1 # *Bacillus* and *Aspergillus* prevalence: an investigation into the indoor microbial quality of built environment at the University of Port Harcourt, Nigeria
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5 ## Abstract

6 **Background**Inadequate indoor air quality poses significant challenges in school environments, stemming from
7 factors such as overcrowded classrooms, insufficient ventilation, and substandard construction and maintenance
8 practices. Bacterial and fungal contaminants in indoor air present serious health risks.

9 **Methods**This institutional-based cross-sectional study focused on selected learning halls to assess bacterial and
10 fungal loads using the settle plate method. Analysis was accomplished through standard cultural methods. Nutrient
11 agar and Potato Dextrose agar plates were exposed for 15 minutes to capture bacteria and fungi, respectively.

12 **Results**The study revealed a total bacterial load of 8300 CFU/m^3 in the morning and 10400 CFU/m^3 in the evening.
13 The highest bacterial load occurred at MBS 25 (3200 CFU/m^3 in the morning and 3700 CFU/m^3 in the evening),
14 while the lowest was recorded at the Arena (310 CFU/m^3 in the morning and 990 CFU/m^3 in the evening). Similarly,
15 fungal loads varied, with the Arena recording the highest (620 CFU/m^3 in the morning and 690 CFU/m^3 in the
16 evening) and the Faculty of Science Auditorium the lowest (310 CFU/m^3 in the morning and 620 CFU/m^3 in the
17 evening). *Bacillus* spp. dominated among bacterial genera (25.9%), while *Aspergillus* spp. was the most prevalent
18 fungal genus (41.7%). Other bacterial genera included *Klebsiella*, *Staphylococcus*, *Enterococcus*, *E. coli*, and
19 *Pseudomonas*, while *Penicillium* and *Candida* were among the fungal genera.

20 **Conclusion**Mitigation strategies should target factors such as temperature, occupancy, and humidity to curb
21 bacterial and fungal proliferation in indoor lecture halls, thus safeguarding the health of students and teachers in the
22 University of Port Harcourt and similar educational institutions.

23 **Keywords**: *Bacillus* spp.; *Aspergillus* spp.; Built environment; Public health; Indoor microbial quality

24 ## 1. Introduction

25 The importance of indoor air quality (IAQ) is increasingly recognized in both occupational and public health
26 spheres. While discussions often focus on chemical pollutants, it's essential not to overlook the health implications
27 of inhaling biological particles. The indoor environment hosts a diverse array of biological materials, as highlighted
28 by [1]. IAQ stands as a critical determinant of human health, given that the air we breathe indoors is rich in
29 microorganisms, collectively known as bioaerosols. Microorganisms, including bacteria and fungi, play vital roles in
30 breaking down complex organic molecules. When provided with moisture and nutrients, microorganisms proliferate
31 rapidly in various indoor settings [2]. Furthermore, outdoor air consistently contains microorganisms and their
32 reproductive structures, with the specific types and populations varying according to local environmental conditions.
33 Doors, windows, and fresh air intakes serve as pathways through which microorganisms readily infiltrate building
34 interiors. It's typical to detect some level of microorganisms in indoor air, although in a standard indoor setting, their
35 abundance should be notably lower than outdoor levels. However, excessive moisture within buildings resulting
36 from leaks, flooding, or other sources can create an imbalanced environment conducive to microbial proliferation.
37 Factors such as viscosity, temperature, lighting, and nutrient availability influence which species thrive, potentially
38 leading to dominance by certain microbial populations [3]. Consequently, elevated quantities of specific
39 microorganisms may trigger adverse health effects among building occupants, including both illnesses and allergic
40 reactions. It is estimated that approximately 30% of indoor air quality-related health issues stem from the human
41 body's reaction to mold exposure.

42 Potential sources of biological contamination in indoor air encompass a variety of sources, including human
43 occupants, organic dust, stored materials within buildings, and air entering from ventilation and air conditioning
44 systems [5]. Numerous studies underscore the importance of efficient ventilation and airtightness in reducing indoor

air contamination compared to spaces lacking such systems. The presence of bacteria and fungi in indoor air presents significant challenges for both health protection and environmental engineering [5]. Indoor air pollution can elevate the risk of irritation, allergic sensitization, acute and chronic respiratory disorders, and impair lung function [6]. Exposure to high levels of airborne microbes often results in allergic reactions, asthma exacerbations, fever, pneumonia, and infections. In recent years, there has been a notable increase in allergic reactions to fungal spores and other microbial indicators. Indicator organisms, chosen for their ability to assess air cleanliness or potential contamination, play a crucial role in evaluating indoor air quality. These organisms, encompassing bacteria, fungi, and viruses, provide insights into the overall microbiological composition of indoor environments [7]. Common viral indicators include Influenza viruses, Rhino- and Enteroviruses, and Adenoviruses. Bacterial indicators comprise *Staphylococcus*, *Streptococcus*, *Legionella*, *Escherichiacoli*, *Clostridium*, and *Bacillus*, while prevalent fungal indicators encompass *Penicillium*, *Cladosporium*, *Aspergillus*, among others.

