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# Microbiological assessment of indoor air quality of some selected private primary schools in Ilishan-Remo, Ogun state, Nigeria

**[*] Enitan SS, Ihongbe JC, Ochei JO, Effedua HI, Adeyemi O, Phillips T**

Department of Medical Laboratory Science, Babcock University, P.M.B. 21244, Ilishan-Remo, Ogun State, Nigeria

**Abstract**
The aim of this study was to determine the indoor air bacterial and fungal density of some selected private primary schools in Ilishan-Remo of Ogun State at different sampling time of the day using the settle plate method. The outcome of this study shows that regardless of the sampling periods, all the classrooms of the three private schools examined for indoor air microbiological quality were more heavily contaminated with bacteria aerosols than with fungal aerosols, with a mean bacterial and fungal count of: 4378.82 CFU/$m^3$ and 178.93 CFU/$m^3$, respectively. There were no significant differences between the mean bacterial counts of School 1 and School 2 ($P>0.05$), whereas the mean bacterial counts of school 1 and 2 were significantly higher ($P<0.05$) than that of School 3. There were no significant differences ($P>0.05$) in the fungal population density between and within the three schools examined. The levels of pollution with bacterial aerosols recorded in this study range from high to very high, while that of fungal aerosols range from very low to high. Aeroflora isolated include: *Staphylococcus aureus, Coagulase negative Staphylococcus species, Micrococcus species* and *Aerococcoccus species, Aspergillus species, Mucor species, Penicillium species, Candida species, Microsporum species, Trichophyton species* and *Rhizopus species.* The concentration of indoor bacterial aerosol observed in this study which was above permissive standard; underscore the importance of this microenvironment for the high exposure of children to bioaerosols.

**Keywords:** indoor air quality, bacteria, fungi, occupancy, temperature, relative humidity

## 1. Introduction
Indoor air quality (IAQ) is vital to human health because most human activities take place in the indoor environment including: classrooms, offices and factories [1]. Primary school education in Nigeria is bisected with myriads of problems including: poor funding, poor educational infrastructures, overcrowding, inadequate classrooms and poor/polluted learning environment [2]. One of the cardinal objectives of primary schools in a society is to provide a safe and conducive learning environment for pupils [3]. However, for many school-aged children, the outcome is different; they acquire communicable diseases in school. The quality of air inside enclosed spaces like the classrooms where they spend a time period of nearly 7 to 8 hours daily while learning in school has become a matter of growing concern today [1]. While, the presence of microbes in air indoors is a problem from the view of health protection; the classroom environment represents a congenial situation where microorganisms and susceptible pupils with their teachers are together indoors. As stated by Riley, "the enclosed atmosphere of a building and its human occupants constitute an ecological unit'' [4]. No doubt, the air within the classrooms may serve as a reservoir for microorganisms thereby contributing to the rate of infection among school aged children who are more susceptible to indoor air pollutants than adults as they are exposed to unidentified amount of indoor air pollutants in school environments [5-7].

According to [8], microorganisms such as bacterial and fungal spores are major indoor biological air pollutants, accounting for 5-34% of indoor air pollution and are almost always present in all indoor locations due to their ubiquity in the environment and in human beings, hence the quality of air inside learning facilities where numerous school aged children and their teachers spend a large part of their life is therefore, an essential determinant of their health, well-being and life expectancy.

The sources of classroom airborne infection or contamination could be traced to a variety of factors. These include the pupil's own normal flora, uniforms, bags, sandals; as well as activity of pupils like sneezing, coughing, talking and yawning [1]. Materials such as cupboards, books and files have been implicated as viable sources [5]. House-keeping activity such as sweeping or using dry dust mops can aerosolize particles that may contain microorganisms. Infectious nuclei in air currents and dust may be inhaled during normal breathing [9].

The number of microorganisms present in classroom will depend on the number of pupils occupying the classroom, the amount of physical activity, the rate of air exchange, the ambient temperature, relative humidity, level of environmental sanitation, type of ventilation, numbers of windows available for cross ventilation amongst others [1].

According to [10], maintaining a healthy environment and, therefore, reducing disease transmission risk should inarguably be one of the key agendas in school operation. It is important to understand the microbial community within public areas and, in particular, within school buildings as poor health in children impacts on wider society. Against this back-drop, the determination of indoor microbial density is necessary, and it is especially important in such populated areas like school settings. There are numerous private primary schools in Ilishan-Remo of Ogun state, but unfortunately,

studies on the microbiological indoor air quality of such private primary schools in Ilishan-Remo, Ogun State is lacking. This study is therefore designed to assess the extent of indoor air microbial contamination in the classrooms of the selected schools, as well as to determine the relationship between microbial density and factors such as occupancy, temperature and relative humidity. It is therefore hoped that the outcome of this study will provide data that will be used to set standards for levels of acceptable microbial population and can also be used to suggest suitable guidelines that will help to decrease microbial density in school indoor air.

## 2. Materials and Methods

### 2.1 Study area

The study was carried out in three selected private primary schools designated as School 1, 2 and 3 located within Ilishan-Remo community of Ogun State. Ilishan-Remo community is one of the geopolitical wards in Ikenne Local Government Area of Ogun state, situated in the tropical area of South-western part of Nigeria, coordinates: 7°29′00″N 2°53′00″E. It has a warm-humid climate characterised by two seasons: wet (April-October) and dry (January, February, March, November and December). It experiences constant high temperatures and relative humidity throughout the year with a diurnal temperature range of minimum 23-27$^{O}$C and maximum 30-34$^{O}$C, with a mean annual relative humidity value of 84%.

### 2.2 Assessment of the physical characteristics of classrooms

Without any form of bias, a structured-checklist was completed by the researcher to collect data on the physical characteristics of each classroom prior to air sampling. Information collected include: School identification number, class level, number of pupils per class, number of seats per class, number of pupils per seat, number of windows per class, number of doors per class, number of ceiling fans per class, if available, area of the classroom in square feet ($ft^2$), number of standard ceiling tiles per classroom, evidence of dampness and molds growing on the classroom wall and ceiling, level of classroom sanitation/hygiene, classroom sweeping and moping routine, availability of well covered waste bins in the classroom etc.

### 2.3 Enumeration of classroom occupants

The numbers of occupants (pupils and teacher) present in each classroom during each sampling period were determined by head count and recorded.

### 2.4 Measurements of meterological parameters

A digital handheld battery-powered temperature-humidity meter (MEXTECH TM-1 digital thermo-hygrometer with an indoor temperature and relative humidity range of: -10$^{o}$C - +50$^{o}$C and 20% - +99%, respectively, held at 2 m above the floor level was used to measure the temperature and relative humidity of each classroom during each sampling period.

