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Microbiological Quality Assessment of Indoor Air of a Private University in Benin City, Nigeria.

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Abstract: *The evaluation of microorganisms present in indoors has become necessary, as how safe the air in our surrounding environment where we spent time is fundamental to our wellbeing. Hence, this study was aimed at assessing the indoor air quality of a private university in Benin City using the settle plate air sampling technique with sampling done morning and evening, once in succession for two weeks. The estimated concentration range of bacterial aerosols in the indoor environments was 7 – 440 cfu/m^3 and 4 – 90cfu/m^3 for week 1 morning and evening sampling respectively, and 72 – 180 cfu/m^3 and 8 - 75 cfu/m^3 for week 2 morning and evening sampling respectively. Also, the fungal aerosols concentration range recorded 11 – 45 cfu/m^3 and 3 – 10 cfu/m^3 for week 1 morning and evening sampling respectively, and 1 - 57 cfu/m^3 and 5 - 45 cfu/m^3 for week 2 morning and evening sampling respectively. Microbial isolates characterized were Micrococcus luteus, Klebsiella pneumonia, Micrococcus sp., Staphylococcus epidermidis, Serratiamarcensces, Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis and Escherichia coli, Neurospora sp., Rhizopus sp., Penicillium sp., Candida sp., Fusarium sp. Aspergillusniger, Alternaria sp., Aspergillusfumigatus, Cladosporiumcladosporoides. Among the microbial isolates, Aspergilusniger had the highest percentage frequency (29%) while Sarretiamarcensces and Candida sp. had the least percentage frequency (4%) each. These isolates are considered potential candidates of 'sick building' syndromes. Thus, attention must be given to control environmental factors which favour microbial growth and multiplication in indoor environment for health safety.*

Keywords: *Indoor air, sample, morning, evening, Staphylococcus aureus*

I. Introduction

Air, as a non-renewable resource, supplies us with the energy production requirement, oxygen which is essential for our bodies to live.Pollution of the air is the introduction of chemicals, particulate matter or biological materials into the atmosphere, capable of causing discomfort, disease or death to humans, damage to other living organisms including food crops. Since air is an important medium for the spread of infectious and allergic triggers which can result to undesirable effects on human beings, the control of the microbial charge became an important key to define the environmental quality of ambient media surrounding wide human populations which are largely exposed to indoor air during their daily activities (Soto *et al*., 2009). Indoor air quality (IAQ), as the name implies, is a term used to assess the quality of the air in indoor environment like offices and other building environments.In a normal indoor environment, the quantity of microorganisms should be significantly lower than outdoor levels. Possible sources of biological contamination of indoor air include: people, organic dust, various materials stored in the buildings, and the air inflowing from the ventilation and air conditioning systems (Kalwasińska*et. al*., 2012).

Depending on the amount of viscosity, temperature, lighting, and food available, different species may become dominant (Dumala and Dudzinska, 2013). The presence of bacteria and fungi in indoor air pose a serious problem from the point of view of health protection and environmental engineering. Dust, a good vehicle of airborne contamination may arise from human activities such as sweeping, movements, waving of handkerchief and bed making. Sneezing has been described as the most vigorous mechanism of generating millions of droplet into the environment with the larger falling to the ground and smaller ones evaporate and remain suspended as nuclei (Awosika*et. al*., 2012). Although pathogenic species are rather scarce in the air, some relevant microorganisms travel by aerial transmission and are involved in serious processes causing pneumonia and other diseases. Aerial fungi are much more important than bacteria as agents for allergic diseases. Air sampling of microorganisms is a popular method of conducting microbial examinations, as it allows a direct toxicological evaluation.The problem of environmental pollution is enormous and various attempts have been made to establish facilities for its control and regulation in various parts of the world.

