

CHARACTERIZATION OF AIRBORNE MYCOFLORA IN ACADEMIC BUILDINGS OF A TERTIARY INSTITUTION IN RIVERS STATE

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Abstract: Atmospheric pollution has become one of the most pressing problems of our age, and the involvement of indoor air poses a potential threat to the health and well-being of the populace. This study therefore, focused on assessing indoor airborne fungi in some buildings in a Tertiary Institution in Rivers State Nigeria. Sampling sites included classrooms, shopping complex, restaurant, and residential quarters. The open plate method called 'Sedimentation Technique' was employed in the analysis of air-borne indoor fungi using Sabouraud Dextrose agar medium. Sampling was carried out weekly for four weeks. Results of total counts of fungi in ten (10) sampling sites ranged between 1.7 and 60.2X10³CFU M-3 while mean counts ranged from 6.5 – 21X10³CFU M-3. Fungi isolated during the period of the study were *Aspergillus flavus*, *A. niger*, *A. nidulans*, *A. terreus*, *Cladosporium resinae*, *Fusarium* species and *Penicillium spinulosum*; some of which have been implicated in respiratory infections. The study revealed that microbial load and types varied from site to site and from week to week throughout the study period. Furthermore, high load of fungi observed in some sampling sites indicated that the requirements regarding building design were not observed during construction of the sampled buildings; hence most of the buildings sampled revealed conditions below living standard.

Keywords: Pollution, potential threat, screening, fungi, indoor air

Introduction

Atmospheric pollution is one of the most pressing problems of our age. This pollution has now reached advanced levels that they pose a potential threat to the health and well-being of the populace. The atmospheric components seem to promote the survival of microorganisms in the air. The air is composed of 75% nitrogen, 21% oxygen, 0.9% argon, 0.03% carbon dioxide and 0.076% other trace gases, very low concentration of organic and inorganic nutrients and free waters at an irregular intervals (Burge and Hoyer, 1990; Jaffal *et al.*, 1997; Dutkiewicz and Augustowska, 2006). The health and well-being of the public are affected by the physical, chemical and biological properties of indoor environment. The quality of indoor environment, however, is not easily defined or controlled and can potentially place human occupants at risk (Burge and Hoyer, 1990; Jaffal *et al.*, 1997).

Microorganisms are the primary source of indoor air contamination (Lewis, 1994; Dutkiewicz and Augustowska, 2006; Gocgeldi *et al.*, 2011). The source and spread of organisms inside room are human related, and usually the body normal flora and organisms found in clothing are shed during human activities (Jaffal *et al.*, 1997; Dutkiewicz and Augustowska, 2006). Environmental organisms and other bacteria from air, dust, soil and bed making, coughing, sneezing and yawning add to indoor microbial collections (Jaffal *et al.*, 1997; Dutkiewicz and Augustowska, 2006). Microorganisms and other particles

are transported and dispersed by air movements. The air-borne microbes that constitute public health concern indoor are those that are causative agents of infectious diseases and allergies.

These agents belong to the viruses, fungi and bacterial groups. Factors such as the number of visitors, and the amount of materials brought in from outside influence microbial population of a given environment (Jaffal *et al.*, 1997; Dutkiewicz and Augustowska, 2006). Also, building designs promote good indoor air or deteriorate it. Ventilation is an important factor that can influence the quality of indoor environment (Wemedeo *et al.*, 2012).

Fungal spores disseminated by air are potential biological hazards; some species have been isolated from indoor air (Gammage *et al.*, 1985; Wemedeo *et al.*, 2012). Previous studies had shown that the microbial flora of indoor air depends on such factors as the number and hygienic standard of the occupants (Dugid *et al.*, 1946; Dutkiewicz and Augustowska, 2006), and the ventilation of the enclosed space. In poor quality and crowded rooms, the higher number of occupants confined to a small space, results in the build-up of air-borne microbes shed from the human body. High humidity level results in condensation on surfaces and growth of biological contaminants such as fungi particularly in places where dusts and dirt accumulated. The health risk is that people who stay in the room may be exposed to the biological pollutants and be at risk of contracting infections (Burge and Hoyer, 1990).