### 2.5 Microbiological air sampling

Settle plate method using three (3) open 8.5 cm diameter Petri dishes (60.0525 $cm^2$ areas) containing different cultures were used as described by [11, 12]. This method allows bacteria or fungi carrying particles to settle on culture media. Bacteria were collected on Nutrient agar (NA) and Blood agar (BA) to which an antifungal agent (Griseofuvin) has been incorporated to inhibit the growth of fungi; while fungi were collected on Sabouraud dextrose agar (SDA) plates to which an antibacterial agent (Chloramphenicol) has been incorporated to inhibit the growth of bacteria. The media plates were placed on a table with the sampling height 2m above the floor which approximated the human breathing zone, while the sampling area was at the center of the selected classrooms. Taking into consideration the variation in environmental factors, the samplings were done at three different periods of the day: 7.30 - 8.00 am (before class session begins in the morning), 11.30 am -12.00 pm (on resumption from recess) and 3.00-3.30 pm (just before school closing time).

A set of 3 plates (NA, BA and SDA) were exposed with their lids open, about 3 meters apart and allowed to stay for 30 minutes. Triplicate samples for each culture medium were collected to ensure sampling accuracy. Afterwards, the plates were covered, kept in tight sealed case and transported to the Microbiology Laboratory unit of the Department of Medical Laboratory Science, Babcock University and incubated at 37°C for 24-48 hours for bacteria and at 25°C for 5-7 days for fungi as described by [13]. To avoid self-contamination of agar plates during air sampling, sterile gloves, mouth masks and protective gown were worn, and before use the agar plates were also checked visually for any microbial growth.

### 2.6 Microbiological analysis

#### 2.6.1 Determination of microbial density

Following incubation of culture plates, bacterial and fungal colony forming units (CFU) were enumerated. Afterwards, the mean colony forming units per cubic meter ($CFU/m^3$) of air of the plates collected in triplicates were determined using the following equation as described by [14, 15]:

$$\mathbf{N = 5a \times 10^4 (bt)^{-1},}$$

Where

- N: microbial $CFU/m^3$ of indoor air;
- a: number of colonies per Petri dish;
- b: dish surface area, $cm^2$;
- t: exposure time of the petri dish, minutes.

After the microbial density of the resultant colonies on each plate has been determined, the colonial morphology of the different colonies formed were noted and identical colonies were sub-cultured into Nutrient Agar (NA) or Sabouraud Dextrose Agar (SDA) plates, incubated appropriately and stored for further identification and characterization.

#### 2.6.2 Identification of bacterial and fungal isolates

Individual bacterial isolates were identified using standard methods (including: colonial morphology, microscopy and biochemical tests) as described by [16]. While, fungal isolates were identified on the basis of microscopic (using Lactophenol cotton blue staining) and macroscopic characteristics (with the aid of an Atlas of Mycology) as described by [17].

### 2.7 Data analyses

Data were presented using tables and graphs. Statistical analyses were carried out with one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test

using SPSS Statistics Software Package (Version 18.0) to test for significant differences in the microbial densities of the classrooms at different sampling time. P-values <0.05 were considered significant. Data were also subjected to Spearman correlation analysis using Graphpad In-stat Software Package to determine the relationship between microbial densities and classroom occupancy, temperature and relative humidity.

## 3. Result and Discussion

This present study assessed the microbiological quality of indoor air of three private primary schools in Ilishan-Remo, Ikenne Local Government Area of Ogun State, Nigeria using settle plate method. The mean indoor bacterial colony counts, temperature and relative humidity of the selected classrooms of School 1 at different sampling periods (7:30 am, 11:30 am and 3:00 pm) of the day are presented in Table 1. There were no significant differences in the bacterial colony counts between and within the classrooms (P>0.05).

**Table 1:** Indoor bacterial colony counts (CFU) per $m^3$ air at different sampling periods of day of School 1

| | Different air sampling time of the day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7.30 am | | | 11:30 am | | | 3:00 pm | | | |
| Study Class | Bacterial colony counts (CFU/$m^3$) | Mean T (ºC) | Mean RH (%) | Bacterial colony counts (CFU/$m^3$) | Mean T (ºC) | Mean RH (%) | Bacterial colony counts (CFU/$m^3$) | Mean T (ºC) | Mean RH (%) | CMBCC/C (CFU/$m^3$) |
| Class 1 | 5308 | 28.7 | 74.3 | 3687 | 32.9 | 65.7 | 4500 | 33.7 | 63.7 | 4498 |
| Class 2 | 5979 | 28.4 | 74.0 | 7389 | 32.9 | 61.0 | 4250 | 33.9 | 61.0 | 5872 |
| Class 3 | 5309 | 28.5 | 73.3 | 3728 | 32.9 | 63.3 | 4375 | 34.2 | 61.7 | 4471 |
| Class 4 | 6129 | 28.4 | 74 | 5733 | 32.9 | 58.3 | 4340 | 34.0 | 61.3 | 5401 |
| Class 5 | 5249 | 28.4 | 75.3 | 7996 | 33.0 | 64.7 | 0 | 33.4 | 62.6 | 4415 |
| CMBCC/SP (CFU/$m^3$) | 5595* | | | 5707* | | | 3493 | | | |
| CMT/SP ($^OC$) | | 28.5 | | | 33.0 | | | 33.8 | | |
| CMRH/SP (%) | | | 74.2 | | | 62.6 | | | 62.1 | |

*P<0.05 is considered statistically significant.

**KEY:** T = Temperature, RH = Relative humidity, CMBCC/CL = Combined mean bacterial colony count per classroom (CFU/$m^3$), CMBCC/SP = Combined mean bacterial colony count per sampling period (CFU/$m^3$), CMT/SP = Combined mean temperature per sampling period ($^OC$), CMRH/SP = Combined mean relative humidity per sampling period (%).

Also, there was no significant difference between the 7:30 am and 11.30 am bacterial colony counts (P>0.05), whereas there were significant differences (P<0.05) between the 7:30 am and 3:00 pm bacterial colony counts; as well as between the 11:30 am and 3: 00 pm bacterial colony counts. While the mean temperature of the classrooms increased non-significantly (P>0.05) across the sampling periods: 7:30 am (28.5$^O$C), 11:30 am (33.0 $^O$C) and 3:00 pm (33.8$^O$C), the mean relative humidity of the classrooms decreased non-significantly (P>0.05) across the sampling periods: 7:30 am (74.2%), 11:30 am (62.6%) and 3:00 pm (62.1%).Furthermore, the mean indoor bacterial colony counts, temperature and relative humidity of the selected classrooms of School 2 at different sampling periods (7:30 am, 11:30 am and 3:00 pm) of the day are presented in Table 2. There were no significant differences in the bacterial colony counts between and within the classrooms (P>0.05). However, there were significant differences between the 7:30 am and 11.30 am bacterial colony counts; as well as between the 7:30 am and 3: 00 pm bacterial colony counts (P<0.05); but, there was no significant difference between the 11:30 am and 3: 00 pm bacterial colony counts (P>0.05).

