Review Article

MICROBIAL ASSESSMENT OF INDOOR AIR OF THE APPLIED MICROBIOLOGY LABORATORY, NNAMDI AZIKIWE UNIVERSITY, AWKA, NIGERIA

ABSTRACT

Air is made up of enormous number of microorganisms mainly fungi and bacteria spore. Their estimation is important as an indication of cleanliness of any particular environment. The present study was carried out to assess microorganisms in indoor air of Applied Microbiology laboratory of Nnamdi Azikiwe University, Awka. Prepared plates of Sabouraud Dextrose agar (SDA), Nutrient agar (NA), and Blood agar were exposed for ten minutes for culturing of microorganisms. The NA and Blood agar plates were incubated at 37^{o}C for 24h while the SDA plate was incubated at room temperature for 48h. A total of ten (10) microorganisms were isolated from the samples. These include six (6) bacteria and four (fungal) species. The bacterial isolates include; *Staphylococcus aureus, Bacillus* sp., *Pseudomonas aeruginosa, Escherichia coli, Streptococcus* sp. and *Proteus* sp. The fungal isolates include *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp. The most frequently isolated bacteria were *Staphylococcus aureus* and *Bacillus* sp. with 25% and 35% occurrence. *Proteus* sp., *Streptococcus* sp., *E. coli*. and *P. aeruginosa* had 20%, 10%, 5% and 5% percentage occurrence respectively. *Aspergillus* and *Penicillium* sp. were the most frequently isolated fungal isolates with 40% and 30% occurrence respectively. *Fusarium* sp. and *Rhizopus* sp. both had 20% and 10% occurrence respectively. With this result, attention must be given to control those environmental factors which favor the growth and multiplication of microbes in indoor air of the laboratory to safeguard the health of users and workers.

Keywords: fungal isolates, microbes, Sick Building Syndrome, indoor air quality

1.0 INTRODUCTION

It is estimated that majority of the world's population spends most of their time indoors: in houses, offices, schools and hospitals, where they encounter environmental factors which compromise their health and physical conditions (Leech *et al.*, 2012).

The World Health Organization (WHO) has described the problems of the quality of air in buildings as significant risk factors for human health in low, middle and high-income countries. All around the world, life style changes have resulted in a shift from open air environments to air tight, energy efficient environments at home and work places, where people spend substantial portion of their time (Molhave, 2011). In these environments, improper maintenance, poor building design or occupant activities often result in a condition called as "**S**ick **B**uilding **S**yndrome" (SBS), where occupants experience adverse health effects that appear to link with the time spent in a building (Zain, 2011).

Indoor air quality is influenced by an unknown number of factors. Many of these are related to the structure and decoration of the building, its ventilation, internal temperature and humidity plus ingress of pollution from outside, and inevitably, contamination by microorganisms, especially fungi. Colonisation by microorganisms, their speciation and quantity, is dependent on the indoor microclimate. They require ideal conditions of temperature, humidity, oxygen, carbon sources, nitrogen and minerals to thrive. Their biological activity, seen as biodegradation and biodeterioration, depends on their enzyme activities, the environmental conditions, the competition phenomenon and the nature of the substrate on which they grow. In places where the fungal concentrations are high, they can cause the onset of symptoms and disease in people, particularly those who suffer from respiratory problems or have a weak immune system. The health effects are dependent on the species present, the concentration of their metabolic products and duration of exposure and individual susceptibility (Horner *et al*., 2015).

Atmospheric air, including indoor air, is a basic factor affecting the proper functioning of the human body. Air pollution constitutes one of the main threats to the environments in which people live. World Health Organization (WHO) and European Environment Agency (EEA) report that environmental risks such as air or water pollution have a significant impact on human health. Air pollution includes all substances in the Earth's atmosphere that are not natural components, as well as natural substances in significantly increased quantities. The Earth's

atmosphere is composed of gases and vapours of chemical compounds, acid rain, airborne ashes, dust, trace elements and biological contaminants (EEA, 2018).

Biological air pollutants, also known as bioaerosols, include pollen, fungi, bacteria and viruses. Most are microorganisms that colonise the soil, water bodies, plant surfaces, rocks and buildings. The components of the bioaerosol that make up the dispersed phase are particles ranging in size from 1 to 200 μm. For example, single bacterial cells with dimensions of 0.5–2.0 μm can constitute *Bacillus* sp. or *Pseudomonas* sp. In turn, many spores of mould are sized between 3.0 and 17 μm. In addition, bacterial toxins, mycotoxins, enzymes and fragments of plant and animal tissues are present in the air. Bioaerosols ranging from 1.0–5.0 μm usually float in the air, while larger ones tend to settle on surfaces. The greatest implication to human health has to deal with the so called respirable bioaerosol, which is defined as fraction smaller than 7 μm. This fraction can penetrate the human respiratory tract with the inhaled air. The smallest particles can even reach pulmonary brioncholes. Larger particles of bioaerosols tend to deposit in the upper airways (Douglas *et al*., 2018).

