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Microbiological Analysis of Outdoor Air Quality of Male and Female Hostels In Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

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Abstract: *The microbiological quality of the air environment of male and female hostel was assessed by determining the concentrations and composition of bacteria and fungi present in the outdoor air. Air samples were collected from five different locations of the sites by plate sedimentation methods which involved exposing media-filled petri plates to the air for 10min and 20 min at different period of the (morning and afternoon). Nutrient agar (NA) was used for the enumeration of total bacterial concentrations while sabouraud dextrose agar (SDA) was used for the enumeration of total fungal isolates. The results obtained showed that concentrations of bacteria is hostel ranged from 7-98 (100%) while the concentrations of fungi ranged from 7-98 (100%). Six bacteria genera and five fungal genera were isolated at varying sampling distance. The qualitative analyses of the microbial flora of the outdoor air of school hostel in Ebonyi State University (EBSU) Campuses have provided information on the airborne microorganisms. The fact that some of the bacteria and fungi species isolated are known to be pathogenic to humans has demonstrated that the micro flora of EBSU has public health implications.*
Keywords: *Outdoor air, Male hostel, Female hostel, Bacteria and Fungi*

I. Introduction

In recent years, research into outdoor air quality has increased because of increasing awareness of the variety of health problems potentially caused by airborne microorganisms. An aerosol is a suspension of microscopic solid and/or liquid particle in air or gas.

Biological aerosols are single microorganisms or clumps of microorganisms attached to solid or liquid particles suspended in the air [1]. The composition of bioaerosol includes: bacteria, yeast, moulds, spores of bacteria and moulds, microbial fragments, toxins, metabolites, viruses, parasites and pollen. Bioaerosols generally range in size from 0.5µm to 50µm in diameter [2]. Microorganisms in bioaerosols may attach to dust particles or may survive as free floating particles surrounded by a coating of dried organic or inorganic material. Location and environmental conditions such as humidity, density and temperature have a great effect on the type of population and amount of microorganisms in enclosed air. Whilst all types of microorganisms can cause problems outdoors. Bacteria and fungi are commonly associated with outdoor air quality complaints..

In any outdoor environment, a variety of species will be present at different times and in different micro-environments. In order for airborne disease transmission to occur from microbes around a building, there must be a source or reservoir for the microbes, that is to say, there are some means for the microbes to multiply and the mechanism for their release and dispersion into air as described by [3].

As seen, especially around Ebonyi State University Hostels, the major outdoor reservoirs are stagnant water or moist exterior surfaces. This can accumulate microbes that enter the building the outdoor air and act as amplifiers for bacteria.

Solomon and Burge (1997), [4], observed that fungi can grow in relatively dry environments (example at relative humidity above 75%). Airborne dispersion is relatively easy for microbes found in building ventilation systems (e.g. fungal and bacterial spores) or contaminant objects. The Northern Territory work health Authority (1993) has stated that measurement of microorganisms in about 1000 CFU (colony forming unit) per cubic meter of air indicates that building environment may need to be investigated for microbiological contamination. However, Burge and Feeley (1991), [3], also stated that exceeding this level does not mean that the air is unsafe or hazardous. Merely using a number to represent CFU per cubic meter is an unreliable indicator of the actual hazard posed on airborne microorganisms because of the universal presence of microorganisms.

It is critically important to obtain an indication of the ratio groups of organisms present. The information will be of greatest value with periodic testing to provide data for a trend analysis of the microbial groups in a particular site. Stuttard (1996), [5], further stated that by differentiating these groups of organisms,

the likely sources, the risk potential and the need for much action can be established; hence the justification for the microbiological analysis of outdoor air quality in both female and male hostel in Ebonyi State University, Abakaliki.

Background of the Study

Ebonyi State University (EBSU), a citadel of knowledge acquisition, is a typical of Nigerian University which amidst holding a large population of staff members and students, activities like animal husbandry, fishery, and businesses of various kind are carried out within the school environment; hence, it is expedient to come up with a work on the microbiological analysis of the outdoor air around the male and female hostel so that knowledge of the variations of the outdoor bioaerosols concentrations can be utilized by both the people with respiratory diseases and those doing heavy activities to plan their daily routine accordingly. Finally, presence of harmful microbes in the air through OAQ can be avoided and or at least their presence should be below pathogenic level.

Statement of Problem

Many students live in school hostel which is within EBSU due to its affordability and proximity. Therefore, it is paramount to microbiologically analyse the outdoor air quality of the male and female hostel in EBSU Abakaliki, Nigeria.