**Table 2:** Indoor bacterial colony counts (CFU) per $m^3$ air at different sampling periods of day at School 2

| | Different air sampling time of the day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7.30 am | | | 11:30 am | | | 3:00 pm | | | |
| Study Class | Bacterial colony counts (CFU/$m^3$) | Mean T (ºC) | Mean RH (%) | Bacterial colony counts (CFU/$m^3$) | Mean T (ºC) | Mean RH (%) | Bacterial colony counts (CFU/$m^3$) | Mean T (ºC) | Mean RH (%) | CMBCC/C (CFU/$m^3$) |
| Class 1 | 6666 | 29.3 | 74.3 | 7299 | 33.6 | 65.0 | 4871 | 33.3 | 65 | 6279 |
| Class 2 | 2709 | 29.3 | 74.3 | 5679 | 33.6 | 61.3 | 4394 | 34.2 | 63.3 | 4261 |
| Class 3 | 1827 | 29.4 | 74.0 | 6298 | 33.3 | 62.3 | 7590 | 33.3 | 64.7 | 5238 |
| Class 4 | 1208 | 29.3 | 74.0 | 9111 | 33.1 | 64.7 | 9860 | 34.2 | 63.7 | 6726 |
| Class 5 | 1932 | 29.4 | 112 | 3873 | 33.5 | 65.3 | 6064 | 33.8 | 58.3 | 3956 |
| CMBCCPP (CFU/$m^3$) | 2868 | | | 6452* | | | 6556* | | | |
| CMT/SP ($^OC$) | | 29.3 | | | 33.4 | | | 33.8 | | |
| CMRH/SP (%) | | | 81.7 | | | 63.7 | | | 63.0 | |

*P<0.05 is considered statistically significant.

**KEY:** T = Temperature, RH = Relative humidity, CMBCC/CL = Combined mean bacterial colony count per classroom (CFU/$m^3$), CMBCC/SP = Combined mean bacterial colony count per sampling period (CFU/$m^3$), CMT/SP = Combined mean temperature per sampling period ($^OC$), CMRH/SP = Combined mean relative humidity per sampling period (%).

While the combined mean temperature of the classrooms increased non-significantly (P>0.05) across the sampling periods: 7:30 am (29.3$^{O}$C), 11:30 am (33.4$^{O}$C) and 3:00 pm (33.8$^{O}$C), the combined mean relative humidity of the classrooms decreased non-significantly (P>0.05) across the sampling periods: 7:30 am (81.7%), 11:30 am (63.7%) and 3:00 pm (63.0%). In addition, the mean indoor bacterial colony counts, temperature and relative humidity of the selected classrooms of School 3 at different sampling periods (7:30 am, 11:30 am and 3:00 pm) of the day are presented in Table 3. There were no significant differences in the bacterial colony counts between and within the classrooms in School 3 (P>0.05). Also, there were no significant differences in the bacterial colony counts between and within the sampling periods of the day (P>0.05). While the mean temperature of the classrooms increased non-significantly (P>0.05) across the sampling periods: 7:30 am (27.9$^{O}$C), 11:30 am (28.3$^{O}$C) and 3:00 pm (33.1$^{O}$C), the mean relative humidity of the classrooms decreased non-significantly (P>0.05) across the sampling periods: 7:30 am (80.1%), 11:30 am (75.8%) and 3:00 pm (62.7%).

**Table 3:** Indoor bacterial colony counts (CFU) per m$^3$ air at different sampling periods of day of School 3

| | Different air sampling time of the day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7.30 am | | | 11:30 am | | | 3:00 pm | | | |
| **Study Class** | **Bacterial colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Bacterial colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Bacterial colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **CMBCC/C (CFU/m$^3$)** |
| Class 1 | 2552 | 28.1 | 76 | 1103 | 29.1 | 76 | 903 | 33.2 | 66 | 1519 |
| Class 2 | 7848 | 27.9 | 83.3 | 2321 | 27.8 | 74.6 | 2835 | 33.5 | 66.7 | 4335 |
| Class 3 | 2594 | 27.9 | 80.3 | 1397 | 28.3 | 75.6 | 3509 | 33.7 | 63 | 2500 |
| Class 4 | 1712 | 27.9 | 79 | 6977 | 28.4 | 76 | 4364 | 34.2 | 61 | 4351 |
| Class 5 | 2258 | 27.7 | 81.7 | 2037 | 28.1 | 77 | 1281 | 30.9 | 57 | 1858 |
| CMBCC/SP (CFU/m$^3$) | 3393* | | | 2767 | | | 2578 | | | |
| CMT/SP ($^{O}$C) | | 27.9 | | | 28.3 | | | 33.1 | | |
| CMRH/SP (%) | | | 80.1 | | | 75.8 | | | 62.7 | |

*P<0.05 is considered statistically significant.

**KEY:** T = Temperature, RH = Relative humidity, CMBCC/CL = Combined mean bacterial colony count per classroom (CFU/m$^3$), CMBCC/SP = Combined mean bacterial colony count per sampling period (CFU/m$^3$), CMT/SP = Combined mean temperature per sampling period ($^{O}$C), CMRH/SP = Combined mean relative humidity per sampling period (%).

On the other hand, the mean indoor fungal colony counts, temperature and relative humidity of the selected classrooms of School 1 at different sampling periods (7:30 am, 11:30 am and 3:00 pm) of the day are presented in Table 4.

There were no significant differences in the fungal colony counts between and within the classrooms in School 1 (P>0.05). Still, there were no significant differences between the 7:30 am and 11:30 am fungal colony counts; but there were significant differences between the 7:30 am and 3:00 pm fungal colony counts, as well as between the 11:30 am and 3:00 pm fungal colony counts (P<0.05). While the mean temperature of the classrooms increased non-significantly (P>0.05) across the sampling periods: 7:30 am (28.5$^{O}$C), 11:30 am (33.0$^{O}$C) and 3:00 pm (33.8$^{O}$C), the mean relative humidity of the classrooms decreased non-significantly (P>0.05) across the sampling periods: 7:30 am (74.2%), 11:30 am (62.6%) and 3:00 pm (62.1%).

Furthermore, the mean indoor fungal colony counts, temperature and relative humidity of the selected classrooms of School 2 at different sampling periods (7:30 am, 11:30 am and 3:00 pm) of the day are presented in Table 5.

**Table 4:** Indoor fungal colony counts (CFU) per m$^3$ air at different sampling periods of day of School 1.

| | Different air sampling time of the day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7.30 am | | | 11:30 am | | | 3:00 pm | | | |
| **Study Class** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **CMFCC/C (CFU/m$^3$)** |
| Class 1 | 0 | 28.7 | 74.3 | 700 | 32.9 | 63.7 | 0 | 33.7 | 65.7 | 233 |
| Class 2 | 245 | 28.4 | 74.0 | 450 | 32.9 | 61 | 0 | 33.9 | 61 | 232 |
| Class 3 | 326 | 28.5 | 73.3 | 967 | 32.9 | 61.7 | 0 | 34.2 | 63.3 | 431 |
| Class 4 | 276 | 28.4 | 74.0 | 0 | 32.9 | 61.3 | 0 | 34.0 | 58.3 | 276 |
| Class 5 | 189 | 28.4 | 75.3 | 462 | 33.0 | 62.6 | 0 | 33.4 | 64.7 | 217 |
| CMFCC/SP (CFU/m$^3$) | 207 | | | 516* | | | 0 | | | |
| CMT/SP ($^{O}$C) | | 28.5 | | | 33.0 | | | 33.8 | | |
| CMRH/SP (%) | | | 74.2 | | | 62.6 | | | 62.1 | |

*P<0.05 is considered statistically significant.