In Nigeria, geographical and regional monitoring of indoor microorganisms has not been extensively studied. Individuals as well as corporate agencies are not mindful of the danger in poor design of buildings. Continuous evaluation of indoor and outdoor atmosphere is of paramount concern to public health practitioners. The aim of this research therefore was to investigate the density and type of air-borne fungi of some buildings in a Tertiary Institution in Rivers State, and to predict the health hazards associated with occupying such residences.

Materials and Methods

Study Area

The study area was Rivers State University Campus, Port Harcourt, Nigeria. Five (5) stations from the sampling area namely: Faculty of Science (FC), Faculty of Engineering (FE), Faculty of Environmental Sciences (FES), Shopping Complex (SC) and Security Village (SV) which were designated as stations 1, 2, 3, 4, and 5 respectively. Two sites were chosen from each station which include FC: site 1 – A classroom in Biology building, and site 2 – a Science Lecture Hall; FE: site 3 and 4 – Engineering classroom-2 and New engineering hall respectively. FES: sites 5 and 6 – level 300 studios and level 400 studios respectively of Department of Urban and Regional Planning. SC: site 7 – Shop F23 and site 8 – Bill box restaurant. SV: site 9 – One room apartment and site 10, a one-room living batcher made up of wooden parts. Sampling was carried out in April for 4 weeks during afternoon period.

Microbiology of Air Samples

Analysis of air for fungi was done by the Open Plate Method called “Kosch’s Sedimentation Technique”. This Air Collection Method involved exposing petri dishes (in duplicates) containing sterile freshly prepared Sabouraud Dextrose agar medium to air; the agar plates were placed one metre above the ground and exposed to the ambient air at the study sites for 30 seconds. After exposure, the plates were covered and aseptically transported to the laboratory and incubated at 28°C for 2 – 3 days. After incubation, fungal colonies were counted and enumerated using Kosch’s sedimentation formula (Latika and Ritu, 2011), and taken as population of fungi enumerated at each site in colony forming units per cubic metre. Colour pigmentation, diameter and colonial characteristics of the colonies were observed and recorded. Pure cultures of fungi were obtained by sub-culturing discrete colonies onto freshly prepared sterile Sabouraud Dextrose agar plates and incubated at 28°C for 2 – 5 days.

Fungi isolated from the air samples were characterized and identified by macroscopic and microscopic examinations of their spores formed in colonies. Macroscopy was carried out by observing the colonial morphology, colour of spores, texture, diameter and surface appearance as well as colour of growth on the reverse plates. Microscopy was done using wet preparation called the needle mount technique and slide culture technique for examination of the fungal spores (Barnett and Hunter, 1972; Winn *et al.*, 2006).

Estimation of fungal population in colony forming units

Fungal colonies were estimated using Kosch's sedimentation formula as follows:

$$A = \frac{a \times 10000}{0.2 \times \pi r^2 \times t} \dots \dots \dots \text{equation 1}$$

$A = \text{CFUM}^{-3}$

a = average number of colonies

r = radius of petri dish

t = time of exposure of the plate

Data Analysis

Data collected were statistically analysed using IBM SPSS version 22 statistical tool. ANOVA without replication was used to check for significant differences between mean of both station and weekly values.

Results

Counts of fungi of the five sampling stations are tabulated in Table 1, and the frequency of the fungal isolates is shown in Tables 2 and 3. Weekly counts of fungi ($\times 10^3 \text{CFUM}^{-3}$) at the sampling sites ranged as follows: site 1: 5.16 – 12.0; site 2: 1.72–60.2; site 3: 5.16 – 25.8, site 4: 3.44 – 10.3; site 5: 5.16 – 15.5; site 6: 3.44 – 15.5; site 7: 3.44 – 10.3; site 8: 3.44 – 31.0; site 9: 5.16 – 17.2; site 10: 1.72 – 13.8. High fungal counts were observed in sites 2, 3 and 8, and in weeks 3 and 4; sites 1, 6, 9 and 10 had moderate counts; low counts were observed in sites 4 and 7, weeks 1 and 2. Eight (8) fungal species isolated, belonging to five (5) genera, were: *Aspergillus flavus*, *A. nidulans*, *A. niger*, *A. terreus*, *Cladosporium resinae*, *Fusarium species*, *Mucor species* and *Penicillium spinulosum*. Some of the species have been implicated in respiratory tract infections.

