Investigation Of Bioaerosols and The Microbiological Indoor Air Quality in an Urban Nursery School in Port Harcourt, Nigeria

.

ABSTRACT

Aims: This study was aimed at investigating the microbial quality of air within the confines of nursery school children. Early exposure to these indoor pollutants can lead to major public health concerns which include acute respiratory tract infections, allergies as well as cancer.

Study Design: Random sampling approach was used in the collection of the samples. Air samples were collected from two different classrooms in a nursery school.

Place and Duration of Study: Air samples were collected within the confines of the nursery section of the Demonstration Primary School in the University of Port Harcourt, Rivers State, Nigeria every other weekday in the month of May 2022.

Methodology: Culture media were placed at the four corners of two classrooms in the nursery section. This nursery class comprised of children between the ages of 4-5 years Nutrient, MacConkey and potato dextrose agar media were used to culture airborne microorganisms during the study. For differential identification of bacteria, citrate, motility, oxidase, indole, catalase, methyl red Voges Proskauer, triple salt iron agar, sugar fermentation tests were carried out.

Results: Both bacterial and fungal species of medical importance, such as *Bacillus, Shigella, Micrococcus, Serratia, Proteus., Yersinia, Enterobacter, Penicilium, Aspergillus, Candida, Microsporum. Exophiala* and *Mucor* spp were isolated in this study. The most predominant bacterial species among the isolates in the study was *Bacillus* sp. with the percentage occurrence of 25%. *Shigella* and *Yersinia* species had the percentage occurrence of 16.67% respectively other species like *Serratia*, *Micrococcus, Enterobacter* and *Proteus* each had percentage occurrence of 8.33% which was the lowest occurrence. All the fungal isolates had similar or equal percentage occurrence (16.67%).

Conclusion: Exposure to microbial aerosols in nursery schools can lead to several health complications. Thus, recognition, control and monitoring of air quality in schools are crucial in limiting the spread of airborne pathogens.

Keywords: Bioaerosols, microorganisms, environment, children, bacteria, fungi.

1. INTRODUCTION

"Bioaerosols consist of airborne particles that consist of living organisms, such as bacteria, fungi and viruses or parts of living organisms, such as plant pollen, spores and endotoxins from bacterial cells or mycotoxins from fungi. Airborne particles which consist of bioaerosols are extremely small thus, not visible to the naked eye as they range in size from 0.02 to 100 micrometers in diameter" [1].

"Schools are environments with a high level of population density and activity of children, where different pollutants originating from both indoor and outdoor sources may be introduced and linger for a long time" [2]. "Furthermore, school buildings are often characterized by irregular interventions for building maintenance and environmental remediation" [3]. "Children are more susceptible to the effects of air contaminants than adults, due to their developing immune and respiratory system, lower body mass index and breathing pattern" [4]. "As a result of the time spent in schools, indoor environmental conditions are among the main contributors of complete exposure for children to several air pollutants" [5].

"The risks posed by bioaerosols have been studied over the years. The results of these link adverse human health effects that are exposed to high concentrations of bioaerosols. Exposure to bioaerosols has been identified with links between respiratory and gastrointestinal illnesses" [6].

"People spend more than 90 % of the day in indoor environments" [7-9]. "In case of younger children, asides the home front, nursery school is the main indoor environment. Studies conducted in the last 20 years by the US Environmental Protection Agency revealed that indoor air is occasionally 70–100 times more polluted than outdoor air” [10]. Consequently, early life exposure to bioaerosols found at nursery schools and their possible roles in airway diseases is a critical area of research.

“ Research has indicated that human activities increase the airborne bacterial loads leaving a distinctly human microbial signal inside buildings” [11]. “Specific activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate air borne biological particulate matter” [12]. “Airborne microbes attach to dust particles, condense and enter the human body directly via inhalation or indirectly by ingestion of contamination foods and water resulting in development of diseases and toxic reactions” [13].

Various microorganisms can be in aerosol form in the atmosphere, including viruses, bacteria, fungi, yeasts and protozoans. To survive in the atmosphere, it is important that these microbes adapt to some of the harsh climatic characteristics of the exterior world, including temperature, gases and humidity. Several microorganisms capable of surviving harsh conditions can form endospores, which can withstand extreme conditions [14]. Most bacteria or bacterial agents are not very potent allergens. Bacterial cell walls components, such as endotoxin (present only in Gram-negative bacteria; and peptidoglycans (most prevalent in Gram-positive bacteria), are agents with important pro-inflammatory properties that may induce respiratory symptoms. The effects of peptidoglycans are assumed to be very similar to those observed with endotoxin exposure; however, this has not been systematically studied.