**KEY:** T = Temperature, RH = Relative humidity, CMFCC/CL = Combined mean fungal colony count per classroom (CFU/m$^3$), CMFCC/SP = Combined mean fungal colony count per sampling period (CFU/m$^3$), CMT/SP = Combined mean temperature per sampling period ($^{O}$C), CMRH/SP = Combined mean relative humidity per sampling period (%).

There were no significant differences (P>0.05) in the fungal colony counts between and within the classrooms in School 2, but there were significant differences between the 7:30 am and 11:30 am fungal colony counts; as well as between the 7:30 am and 3:00 pm fungal colony counts. Howbeit, there was no significant difference between the 11:30 am and 3:00 pm fungal colony counts (P<0.05). While the mean temperature of the classrooms increased non-significantly (P>0.05) across the sampling periods: 7:30 am (29.3$^{O}$C), 11:30 am (33.4$^{O}$C) and 3:00 pm (33.8$^{O}$C), the mean relative humidity of the classrooms decreased non-significantly (P>0.05) across the sampling periods: 7:30 am (81.7%), 11:30 am (63.7%) and 3:00 pm (63.0%). Still, the mean indoor fungal colony counts, temperature and relative humidity of the selected classrooms of School 3 at different sampling periods (7:30 am, 11:30 am and 3:00 pm) of the day are presented in Table 6.

There were no significant differences in the fungal colony counts between and within the classrooms (P>0.05). There were no significant differences between and within the sampling periods (P>0.05).

**Table 5:** Indoor fungal colony counts (CFU) per m$^3$ air at different sampling periods of day of School 2.

| | Different air sampling time of the day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7.30 am | | | 11:30 am | | | 3:00 pm | | | |
| **Study Class** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **CMFCC/C (CFU/m$^3$)** |
| Class 1 | 609 | 29.3 | 74.3 | 21 | 33.6 | 65.0 | 42 | 33.3 | 65.0 | 224 |
| Class 2 | 294 | 29.3 | 74.3 | 42 | 33.6 | 61.3 | 63 | 34.2 | 63.3 | 133 |
| Class 3 | 54 | 29.4 | 74.0 | 63 | 33.3 | 62.3 | 0 | 33.2 | 64.7 | 39 |
| Class 4 | 32 | 29.3 | 74.0 | 0 | 33.1 | 64.7 | 0 | 34.2 | 63.7 | 11 |
| Class 5 | 420 | 29.4 | 112 | 42 | 33.5 | 65.3 | 0 | 33.8 | 58.3 | 154 |
| MFCC/SP (CFU/m$^3$) | 282* | | | 34 | | | 21 | | | |
| CMT/SP ($^{O}$C) | | 29.3 | | | 33.4 | | | 33.8 | | |
| CMRH/SP (%) | | | 81.7 | | | 63.7 | | | 63.0 | |

*P<0.05 is considered statistically significant.

**KEY:** T = Temperature, RH = Relative humidity, CMFCC/CL = Combined mean fungal colony count per classroom (CFU/m$^3$), CMFCC/SP = Combined mean fungal colony count per sampling period (CFU/m$^3$), CMT/SP = Combined mean temperature per sampling period ($^{O}$C), CMRH/SP = Combined mean relative humidity per sampling period (%).

While the mean temperature of the classrooms increased non-significantly (P>0.05) across the sampling periods: 7:30 am (27.9$^{O}$C), 11:30 am (28.3$^{O}$C) and 3:00 pm (33.1$^{O}$C), the mean relative humidity of the classrooms decreased non-significantly (P>0.05) across the sampling periods: 7:30 am (80.1%), 11:30 am (75.8%) and 3:00 pm (62.7%).

The ranges of microbial population density in the three private primary schools are presented in Table 7. On one hand, the bacterial population density at School 1, 2 and 3 ranges from 0-7996 CFU/m$^3$, 1208-9860 CFU/m$^3$, and 903-7848 CFU/m$^3$, respectively. The highest and lowest mean bacterial population density was recorded at School 2 and School 3, 5292.17 CFU/m$^3$ and 2912.73 CFU/m$^3$, respectively. There was no significant difference (P>0.05) between the mean bacterial population density at School l and 2. However, the mean bacterial population density at School 1 and 2 were significantly higher than those of School 3 at P value <0.05. The total bacterial population density for the three schools range between 2913.73-529.17 CFU/m$^3$ with a mean bacterial count of 4378.82 CFU/m$^3$.

**Table 6:** Indoor fungal colony counts (CFU) per m$^3$ air at different sampling periods of day of School 3.

| | Different air sampling time of the day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7.30 am | | | 11:30 am | | | 3:00 pm | | | |
| **Study Class** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **Fungal colony counts (CFU/m$^3$)** | **Mean T (ºC)** | **Mean RH (%)** | **CMFCC/C (CFU/m$^3$)** |
| Class 1 | 358 | 28.1 | 76 | 542 | 29.1 | 76 | 0 | 33.2 | 66 | 300 |
| Class 2 | 0 | 27.9 | 83.3 | 403 | 27.8 | 74.6 | 524 | 33.5 | 66.7 | 309 |
| Class 3 | 102 | 27.9 | 80.3 | 147 | 28.3 | 75.6 | 200 | 33.7 | 63 | 150 |
| Class 4 | 36 | 27.9 | 79 | 55 | 28.4 | 76 | 42 | 34.2 | 61 | 44 |
| Class 5 | 96 | 27.7 | 81.7 | 250 | 28.1 | 77 | 0 | 30.9 | 57 | 115 |
| CMFCCPP (CFU/m$^3$) | 118 | | | 279 | | | 153 | | | |
| CMT/SP ($^{O}$C) | | 27.9 | | | 28.3 | | | 33.1 | | |
| CMRH/SP (%) | | | 80.1 | | | 75.8 | | | 62.7 | |

*P<0.05 is considered statistically significant.

**KEY:** T = Temperature, RH = Relative humidity, CMFCC/CL = Combined mean fungal colony count per classroom (CFU/m$^3$), CMFCC/SP = Combined mean fungal colony count per sampling period (CFU/m$^3$), CMT/SP = Combined mean temperature per sampling period ($^{O}$C), CMRH/SP = Combined mean relative humidity per sampling period (%).

On the other hand, the fungal population density at School 1, 2 and 3 ranges from 0-967 CFU/$m^3$, 0-609 CFU/$m^3$ and 0-542 CFU/$m^3$, respectively. The highest and lowest mean fungal population density was recorded at School 1 and 2, 241.00 CFU/$m^3$ and 112.13 CFU/$m^3$, respectively. There were no significant differences in the mean fungal population density between and within the three schools studied. The total fungal population density for the three schools range between 112.13-241.00 CFU/$m^3$ with a mean fungal count of 178.93 CFU/$m^3$. Assessment of air quality in the selected classrooms of the three private Primary schools in Ilishan-Remo according to the sanitary standards for non-industrial premises is indicated in Table 8. The degree of air pollution by bacteria population across the various classrooms of the three private primary schools ranges largely between high to very high; while the degree of air pollution by fungal population across the various classrooms of the three private primary schools ranges largely between very low to high, indicating that the indoor air of these schools were predominantly polluted by bacteria population.