Aim of the Study

The aim this study was to analyse the microbiological quality of outdoor air around male and female hostel in EBSU.

II. Materials And Methods

Study Area

This research was carried out in EBSU, a Nigerian University with large population and beehive of activities, is divided into four campuses-Presco, CAS, Ishieke and Permanent site Campuses. Two of the Campuses-Presco and CAS were selected for outdoor air quality (OAQ) assessment. EBSU lies in Abakaliki, the state capital, which covers an area of 5,932 square kilometers and is geographically located at longitude $7^{0}3^{1}$N and latitude $5^{0}4^{1}$ E.

Methods

Sampling Sites

The two Campuses, CHS (College of Health Science) and CAS (College of agricultural Science), of EBSU selected for this study hold a large population of inhabitants. Air samples were taken from the following sites in both of the Campuses: male and female hostel. At both sites, different time periods were used: 8:00-8:10 am and 8:00-8:20 am in the morning period while 4:00-4:10 pm and 4:20 pm time ranges for afternoon period. The exposure time was for the air microorganisms to settle gravitationally directly on the media surfaces of the plates, a method called plate sedimentation method. Air sampling, for detection and isolation of viable counts of bacteria and fungi, was carried out for a period of five months (January to May).

Sample Collection

Culturable microorganisms (bacteria and fungi) were sample by exposing prepared nutrient and sabouraud dextrose agar (SDA) media to air 10 and 20 minutes as employed by [6 and 7]. The sampling time was to avoid drying of the agar surface and overloading of the media plates [8]. The air sampler was set up at a height representative of the normal human breathing zone, that is, above ground level [9].

Sample Processing

Each collected specimen was immediately taken to microbiology section of Ebonyi State University, Abakaliki for microbiological analysis. The following processing techniques were employed:

The 24 hour media plates, used for air sampling, that had shown growth, were all sub-cultured to make sure that colonies seen were not contaminants.

The plates were incubated overnight at 37°C and examined. Bacterial isolates were first differentiated by macroscopic examination of the colony. The colonies were differentiated based on size, colour, pigmentation, elevation surface texture and margin.

Gram Staining
Gram stain classifies bacterial isolates into two main groups namely: Gram-positive and Gram-negative on the basis of differential interactions of Gram's reagent with the varying cell wall components of these two groups of bacteria.

Procedure: A drop of normal saline was placed on a well labelled clean grease-free glass slide using a sterile inoculating lop; a colony of an overnight culture of the bacterial isolate was emulsified with the normal saline to make a thin smear. The smear was air dried and then heat fixed. The slide was flooded with crystal violet (primary stain) for 60 s after which the stain was rinsed from the slide with water. The smear was flooded with Lugol's iodine (mordant) to fix the primary stain. The iodine was rinsed with water after 60 s. the slide was then flooded with a decolourizer (acetone) and rinsed off almost immediately. The counter stain; safranin was added and left for 60 s before being rinsed off. The stained smear was air dried, and then observed under the microscope using X_{100} oil immersion objective lens of the microscope. A cluster of purple colonies was indicative of staphylococci for most of the microbial isolates. Gram staining was done as described in [10].

Microscopy:
It was done to further analyse the organisms according to the method described in [11].

Fungal Identification
Identification of all fungal isolates was also carried out using standard methods based on macroscopic and microscopic features as described by [10].

Lactophenol (Cotton blue test)
On a clean slide, a drop of methanol was placed and a portion of fungi growth was cut with the aid of surgical blade and tested in the methanol. A drop of lactophenol cotton blue was added. A cover slip was placed on it gently and observed under the microscope with X_{40} objectives; the picture seen was compared with an identification chart (atlas) [10].

Biochemical Tests
Several biochemical tests were carried out to further identify the various bacteria isolated according to the standard microbiological methods as described by [12].

Catalase Test
Catalase test was carried out as described by [13]. Staphylococci produce catalase, an important virulence factor which degrades the microbicidal H_2O_2 into O_2 and H_2O. This ability differentiates staphylococci from streptococci.

Procedure: a drop of 3% hydrogen peroxide was placed on a clean grease-free glass slide. About 2 colonies of the bacteria were picked from a culture plate using a sterile wire loop and placed on the hydrogen peroxide; presence of bubbles indicated a positive catalase test for most of the microbial isolates.

Oxidase Test
Procedure: A piece of filter was placed in a clean Petri dish. Made few drops of oxidase reagent (tetramethyl-p-phenyldiamine) on afiltered paper such that it soak edcompletely
A small portion of the suspected colony was brought using the edge of a slide and observed for the presence of blue colour. Absence of blue colour indicates oxidase negative.

