

Assessment of the Indoor Air Quality of Majors' Biological Laboratories in Ofrima Complex, University of Port-Harcourt, Nigeria

Mbakwem-Aniebo C¹, Stanley HO^{1*} and Onwukwe CD²

¹Department of Microbiology, Faculty of Biological Sciences, University of Port Harcourt, P. M. B. 5323, Choba, 500004 Port Harcourt, Rivers State, Nigeria

²Department of Microbiology and Technology, School of Science, Laboratory of Technology, University of Port Harcourt, P. M. B. 5323, Choba, 500004 Port Harcourt, Rivers State, Nigeria

Abstract

The study was carried out to assess the genera of airborne micro-organisms present in the indoor environment of Microbiology and Animal/Environmental Biology Majors' laboratories in University of Port Harcourt; which could cause infection to laboratory users and contamination of culture. Indoor air was assessed (at 9 am, 12 pm and 4 pm) for five days and the basic parameters in this study were number of individuals present at each exposure time, time of sampling and duration. The settling plate technique was employed as sample collection method. The total heterotrophic bacterial and total fungal count from Microbiology Majors' laboratory was 987 CFU/m²/h (Colony Forming Unit per square meters per hour) (80.18%) and 244 SFU/m²/h (Spore Forming Unit per square meters per hour) (19.82%) respectively while the total heterotrophic bacterial and total fungal count from Animal/Environmental Biology Majors' laboratory was 677 CFU/m²/h (79.27%) and 177 SFU/m²/h (20.73%) respectively, reported as contamination flow. In the examined area, the predominant culturable species of air borne microflora were members of bacteria genera; *Bacillus*, *Staphylococcus*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Serratia*, *Acinetobacter* and fungi; *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Rhodotorula*, *Trichophyton*, *Candida*, *Mucor*, *Rhizopus*, *Scopulariopsis* and *Trichoderma*. Despite the high number counted plates in both laboratories, the data proves to be statistically insignificant at P=0.05 for 9 am and 4 pm while significant at 12 noon for laboratories 1 and 2. Both laboratories are were undergraduate science students of the University of Port Harcourt in the faculty of biological sciences carry out their resesearch and study.

Keywords: Assessment; Air quality; Laboratory environment; Ofrima complex; University of Port Harcourt

Introduction

Air is a carrier of particulate matter, dust and droplets which remain generally laden with microorganism but not a natural medium for microorganisms. Air transports the microorganisms and the ultimate fate of such microorganisms depends on factors such as sunlight, temperature, humidity, size of microorganism, laden particulate degree of susceptibility or resistance of a particular microorganism to form resistant spores or cyst [1]. Airborne microorganisms originate from different sources such as soil, animals and humans [2-4].

In many human activities, micro-organisms in the environment represent a hidden but dangerous risk factor [5].

The activities of men such as sewage treatment plants, animal rendering, fermentation processes, construction works and agricultural activities play a major role in emitting microorganisms into the air [6,7].

Exposure to bio-aerosol in the occupational environment are associated with a wide range of health effects with major public health impact, including infectious disease, acute toxic effects, allergies and cancer.

The microbial quality of indoor air in a given space at a particular time period is said to be determined by the quality of air entering the space, the number of occupants in the space, their physical activities and resultant aerosol generation, human traffic and ventilation of the area [8].

Indoor air quality (IAQ) is becoming an increasingly important issue for occupational and public health [9,10].

Droplets can be propelled up to 6-12 feet depending on the size

of the droplets and the force of expulsion. The largest droplets are more likely to fall to the floor or ground fairly rapidly. Medium sized pathogenic droplets are more likely to enter the nasopharynx of someone nearby, and can adhere to nasopharyngeal epithelial cells where they can cause infection [11].

Diseases associated with inhalation of fungal spores include toxic pneumonitis, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, kidney failure, and cancer [12].

Aspergillus fumigatus is the most prevalent cause of severe pulmonary allergic disease, including allergic bronchopulmonary aspergillosis (ABPA), known to be associated with chronic lung injury and deterioration in pulmonary function in people with chronic asthma and cystic fibrosis (CF) [13].