Bacillus anthracis can resist environmental stressors. It is a Gram-positive rod-shaped bacterium which utilizes spore formation to resist environmental stresses. The spore is a dehydrated cell with extremely thick cell walls which can remain inactive for many years. This spore makes *Bacillus anthracis* a highly resilient bacterium, enabling it to survive extreme temperatures, chemical contamination, as well as low nutrient concentrations [15] These species of bacteria are associated with Anthrax, which is a severe respiratory disease that infects humans.

According to Selman *et al*., [16], fungi are well-known sources of allergens that play a role in the development of Hypersensitivity pneumonitis (HP). The species involved include many common genera such as *Penicillium* and *Aspergillus*, which occur in some work environments usually at very high levels (e.g., composting facilities, farms, etc.). Hay contaminated with thermophilic bacteria such as *Saccharopolyspora rectivirgula* or *Thermoactinomycetes vulgaris* is the source of allergens causing farmer's lung or HP [17], and similar disorders have been observed among mushroom growers and, incidentally, among compost workers [16].

A specific exposure with high risk to occupational disease is that to *Aspergillus fumigatus*, a fungus that not only induces allergic sensitization and symptomatic allergic lung disease but can also cause an infectious mycosis (Broncho-pulmonary aspergillosis), especially in immuno-compromised subjects. Many fungal species have also been described as producers of type I allergens (IgE binding allergens), and IgE sensitization to common outdoor and indoor fungal genera like *Penicillium* and *Aspergillus* are strongly associated with allergic respiratory disease, especially asthma [18].

Another microorganism that can resist environmental stresses is *Aspergillus fumigatus*, it is a major airborne fungal pathogen [19]. This pathogen has the propensity to illicit human diseases when conidia are inhaled into the lungs. While *A. fumigatus* lacks virulence traits, it is very adaptable to changing environmental conditions and therefore is still capable of mass infection. [19]. Hence this study was carried out to investigate the microbial air quality within the confines of nursery school children in Port Harcourt, Rivers State, Nigeria.

2. METHODOLOGY

2.1 Media preparation

The different media used during the research: MacConkey, nutrient and potato dextrose agar were prepared in accordance with the manufacturer's specification.

2.2 Sampling Area

Two classrooms from the nursery section of University of Port Harcourt Demonstration Primary School Choba were used as sampling areas for the study.

2.3 Sampling Method

Indoor air was sampled in two different nursery classrooms by gravitational (drop plate) method. This was carried out by placing open sterile petri dishes containing already prepared sterile Nutrient, MacConkey, and Potato Dextrose Agar respectively, exposed to air. This exercise covered two different nursery classrooms, A and B, 1 meter above the ground for 30 minutes at separate time intervals: 9:00 – 9:30 am and 12:00 – 12:30 pm respectively. The Nutrient Agar and MacConkey Agar were used for the isolation of culturable heterotrophic bacteria, while the Potato Dextrose Agar with drops of lactic acid was used for the isolation of fungi. Thereafter, the plates were covered aseptically and transported to the University of Port Harcourt microbiological laboratory where they were incubated appropriately.

2.4 Microbial analysis of the Air samples

Total bacterial and fungal counts were enumerated and reported as colony forming units per plate per time (Cfu /plate/time). Omeliansky formula [20] was used in the calculation of the cfu/plates for fungi and bacteria for the indoor air quality.

Formula:

$$N=5ax104\ (bt^{-1})$$

N – Microbial cfu/m^3 of indoor air

a – No of colonies per plates

b – Dish surface (m^2)

t – Exposure time (min)

2.4 Isolation of Bacteria and Fungi from the Air samples

Growths from nutrient and MacConkey agar (BIOLIFE) were isolated into freshly prepared nutrient agar by streaking a loopful of each sample onto duplicate nutrient Agar (NA) (Hardy Diagnostics USA) plates after 24hrs of incubation at 25^{o}C, while fungi were isolated on potato dextrose agar acidified with lactic acid (APDA) plates and incubated at 37^{o} C for 72 hrs. Distinct colonies were sub-cultured accordingly by streaking loopful of a single colony on nutrient agar duplicated and incubated at 37^{o}C for 24 hrs after which they were stored on agar slants at 4^{o}C and preserved for biochemical identification.

2.5 Biochemical identification

Biochemical tests such as citrate, motility, oxidase, indole, catalase, Methyl Red and Voges Proskauer, triple salt iron agar, sugar fermentation were applied for the identification of the isolates [12].

2.6 Statistical Analysis

All experiments were carried out in triplicates, data obtained was subjected to AVOVA and mean were separated with Duncan multiple range test using SPSS version.

3. RESULTS

Table 1 shows the percentage occurrence of the bacterial isolates. The most predominant species among the isolates in the study was *Bacillus* sp with the percentage occurrence of 25%%. *Shigella* and *Yersinia* species had the percentage occurrence of 16.67% each. Other species like *Serratia*, *Micrococcus, Enterobacter* and *Proteus* each had percentage occurrence of 8.33% which was the lowest occurrence.