The distribution of aero-flora in the classrooms of the three private primary schools, in Ilishan-Remo studied is present in Table 9.

**Table 7:** The ranges of microbial population density in the three private primary schools

| Study Area | N | Minimum | Maximum | Median | Mean | Standard error of mean |
|---|---|---|---|---|---|---|
| **SCHOOL 1** | | | | | | |
| Bacteria Count (CFU/$m^3$) | 15 | 0 | 7996 | 5249 | *4931.57 | 476.18 |
| Fungi Count (CFU/$m^3$) | 15 | 0 | 967 | 189 | 241 | 77.09 |
| **School 2** | | | | | | |
| Bacteria Count (CFU/$m^3$) | 15 | 1208 | 9860 | 5679 | *5292.17 | 683.3 |
| Fungi Count (CFU/$m^3$) | 15 | 0 | 609 | 42 | 112.13 | 46.94 |
| **School 3** | | | | | | |
| Bacteria Count (CFU/$m^3$) | 15 | 903 | 7848 | 2321 | 2912.73 | 529.31 |
| Fungi Count (CFU/$m^3$) | 15 | 0 | 542 | 102 | 183.67 | 48.93 |
| **School 1, 2 & 3 Combined** | | | | | | |
| Bacteria Count (CFU/$m^3$) | 3 | 2913.73 | 5292.17 | 4931.57 | 4378.82 | 740.40 |
| Fungi Count (CFU/$m^3$) | 3 | 112.13 | 241.00 | 183.67 | 178.93 | 37.28 |

*P value <0.05 is considered statistically significant

In all, four (4) bacteria types were isolated which include: *Staphylococcus aureus (SA), Coagulase negative Staphylococcus (CoNS) species, Micrococcus (MC) species* and *Aerococcoccus (AE) species;* while seven (7) fungal types were isolated which include: *Aspergillus (AS) species, Mucor (MU) species, Penicillium (PE) species, Candida (CA) species, Microsporum (MS) species, Trichophyton (TR) species* and *Rhizopus (RH) species. Penicillium spp.* and *Trichophyton spp.* were exclusively absent in School 1. Also, *Mucor spp.* and *Penicillium spp.* were exclusively absent in School 2; while, *Candida spp., Microsporum spp., Trichophyton spp.* and *Rhizopus spp.* were exclusively absent in School 3. School 1 recorded the highest number of isolates (32), followed by School 2 (31), while School 3 recorded the lowest number of isolates (25).

**Table 8:** Assessment of air quality in the selected classrooms of the three private Primary schools in Ilishan-Remo according to the sanitary standards for non-industrial premises

| Study area | Range of values (CFU/$m^3$) | Degree of Air Pollution | Sampling Sites and time | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Primary 1 | | | Primary 2 | | | Primary 3 | | | Primary 4 | | | Primary 5 | | |
| | | | 7: 30 am | 11: 30 am | 3: 00 pm | 7: 30 am | 11: 30 am | 3: 00 pm | 7: 30 am | 11: 30 am | 3: 00 pm | 7: 30 am | 11: 30 am | 3: 00 pm | 7: 30 am | 11: 30 am | 3: 00 pm |
| **School 1** | | | | | | | | | | | | | | | | | |
| Bacteria | <25 | Very Low | | | | | | | | | | | | | | | √ |
| | 25-100 | Low | | | | | | | | | | | | | | | |
| | 100-500 | Intermediate | | | | | | | | | | | | | | | |
| | 500-2000 | High | | | | | | | | | | | | | | | |
| | >2000 | Very high | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | |
| Fungi | <25 | Very Low | √ | | √ | | | √ | | | √ | | √ | √ | | | √ |
| | 25-100 | Low | | | | | | | | | | | | | | | |
| | 100-500 | Intermediate | | | | √ | √ | | √ | | | √ | | | √ | √ | |
| | 500-2000 | High | | √ | | | | | | √ | | | | | | | |
| | >2000 | Very high | | | | | | | | | | | | | | | |
| **School 2** | | | | | | | | | | | | | | | | | |
| Bacteria | <25 | Very Low | | | | | | | | | | | | | | | |
| | 25-100 | Low | | | | | | | | | | | | | | | |
| | 100-500 | Intermediate | | | | | | | | | | | | | | | |
| | 500-2000 | High | | | | | | | √ | | | √ | | | √ | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | >2000 | Very high | √ | √ | √ | √ | √ | √ | | √ | √ | | √ | √ | | √ | √ |
| Fungi | <25 | Very Low | | √ | | | | | | | √ | | √ | √ | | | √ |
| | 25-100 | Low | | | √ | | √ | √ | √ | √ | | √ | | | | √ | |
| | 100-500 | Intermediate | | | | √ | | | | | | | | | √ | | |
| | 500-2000 | High | √ | | | | | | | | | | | | | | |
| | >2000 | Very high | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| **School 3** | | | | | | | | | | | | | | | | | |
| Bacteria | <25 | Very Low | | | | | | | | | | | | | | | |
| | 25-100 | Low | | | | | | | | | | | | | | | |
| | 100-500 | Intermediate | | | | | | | | | | | | | | | |
| | 500-2000 | High | | √ | √ | | | | | √ | | √ | | | | | √ |
| | >2000 | Very high | √ | | | √ | √ | √ | √ | | √ | | √ | √ | √ | √ | |
| Fungi | <25 | Very Low | | | √ | √ | | | | | | | | | | | √ |
| | 25-100 | Low | | | | | | | | | | √ | √ | √ | √ | | |
| | 100-500 | Intermediate | √ | | | | √ | | √ | √ | √ | | | | | √ | |
| | 500-2000 | High | | √ | | | | √ | | | | | | | | | |
| | >2000 | Very high | | | | | | | | | | | | | | | |

***NB:*** *≤ 500 CFU/m³ is the permissive standard*

Figures 1-6 are graphs showing the correlations between human/environmental factors (classroom occupancy, indoor temperature and relative humidity) and microbial counts of the three private primary schools.

**Table 9:** Distribution of aero-flora in the classrooms of the three private primary schools, in Ilishan-Remo, Ogun State.

| | **Bacterial isolates** | | | | **Fungal isolates** | | | | | | | **No. of isolates** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***SA*** | ***CoNS*** | ***MC*** | ***AE*** | ***AS*** | ***MU*** | ***PE*** | ***CA*** | ***MS*** | ***TR*** | ***RH*** | **Per class** |
| **School 1** | | | | | | | | | | | | |
| Primary 1 | + | + | - | + | + | - | - | - | + | - | + | 6 |
| Primary 2 | + | + | + | + | + | + | - | + | + | - | + | 9 |
| Primary 3 | + | - | + | + | + | - | - | + | + | - | + | 7 |
| Primary 4 | + | - | + | + | - | - | - | + | - | - | + | 5 |
| Primary 5 | - | + | + | - | + | - | - | - | + | - | + | 5 |
| | | | | | | | | | | **Total =** | | **32** |
| **School 2** | | | | | | | | | | | | |
| Primary 1 | + | + | + | - | + | - | - | + | + | + | - | 7 |
| Primary 2 | + | + | + | + | + | - | - | + | + | + | - | 8 |
| Primary 3 | + | + | + | - | + | - | - | - | + | - | + | 6 |
| Primary 4 | + | + | - | - | + | - | - | - | + | - | + | 5 |
| Primary 5 | + | + | - | - | + | - | - | - | + | + | - | 5 |
| | | | | | | | | | | **Total =** | | **31** |
| **School 3** | | | | | | | | | | | | |
| Primary 1 | + | - | + | - | + | + | + | - | - | - | - | 5 |
| Primary 2 | + | + | - | + | + | + | + | - | - | - | - | 6 |
| Primary 3 | + | + | - | + | + | - | + | - | - | - | - | 5 |
| Primary 4 | + | + | + | - | + | - | - | - | - | - | - | 4 |
| Primary 5 | - | + | - | + | + | + | + | - | - | - | - | 5 |
| | | | | | | | | | | **Total =** | | **25** |