Understanding the types of microorganisms present in indoor environments is crucial for assessing indoor air quality, which can foretell potential health risks, and instigate implementing appropriate mitigation strategies to ensure the well-being of occupants. Information relating to indoor microorganisms of classrooms is limited in the public domain. It is on this note we direct our effort to study the fungal and bacterial organisms that constitute indoor quality of some selected lecture rooms of the university of Port Harcourt, Nigeria. Our study shows that *Staphylococcus* and *Aspergillus* dominate indoor microbial quality of the selected lecture halls. The culturally identified bacteria and fungi genera provide valuable information about the general microbiological quality of air, they might not directly correlate with specific health risks.

2. Materials and Methods

2.1 Study site

The research was conducted at specific sites within the University of Port Harcourt, Rivers State, as outlined in Table 1. A total of sixteen (16) samples were aseptically collected from three (3) distinct lecture halls and the open Arena, utilized as a control, at the Abuja campus of the University of Port Harcourt, Choba, Rivers State.

Table 1. Description of sample collection location

S/N	Location	Description
1	A	MBS 24 lecture hall
2	B	MBS 25 lecture hall
3	C	Faculty of Science Auditorium (FOS)
4	D	Arena (Control)

2.2 Sampling

Petri dishes containing Nutrient Agar and Potato Dextrose Agar media were utilized to capture bacteria and fungi from the air, respectively. The exposure duration was set at fifteen minutes. Sampling occurred at two distinct time points during the day: morning and evening. The initial set of Petri dishes, each containing the appropriate medium, was positioned at the sampling site before the commencement of lectures (8-8:15 am), with a repeat procedure for the second set in the evening (6-6:15 pm). Following exposure to indoor air, the Petri dishes were transported to the laboratory and incubated at room temperature on the laboratory bench for 24 to 48 hours for bacteria and 3-5 days for fungi. Subsequently, isolated samples were inoculated into sterile bijou bottles containing nutrient agar slants and incubated at 37°C for 24 hours before refrigeration at 4°C. The average colony-forming units (CFU) of both bacteria and fungi were determined and converted to organisms per cubic meter of air, as described by [8]. The formula used to calculate the colony-forming unit per cubic meter is expressed as:

CFU/m3 = a * 10000 ÷ p * t * 0.2 ($1CFU/m^3 = a10000 \div p \times t \times 0.2$ (1)

Where:

a = the number of colonies on the petri dishes;

p = surface of the petri dishes;

t = the time of petri dish exposure

2.3 Isolation and Characterisation of Isolates

Sterile wire loops were utilized to retrieve pure cultures from the stocked cultures, which were subsequently streaked onto suitable prepared media and then incubated for 24 hours for bacteria and 3-5 days for fungi, in accordance with the methods outlined by Toppo and Naik [9]. The pure isolates were then evaluated based on their morphological characteristics, including shape, size, color, edge, opacity, and elevation. Additionally, they underwent gram staining and biochemical tests for further characterization.

2.4 Gram Staining

This test aimed to classify bacterial isolates into two categories: gram-positive and gram-negative organisms. Gram staining achieves this by discerning the chemical and physical characteristics of bacterial cell walls, particularly the presence of peptidoglycan, which is prominently found in the thick cell wall of gram-positive bacteria [10]. A smear of the bacterial test culture was prepared and heat-fixed onto a grease-free slide. The slide was then flooded with crystal violet for one minute, gently washed with tap water, and drained. Subsequently, it was exposed to Gram's iodine for one minute, washed with 75% alcohol for 30 seconds, rinsed with tap water, and air-dried. Next, the slide was counter-stained with 0.25% safranin for 30 seconds, washed, drained, air-dried, and examined under a microscope using an oil-immersion lens (x100). A purple coloration indicated a gram-positive organism, signifying retention of the primary stain (crystal violet), while a pink coloration resulting from the counter stain (safranin) indicated a gram-negative organism, indicative of the inability to retain the primary stain.

2.5 Biochemical Tests to Identify Bacterial Isolates

2.5.1 Oxidase test

This test aimed to assess the isolates' capacity to produce the oxidase enzyme. The dry filter paper method, outlined by Al-Dhabaan [11] was employed for this purpose. A filter paper soaked with a reagent solution was allowed to dry before being smeared with a colony of the test organism. The observation for the formation of a purple color ensued. A positive result was denoted by the appearance of a purple color within 2 minutes, while isolates without such coloration within this timeframe were considered negative.