Table 1: Distribution of bacterial isolates within the classrooms

Bacteria	**Classroom A No. (%)**	**Classroom B No. (%)**	**Occurrence rate (%)**
Bacillus	2(25)	1(25)	25
Serretia	1(12.5)	0(0.0)	8.3
Micrococcus	0(0.0)	1(25)	16.7
Proteus	1(12.5)	0(0.0)	8.3
Enterococcus	1(12.5)	0(0.0)	8.3
Micrococcus	0(0.0)	1(25)	8.3
Yersinai	2(25)	0(0.0)	16.7
Shigella	1(12.5)	1(25)	16.7
Total	8(66.7)	4(33.3)	100

The percentage occurrence of the fungal isolates obtained during the study are presented in Table 2. *Penicillium, Aspergillus, Candida, Microsporum. Exophiala* and *Mucor* species were identified as the likely fungal organisms in the study. All the fungal isolates had similar or equal percentage occurrence (16.67%).

Table 2: Distribution of fungal isolates within the classrooms

Fungi	Classroom A No.(%)	Classroom B No.(%)	Occurrence rate (%)
Aspergillus	1(33.3)	0(0.0)	16.7
Penicillium	1(33.3)	0(0.0)	16.7
Microsporum	0(0.0)	1(33.3)	16.7
Exophiala	1(33.3)	0(0.0)	16.7
Mucor	0(0.0)	1(33.3)	16.7
Candida	0(0.0)	1(33.3)	16.7
Total	3(50)	3(50)	100

Figure 1 shows the result of the comparison of the total bacterial load in classroom A and B on nutrient agar at (9:00am - 9:30am) within 8 days. Results obtained showed that the highest bacterial load was observed in day 3 in classroom A. whereas; the lowest bacterial load was observed in day 1 classroom B. Classroom B, had a relatively lower bacterial load compared to A, the highest bacterial load was observed in day 5; whereas the lowest bacterial load was recorded in day 1.

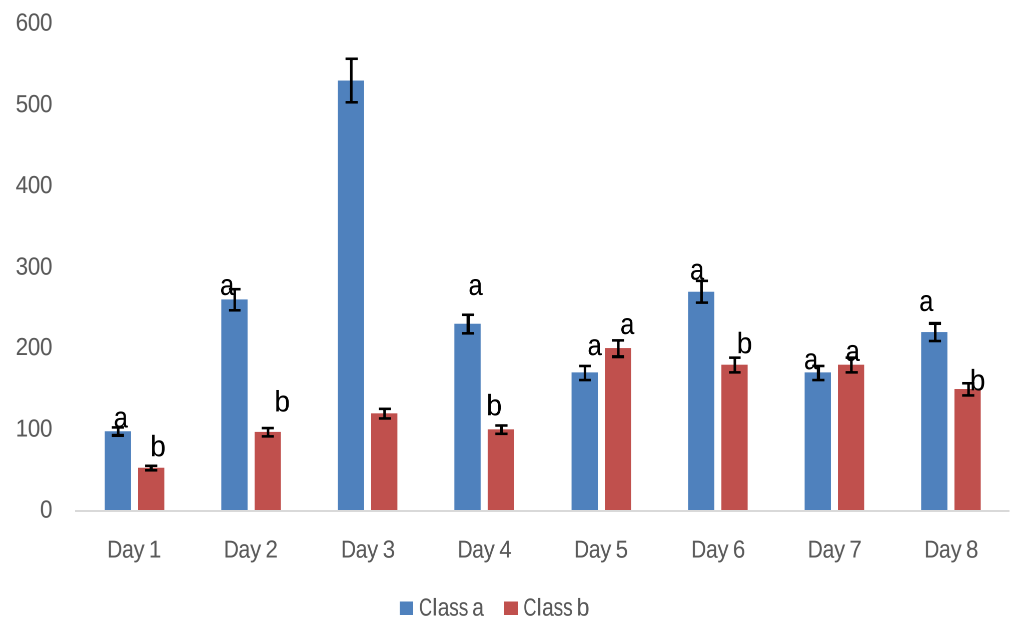

Figure 1: Comparison of the total heterotrophic bacterial loads in air of classrooms A and B during morning class (9:00am - 9:30am).

Different letters on bars highlights significant difference between classrooms per time, *P*≤0.005.

Figure 2 shows the contrast in the total heterotrophic bacterial loads in classrooms A and B on nutrient agar at (12:00pm – 12:30pm) within 8 days. From the result, the highest bacterial load was obtained in air on day 2 classroom in A, whereas; the lowest bacterial load was obtained on day 1 classroom A. The highest bacterial load in classroom B was obtained on day 2, whereas the lowest was obtained on day 1.