**KEYS:** SA = S. aureus, CoNS = Coagulase Negative Staphylococcus, MC = Micrococcus spp., AE = Aerococcus spp., AS = Aspergillus spp., MU = Mucor spp., PE = Penicillium spp., CA = Candida spp., MS = Microsporium spp., TR = Trichophyton spp., RH = Rhizopus spp., + = Present, - = Absent.

The mean bacterial count (4378.82 CFU/m$^3$) recorded in this study was higher than the ones previously reported by other researchers. For instance, [18] reported a mean bacterial count of 1538 CFU/m$^3$/carpets and 840 CFU/m$^3$/no carpets in the rooms of 15 schools and day-care centers in Denmark [19]. reported a mean bacterial count of 519 CFU/m$^3$ for 10 schools in the same Denmark. In Sweden, [20] reported a mean bacterial count of 900 CFU/m$^3$ for 38 schools.

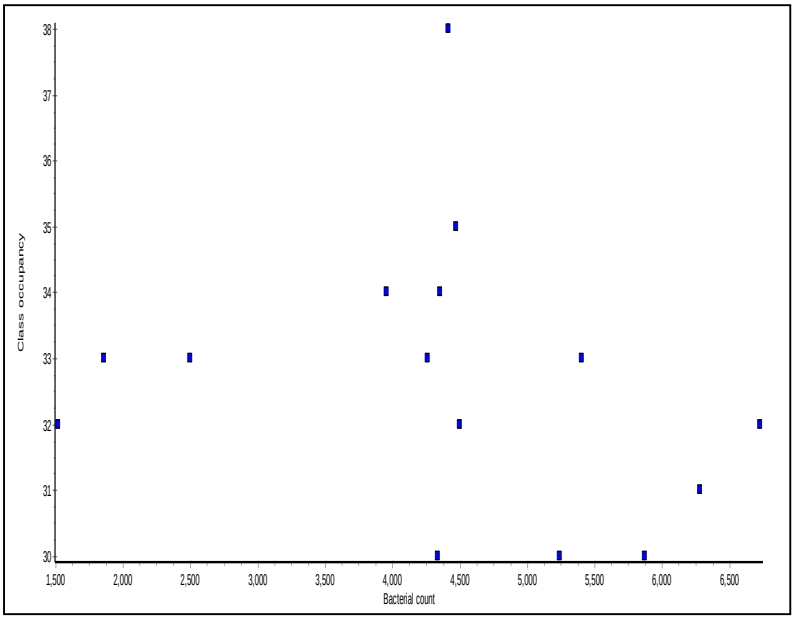


**Fig 1:** Graph showing the correlation between the combined classroom occupancy and bacterial count (CFU/$m^3$) of the three private Primary Schools. Classroom occupancy correlates with bacterial count by a correlation coefficient (r) value of 0.2336. The two-tailed P value is 0.4021, considered not significant.

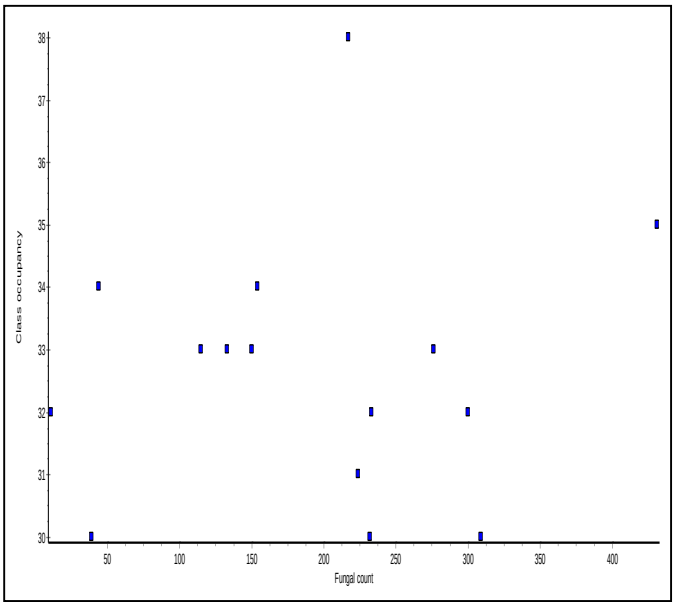


**Fig 2:** Graph showing the correlation between the combined classroom occupancy and fungal count (CFU/$m^3$). of the three Private Primary Schools. Classroom occupancy correlates with fungal count by a correlation coefficient (r) value of 0.1078. The two-tailed P value is 0.7023, considered not significant.

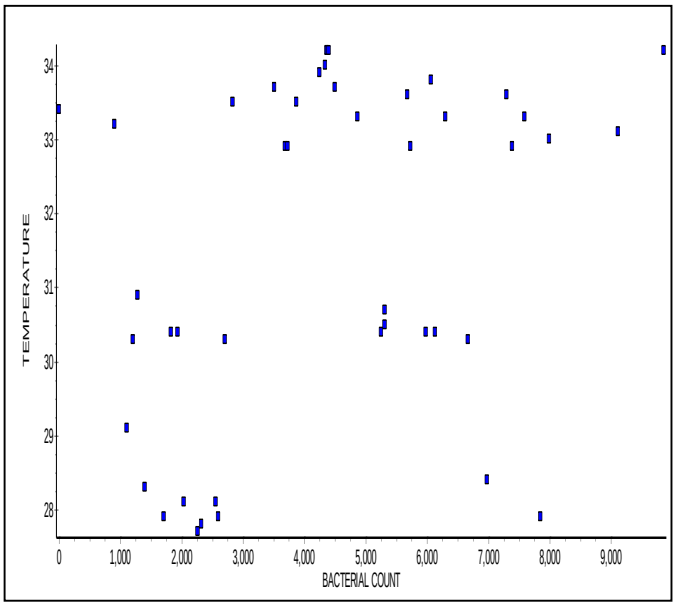


**Fig 3:** Graph showing the correlation between the combined indoor temperature ($^oC$) and bacterial count (CFU/$m^3$) of the three Private Primary Schools. Indoor temperature correlates with bacterial count by a correlation coefficient (r) value of 0.3368, the two-tailed P value is 0.0237, considered significant).