Results have shown from various studies that microbial characterisation of different indoor air environments differ greatly in time, season, location and ventilation. According to (Udochukwu*et. al*., 2015);

major bacterial and fungal strains found in the University of Port Harcourt dormitories include *Micrococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *Serratia* spp., *Bacillus* sp.and *Kiebsiella* spp. Fungi: *Aspergillus* sp.,*Fusarium* sp., *Altenaria* sp., *Penicillium* sp. and *Cladosporium* sp. (Stryjakowska-Sekulska*et al.* 2007) had also recorded similar results of bacterial and fungal load in university rooms in Poland, he also reported that *Escherichia* genus was dominant in toilet indoor air and some species of *Cladosporium*, *Alternaria*, *Mucor*, *Rhizopus* and *Epicoccum*prevailed in a canteen and a corridor. This is mainly affected by the inflow of students in a new academic session with an inflow of different students from different backgrounds with different internal microbial characteristics resulting in the release of such through sneezing, coughing or yawning.This current study will try to develop a trend in microbial load in university rooms.

II. Materials And Method

Study Site

This study was carried out in Wellspring University, Benin City, Nigeria. Wellspringis a private tertiary institution, licenced and approved by the Federal Government of Nigeria. The institution is located in Benin City, Edo State, in the Southern region of Nigeria. Ten (10) sampling locations in the university were used in this study and they include Sickbay, Library, Administrative block, Male hostel, Female hostel, Lecture hall 1 and 2, Staff office, Canteen and Laboratory.

Sampling Procedure

Samples were taken in the school environments. Passive monitoring, using the settle plate method was done by exposing the petri dishes containing culture media at the different sampling location to be examined in the school. Sampling was done in the morning at 8:00am and evening at 4:00pm. Air samples were collected once a week for two weeks consecutively in the month of July at ten (10) sampling locations for bacterial and fungal; giving a total of 80 samples collected. The plates containing nutrient agar (NA) and potato dextrose agar (PDA) were used for the isolation of bacterial and fungal isolates respectively. An antifungal agent (Griseofulvin) was impregnated into the nutrient agar medium for the inhibition of fungi while antibiotic (Chloramphenicol) was impregnated into the potato dextrose agar medium for the inhibition of bacteria growth. Each plate was exposed on a table above ground level for a period of 30 minutesfor air sampling. The bacterial culture plates were incubated at a temperature of 37^0C for 48hours in an incubator while the fungal culture plates were left on the laboratory working bench at room temperature (20-28^0C) for 5-7 days.

Microbiological Examination

After incubation, all the nutrient agar plates incubated at 37^0C for 48hours were observed, and total number of bacterial colony forming units per cubic meter were counted and recorded. The colonial morphology of the colonies formed was noted; distinctand identical colonies were sub-cultured and incubated at 37^0C for 24 hours and stored for identification and characterizationaccording to Bergey's Manual of Systematic Bacteriology (Sneath*et al.*, 1986.) The fungal colonies were enumerated after which morphological and colonial characteristics of each colony was identified according to the manual of Barnet and Hunter 1972. The identification of fungal isolates was done according to standard methods (Chessbrough, 1991).

III. Results

Results below revealed the microbiological quality of the sampled indoor air from ten (10) different location sites in the study location. Tables 1 - 4 revealed the microbial load of the indoor air sampled while tables 5 and 6 showed the different microbial species of the sampled indoor air and their occurrence. Also, Figures 1 – 8 showed the percentage frequency of the microbial speciesas isolated from the indoor air sampled.

Table 1: Week 1 Concentration of bacteria isolates of sampled indoor air

SAMPLING SITE	SAMPLING TIME	
	8.00am	4.00pm
Sickbay	80	25
Library	14	12
Admin block	24	15
Female hostel	440	90
Male hostel	23	35
Lecture hall 1	7	4
Lecture hall 2	10	5
Office	17	12
Canteen	14	15
Laboratory	20	9

Table 2: Week 1 concentration of fungi isolates of sampled indoor air

SAMPLING SITE	SAMPLING TIME	
	8.00am	**4.00pm**
Sickbay	30	6
Library	14	4
Admin block	35	5
Female hostel	45	10
Male hostel	11	3
Lecture hall 1	27	6
Lecture hall 2	22	10
Office	25	5
Canteen	20	3
Laboratory	30	4