2.5.2 Methyl Red (MR) Test

This step aimed to assess the test organism's capacity for mixed acid fermentation. Organisms demonstrating this ability produce sufficient acid to overcome the buffering capacity of the broth medium, thereby reducing its pH from 7.5 to approximately 4.4 or lower (Madigan and Martinko, 2008). Subsequently, 5 drops of 0.05% methyl red solution were added to one of the 2.5 ml MR-VP broth tubes. The development of a red coloration, resulting from heightened acid production and a subsequent decrease in the pH of the culture medium to 4.4, indicated a positive result. Conversely, the formation of a yellow coloration, indicative of a less acidic medium with a pH above 6.0, signaled a negative result [12].

2.5.3 Voges Proskauer (VP) Test

This test aimed to assess the isolates' capacity to produce acetylmethyl carbinol acetoin, a fermentation byproduct. Following the incubation period, 0.6 ml (approximately 12 drops) of 5% (w/v in ethanol) α-Naphthol and 0.2 ml (approximately 4 drops) of 40% (w/v in distilled water) KOH solution were added to the test tube. The mixture was vigorously shaken and then set aside in a slanted position to maximize exposure to oxygen for approximately 1 hour. The appearance of a red coloration indicated a positive result, while a yellow coloration implied a negative result [13].

2.5.4 Citrate Test

This experiment aimed to assess the isolates' capability to utilize sodium citrate as the sole carbon source. The protocol outlined by MacWilliams [13] was followed. Under aseptic conditions, the test isolates were inoculated into test tubes containing Simmon's citrate agar, which were covered with cotton wool to facilitate oxygen supply necessary for the citrate test. The inoculated tubes were then incubated for 48 hours at 37°C. The medium contained bromothymol blue as a pH indicator. Test tubes retaining a green color were considered negative, while those exhibiting a color change to blue indicated positive results. The shift in color from green to blue occurred due to an increase in pH above 7.6.

2.5.5 Sugar Fermentation Test

This test, conducted following the previously outlined procedure by Kockova ***etal.***[15], aimed to evaluate the isolates' ability to metabolize specific sugars, including glucose, lactose, and sucrose. Initially, 105 g of peptone water powder was precisely weighed and transferred into a conical flask. Subsequently, 1g of each sugar was accurately weighed and added to the flask. To this mixture, 1ml of Bromo-thymol blue indicator was introduced, followed by the addition of 100 ml of water. The resulting broth was thoroughly mixed and dispensed into bijou bottles or test tubes containing inverted Durham tubes. The peptone water broth in the bijou bottles or test tubes was then sterilized in an autoclave at 115°C for 15 minutes. This process was repeated for the other sugars. Following sterilization, the peptone water broth was allowed to cool before inoculation with the culture. Subsequently, the inoculated broth was incubated at room temperature for 24 hours. The ability of the isolates to utilize sugar was indicated by a color change to light blue and, in some instances, the production of gas in the Durham tubes.

2.5.6 Motility Test

This test was conducted to ascertain the motility of organisms. The procedure outlined by Al-Aabideen [16] was followed. A semi-solid medium was prepared by dissolving 14 g of nutrient agar in one liter of distilled water. Subsequently, 10 ml of the semi-solid nutrient agar was dispensed into each test tube and allowed to solidify. Using a sterile inoculating needle, the test organism was aseptically picked and stabbed into the semi-solid agar. The inoculated tubes were then incubated for 48 hours at 37°C, and growth was observed for any deviation from the original needle stab line. Deviation from the line indicated motility, whereas straight-line growth suggested non-motile organisms.

2.5.7 Indole Test

This test was conducted to assess the isolates' ability to metabolize tryptophan for nutritional purposes, utilizing the enzyme tryptophanase and producing indole, which was detected using Kovac's reagent. The procedure was performed in accordance with the method described by MacWilliams [17]. The test organism was incubated in a test tube containing peptone water for 48 hours at 37°C. Following incubation, 0.5 ml (approximately 5 drops) of Kovac's reagent was added to the test tube and observed after agitation for one minute. A positive indole test was characterized by the rapid formation of a pink to red (cherry-red ring) in the reagent layer on top of the medium, while the formation of a yellow or slightly cloudy color indicated a negative result.

2.5.8 Catalase Test

This test aimed to distinguish between catalase-producing and non-catalase-producing bacteria, following the protocol outlined by Reiner [18]. The slide test method was employed for this purpose. Initially, a drop of hydrogen peroxide (H_2O_2) solution was dispensed onto a sterile glass slide using a sterile wire loop. Subsequently, a colony of each isolate was collected and transferred onto another sterile glass slide, which acted as a cover slip. The cover slip was then inverted and positioned atop the hydrogen peroxide solution. After observation for 10 seconds, the

175 formation of bubbles indicated a positive catalase result, whereas the absence of bubble formation denoted a
176 negative result.

