19.3	14.3
***Trichophyton* spp.**	12.5	10.3	-	15	-	-
***Fusarium* spp.**	-	10.3	-	5	-	-
***Candida* spp.**	-	-	16.7	-	-	-
***Chaetomium* spp.**	-	-	-	-	1.8	14.2
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**

AC = air conditioned buildings; **NV** = naturally ventilated building

The average fungal count in the samples of air conditioned building was highest in the Eatery with average CFU of (56.7 CFU m^{-3} air) followed by the Laboratory (11.5 CFU m^{-3} air) and lowest in the Supermarket (7.8 CFU m^{-3} air). On the other hand, for the building with natural ventilation, the number of fungi CFU were (13.5 CFU m^{-3} air), (19.6 CFU m^{-3} air) and (28.6 CFU m^{-3} air) for Eatery, Laboratory, and Supermarket respectively. It was observed that in all the sites the natural ventilation building indoor air had a higher number of fungal isolates when compared with the air conditioned indoor air except in the Eatery (Table 4).

Table 4: Average fungal count from different locations

Samples	Supermarket CFU m^{-3} air	Laboratory CFU m^{-3} air	Eatery CFU m^{-3} air
AC	7.8	11.5	56.7
SE	1.2	1.3	4.0
NV	28.6	19.6	13.5
SE	1.1	2.9	1.8

AC = air conditioned buildings; **NV** = naturally ventilated building; SE=standard error

4.1 Discussion

Microbiological assessment of indoor air study is one of the most vital investigations to determine the indoor air quality. The concentrations of fungi and bacteria aerosols in the indoor environment of two ventilation system was not performed in response to occupant complaints but rather to compare the building's air quality over time. The naturally ventilated buildings (NV) had more bacterial load at 54.3, 95.7 and 134.3 CFU/m^3 for Supermarket, Laboratory and Eatery respectively than the air conditioned buildings (AC) which had 24.0, 29.2, 51.0 CFU/m^3 air for Supermarket, Laboratory and Eatery respectively). The air in NV had higher loads of microbial pollutants before it relies solely on the natural process of air flow through a building, which is usually not efficient. It was observed that Supermarket with both the AC and the NV had the lowest bacterial populations followed by the Laboratory, but the Eatery had the highest bacterial populations as shown in (Table 1). The Eatery had the highest bacterial population probably due to the high amount of human activities during sample collection. This is agreement with findings of Bomala *et al*. [16] which suggested that areas characterized by a large circulation of people usually have the highest level of air contamination.

According to the sanitary standards for non-industrial premises (CEC, 1993), the level of bacterial pollution in the air conditioned buildings (AC) was very small (<50 CFU m^{-3} air) for Supermarket and Laboratory, while that of the Eatery was small (50-100 CFU m^{-3} air). For the naturally ventilated buildings (NV), the degree of pollution was small (50-100 CFU m^{-3} air) for Supermarket and Laboratory, while the Eatery is considered moderately contaminated (100-150 CFU m^{-3} air). This shows that the buildings with air conditioning systems were less contaminated with bacteria compared to those relying on naturally ventilated systems, which is in congruence with the findings of Lugauskas *et al*. [17] and Gorny [18].

Following the same classification, the degree of pollution with respect to fungal contamination for air conditioned buildings (AC) was very small (<25 CFU m^{-3} air) for the Supermarket and Laboratory, and small (50-100 CFU m^{-3} air) for the Eatery. For the natural ventilated buildings (NV), the degree of population was general very small. The results revealed that the fungal count in the Eatery was higher in the air conditioned building than in the naturally ventilated buildings. The reason was probably due to the high contamination from the air conditioning system in the eatery which was very old and poorly maintained and in condition which provided high humidity which were favourable for fungi and mould growth. Again, the air conditioning system in the eatery was found to be of old design with dirty filter coil that trapped microbial contaminants like fungal spores which are then circulated within the indoor air environment.

It is true that air coming into air conditioned environment are filtered, holding most air pathogens in the filter. But when the filter is not cleaned at specific intervals, it becomes source of biological indoor air pollution. This research demonstrated that *Staphylococcus* spp. and *Micrococcus* spp., were the most commonly found bacteria in indoor air, in agreement with Gorny and Dutkiewicz [19]. As averred by Cox and Waters [20] *Staphylococcus* spp. and *Micrococcus* spp. are significant contributors to emissions in environment with people and animals. The predominant fungal isolates include *Penicillium* spp. and *Aspergillus* spp. These fungal isolates are recognized as opportunistic pathogens for humans and often associated with clinical manifestations of allergy, rhinitis, asthma and conjunctivitis, and are considered potential candidates involved in the establishment of sick building syndromes [21].