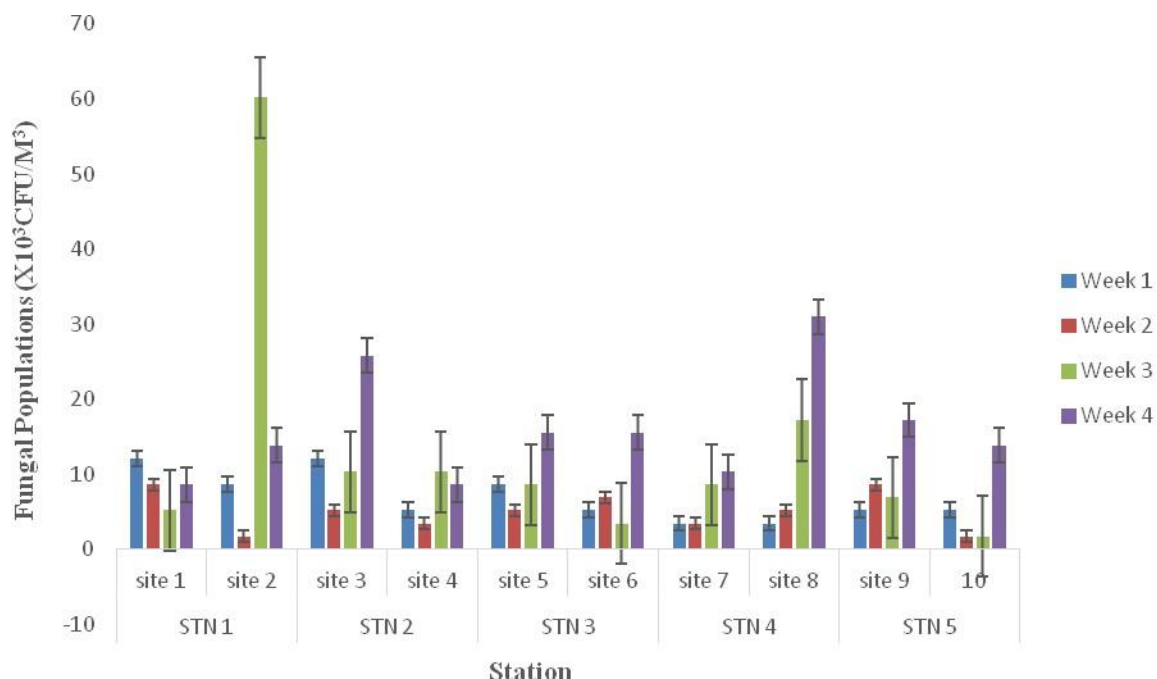


Fig. 1: Densities of Fungi of Indoor Air of the Sampling Sites in four weeks

Table 1: Frequency of occurrence of fungi at the sampling stations

Fungal Organism	Frequency of occurrence at the sampling stations					
	STN 1	STN 2	STN 3	STN 4	STN 5	No. of Occurrence
<i>Aspergillus species</i>	5	6	4	6	4	25(30.9)
<i>Cladosporium resinae</i>	5	4	3	5	3	20(24.7)
<i>Fusarium species</i>	4	2	2	4	3	15(18.5)
<i>Mucor species</i>	3	2	1	2	1	9(11.1)
<i>Penicillium spinulosum</i>	4	2	1	3	2	12(14.8)
No. of Occurrence	21(25.9)	16(19.8)	11(13.6)	20(24.7)	13(16)	81(100)

Percentage occurrence in parenthesis

Table 2: Frequency of occurrence of *Aspergillus* species at the sampling stations

Fungal Organism	Frequency of occurrence at the sampling stations					
	STN 1	STN 2	STN 3	STN 4	STN 5	No. of Occurrence
<i>Aspergillus flavus</i>	2	3	0	2	2	9(36)
<i>A. nidulans</i>	0	1	2	3	1	7(28)
<i>A. niger</i>	1	2	1	1	0	5(20)
<i>A. terreus</i>	2	0	1	0	1	4(16)
No. of Occurrence	5(20)	6(24)	4(16)	6(24)	4(16)	25(100)