Since air can play a central role as a reservoir for microorganisms, regular microbial monitoring is useful to assess air quality and identify critical situations [14].

This study is therefore aimed at assessing the airborne microorganisms present in the indoor laboratory environment that

*Corresponding author: Stanley HO, Department of Microbiology, Faculty of Biological Sciences, University of Port Harcourt, P. M. B. 5323, Choba, 500004 Port Harcourt, Rivers State, Nigeria, E-mail: okehstanley@yahoo.com

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could possibly cause contamination of culture media and infection of laboratory workers and students as they often make use of the laboratory.

Methods

Sample collection and number of plates exposed

The settling plate technique also known as sedimentation method was used as sample collection method [15]. In this technique, standard 90 mm diameter Petri dishes containing 20 ml of sterile culture media (Nutrient Agar and Potato Dextrose Agar) were opened at the various sampling locations for 1 hour. After which the Petri dishes containing nutrient agar were closed and placed in the incubator at 37°C for possible bacterial growth within 18 to 24 hours, whereas Petri dishes containing potato dextrose agar were incubated at room temperature for 5 days.

This technique of sample collection is known as 1/1/1 scheme, 1 hour of exposure, 1 metre from the floor and 1 meter from the wall or obstruction as described by Pasquarella [5].

A total number of 16 plates were exposed at each exposure time; these comprised of 8 Nutrient agar plates (4 in Animal and Environmental Biology Majors' laboratory (designated as Lab 2) and 4 in Microbiology Majors' laboratory (designated as Lab 1) and 8 Potato Dextrose agar plates (4 in Animal and Environmental Biology Majors' laboratory and 4 in Microbiology Majors' laboratory and this was carried out 3 times in a day (9 am - when the number of students and staff present are minimal; 12 pm- when the work force in the laboratory is at its peak and 4 pm - when the day's work is done and students and staff are about leaving the laboratory). The total number of individuals present in the different laboratory at each time of exposure was noted. Isolates were identified by their cultural, morphological and biochemical characteristics as described by many researchers [16,17].

Colony forming unit (CFU) and Spore forming unit (SFU) of the bioaerosols (bacteria and fungi respectively) were assessed (3 times a day) for five days, taking into consideration the number of individuals present at each exposure time at the sampling sites and the weather conditions at such points.

Results

The result obtained showed that there were more suspensions of bio aerosols in the indoor air at noon which correlates to the period when more persons were found at the sampling sites. The activities of persons at the sampling sites kept the aerosols airborne at varying dispersal rates and the ambient weather condition which was rainy and not windy at the time of study did not exert much influence on the results obtained.

Table 1 shows the Total heterotrophic bacterial count 987 CFU/m²/h (59.30% for Lab 1) and 677 CFU/m²/h (40.69% for Lab 2). Day five had the highest heterotrophic bacteria count in both laboratories. 314 CFU/m²/h and 145 CFU/m²/h for Lab 1 and Lab 2 respectively and Total Fungal count 244 SFU/dm²/h (57.96% for Lab 1) and 177 SFU/dm²/h (42.04% for Lab 2).

The total heterotrophic bacterial and total fungal count from Microbiology Majors' laboratory was 987 CFU/m²/h (80.18%) and 244 SFU/m²/h (19.82%) respectively while the total heterotrophic bacterial and total fungal count from Animal/Environmental Biology Majors' laboratory was 677 CFU/m²/h (79.27%) and 177 SFU/m²/h (20.73%) fungal count respectively, reported as contamination flow. Tables 2a and 2b show the ANOVA for heterotrophic bacteria and fungal plate count obtained from both laboratories.

Data represent mean ± S.D of triplicate exposure result from Lab 1 and Lab 2. Anova analysis was conducted to check if the data obtained were statistically significant between the data horizontally and values with the same superscript are not statistically significant at P=0.05, using SPSS Version 17.0.

The most presented microorganisms are *Aspergillus* spp., *Bacillus* spp., *Staphylococcus* spp. in Lab 1 and Lab 2 with different percentages; 37% and 25%, 33.3% and 18.2%, 18.2% and 17.9% respectively and *Escherichia* spp. 25% only in Lab 1.