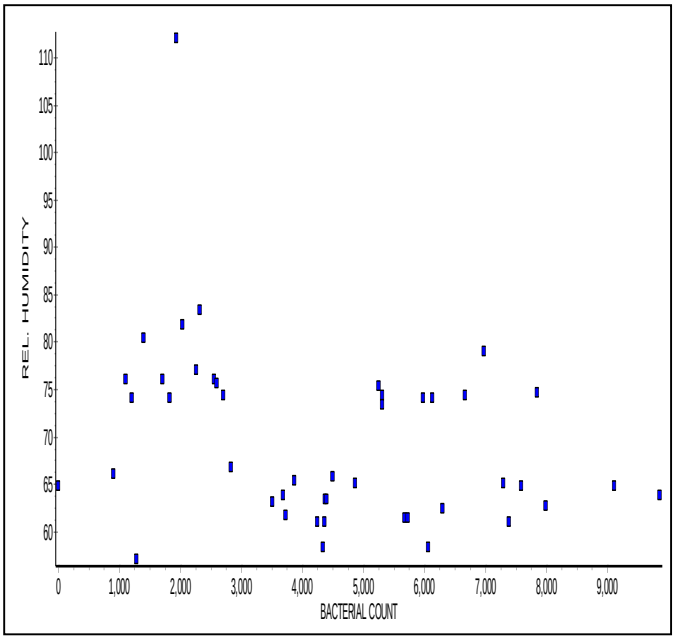


**Fig 4:** Graph showing the correlation between the combined indoor relative humidity (%) and bacterial count (CFU/$m^3$) of the three Private Primary Schools. Relative humidity correlates with bacterial count by a correlation coefficient (r) value of - 0.3139, the two-tailed P value is 0.0357, considered significant.

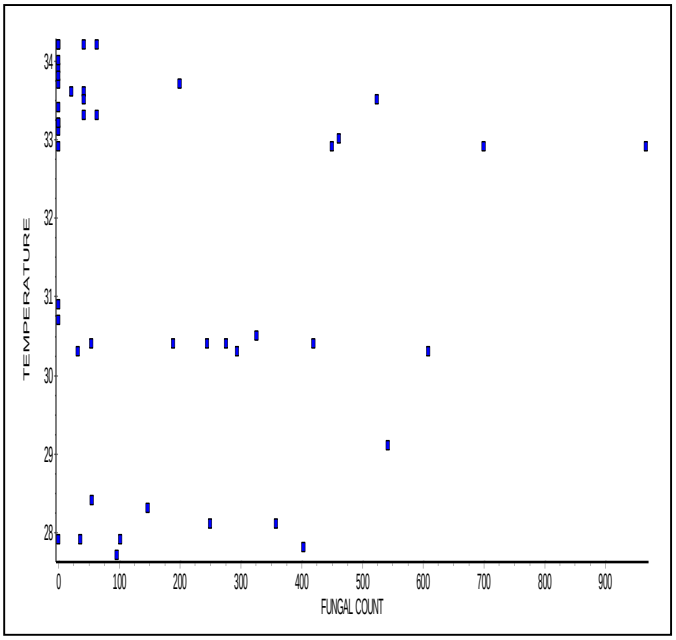


**Fig 5:** Graph showing the correlation between the combined indoor temperature ($^OC$) and fungal count (CFU/$m^3$) of the three Private Primary Schools. *Temperature correlates with fungal count by a correlation coefficient (r) value of -0.1639, the two-tailed P value is 0.2819, considered not significant*).

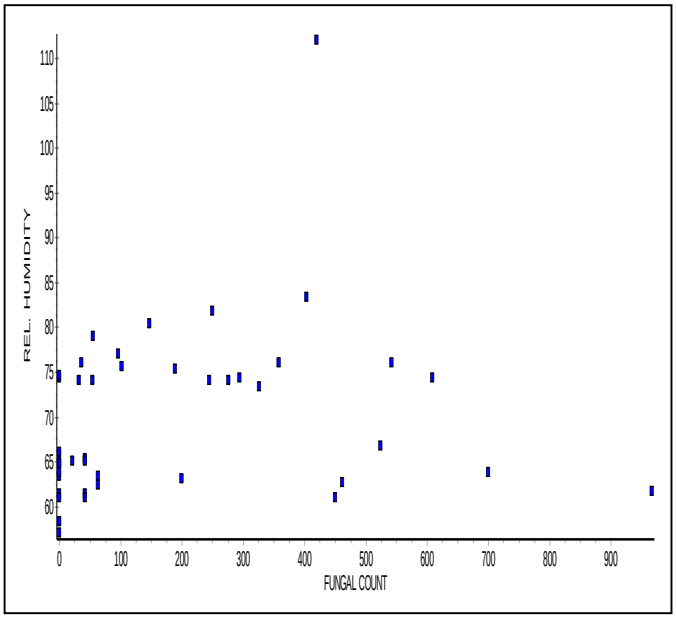


**Fig 6:** Graph showing the correlation between the combined indoor relative humidity (%) and fungal count (CFU/$m^3$) of the three Private Primary Schools. Relative humidity correlates with fungal count by a correlation coefficient (r) value of 0.2219, the two-tailed P value is 0.1429, considered not significant.

Still, [21] reported mean bacterial concentrations of 782 and 621 CFU/$m^3$ in a science classroom and in an art classroom, respectively, from an USA school [22]. Reported an average bacterial concentration of 1025 CFU/$m^3$ in indoor air of 5 primary schools from Malaysia. While [23] registered a mean winter indoor value of 1984 CFU/$m^3$ and 2784 CFU/$m^3$ for primary schools in rural and urban settings, respectively.

Furthermore, the mean fungal count (178.93 CFU/$m^3$) recorded in this study varied when compared with those of previous studies. On one hand, the mean fungal count was higher than the ones previously reported by some researchers. A work conducted by [18] in 15 schools and day-care centers in Denmark reported a mean fungal level of 155 CFU/$m^3$/no carpet in the rooms. While, [24] reported a mean fungal level of 100 CFU/$m^3$ for a research conducted in 10 schools in Paris. According to [25], the fungal concentration was <30 CFU/$m^3$ for 7 schools in Norway.

On the other hand, the mean fungal count recorded in this study was found to be lower than some previous reports. For instance, [18] reported a mean fungal level of 291 CFU/$m^3$/carpets in 15 schools and day-care centers in Denmark [26]. Investigated air-bone fungi in 13 classrooms of 6 Florida schools during summer and reported indoor fungal levels with mean value of 475 CFU/$m^3$. Still, a study conducted by [20] in 96 classrooms in 38 randomly selected schools in Sweden, reported mean values of 500 CFU/$m^3$ [21].

Reported fungi concentrations of 561 and 811 CFU/$m^3$ in a science classroom and in an art room, respectively, from an USA school. Also, [27] registered an average of 10375 CFU/$m^2$/h in the indoors of the studied classrooms. While [28] reported culturable fungal counts ranging from 268-2089 cfu/m3 in the three schools studied [23]. The last, but not the least reported an average of 717 CFU/$m^3$ and 2248 CFU/$m^3$ indoor level of fungi measured in winter and summer, respectively.