Indole Test
This test is for the production of indole by bacteria on a medium which contains tryptophan as described by [10].

Procedure: an organism was inoculated in 3 ml sterile tryptone water. Incubated at 35-37^{0}C for up to 48h; 0.5 ml of kovac's reagents was added to test for indole production. After a gentle shake, red colour in the surface layer within 10 min is indicative for a positive result. The red colour seen is as a result of the reaction between 4-(p)- dimethylamino-benzaldehyde in kovac' reagent and indole.

Statistical Analysis
Charts demonstrating the percentage distribution of the bio aerosols isolated are shown in page 32-34.

III. Results

Identification and Characterization of Fungal Isolates

A total of 318 colonies were isolated for both fungi and bacteria. The genera of isolated airborn fungi depending on mean frequency distribution in number of colony counts were classified as predominant and as isolates of no concern. The dominant species were members of the genera *Aspergillus SP*. (46.40% am and 3.55% pm). *Fusarium* sp. (41.30% am and 8.70% pm) *Penicillium sp*. (36.35% am and 13.65% pm) for both afternoon and morning periods while *Mucor sp*. (50.00% am and 0.00% pm) and *Rhizopus sp*. (0.00% am and 50.00% pm) were less frequent as shown in table 1 and

Table 1: Identification and Characterization of Fungal Isolates

		Colony	Morphology		Time			
s/n	unit	Colour on SDA	Appearance	10am	20am	10pm	20pm	Suspected organisms
1	SH	Black	Brown, Hyphae with spores borne around it	56	35	6	1	*Aspergillus sp.*
2	SH	White	Dense smooth spores born around the hyphae	8	11	2	2	*Fusarium sp.*
3	SH	Whitish grey	No rhizoids, spores borne within the hyphae	2	7	nil	nil	*Mucor sp.*
4	SH	Yellow	Conidia in chains, spores borne around the hyphae	7	9	4	2	*Penicilium sp.*
5	SH	Brown	Spores around nil the hyphae	Nil	2	5	Rhizopus spp.	

Key:
SDA = sabaroud dextrose agar (solid medium)
SH = School Hostel

Percentage Distribution of Fungal isolates

Table 2: Percentage Distribution of Fungal isolates

	Morning		Afternoon		
Fungal isolates	10min	20min	10min	20min(%)	Total
	(%)	(%)	(%)		
Aspergillus sp.	56(57.1%)	35(35.7%)	6(6.1%)	1(1.0%)	98(100%)
Fusarium sp.	8(34.8%)	11(47.8%)	2(8.7%)	2(8.7%)	23(100%)
Mucor sp.	2(22.2%)	7(77.8%)	0(0.0%)	0(0.0%)	9(10``0%)
Penicillium sp.	7(31.8%)	9(40.9%)	4(18.2%)	2(9.1%)	22(100%)
Rhizopus sp.	0(0.0%)	0(0.0%)	2(28.6%)	5(71.4%)	7(100%)

Identification and Characterization of Bacterial Isolates

For bacterial isolates, the dominant species were members of the genera *Escherichia coli* (25.00% am and 25.00%pm), *Micrococcus sp.* (33.80% am and 16.20% pm) and *Klebsiella sp*. (23.65% am and 26.35%pm) for both afternoon and morning period while *Corynebacterium spp*. (6% am and 0% pm) *Pseudomonas sp*. (50.00% am and 0.00% pm) *Staphylococcus spp*. (0.00% am and 50.00% pm) were less frequent as shown in table 3 and 4.

Table 3: Identification and Characterisation of Bacterial Isolates

S/N	Unit	CC	Morphologyon NA media	Time (of agar plate exposure)				GR	Biochemical tests			Suspected organisms
									CT	OT	IT	
				10am	20am	10pm	20pm					
1	SH	Y	Rod (singly)	12	14	9	17	-	-	-	+	*Escheri-chia coli*
2	SH	Y	Cccci(clustered)	8	1		4	+	+	-	+	*Micrococcus sp*
3	SH	W	Rod(in chains)	17	10	18	12	-	+	-	+	*Klebsiella sp.*
4	SH	B	Rod (singly)	3	2	nil	nil	+	+	-	-	*Coryne-bacteriums pp*
5	SH	C	Rod (singly)	3	2	nil	nil	_	+	+	+	*Pseudomonas sp*
6	SH	P	Cocci(clustered)	Nil	nil	2	4	+	+	-	+	*Staphylococcus spp.*