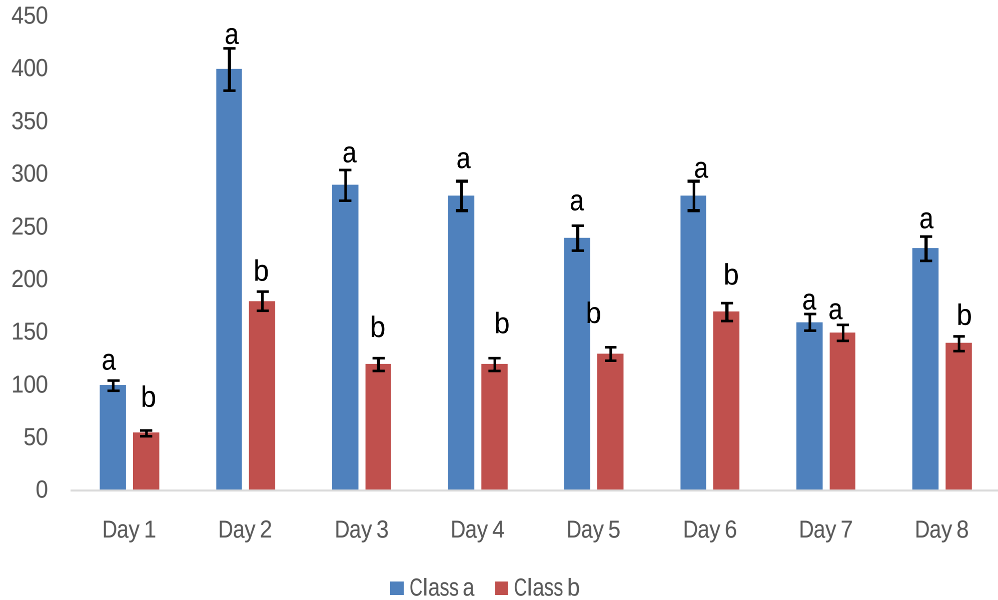

Figure 2: Comparison of the total heterotrophic bacterial loads in air of classrooms A and B during afternoon class (12:00 - 12:30pm)

Different letters on bars indicate significant difference between classrooms per time, *P*≤0.005.

.

Figure 3 shows the result of the comparison of the total fungal load in classrooms A and B on potato dextrose agar during the morning class (9:00am - 9:30am). From the result, the highest fungal load obtained in air of classroom A on day 7, whereas; day 3 and day 5 had the lowest fungal load for classroom A. The result of the total fungal loads in classroom B, indicated that the highest load was obtained on day 1, whereas day 4 had the lowest fungal load.

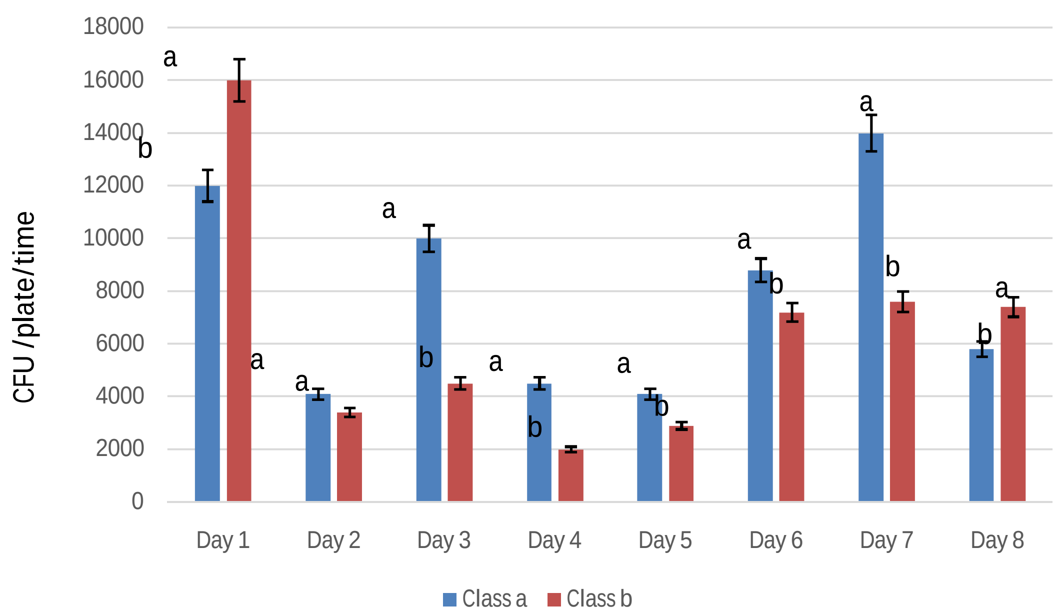

Figure 3: Comparison of the total fungal loads in air of classrooms A and B during the morning class (9:00am - 9:30am)

Different letters on bars indicate significant difference between classrooms per time, *P*≤0.005.