177 **2.6 Isolate characterisation**

178 Data obtained from gram staining and biochemical tests were utilized to classify the isolates according to the
179 guidelines provided in Bergey's Manual of Determinative Bacteriology [19].

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181 ## 3. Results

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183 ### 3.1 Total bacterial count

184 The result of the total bacteria count of the various air samples are shown in Fig. 1. The count ranged from 3.1×10^2
185 to 3.7×10^3 CFU/m^3, with Arena-Morning having the lowest value (log10 value of 2.50) and MBS 25-Evening
186 having the highest value (Log10 value 3.57. A total of 18700 (8300 morning; 10400 evening) isolates were found
187 with a mean value of 2337.5. The result of biochemical characterisation is displayed in Table 1 while the percentage
188 distribution of the culturally characterised bacteria is represented in Fig. 2. Six different bacterial genera were
189 characterised out of the 27 isolates. These bacterial genera are *Bacillus*, *Klebsiella*, *Staphylococcus*, *Enterococcus*,
190 *Escherichiacoli* and *Pseudomonas*. The percentage distribution shows that *Staphylococcus* had the highest share
191 with 22.20% while *Pseudomonas*, *Klebsiella* and *Escherichiacoli* had the lowest percentage with a value of 11.10%.
192 *Bacillus* had 25.90%, and *Enterococcus* 14.80

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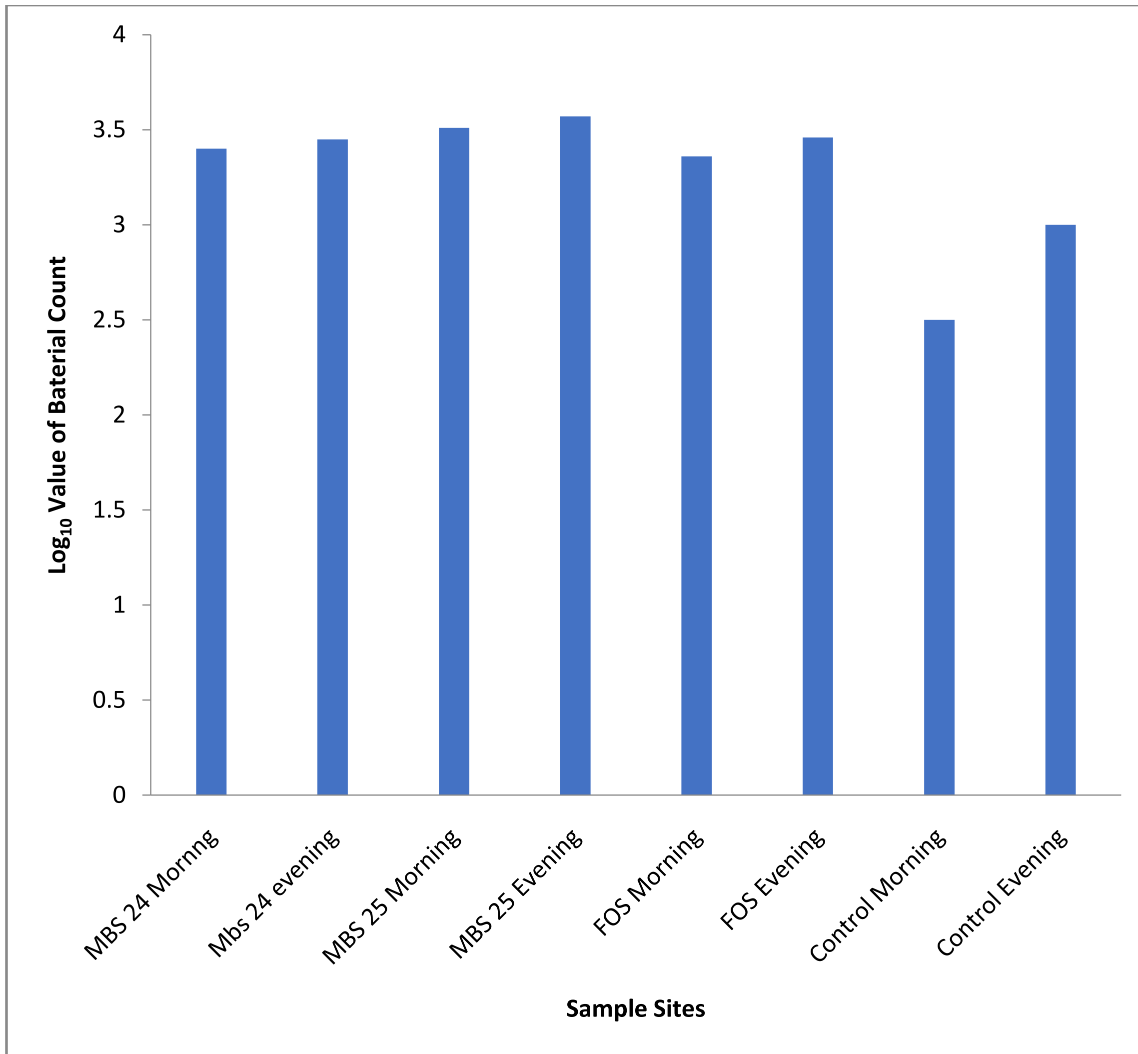