Discussion

The alterations in quality of indoor air in Microbiology Majors' Laboratory (Lab 1) and the Animal and environmental biology majors'

| Day | Time | Mean Bacterial Plate Count (CFU/m ² /h) | Mean Fungal Plate Count (CFU/m ² /h) | Mean Bacterial Plate Count (CFU/m ² /h) | Mean Fungal Plate Count (CFU/m ² /h) |
|---|-------|--|---|--|---|
| Microbiology Majors' Laboratory (Lab 1) | | | Animal/Environmental Biology Majors' Laboratory (Lab 2) | | |
| 1 | 9 am | 33 | 10 | 16 | 9 |
| 1 | 12 pm | 50 | 22 | 24 | 17 |
| 1 | 4 pm | 44 | 13 | 17 | 7 |
| 2 | 9 am | 15 | 10 | 19 | 7 |
| 2 | 12 pm | 81 | 17 | 38 | 19 |
| 2 | 4 pm | 49 | 19 | 11 | 16 |
| 3 | 9 am | 27 | 22 | 5 | 5 |
| 3 | 12 pm | 128 | 42 | 40 | 4 |
| 3 | 4 pm | 21 | 25 | 17 | 8 |
| 4 | 9 am | 17 | 6 | 20 | 4 |
| 4 | 12 pm | 27 | 10 | 72 | 17 |
| 4 | 4 pm | 16 | 18 | 107 | 20 |
| 5 | 9 am | 49 | 8 | 27 | 11 |
| 5 | 12 pm | 314 | 5 | 145 | 19 |
| 5 | 4 pm | 116 | 17 | 119 | 14 |
| Total | | 987 (59.30%) | 244 (57.96%) | 677 (40.69%) | 177 (42.04%) |
| Note: Sum total heterotrophic bacterial count from both Laboratories =1664 CFU and Sum total of fungal count from both Laboratories = 421 SFU. | | | | | |

Table 1: Total heterotrophic bacterial and fungal count.

| Day | Time | Laboratories | |
|-----|-------|------------------------------|------------------------------|
| | | Mean ± S.D Lab 1 | Mean ± S.D Lab 2 |
| 1 | 9 am | 33.00 ± 1.00 ^{a,d} | 16.00 ± 1.00 ^{a,e} |
| | 12 pm | 50.00 ± 1.00 ^{b,d} | 24.00 ± 1.00 ^{b,e} |
| | 4 pm | 44.00 ± 1.00 ^{c,d} | 17.00 ± 1.00 ^{a,e} |
| 2 | 9 am | 15.00 ± 1.00 ^{a,d} | 19.00 ± 1.00 ^{a,e} |
| | 12 pm | 80.00 ± 1.00 ^{b,d} | 38.00 ± 1.00 ^{b,e} |
| | 4 pm | 49.00 ± 1.00 ^{c,d} | 11.00 ± 1.00 ^{c,e} |
| 3 | 9 am | 27.00 ± 1.00 ^{a,d} | 5.00 ± 1.00 ^{a,e} |
| | 12 pm | 128.00 ± 1.00 ^{b,d} | 39.67 ± 0.5 ^{b,e} |
| | 4 pm | 21.00 ± 1.00 ^{c,d} | 17.00 ± 1.00 ^{c,e} |
| 4 | 9 am | 17.00 ± 1.00 ^{a,d} | 20.00 ± 1.00 ^{a,e} |
| | 12 pm | 27.00 ± 1.00 ^{b,d} | 72.00 ± 1.00 ^{b,e} |
| | 4 pm | 16.00 ± 1.00 ^{a,d} | 107.00 ± 1.00 ^{c,e} |
| 5 | 9 am | 49.00 ± 1.00 ^{a,d} | 27.00 ± 1.00 ^{a,e} |
| | 12 pm | 314.00 ± 2.00 ^{b,d} | 145.00 ± 1.00 ^{b,e} |
| | 4 pm | 116.00 ± 2.00 ^{c,d} | 119.00 ± 1.00 ^{c,e} |

Data represent mean ± S.D of triplicate exposure result from both Laboratories. Anova analysis was conducted to check if the data obtained were statistically significant between the data vertically and horizontally and values with the same superscript are not statistically significant while mean with different superscripts are statistically significant at P=.05, using SPSS Version 17.0. Superscript a,b,c compares microbial count with time(hours)along the rows while superscript d,e compares microbial count in both Laboratories. AEB= Animal/Environmental Biology.