Percentage occurrence in parenthesis

Discussion

Quantitative estimation of fungi in this study showed that all the sampling sites had certain levels of fungal loads. Fungal counts varied from sites to sites and from week to week. Some sites had low fungal densities and other sites had high fungal counts. Generally, site 2 had the highest fungal counts at week 3 while equal lowest counts were recorded in sites 2 and 10 at week 2. In station 1, fungal loads were highest in site 2 at week 3 and lowest in site 2 at week 2. Station 2 had highest counts at site 3 in week 4, and lowest counts at site 4 in week 2. In station 3, fungal counts were highest in sites 5 and 6 at week 4 and lowest in site 6 at week 3. Station 4 had highest fungal counts at site 8 in week 4 and lowest equal counts at sites 7 and 8 in week 1 and at site 7 in week 2. In station 5, the highest fungal loads were observed at site 9 in week 4 and at site 10 in week 3 while the lowest loads occurred at site 10 in week 2. Statistical analysis showed that there is no significant difference ($p \leq 0.005$) between the fungal populations of the study sites and between weekly counts. However, numerical data revealed variations in the site-to-site and week-to-week fungal populations. Mean data for the four weeks of study at each site showed that fungal densities were lowest at sites 4 and 7, and highest at site 2. Considering the weekly mean of all the sampling sites, week 4 had the highest fungal counts and week 2 had the lowest counts. Week 1 counts were in close range to week 2 counts while counts for week 3 were in close range to counts of week 4.

The variations in fungal densities at the sampling sites which resulted in some sites having somewhat high fungal densities and other sites having low densities could be attributed to certain conditions existing in the indoor environments sampled. These conditions could range from overcrowding/number of people occupying the room, human activities, building construction such as ventilation, surrounding of the building, dirtiness of the room and other factors. The weekly variations in fungal counts could probably be due to changes in weather conditions; sampling in weeks 3 and 4 was done during high rainfall period which resulted in dampness of the rooms that encouraged more growth of fungi indoor.

A total of 5 fungal organisms occurred in the indoor rooms sampled. Of the 8 species of fungi isolated during the study 4 were *Aspergillus* species while the other 4 belong to other genera. *Aspergillus* species was the most prevalent and had the highest occurrence rate with a percentage of 25%. It was followed by *Cladosporium* species which had percentage of 20%. *Fusarium* and *Penicillium* species were less prevalent with percentage occurrence rate of 15% and 12% respectively. *Mucor* was the least prevalent with percentage occurrence of 9%. Some of the fungal genera isolated during this study have been shown by other researchers to be amongst the common fungal species often isolated from the air

environment (Jaffal *et al.*, 1997; Wemedo *et al.*, 2012). The results of this study confirm the report of previous workers that human population and activities influenced concentration of microorganisms in air (Okhuoya and Okaraedje, 1992). *Aspergillus species* have been noted to be the most prevalent organism among the fungi isolated. This agreed with the work of Jaffal *et al* (1997) and Pickering *et al* (1976) who reported that *Aspergillus*, *Chaetomium* and *Alternaria* were the most common fungal genera frequently isolated from indoor air environments and air conditional systems. *Aspergillus species* has been implicated in an incidence of aspergillosis, ear and skin infections. Many of the other species isolated during this study have been implicated in certain air-borne microbial infections. Of the *Aspergillus species* isolated, *A. flavus* had the highest frequency, followed by *A. nidulans*, *A. niger* and *A. terreus* in descending order. This could mean that *A. flavus* would be mostly implicated in air related aspergillosis more than the other strains in the study area.

There is therefore the need to regularly evaluate the quality of the air we breathe whether indoor or outdoor especially in public places like the school environment and residential buildings particularly of low income occupants. The number and types of airborne microorganisms, as was the case in this study, could be used to determine the level of health risk associated with such environments. This is because population of indoor air microbes may be low at present and be high in the nearest future if not regularly monitored. In this study, sites that had somewhat high numbers of air-borne fungi could pose high risk in air related infections than those sites with low load of fungi.

In conclusion, this study showed that the indoor air sampled contained certain levels of fungal spores in terms of numbers and types which can create a potential public health danger. Because of the need for systematic and universally applicable approach to indoor air safety, personal cleanliness and adoption of proper sanitation habit are very important. The five (5) frequently isolated fungal species in this study were the common air contaminants in all the stations sampled which do not only pose health challenge to the regular occupants but also other people who may visit the sites.

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