Conclusion

This study demonstrated that buildings with air conditioning systems were less contaminated than buildings with naturally ventilated systems. It is pertinent to note that air conditioning systems may be a source of biological contamination if not properly maintained and can give rise to higher degree of contamination as shown in this study. Thus, control of indoor microbial contamination in air conditioned buildings can be achieved by cleaning the filter in the AC chamber to prevent over growth of fungi and bacteria as these organisms constitute the major biological contamination of indoor air.

Although in the investigated buildings, the degree of contamination was observed to the small and posed no immediate risk, it is important to note the usefulness of an IAQ preventive evaluation to avoid occupant complaints or health effects. Even if the preventive investigation may be initially more expensive and time consuming, it may prove to be useful by preventing building environmental problems whose solution could be very difficult and need greater investment.

References

1. United States Environmental Protection Agency (USEPA). *An introduction to Indoor Air Quality*. 2000.

2. Jain AK. Survey of bioaerosol in different indoor working environments in central India. *Aerobiologia.* 2000;16: 221-225.

3. Brochu P, Ducré-Robitaille JF, and Brodeur J. Human Ecology Risk Assess. 2006; (2: 675-701.

4. Graudenz GS, Oliveira CH, Tribess A, Mendes C, Latorre MRDO Kalil J. Association of air-conditioning with respiratory symptoms in office workers in tropical climate. *Indoor Air* 2005; 15: 62–66.

5. American Society of Heating Refrigerating and Air Conditioning Engineers (ASHRAE). Ventilation for acceptable indoor air quality. 2001; 62: 92-111.

6. Carrer P. Co-Ordination Action On Indoor Air Quality And Health Effects: Policies On Indoor Air Quality: Assessment And Needs. 2008.

7. Lewtas J. Air Pollution Combustion Emissions: Characterization Of Causative Agents And Mechanisms Associated With Cancer, Reproductive, And Cardiovascular disease. 2007.

8. Samet JM. Indoor Air Pollution: A Public Health Perspective. *Indoor Air.* 1993; (3) 219-226.

9. World Health Organization. WHO guidelines for indoor air quality: dampness and mould. Copenhagen, Denmark: World Health Organization; (2009).

10. Wang Z, Bai Z, Yu H, Zhang J, Zhu T. Regulatory standards related to building energy conservation and indoor air-quality during rapid urbanization in China. *Energy Buildings*. 2004; 36, 1299–1308.

11. Commission of the European Communities (CEC). Nutrient and Energy Intakes For the European Community. Reports of the Scientific Commitee For Food. Thirty-first series, Office For Official Publication of the European Communities, Luxembourg. 1993.

12. Seppanen O, Fisk WJ. Association of ventilation system type with sick building syndrome symptoms in office workers. *Indoor Air.* 2002;12: 98–112.

13. Awad AHA, Mawla HA. Sedimentation with the omeliansky formula as an acceptable technique for quantifying airborne fungi. *Polish Journal of Environmental Studies*. 2012; 21(6): 1539-1541.

14. Bergey D H, Holt JG. Bergey's Manual of Determinative Bacteriology. 1994.

15. Cheesbrough M. *Medical Laboratory Manual. Tropical Health Technology.* Low priced Edition. Doddington, Cambridgeshire, England. 2003, Pp 20 - 35.

16. Bomala K, Saramanda G, Reddy B, Kaparapu J. microbiological indoor and outdoor air quality of selected places in Visakhapatnam City, India. *International Journal of Current Research.* 2016;8(4):29059-29062.

17. Lugauskas A, Krikštaponis A. Microscopic fungi found in libraries of Vilnius and factors affecting their development. *Indoor Built Environment.* 2004;(13): 169-182.

18. Gorny RL. Fungal and bacterial propagules as indoor contaminants: characteristic, release mechanisms, detection. Sosnowiec. Poland, *Instit Occup Med & Environl Health Publ.,* 2004.

19. Gorny RL, Dutkiewicz J. Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Ann. Agric. Environ. Med.* 2002;9:17–23.

20. Cox CS, Waters CM. Bioaerosols handbook. Lewis Publishers, New York. 1995.

21. Schwab CJ, Straus DC. The roles of *Penicillium* and *Aspergillus* in sick building syndrome. *Advances in Applied Microbiology*. 2004;55: 215-238