Table 2a: ANOVA for mean bacterial count from both Laboratories (CFU/ml).

| Days | Time | Laboratories | |
|------|-------|-----------------------------|-----------------------------|
| | | Mean ± S.D Lab 1 | Mean ± S.D Lab 2 |
| 1 | 9 am | 10.00 ± 2.00 ^{a,d} | 9.00 ± 1.00 ^{a,d} |
| | 12 pm | 22.00 ± 1.00 ^{b,d} | 17.00 ± 1.00 ^{b,e} |
| | 4 pm | 13.00 ± 1.00 ^{a,d} | 7.00 ± 1.00 ^{a,e} |
| 2 | 9 am | 10.00 ± 1.00 ^{a,d} | 7.00 ± 1.00 ^{a,d} |
| | 12 pm | 17.00 ± 1.00 ^{b,d} | 19.00 ± 1.00 ^{b,d} |
| | 4 pm | 19.00 ± 1.00 ^{b,d} | 16.00 ± 1.00 ^{c,d} |
| 3 | 9 am | 22.00 ± 1.00 ^{a,d} | 5.00 ± 1.00 ^{c,d} |
| | 12 pm | 42.00 ± 1.00 ^{b,d} | 4.00 ± 1.00 ^{a,e} |
| | 4 pm | 25.00 ± 1.00 ^{c,d} | 8.00 ± 1.00 ^{b,e} |
| 4 | 9 am | 6.00 ± 1.00 ^{a,d} | 4.00 ± 1.00 ^{a,d} |
| | 12 pm | 10.00 ± 1.00 ^{b,d} | 17.00 ± 1.00 ^{b,e} |
| | 4 pm | 18.00 ± 1.00 ^{c,e} | 20.00 ± 1.00 ^{c,e} |
| 5 | 9 am | 8.00 ± 1.00 ^{a,d} | 11.00 ± 1.00 ^{a,e} |
| | 12 pm | 5.00 ± 1.00 ^{a,d} | 19.00 ± 1.00 ^{b,e} |
| | 4 pm | 17.00 ± 1.00 ^{c,d} | 14.00 ± 1.00 ^{c,e} |

Data represent mean ± S.D of triplicate exposure result from both Laboratories. Anova analysis was conducted to check if the data obtained were statistically significant between the data vertically and horizontally and values with the same superscript are not statistically significant while mean with different superscripts are statistically significant at P =.05, using SPSS Version 17.0. Superscript a,b,c compares microbial count with time(hours)along the rows while superscript d,e compares microbial count in both Laboratories.

Table 2b: ANOVA for mean fungal count from both Laboratories (SFU/ml).

laboratory (Lab 2) in Ofrima Complex of University of Port Harcourt showed increase with increasing human population and activities.

Bacterial isolates were presented more on day 5 (Lab 1 and Lab 2) (Figure 1), while Fungal isolates were presented more on day 3 (Lab 1). This could be as a result of human activities which caused the organism to be airborne. On the average, the study sites were more occupied by research students and staff at noon and the activities of these individual and the flora carried by them influenced the plate counts as organisms

were counted more at noon. At all times, Lab 1 was more populated compared to Lab 2, and corresponding to this, it also had more count of organisms on the average (Figure 2a).

Biochemical characterization of bacterial isolates (flora composition) of (Lab 1) sampling station revealed the bacterial species present as shown above with, *Bacillus*, *Staphylococcus*, *Escherichia*, *Micrococcus*, *Pseudomonas* and *Serratia* being the predominant genera in (Lab 1) and *Acinetobacter*, *Bacillus*, *Escherichia* and *Staphylococcus* being the most dominant in Animal and environmental biology laboratory (Lab 2). These isolates are commonly found bio aerosols and pathogens in the air. This is because the carriers are commonly present in the area under study. This supports previous studies which state that *Staphylococcus* sp is the most commonly found pathogen in air [15,18].