Table 3: Week 2 concentration of bacteria isolates of sampled indoor air

SAMPLING SITE	SAMPLING TIME	
	8.00am	**4.00pm**
Sickbay	100	40
Library	100	37
Admin block	76	33
Female hostel	180	75
Male hostel	105	65
Lecture hall 1	86	39
Lecture hall 2	93	8
Office	93	11
Canteen	72	25
Laboratory	73	42

Table 4: Week 2 concentration of fungi isolates of sampled indoor air

SAMPLING SITE	SAMPLING TIME	
	8.00am	**4.00pm**
Sickbay	21	26
Library	23	18
Admin block	53	27
Female hostel	57	23
Male hostel	1	17
Lecture hall 1	48	30
Lecture hall 2	52	45
Office	41	5
Canteen	50	32
Laboratory	45	26

Table 5: Bacteria isolates occurrence in indoor air sample

BACTERIA ISOLATES	SAMPLE SITES									
	A	B	C	D	E	F	G	H	I	J
Micrococcus luteus	+	+	+	+	+	-	+	+	+	+
Klebsiella pneumonia	+	+	-	+	+	+	+	-	-	-
Micrococcus sp.	+	+	+	+	+	+	+	+	+	+
Staphylococcus epidermidis	-	-	-	+	+	-	-	-	-	-
Serratiamarcensces	-	-	-	+	-	-	+	-	+	+
Streptococcus pyogenes	+	+	+	+	+	+	+	+	+	-
Bacillus subtilis	+	+	+	+	+	+	+	+	+	+
Staphylococcusaureus	+	+	+	+	+	+	+	+	+	+
Proteusmirabilis	-	-	-	-	+	-	+	-	-	-
Escherichiacoli	+	+	+	+	+	-	+	-	-	-
Pseudomonas aeruginosa	-	-	-	+	-	-	-	-	-	-

KEY: A= Sickbay, B=Library, C=Admin Block, D=Female hostel, E=Male hostel, F=Lecture hall I, G=Lecture hall II, H=Office, I=Canteen, J=Laboratory, + means present, - means absent

Table 6: Fungi isolates occurrence in indoor air sample

FUNGI ISOLATES	SAMPLE SITES									
	A	B	C	D	E	F	G	H	I	J
Aspergillus fumigates	+	-	-	-	-	+	+	-	-	+
Cladosporiumcladosporoides	-	-	+	+	-	-	-	+	+	+
Neurospora sp.	-	+	-	+	+	+	-	-	-	+
Penicillium sp.	+	+	+	+	-	+	-	+	+	+
Rhizopus sp.	+	+	-	+	+	-	+	-	-	+
Alternaria sp.	+	+	+	+	+	+	+	+	-	-
Aspergillusniger	+	+	+	+	-	-	+	-	-	+
Candida sp.	+	-	-	-	+	-	-	-	+	+
Fusarium sp.	+	-	-	+	-	+	-	-	-	-

KEY: A= Sickbay, B=Library, C=Admin Block, D=Female hostel, E=Male hostel, F=Lecture hall I, G=Lecture hall II, H=Office, I=Canteen, J=Laboratory, + means present, - means absent