Key:
SH = School Hostel
Y = yellow O = Orange W = White B = Black C = Chalky P = Pink
CC = colony colour NA = nutrient agar GR = Gram reaction
+ = positive tests = negative CT = catalase OT = oxidase test

Table 4: percentage distribution of bacterial isolates

	Morning		Afternoon		
Bacterial isolates	10min (%)	20min (%)	10min (%)	20 min(%)	Total
Escheri-chia coli	12(25.1%)	14(26.9%)	9(17.3%)	17(32.7%)	52(100%)
Micrococcus sp	8(23.5%)	15(44.1%)	7(20.6%)	4(11.8%)	34(100%)
Klebsiella sp.	17(29.8%)	10(17.5%)	18(31.6%)	12(21.1%)	57(100%)
Coryne-bacteriums pp	3(60%)	2(40%)	0(0.0%)	0(0.0%)	5(100%)
Pseudomonas sp	3(60%)	2(40%)	0(0.0%)	0(0.0%)	5(100%)
Staphylococcus spp.	0(0.0%)	0(0.0%)	2(33.3%)	4(66.7%)	6(100%)

IV. Discussion

This is unique because it described culturable fungi and bacterial obtained from outdoor air of students' hostels and sit-outs, as little or no study have been carried out on this in this part of the world.

Results of the research into the percentage concentration of bioaerosol present in the air outside the EBSU library building are presented in Table 2 and 4.

The results indicated that the total amount of fungi ranged from 7-98 (100%) while that of bacteria found in the outdoor air ranged from 5-57 (100%).

The percentage concentration range of fungi [62-73 (100%) in the outdoor air was higher than bacteria which maintained a constant concentration of 43 (100%) in the morning, but fluctuated, that is, 10-73 (100%) in the afternoon against that of bacteria [36-37 (100%). The overall percentage concentration for both fungi and bacteria aerosol was surprisingly the same, [159 (100%)], which suggested a balance in bioaerosols within the library air ecosystem.

Comparison between bioaeresol concentrations determined during the investigations carried out on the premises of the University Library in EBSU and the results obtained by other researchers who investigated the library environment posed some difficulty due to the small number of available publications and some methodological limitations. The tests were conducted using the plate sedimentation method which, in view of recent studies, can be used for qualitative rather than quantitative assessment of the presence of microorganisms in the air [14].

Studies conducted by ACGH (1989) states that outdoor airborne fungi concentration routinely exceeds 1000 CFU/m^3 and may average near 10,000 CFU/m^3 in summer months. ACGIH (1989) also show that concentrations of less than 100 CFU/m^3 may be unhealthy to immunosuppressed people; by comparison with the above data, it can be concluded that the concentrations of bioaerosols determined during the investigations in the University Library in EBSU do not fall within the range normally observed in such areas-has a higher percentage bioaerosol occurrence.

A relatively high concentration of *fusarium sp*. (a plant pothogen) and *rhizopus sp.* Observed in library air quality assessment was because of the farming activities that go on around and within the library ecosystem which is in accordance with [14]. Remarkably, air contamination reached the highest level in areas characterized by a large circulation of people-the region around the front of the library-produced large amounts of microorganisms in the air. Vehicular and human movement constituted an additional source of air contamination since they are responsible for large amounts of dust around the library.

A quantitative interpretation of the results describing that air quality in the library is difficult due to the lack of widely accepted normative and reference values. Evaluation of the air quality in the premises of the University Library in EBSU was based on the sanitary standards for non-industrial premises formulated by the European Commission in 1993.

V. Recommendation

To mitigate adverse effects of bioaerosols, school management should ensure that the refuse dump in proximity to school hostels should be properly attended and mechanical aids or powered machinery should be made available to garbage clearers to reduce physical exertion and shorten the duration of outdoor work.

Medical advice, especially for those with existing heart or respiratory illnesses, should be taken into account in assigning outdoor work, particularly, heavy manual work while persons in doubt of their health condition or feel chest or breathing discomfort should seek medical advice to know their health status.

VI. Conclusion

Even though rankings should not be used to define safe or unsafe concentrations of bioaerosols, determing the number and type of airborne microorganisms can be used to determine the degree of cleanliness as a means of determining the source of human discomfort and certain airborne microbial infections. The prevalence of *corynebacterium sp. Pseudomonas sp., staphylococcus, aspergillus, rhzopus* and *mucor sp.* after analysis in this study was relatively very high, therefore, it is important to develop low cost screening tools that can be used to identify the probable source of the bioaerosols; this is because knowledge of the hourly variations

of the bioaerosols concentrations outdoor can help the people with respiratory diseases and those doing heavy activities to plan their daily routine accordingly.

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