Staphylococcus is found in all individuals and usually expelled from the respiratory tract through the nose and mouth which may also account for their presence in the environment and can cause bacteremia and gastrointestinal infections [19]. *Bacillus* species are persistent and resistant in the environment because of the formation of spores. Spores have since been recognized as the hardest known form of life on Earth [20].

The production of spores enables this organism to withstand unfavourable conditions such as low temperatures or heat and may improve the chances of *Bacillus* to be present in high numbers in the air [21]. *Escherichia* spp. can be found in the normal intestinal flora of humans and animals but can also be an important cause of enteric illness and constitute the major etiologic agent of sporadic and epidemic diarrhea both in children and adults [22].

The laboratory analysis also showed that *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Rhodotorula* and *Trichophyton* species were predominant fungal isolates in (Lab 1) and *Aspergillus*, *Candida*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis* and *Trichoderma* species were predominant in (Lab 2) (Figure 2b).

These genera of bacteria and fungi have been shown to be amongst the most common bacterial and fungal genera often isolated from air. Previous studies have also shown that people occupying or visiting enclosed spaces play a dominating role in the creation of indoor microbiological environment [23]. *Aspergillus fumigatus* is widely distributed in the environment and airborne asexual conidia serves as the main mode of transport for pulmonary lung infection [7,24-28].

The following bacterial and fungal genera; *Aeromonas*, *Enterobacter*, *Klebsiella*, *Serratia*, *Cladosporium*, *Fusarium*, *Rhodotorula*, and *Trichophyton* were isolated only from the indoor environment of (Lab 1) but were not found in (Lab 2) indoor air and *Acinetobacter*, *Salmonella*, *Streptococcus*, *Geotrichum*, *Mucor*, *Scopulariopsis* and *Trichoderma* were found only in Animal/ Environmental Biology Majors' Laboratory (Lab 2) and were not found in Microbiology Majors' Laboratory (Lab 1) [29-32]. The Microbiology Majors' Laboratory (Lab 1) had a higher bacterial and fungal percentage count as against the Animal/Environmental Biology Majors' Laboratory (Lab 2).

This could probably be attributed to the more number of individuals usually present in Microbiology Majors' Laboratory and their various research works, which could introduce more organisms to the indoor air. Despite the high number of bacterial and fungal plate count in both laboratories, the data proves to be statistically insignificant at P=0.05 for Microbiology Majors' Laboratory. While the fungal plate and bacterial plate count in Animal/Environmental Biology Majors' Laboratory are statistically significant at P=0.05. From this study, the

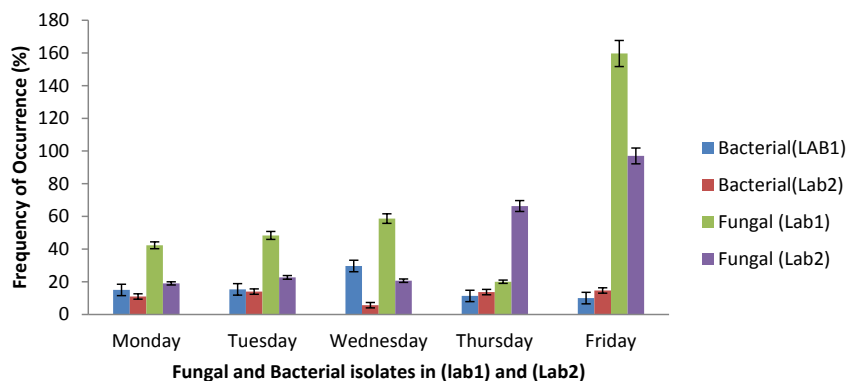


Figure 1: Cumulative distribution of fungal and heterotrophic bacterial isolates in both laboratories on daily basis with percentage error bars.