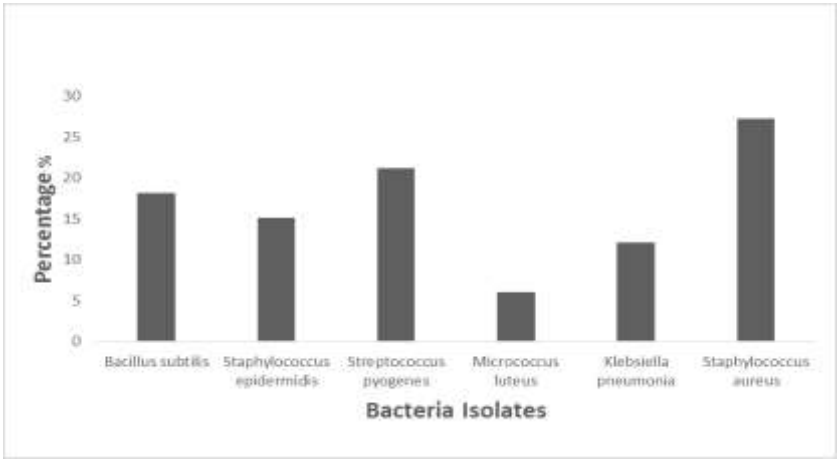

Figure 1: Percentage frequency of bacterial isolates in week 1 morning samples

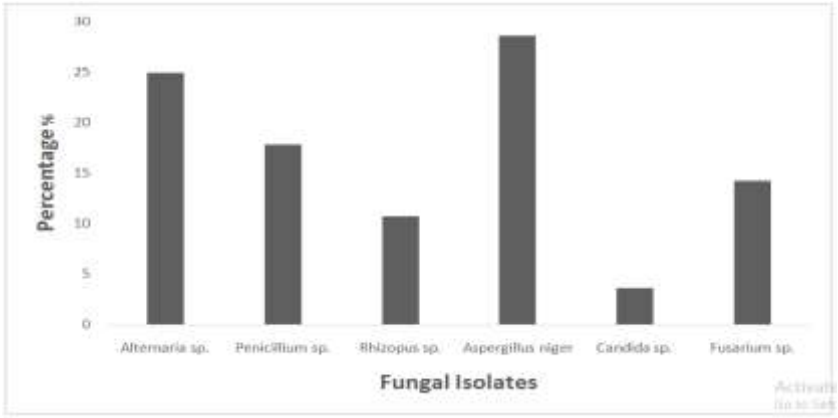

Figure 2: Percentage frequency of fungal isolates in week 1 morning samples

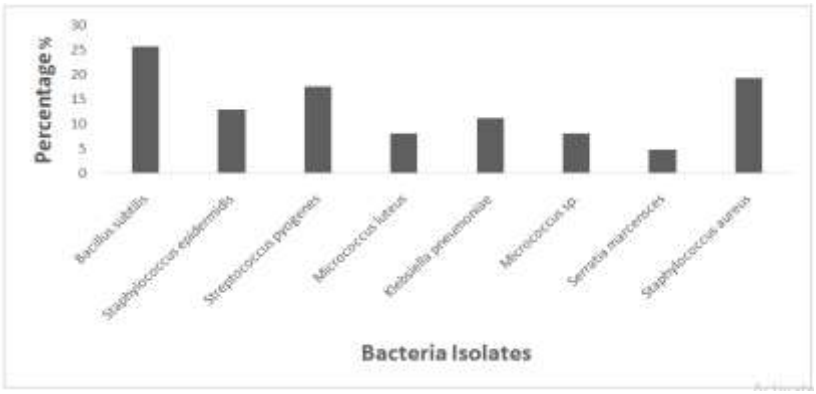

Figure 3: Percentage frequency of bacterial isolates in week 1 evening samples

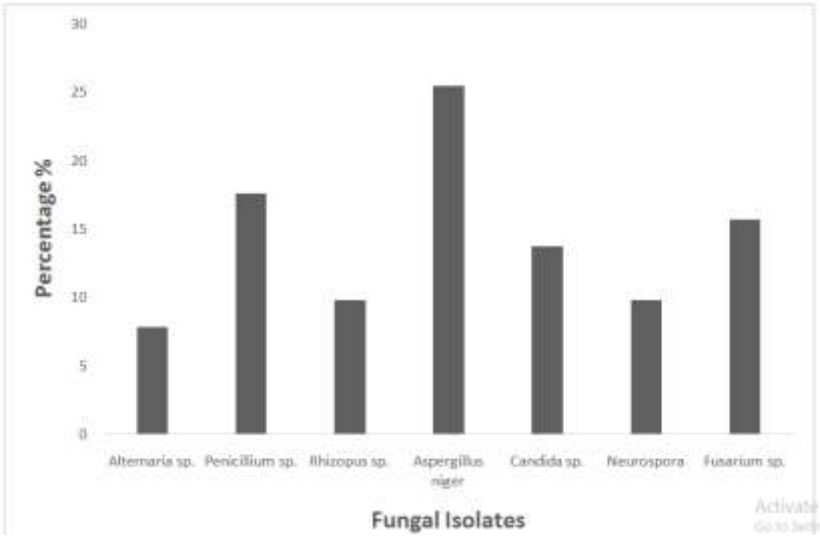

Figure 4: Percentage frequency of fungal isolates in week 1 evening samples

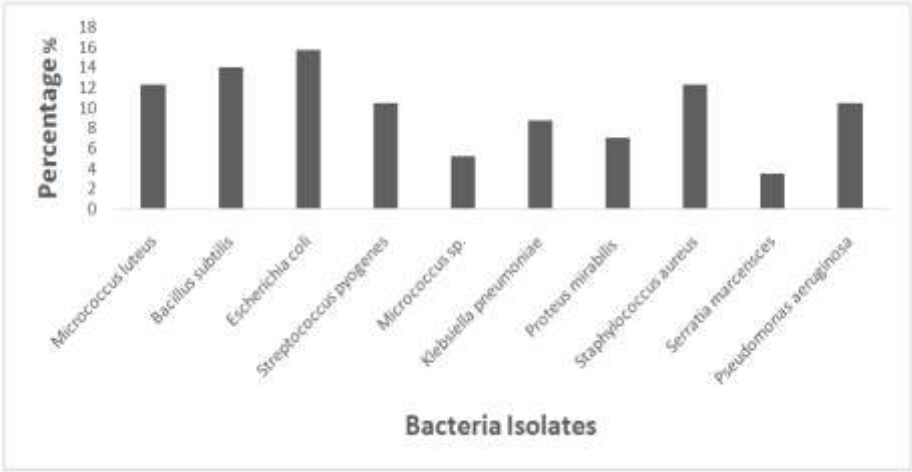

Figure 5: Percentage frequency of bacterial isolates in week 2 morning samples

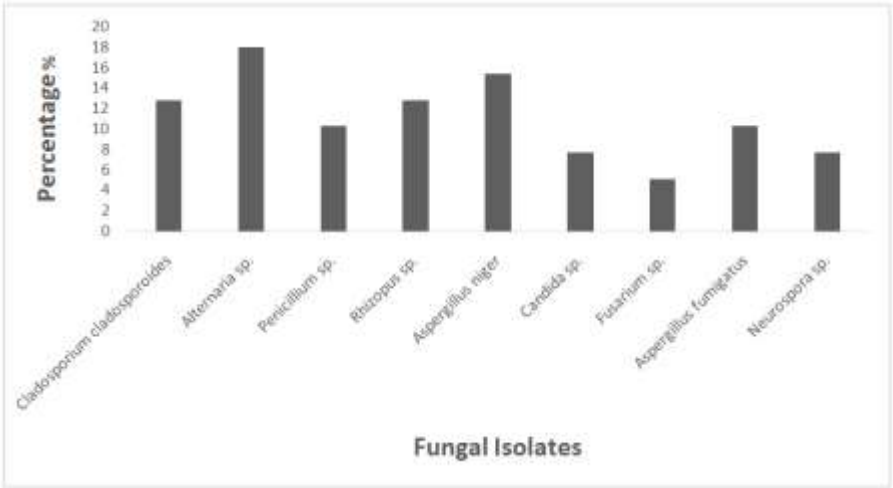

Figure 6: Percentage frequency of fungal isolates in week 2 morning samples

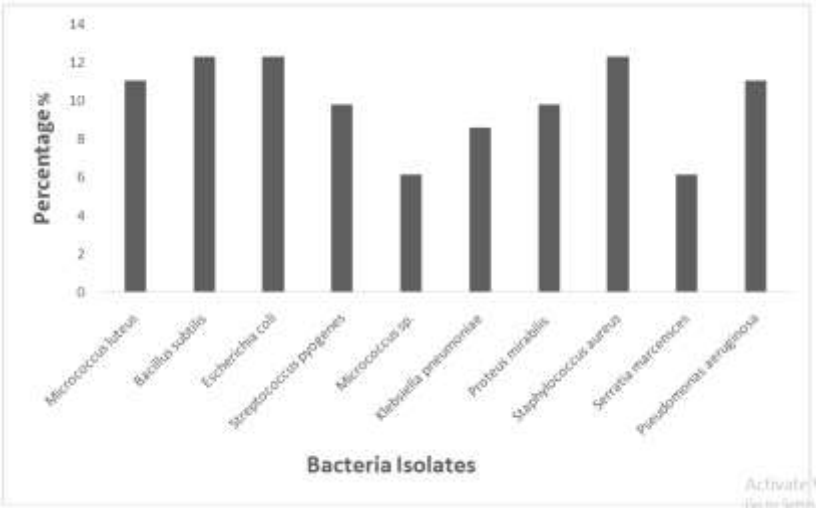

Figure 7: Percentage frequency of bacteria isolates in week 2 evening samples

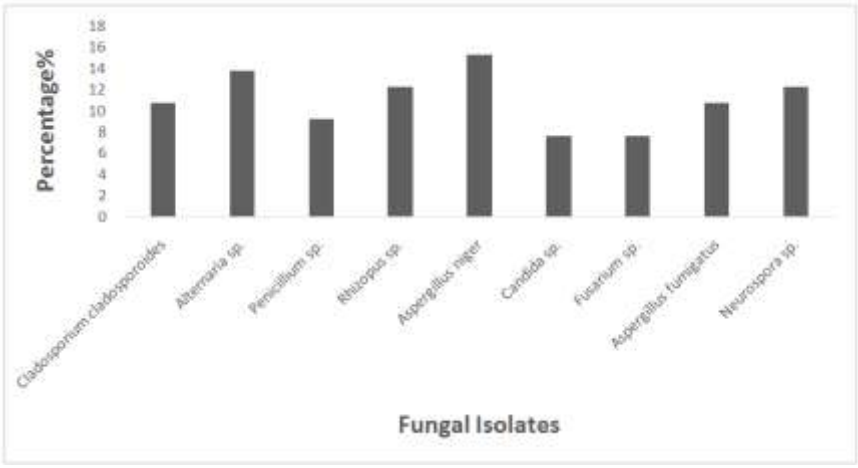

Figure 8: Percentage frequency of fungal isolates in week 2 evening samples

IV. Discussion