194 Fig. 1: Log_{10} value of total Bacterial Count.

195 Table 1. Biochemical characterization of isolated bacteria

Sample Locations	Isolates	Catalase	Oxidase	Indole	Coagulase	Citrate	TSIAButt	TSIAslant	Gas	H_2S	Sucrose	Glucose	Lactose	Gram Reaction	Motility	Possible organism
MBS 24	T1	-	-	-	-	-	Y	Y	-	-	-	-	-	+ Cocci	-	*Enterococcus* spp.
	T2	-	-	-	-	+	Y	Y	-	-	+	+	+	- Rod	-	*Klebsiella* spp.
	T3	-	-	+	-	-	Y	Y	-	-	+	+	+	- Rod	-	*Escherichia coli*
	T4	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.
	T5	+	-	-	+	-	Y	Y	-	-	+	+	+	+ Cocci	-	*Staphylococcus* spp.
	T6	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.
	T7	-	-	+	-	-	Y	Y	-	-	+	+	+	- Rod	-	*Escherichia coli*
MBS 25	T8	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.
	T9	-	-	-	-	-	Y	Y	-	-	-	-	-	+ Cocci	-	*Enterococcus* spp
	T10	+	-	-	+	-	Y	Y	-	-	+	+	+	+ Cocci	-	*Staphylococcus* spp.
	T11	-	-	+	-	-	Y	Y	-	-	+	+	+	- Rod	-	*Escherichia coli*
	T12	-	+	-	-	-	Y	R	-	-	-	-	-	- Rod	-	*Pseudomonas* spp.
FOS HALL	T13	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.

	T14	+	-	-	+	-	Y	Y	-	-	+	+	+	+ Cocci	-	*Staphylococcus* spp.
	T15	-	-	-	-	+	Y	Y	-	-	+	+	+	- Rod	-	*Klebsiella* spp.
	T16	+	-	-	+	-	Y	Y	-	-	+	+	+	+ Cocci	-	*Staphylococcus* spp.
	T17	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.
	T18	-	-	-	-	-	Y	Y	-	-	-	-	-	+ Cocci	-	*Enterococcus* spp
	T19	-	-	-	-	+	Y	Y	-	-	+	+	+	- Rod	-	*Klebsiella* spp.
Arena (Control)	T20	-	+	-	-	-	Y	R	-	-	-	-	-	- Rod	-	*Pseudomonas* spp.
	T21	+	-	-	+	-	Y	Y	-	-	+	+	+	+ Cocci	-	*Staphylococcus* spp.
	T22	-	-	-	-	-	Y	Y	-	-	-	-	-	+ Cocci	-	*Enterococcus* spp
	T23	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.
	T24	-	+	-	-	-	Y	R	-	-	-	-	-	- Rod	-	*Pseudomonas* spp.
	T25	+	-	-	+	-	Y	Y	-	-	+	+	+	+ Cocci	-	*Staphylococcus* spp.
	T26	-	-	-	-	-	Y	Y	-	-	-	-	-	+ Cocci	-	*Enterococcus* spp
	T27	-	-	-	-	-	Y	Y	-	-	+	+	+	+ Rod	-	*Bacillus* spp.