Figure 4 shows the contrast between the total fungal loads of air in classrooms A and B during the morning class (at 9:00am – 9:30am). The results obtained revealed that for classroom A, the highest fungal counts were observed on days 1 and 6 respectively whereas the lowest fungal counts were observed in days 4 and 5 respectively. For classroom B, the lowest fungal count was observed on day 7, whereas the lowest counts were observed on day 5.

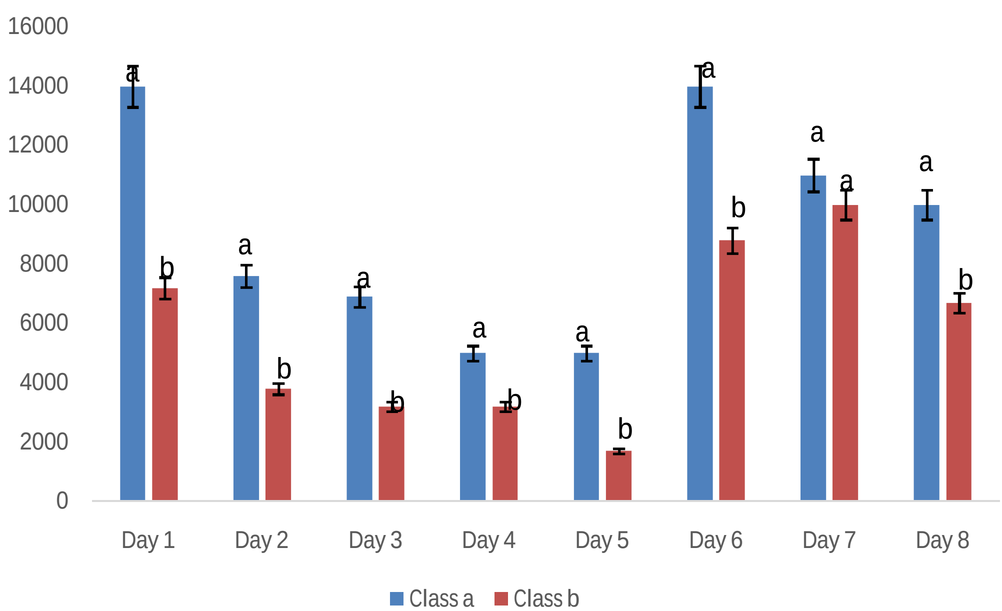

Figure 4: Comparison of the total fungal loads in air of classrooms A and B during afternoon class (12:00 - 12:30pm)

Different letters on bars indicate significant difference between classrooms per time, *P*≤0.005.

Figure 5 shows the result of the comparison between the total bacterial loads in air of classrooms A and B during the afternoon class session (9:00 - 9:30am). From the result, for classroom A, on day 3 had the highest bacterial load, whereas the lowest bacterial load was obtained on day 8. For classroom B, the highest bacterial load during the study was obtained on day 8, whereas the lowest bacterial load was obtained on day 2.

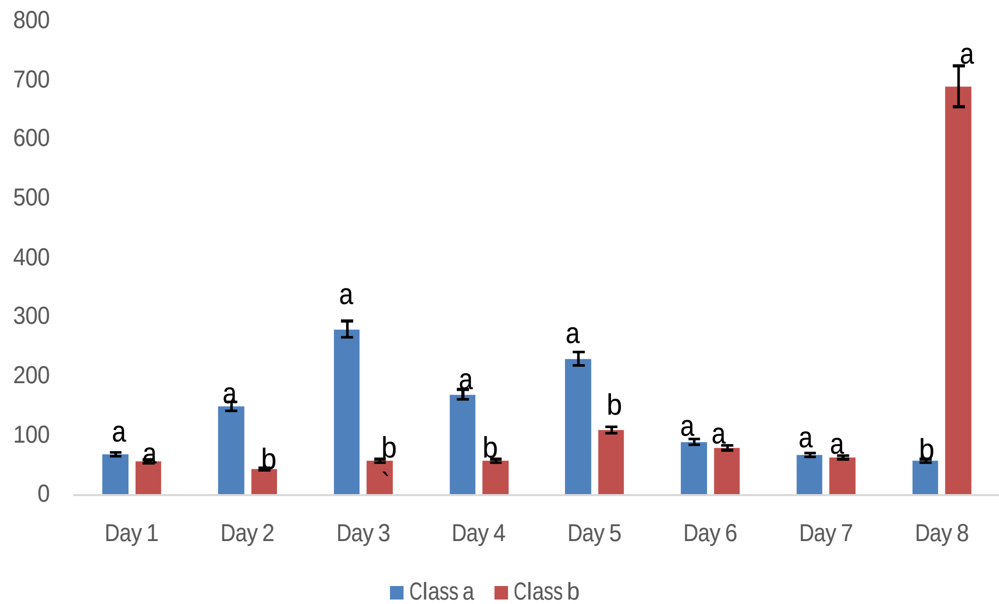

Figure 5: Comparison of the coliform bacterial loads of air in classrooms A and B during the afternoon class session (9:00 - 9:30am)

Different letters on bars indicate significant difference between classrooms per time, *P*≤0.005.