Microbiological quality assessment of indoor air study is one of the most vital investigations to determine the microbial indoor air pollution. The information on the indoor microbial concentrations of airborne bacteria and fungi is necessary both to estimate the health hazards and to create standard for indoor air quality control. The concentrations of bacteria and fungi aerosols in the indoor air environment as sampled in this research work carried out in Wellspring University, estimated with the use of settle plate method ranged between 7 – 440 cfu/m^3(colony forming unit per cubic metre) and 11 – 45cfu/$m^3$for bacteria and fungi respectively for week 1 morning sample; 4 – 90cfu/m^3 and 3 – 10 cfu/m^3 for bacteria and fungi respectively for week 1 evening sample; 72 - 180 cfu/m^3 and 1 - 57 cfu/m^3 for bacteria and fungirespectively for week 2 morning sample; and 8 - 75 cfu/m^3 and 5 - 45 cfu/m^3 for bacteria and fungi respectively for week 2 evening sample. There was variation in concentration of bacteria and fungi across the various sample sites as hostels generally recorded the highest count while office recorded the least count. This could be attributed to the variation in density of human population activities taking place before and during sampling time as well as the variation of ventilation conditions. These findings do agree with earlier reports by Graduenz*et al.,* 2005, Wamedo*et al.,* 2012. However, there was a slight significant difference between the morning and the evening counts, with an observable increase in the morning counts over the evening counts. This difference could be attributed to the fact that there was less ventilation in the morning due to the shutdown of the doors and windows arising from the previous day human activities. This finding is in agreement with the work done by Hayleeyesus and Manaye, 2014 where the microbial load was more in the morning compare to evening. Another determinant factor in the characteristics of microbial load in enclosed air that was not taken into consideration in this study is atmospheric conditions. This could have played an important role in the determination of internal air quality.