It is very important to mention here that, there is no uniform international standard available on levels and acceptable maximum bioaerosol loads [29], besides, different countries have different standards. The work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments has set the guidelines of bioaerosol counts at 500 cfu/$m^3$, if higher than this, the environment is considered as contaminated [30]. The quantitative interpretation of the results (Table 8) describing the indoor air quality in the classrooms of the three private schools were evaluated based on the sanitary standards for non-industrial premises formulated by the European Commission in 1993 [31]. According to this classification, all the classrooms of the three private schools examined for bacterial aerosols at different time of the day were not in hygienic conditions. Most of them were rated to be either highly or very highly polluted as the values obtained clearly exceed the permissive standard of 500 CFU/$m^3$ for indoor environments, except for class 5 of School 1 with no bacterial growth recorded at 3:00 pm. However for fungal aerosols, the levels of pollution range from very low to high.

The number of pupils per classroom in relation to the classroom area, ambient temperature, relative humidity, poor ventilation, increased indoor traffic, together with poor sanitary measures as at the time of this study might be responsible for the level of air pollution recorded in this study.

Regarding the relationship between the density of occupants and the concentration of indoor aeroflora, a correlation between bacteria count and number of persons in a classroom has been previously reported by [32]. However in this present study, there was no significant correlation between classroom occupancy and microbial counts. It is worth knowing that the allowable number of pupils per classroom varies from one country to another. In Nigeria, for the purpose of effective teaching and learning, the allowable number of pupils for a standard classroom with an area of 50$m^2$ or 538 $ft^2$ according to the National Policy on Education of the Federal Republic of Nigeria [33], is officially pegged at 35, although some well populated schools especially in urban areas may be close to 50 if not more [34]. In this study, it was observed that most of the classrooms examined had less than 35 pupils each, only one classroom in School 1 had exactly 35 pupils, while another had above 35. Howbeit, none of the classroom examined had a standard area of 538 $ft^2$. The classroom area of School 1 was 181 $ft^2$, while that of School 2 and 3 ranges between 120-459 $ft^2$ and 289-378 $ft^2$, respectively. These classroom areas appear not to be proportionate with the occupant density and hence, could be partly responsible for the concentration of indoor aeroflora recorded in this study.

Furthermore, airborne bacterial counts have been found to be directly associated with temperature and relative humidity [35]. Also, it is an established fact that temperature and relative humidity are two important factors for fungal spore generation, release and dispersal; particularly in indoor environments. The indoor temperature range suggested by the ASHRAE standard 55 [36, 37] I must be between 23 and 26°C, while, the recommended indoor relative humidity must be between 30% and 70%. The combined mean indoor temperature values of the three private primary schools obtained in this study at different sampling time of the day: 7:30 am (28.5$^O$C), 11:30 am (31.6$^O$C) and 3:00 pm (33.6$^O$C); were not above the maximum acceptable value (34$^O$C), but they were also not within comfort range. On the other hand, the combined mean relative humidity values of 11:30 am (67.4%) and 3:00 pm (62.6%) were within comfort range, except for the 7:30 am (78.7%), which still, did not exceed the maximum acceptable value (84%). The outcome of this study shows that bacterial count, rather than fungal count is strongly associated with indoor temperature and relative humidity with P-value <0.05. This finding agrees with the reports of [38, 39]. However, in a study by [40], they found out that relative humidity exaggerated fungi levels more than temperature did.

With regards to ventilation, most of the classrooms examined were either moderately or poorly ventilated. The classrooms of School 1 had either 2 or 4 windows and between 1-3 ceiling fans. While the classrooms of School 2 had between 1-3 windows, with no single ceiling fan. Also all the classrooms examined in School 3 had 4 windows each, but no ceiling fans. Inadequate ventilation has been reported to be one of the causes of poor indoor air quality of classrooms [41, 42]. It leads to the accumulation of pollutants from different sources and may increase the incidence of symptoms among building occupants. Poor ventilation may also indirectly contribute to moisture damage in a building by increasing the risk of condensation of water [43].

Another remote factor that can impact on indoor air quality is poor sanitary measures. The level of classroom sanitation in School 1 was rated between intermediate and high. The classrooms are swept and mopped daily. Waste bin was particularly lacking in one of the classrooms and where available, it may be left uncovered. On the other hand, the level of classroom sanitation in School 2 was rate between low and intermediate. Although the classrooms are swept daily, routine mopping of the floor is not been done. Similarly, waste bins were either lacking or available, with or without cover. The level of classrooms sanitation in School 3 was also rated between low and intermediate, with features similar to those of School 2.

In addition, physical assessment of the classrooms also shows that one or two classrooms in School 1and 2 had evidences of dampness on the wall or ceiling. Also, one of the classrooms in School 2 and 3 had evidence of molds growing on the wall or ceiling. This agrees with the report of [44, 45], who both made the observation that moisture is a predisposing factor to fungal growth on school buildings.

Regarding the distribution of aero-flora in the classrooms of the three private primary schools, in Ilishan-Remo studied, four (4) bacteria types were isolated which include: *Staphylococcus aureus (SA), Coagulase negative Staphylococcus (CoNS) species, Micrococcus (MC) species* and *Aerocococcus (AE) species;* while seven (7) fungal types were isolated which include: *Aspergillus (AS) species, Mucor (MU) species, Penicillium (PE) species, Candida (CA)*

*species, Microsporum (MS) species, Trichophyton (TR) species* and *Rhizopus (RH) species.* The outcome of this present study partly agrees with the work of [46], who reported *Micrococcus,* but disagrees with the same, who also reported *Bacillus* species and pigmented gram negative rods such as *Flavobacterium* species and coryneforms. It also agrees with the work of [47] who reported principally species of *Staphylococcus* and *Micrococcus*, except for *Difteroides* in Italian classrooms. It also agrees with the report of [24], on *Staphylococcus auerus*, except for *Escherichia coli* and *Streptococcus D*. Similarly, it agrees with the report of [48] on *Staphylococcus*, but disagrees with the same on *Streptococcus, Pseudomonas, Klebsiella* and *Escherichia* as the dominant bacteria genera.

On the other hand, regarding the distribution of fungal isolates, this present study agrees with the reports of [32] and [49] who both isolated moulds belonging to the genera: *Penicillium* and *Aspergillus* from the indoor air of some Norwich schools and Danish schools, respectively. It also agrees with the report of [44], on *Penicllium,* Yeasts, and *Aspegillus,* except for *Cladosporium*. It further agrees with the report of [50] on *Penicllium,* Yeasts and *Aspegillus* as the most common fungal genera isolated, except for *Cladosporium* and Actinobacteria. Still, it agrees with the reports of [8, 51], on *Aspergillus spp., Penicillium spp.* and *Rhizopus spp.,* except for *Cladosporium spp.* and *Alternaria spp* as the dominant fungal isolates.

## 4. Conclusion

The results obtained in this study clearly suggest that regardless of the time of the day, indoor environment allows aerosols build up which could serve as potential risk factors for spread of infections in the classrooms of these private schools. The concentrations of aeroflora above permissive standard recorded in this study, underscore the importance of this microenvironment for the high exposure of children to bioaerosols. Immediate interventions are therefore needed to control both human and environmental factors which favor the growth and multiplication of microorganisms.

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