Figure 6 shows the result of comparison of the total coliform bacterial counts in classrooms A and B during the afternoon class session (12:0 0 - 12:30pm). From the result, it was observed that day 7 had the highest bacterial counts, whereas day 8 had the lowest bacterial load for classroom A. For classroom B, day 8 recorded the highest bacterial counts whereas, day 4 had the lowest bacterial counts.

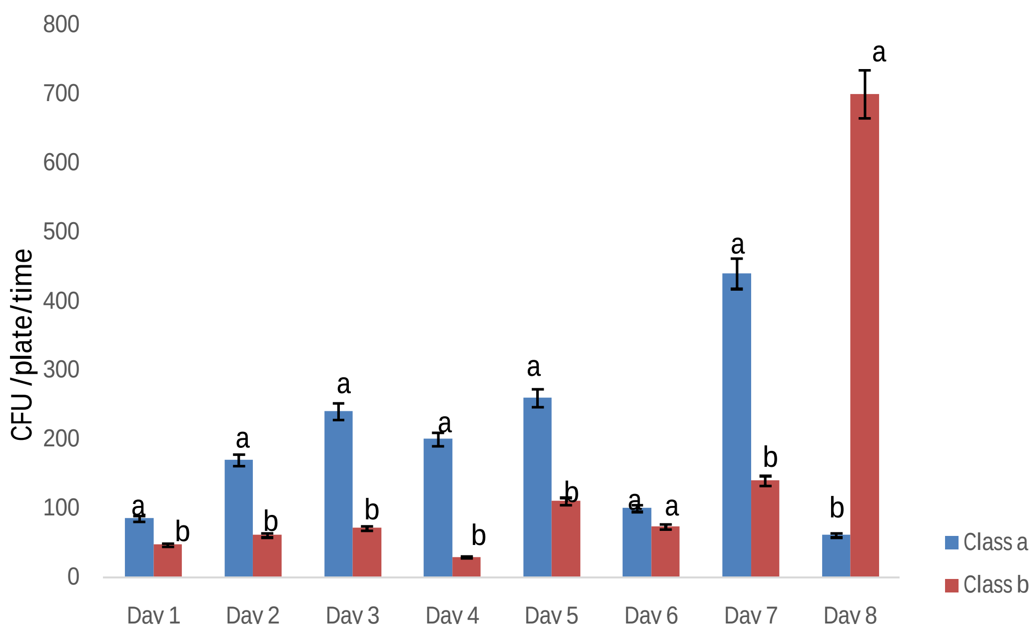

Figure 6: Comparison of the total coliform bacterial loads of air in classrooms A and B during the afternoon session (12:00 - 12:30pm)

Different letters on bars indicate significant difference between classrooms per time, *P*≤0.005.

3.1 Discussion

Schools are places with a high level of activity and population density of children and where different pollutants from both indoor and outdoor sources may be introduced and persist for a long time [21]. School buildings and facilities provide children with ideal places for their learning and development. Outside of the home, children spend most of their time indoors while at school. The air quality of any learning environment determines the health status, and the degree of comfort children derive from such environment. Indoor air quality (IAQ) in school buildings is characterized by various pollutants, such as volatile organic compounds (VOCs), aldehydes, particulate matter, fungi and bacteria [22-24].

Microbial contamination of air in schools is one of the most important high-risk factors of infections. Therefore, recognition, monitoring and control of air microbial contamination in schools are very necessary especially for airborne pathogens. This can be done routinely by microbiological sampling [25]

The bacterial species isolated in this study were also isolated in a similar study by Ki-Hyun *et al*. [26] (2018). Most of the bacterial isolates in this study are known to be pathogenic, and as such, could pose a whole lot of health challenges to humans, especially children. For instance, *Bacillus* sp which is known to be associated mainly with food poisoning, has been increasingly reported to be a cause of serious and potentially fatal non- gastrointestinal-tract infections [27]. *Shigella flexneri* causes diarrhea that is usually self-limiting, which may result to life threatening disease [28]. However, in the absence of adequate medical care or in immunocompromised patients, it can be fatal. Other bacterial isolates that were obtained during the study that could pose serious threat to human health are *Micrococcus, Serratia, Proteus., Yersinia and Enterobacter* spp*.*

The fungal species that were isolated from the indoor air during the study are *Penicilium, Aspergillus, Candida, Microsporum. Exophiala* and *Mucor* spp. Some of these fungi were also isolated by Amemeh *et al.* [29] in a related study. The presence of *Aspergillus* and *Penicillium* could be because they have high growth ability in different climatic conditions and by producing small light spores which remain in the air [30].