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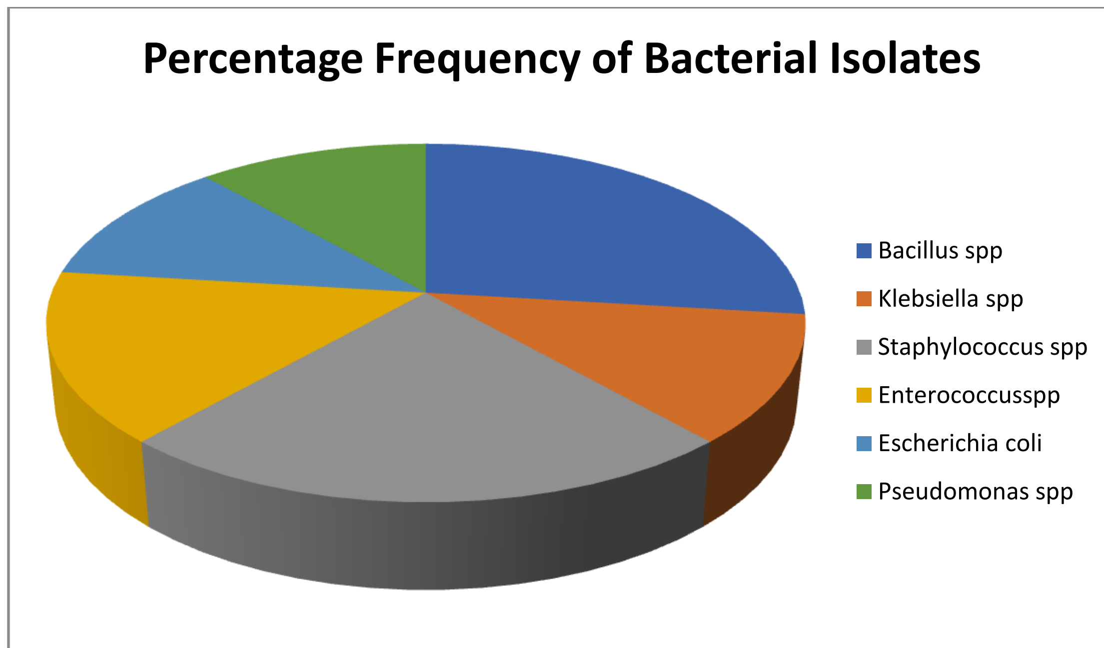

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198 Fig: 2. Percentage frequency of bacterial Isolates

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212 **3.2 Total Fungal Count**

213 The result of the total fungal count of the various sample sites are shown below, the count ranged from 3.1×10^2 to
214 $6.9\text{x}10^2$ CFU/m^3 with FOS-Morning having the lowest value and Arena-Evening having the highest value (see Fig.
215 3). A total of 3930 isolate were found with a mean value of 491.25. The 12 isolates were characterized (as displayed
216 in Table 2) into three fungal genera: *Aspergillus* (41.70%), *Penicillium* (33.3%) and *Candida* (25.00%), following
217 macroscopic and microscopic examination. The percentage distribution is shown in Fig. 4.

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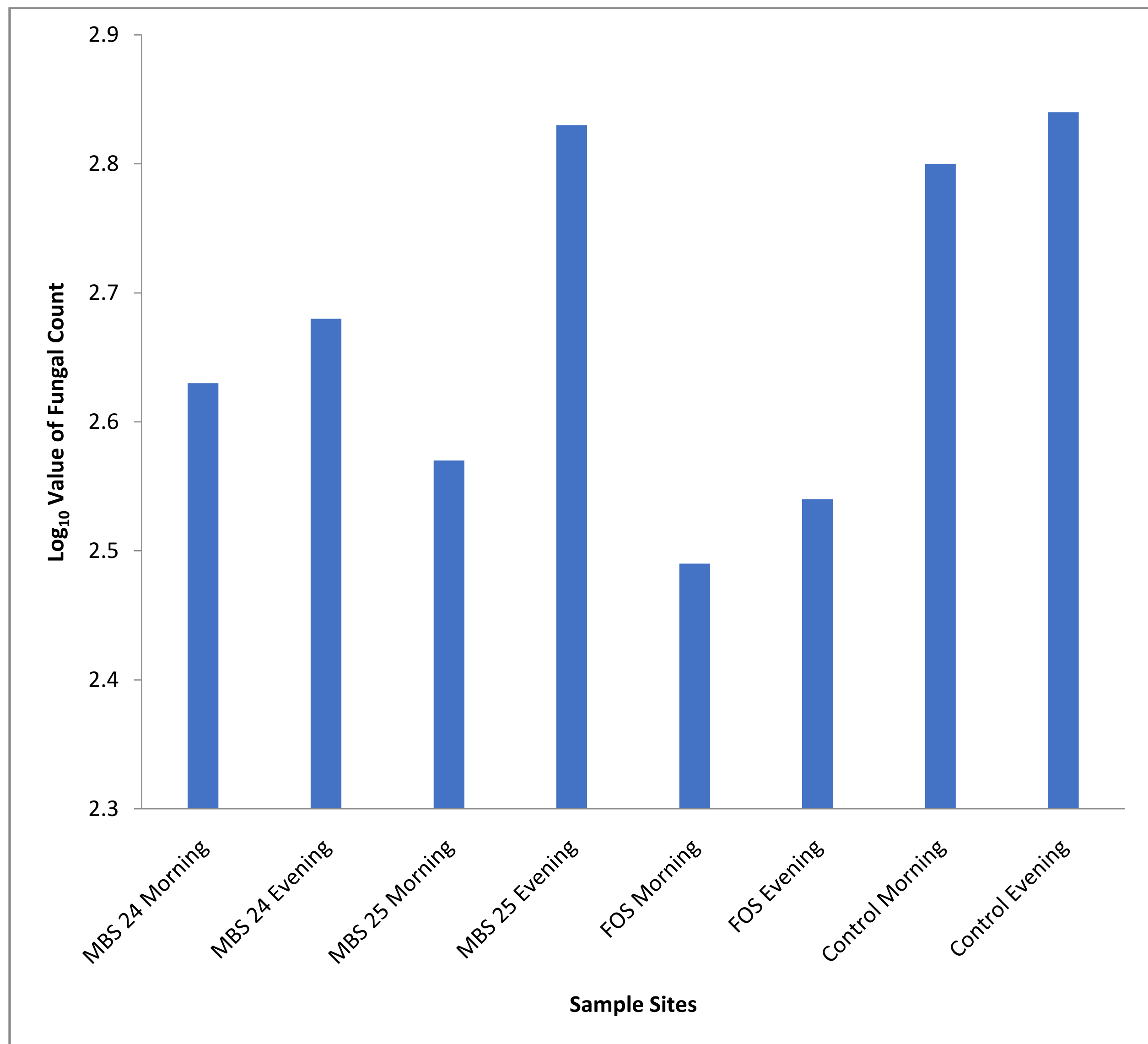