The both lecture halls in both weeks recorded the least microbial concentration. In lecture hall I, the bacterial count ranged from 4-86 cfu/m3 while the fungal count ranged from 6- 48 cfu/m3. While the lecture hall II

recorded a bacterial count of 5 - 93 cfu/m^3, the fungal count ranged from 10-52 cfu/m^3. The female hostel in the both weeks had the highest microbial concentration with the bacterial count ranging from 75 - 440 cfu/m3 and the fungal count was 10 - 57 cfu/m^3. However, the bacteria and fungi counts were found to be higher in the morning than in the evenings. The bacterial counts in sickbay, administrative block, canteen, library and laboratory ranged between 25 - 100 cfu/m^3, 15 - 76 cfu/m^3, 25 - 72 cfu/m^3, 12 - 100 cfu/m^3 and 9 - 73 cfu/m^3 respectively. Similarly, the fungal counts in sickbay, administrative block, canteen, library and laboratory ranged between 6 - 30 cfu/m^3, 5 - 53 cfu/m^3, 3 – 50 cfu/m^3, 4 - 23 cfu/m^3 and 4 - 45 cfu/m^3.The nature/type of activity that holds in the respective sampling locations also contributed to the characteristics difference in the microbial load during this study. As shown from the results, female hostels and lecture halls had high levels of bacteria and fungi as opposed to less populated offices and libraries. This was observed by Stryjakowska-Sekulska e*t al.*, 2007 who recorded an elevated amount in bacterial load in lecture halls at the start of the session. He also reported the importance of ventilation in the determination of indoor air quality. In the study, a well-ventilated lecture room showed a reduced amount of moulds in the afternoon when compared to other investigated spaces. This is another important aspect of the study that can be taken into consideration for future research; including the frequency and number or personnel in the investigated open space. The bacterial genera and species isolated and characterized from all sample were *Micrococcusluteus, Klebsiellapneumonia, Micrococcus* sp., *Staphylococcusepidermidis, Serratiamarcensces, Streptococcuspyogenes, Bacillussubtilis, Pseudomonasaeruginosa, Staphylococcusaureus*, *Proteusmirabilis* and *Escherichiacoli*; while the fungal genera and species isolated and characterized were *Neurospora* sp., *Rhizopus* sp., *Penicillium* sp., *Candida* sp., *Fusarium* sp. *Aspergillusniger, Alternaria* sp.,*Aspergillusfumigatus*and*Cladosporiumcladosporoides*.Results of this research showed that the most common fungi were moulds from the genera *Aspergillusniger*(29%). Similar findings was also reported by Udochukwu*et al.*, 2015, who recorded the abundance of fungi species of *Aspergillus* (52.3%), who also reported the common genera of fungi frequently isolated from the hospital air as *Aspergillus*, *Alternaria* and *Penicillium*. Meanwhile, the least percentage frequency (4%) of fungi species was recorded as *Candida* sp. The study of airborne fungal spores is important to understand the dissemination, spread, and movement of the microbes, particularly the pathogenic ones in the atmosphere (Udochukwu*et al.*, 2015). Thus, it can be posited from this investigation that occupants within the investigated enclosed environment are frequently been exposed to health hazards associated with *Aspergillus* infections. Therefore, students may suffer from (if not controlled) different respiratory diseases. Further studies will be necessary to determine which enclosed space within the school environment contributes a higher amount of a specific microbial species. On the other hand, *Staphylococcus aureus*recorded the highest percentage frequency (27%) of bacteria isolated while *Sarretiamarcensces* had the least percentage frequency (4%) of bacteria isolated.

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