Considering the bacterial load on the various media used in classrooms A and B, at 9:30 am and 12:30 pm, it was revealed that classroom A had more bacterial load compared to classroom B. However, classroom B had substantially high total fungal load on day 1 (9:00 am – 9:30 am), likewise the total bacterial loads observed in day 1 (9:00 am – 9:30 am and day 8, 9:00 – 9:30 am as well as 12:00pm – 12:30pm). It is important to note that bioaerosols pose serious health hazards for people and animals living in their vicinity. Environmental pollution is spread in the form of bioaerosols containing viruses, bacteria, actinomycetes and fungi [31].

4. CONCLUSION

This study identified different airborne microorganisms within the confines of a nursery school in an urban area. Some of these microorganisms have distinct signatures with reference to the species associated with them. It is expedient to note that exposure to indoor pollutants can lead to a variety of health challenges such as respiratory illnesses, allergies, asthma and many other ailments in children. Certain species of fungi like *Candida* are associated with the human skin and may be released as bioaerosols upon shedding. Therefore, there is need for nursery school buildings to be monitored closely in order to improve the indoor air quality to ensure the health and well-being of children. Factors such as: temperature, humidity, occupancy, ventilation, and cleaning can all affect the air quality. And so, understanding these factors and implementing measures to improve air quality, we can ensure a safe and healthy environment for the children in nursery schools.

ACKNOWLEDGEMENTS

The authors warmly acknowledge the management and staff of the Demonstration Primary School in the University of Port Harcourt for granting us access into the nursery section to carry out this study.

COMPETING INTERESTS

The authors declare that there is no competing interests regarding the publication of the paper.

AUTHORS' CONTRIBUTIONS

Ughala Ezinwanne took the lead in the study design and writing the manuscript while Okoro Chisom, Loveth performed the laboratory experiments.

REFERENCES

1 Walser,.R.D., Gavert, D.W., Karlin, B.E., Trockel, M., Ryu, D.M. & Taylor, C.B. (2015). Effectiveness of Acceptance and Commitment Therapy in treating depression and suicidal ideation in veterans. *J. Behav. Res. and Ther., 74,* 25-31.

2 Vornanen-Winqvist, C., Järvi, K., Andersson, M. A., Duchaine, C., Létourneau, V., Kedves, O., & Salonen, H. (2020). Exposure to indoor air contaminants in school buildings with and without reported indoor air quality problems. *Envirn. Int.*, *141*, 105781.

3 Ruggieri, S., Longo, V., Perrino, C., Canepari, S., Drago, G., L'Abbate, L., & Rizzo, G. (2019). Indoor air quality in schools of a highly polluted south Mediterranean area. *Indoor Air*, *29*(2), 276-290.

4 Yassin, M. F., & Pillai, A. M. (2019). Monitoring of volatile organic compounds in different schools: a determinant of the indoor air quality. *Int.l J. Environ. Sci. Technol.*, *16*, 2733-2744.

5 Bennett, J., Davy, P., Trompetter, B., Wang, Y., Pierse, N., Boulic, M., ... & Howden-Chapman, P. (2019). Sources of indoor air pollution at a New Zealand urban primary school; a case study. *Atmos. Pollut. Res.*, *10*(2), 435-444.

6 Pearson, C., Littlewood, E., Douglas, P., Robertson, S., Gant, T. W., & Hansell, A. L. (2015). Exposures and health outcomes in relation to bioaerosol emissions from composting facilities: a systematic review of occupational and community studies. *J. Toxicol. Environ. Health, Part B*, *18*(1), 43-69.

7 Ashmore, M.R & Dimitroulopoulou, C. (2009). Personal exposure of children to air pollution. *Atmos. and Environ.*, *43*, 128–141.

8 Lee, J.H., Kim, Y.J. & Kim, S. H. (2018). Indoor air quality and respiratory symptoms in a daycare center. *Environ. Res.*, *161*, 536-543.

9 Witchmann, J., Lin, T., Nilsson, M.A.M. & Bellander, T. (2017). PM2.5, soot and NO_2 indoor outdoor relationships at homes, Pre-schools and schools in Stockholm, Sweden. *Atmos. and Environ.*, *148*, 182-189

10 Kotzias, D. (2005) Indoor air and human exposure assessment needs and approaches. *Exp. Toxicol. Pathol.*, *57*, 5–7.

11 Srikanth, P., Sudharsanam, S. & Steinberg, R. (2008). Bioaerosols in indoor environment: Composition, health effects and analysis. *Indian J. Med. Microbiol.*, *26*(4), 302-312.