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220 Fig. 3. Chart showing Log_{10} Value of Fungal Count

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223 Table 2. Morphology of Fungal Isolates

Sample Location	Macroscopy	Microscopy	Possible Organism
MBS 24 (Morning)	White muccor growth with brown reverse	Long rod-like cells	*Candida* spp.
	Brown cottony growth, white periphery and brown radial reverse	Septate hyphae round columinal head spores	*Aspergillus* spp.
MBS 24 (Evening)	Green variety growth with brown radial reverse	Septate branching hyphae, chain-like conidia, spore	*Penicillium* spp.
	Black cottony growth, brown radial reverse	Sptate hyphae, round head conidia spore present	*Aspergillus* spp.
MBS 25 (Morning)	White Muccor growth with brown reverse	Long rod-like cells	*Candida* spp.
FOS HALL (Morning)	Black cottony growth, brown radial reverse	Septate hyphae, round head conidia spore present	*Aspergillus* spp.
	Green variety growth with brown radial reverse	Septate branching hyphae, chain-like conidia, spore	*Penicillium* spp.
FOS HALL (Evening)	Black cottony growth, brown radial reverse	Septate hyphae, round head conidia spore present	*Aspergillus* spp.
	Green variety growth with brown radial reverse	Septate branching hyphae, chain-like conidia, spore	*Penicillium* spp.
Control (Arena)	Milk colour, muccor growth and brown reverse	Oval shape cell	*Candida* spp.
	Black cottony growth, brown radial reverse	Septate hyphae, round head conidia spore present	*Aspergillus* spp.
	Green variety growth with brown radial reverse	Septate branching hyphae, chain-like conidia, spore	*Penicillium* spp.

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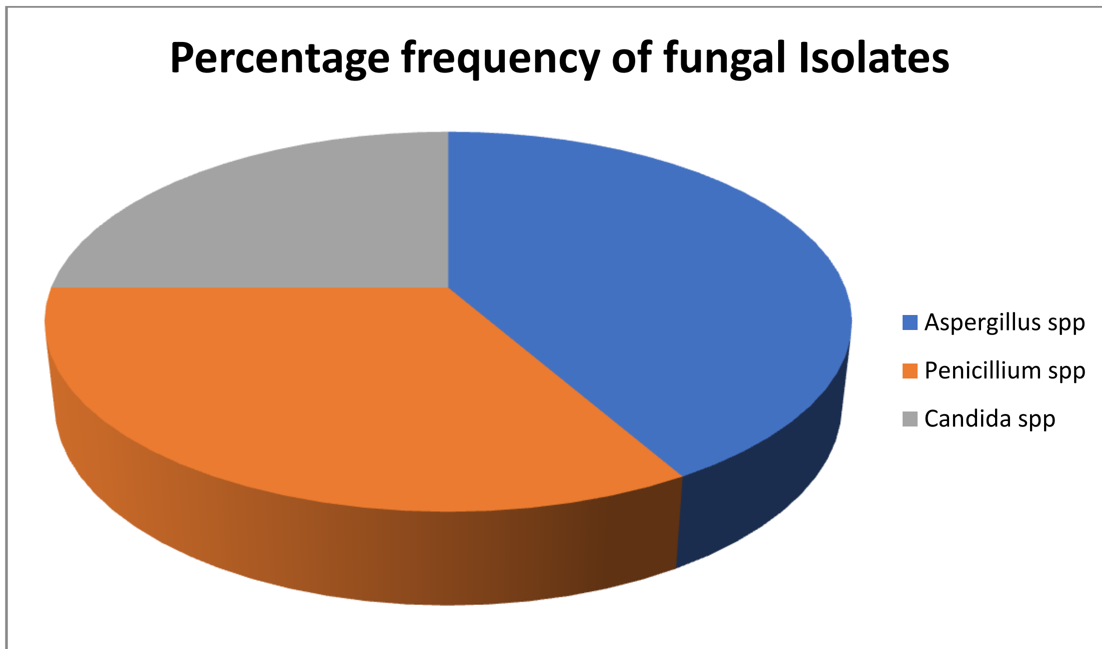