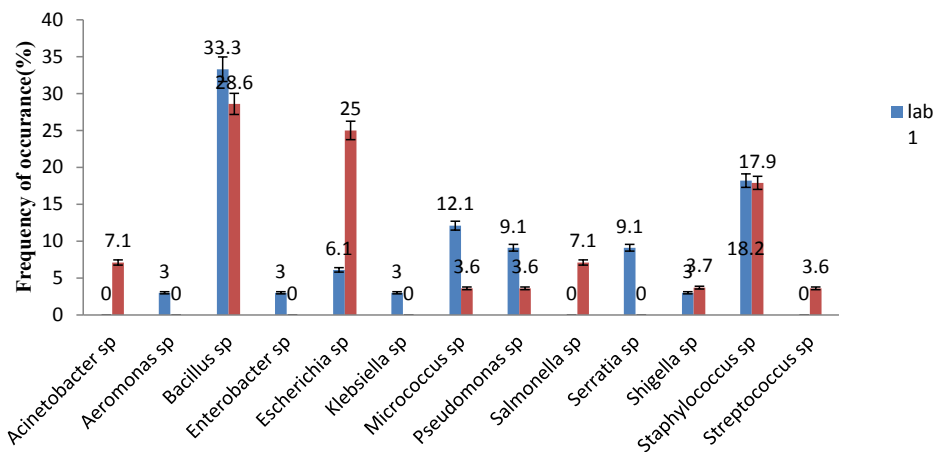


Figure 2a: Frequency of bacterial isolates in microbiology majors' laboratory (Lab 1) and animal and environmental biology majors' laboratory (Lab 2), University of Port Harcourt with percentage error bars.

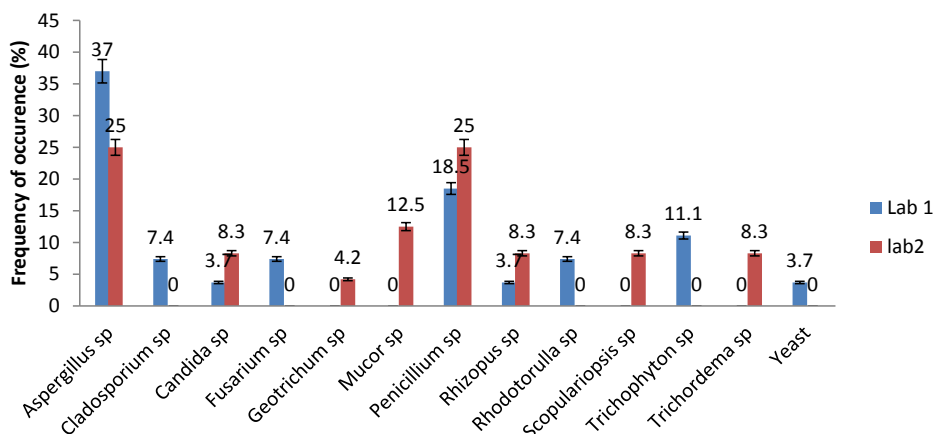


Figure 2b: Frequency of fungal isolates in microbiology majors' laboratory (Lab 1) and animal and environmental majors' laboratory (Lab 2), University of Port Harcourt with percentage error bars.

indoor air of Microbiology Majors' Laboratory (Lab 1) is more laden with micro organisms compared to Animal/Environmental Biology Majors' Laboratory (Lab 2).

Conclusion and Recommendations

This research has shown that the sample sites are laden with micro

organisms and most of which are pathogenic to human and can result in mild or adverse health conditions based on individual health status and dose response. These organisms pose threats to research work as they constantly contaminate plates if proper aseptic technique is not efficiently employed. Therefore, health challenged or immune compromised researchers should ensure to work with nose masks if

they must use the laboratory and proper aseptic techniques should be employed in discarding used media plates (autoclave before discarding). Adequate Sanitary measures by laboratory users can help reduce the level of bioaerosols, measures such as disinfecting work benches before and after use. Avoid the practice of sweeping with brooms and use vacuum cleaners or wet mop instead. These technique will help reduce the microbial load of the environment instead of pushing bioaerosols off to the air as observed in the case of sweeping practice.

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