12 Samuel, F. H. & Abayneh,, M. M. (2014). Microbiological Quality of Indoor Air in University Libraries. *Asian Pac. J. Trop. Med. 4* (1), 312-317.

13 Frankel, M., Gabriel, B., Michael, T., Sine, G., Erik, W. H. & Anne, M. M. (2012). Seasonal Variations of Indoor Microbial Exposures and Their Relation to Temperature, Relative Humidity, and Air Exchange Rate. *Appl. Environ. Microbiol. 78* (23), 8289–8297.

14 Viegas, C.,, Viegas., S., Gomes, A., Taubel., M. & Sabino, R. (2017). Indoor Microbial Aerosol and its Health Effects: Microbial Exposure in Public Buildings- Viruses, Bacteria and Fungi. *J Health Res. Sci.,* 237-252.

15 Gatchalian, M. L. (2010). An in-depth analysis of the entrepreneurship education in the Philippines: An initiative towards the development of a framework for a professional teaching competency programme for entrepreneurship educators. *Int. J. Res. Rev., 5,* 51-73.

16 Selman., M., Lacasse., Y., Pardo, A. & Carmier, Y. (2009). Hypersensitity Pneumonitis caused by Fungi. *Proc. Am Thorac. Soc.,7*(3).

17 Reboux, G., Piarroux, R., Mauny, F., Madroszyk, A., Millon, L., Bardonnet, K., & Dalphin, J. C. (2001). Role of molds in farmer's lung disease in Eastern France. *Am. J. Respir. Criti. Care Med., 163*(7), 1534-1539.

18 Ostro, B., Lipsett, M., Mann, J., Braxton-Owens, H., & White, M. (2001). Air pollution and exacerbation of asthma in African-American children in Los Angeles. *Epidemiol.*, 200-208.

19 McCormick, A., Jürgen L., & Frank E. (2010):. "Aspergillus Fumigatus: Contours of An Opportunistic Human Pathogen*." Cell. Microbiol., 12,* 1535-1543.

20 Abel, H.A.A. & Hanan, A.M. (2012). Sedimentation with the Omeliansky Formula as an Accepted Technique for Qualifying Airborne Fungi. *Pol. J. Environ. Stud, 21*(6), 1539-1541.

21 Chithra, V.S., Nagendra, S.M.S. (2013) Chemical and morphological characteristics of indoor and outdoor particulate matter in an urban environment.,. *Atmos. Environ.,* 77, 579-587.

22 Madureira, J., Paciência, I., Pereira, C., Teixeira, J.P., Fernandes, E.D.O. (2015). Indoor air quality in Portuguese schools: levels and sources of pollutants. *Indoor Air*, 1-12.

23 Salonen, H., Duchaine, C., Mazaheri, M., Clifford, S., Lappalainen, S., Reijula, K., & Morawska, L. (2015). Airborne viable fungi in school environments in different climatic regions–A review. *Atmos. Environ.*, *104*, 186-194.

24 Madureira, J., Paciência, I., Pereira, C., Teixeira, J.P., Fernandes, E.D.O. (2015). Indoor air quality in Portuguese schools: levels and sources of pollutants. *Indoor Air*, 1-12.

25 Javed,, A., Aamir, F., Gohar, U.F. & Mukhtar, H. (2021). The Potential Impact of Smog Spell on Human's Health Amid COVID-19 Rages. *Int. J. Environ. Res. Pub. Health, 18*, 11408.

26 H., Kim, Y.J. & Kim, S. H. (2018). Indoor air quality and respiratory symptoms in a daycare center. *Environ. Res.*, *161*, 536-543.

27 Edward, J.B.(2010). *Bacillus cereus*, a Volatile Human Pathogen. *Chem. Microbiol. Revs.*

28 Hans, H.N. & Janos, M. (2018). Epithelial and Human Infectious Diseases. *Epig. Human Dis.*

29 Amemeh, Y.Z., Afshin, M., Saeed, D.A., Ebrahim, D., Manochehr, A., Ebrahim, M., Van, T.T., Rasoul, N.K. & Hajar, K..(2022). Evaluation of bioaerosols type, density and modeling of dispersion in inside and outside of different wards of educational hospitals. *Environ. Sci. Pol. Res. Int., 29* (10),14143-14157.

30 Fernando, N.L. & Fedorak, P.M. (2005). Changes at an activated sludge sewage treatment plant alters the numbers of airborne aerobic microorganisms. *Water Res.*, *39*,4597.