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236 Fig. 4. Percentage Frequency of Fungal Isolates

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4. Discussions

Cultural method relevance

Microbiological analysis was carried out on sixteen (16) samples obtained from selected lecture Halls in the University of Port Harcourt. The bacterial load and diversity of the air samples were determined using traditional approach. All the sixteen (16) samples collected in this current study yielded bacterial growth on nutrient agar and fungal growth on Potato dextrose agar. Though, have more disadvantages in comparison to molecular methods, cultural methods are still used to charcaterise bacteria and fungi especially in medical microbiology [20, 21, 22]. Cultural methods are important because it is less expensive, provide the prerequisite for further analysis like gram staining, biochemical tests and serotyping [23, 24]. Diverse of health-implicated bacteria and fungi indicators in built environment has been isolated and characterized using cultural methods [25]. Akinrotoye [26] identified *Bacillus* spp. in public schools while Lee *etal*. [27] identified *Klebsiella* and *Staphylococcus* in public-use facilities using cultural methods. The identified bacteria in this study have been implicated with public health. For instance, *Bacillus* shows to be a virulence factor for septicemia [28]; *Staphylococcus* causes food poisoning [29], *Klebsiella* aggravate respiratory diseases [30]. *Candida*, *Aspergillus* and *Penicillium* fungi genera, identified in this study have been shown to have public health issues [31]. The loads of these indicator microorganisms are not equally distributed according to our result.

Microbial loads appear higher in the evening

The microbial loads recorded in the morning were lower than the loads recorded in theevening in most cases. This result is predicated on the complex interaction of temperature, occupancy, humidity and ventilation. The first three factors increase microbial load while the last factor reduce microbial diversity load [32, 33]. Temperature between 25 – 37 ^{0}C positively influence the microbial growth rate. This temperature range control the vicinity of the study site but the optimum temperature is closer to the upper temperature value [34] during early evening while in the early morning the lower limit predominates. Andualem *etal*. [16] demonstrated a positive correlation between that temperature and microbial diversity of bacteria and fungi in public primary schools. Occupancy contributes to increased microbial diversity as was demonstrated by Zhang *etal*. [35] and Li *etal*. [36] in built environment in public university. Vacation of halls between evening and the next morning allow enough ventilation, which abate the microbial loads. This was the observation of Lestinen *etal*. [37] during research conducted in educational buildings, where non-activity period is long enough to freshen the indoor air before occupancy. Relative humidity to some extent correlates with selective microorganisms. High humidity promotes mold and fungal growth, while low humidity can limit bacterial survival. Kravchenko *etal*. [38] show that humidity has the most influence on fungal growth and temperature on bacteria. The interaction of these factors justifies our findings that indoor microbial quality tends to increase immediate after the classes in the evening than before the start of class in the morning.

Dominance of *Bacillus* and *Aspergillus*

The interaction of predominant factors in the study site give rise to the dominance of *Bacillus* and *Aspergillus* in the indoor microbial quality. *Bacillus* species pose threat to humans in public spaces; thus, their presence warrants careful management to mitigate potential risks to public health and environmental quality. Exposure to Aspergillus can lead to opportunistic diseases, allergic reactions and food spoilage. Effective strategies include implementing hygiene measures, proper waste management practices, regular monitoring, and targeted interventions to ensure the safe coexistence of *Bacillus* and *Aspergillus* with humans in shared environments.

5. Conclusion

The research concludes that individuals in the observed spaces are regularly exposed to health risks from bioaerosols. The findings indicate that regardless of the time of day, indoor environments tend to accumulate bioaerosols, potentially facilitating the rapid spread of infections within lecture halls. The study recommends addressing physical factors conducive to bacterial growth indoors in lecture halls to protect the health of students and lecturers. Overall, insights into microbial indoor air quality in university lecture halls would help alert school management to potential hazards and their impact on academic activities.

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