Microorganisms are found in virtually every environment, including at extreme temperatures, pressures, salinity and acidity. The atmosphere has been described as one of the last biological limits on Earth. The composition and biodiversity of the microbial community in the atmosphere is still poorly researched. Interestingly, bacteria and fungi have been detected in various atmospheric layers, such as the boundary layer (up to 1.5 km high), the upper troposphere (up to 12 km high) and even the stratosphere at an altitude of over 20 km above sea level. In addition, the fungi *Penicillium notatum* have been collected at an altitude of 77 km, and *Micrococcus albus* and *Mycobacterium luteum* bacteria at an altitude of 70 km. The movement of airborne microorganisms at different distances and through wind and precipitation means that they can spread in all ecosystems. Some may be pathogens or transmit allergens and consequently endanger the health of the population (Griffin, 2014).

As we know, one of the main factors influencing human health is air quality. Therefore, the presence of microorganisms, in particular those causing infectious diseases, in both ambient air and indoor environments can be particularly dangerous. Scientists have demonstrated that most often, microorganisms living in the air are responsible for irregularities in the immune system, such as allergies and infections. WHO alerts that the exposure to mould and other dampness-

related microbial agents increases the risks of hypersensitivity pneumonitis, allergic alveolitis or chronic rhinosinusitis which is supported by *in vivo* and *in vitro* studies (WHO, 2009).

Fungi are able to grow in indoor environments where there is sufficient moisture and a nutrition source, such as wood, paint and insulation and release spores as part of their reproductive process. Both temperature and water availability affect growth and sporulation characteristics of airborne fungi, with higher ambient temperatures and available water favouring faster growth. Release of spores is subsequently increased by intermittent periods of dryness where spores are dispersed, and moisture allowing for further growth and sporulation. Fungal species found indoors usually reflect those in the outdoor environment, although concentrations may change seasonally or locally where the indoor environment is favourable to the growth of particular species (Taylor *et al*., 2013). The most common genera are saprophytes, including those living on decaying plant material, *Cladosporium*, *Alternaria*, *Epicoccum*and *Aureobasidium*, while soil-based species, such as *Aspergillus* and *Penicillium*, are relatively low in number in outdoor air but are found at increased levels indoors. *Aspergillus* sp. and *Penicillium* sp. have been recognized as significant indoor air allergens. Fungi typically enter a building through heating, air conditioning and ventilation systems, windows, doors, and as contaminants on building materials. Prolonged high moisture levels in a building then provide the necessary conditions for fungal growth and sporulation to occur and mechanical disturbances can cause the spores to become airborne. To minimise the potential for exposure, it is essential to remediate an indoor space with visible fungal contamination. The remedial process involves the removal of visibly contaminated building material and the use of an antifungal product to treat surfaces, in conjunction with steps to modify the indoor environment to prevent future fungal growth (Chakravarty and Kovar, 2013). The use of a HEPA vacuum cleaner is recommended in combination with damp wiping non-porous surfaces to remove the dispersed spores in buildings (Morey, 2011).

Information on the presence of microorganisms in the air remains incomplete and numerous questions still need to be answered. The recent report of European Environment Agency (EEA) (2018) describes sources and types of air pollution along with the potential health and ecosystem impacts, however it does not refer to biological agents in the air. Research on microorganisms in air have emerged only recently as documented by number of scientific publication. This work

therefore is aimed at assessing microorganisms in indoor air of Applied Microbiology laboratory of Nnamdi Azikiwe University, Awka.

Materials and Methods

1. **Sample collection**

   Plate exposure method, which requires that plates with specific culture media be opened for a specified duration, was used for this study (Ekhaise *et al.*, 2010). This method allows bacteria or fungi in the air to settle on the respective culture media. Prepared plates of Sabouraud Dextrose agar (SDA), Nutrient agar, and Blood agar were exposed for ten minutes for culturing of microorganisms. The plates were kept on the floor (Sample A), the workbench (Sample B) and breathing level (Sample C) and exposed from 10:00 am to 10:10 am. After sampling, the plates were incubated in the laboratory.

2. **Isolation of Microorganisms**

   The plates were incubated at 37°C for 24 hours and 28±1°C respectively for 48 hours (visible fungi colony could take more than 24 hours). Streaking method was used for bacteria while stab inoculation was used for fungi in the isolation of these discrete colonies. The discrete colonies were re-inoculated into appropriate media slants and were kept at 4°C for identification purpose (Cheesbrough, 2010)

3. **Identification of Isolates**

   The identification of bacteria was carried out using standard microbiological methods. Microscopic and biochemical tests done using standard methods include; Gram staining, motility, catalase, coagulase, oxidase and indole tests. In identifying fungi, microscopic and macroscopic examinations including staining for morphological characteristics were carried out on fungal isolates. Identification was done based on the comparison of these characteristics using fungal atlas, so as to identify the fungi isolates to genus level.

4. **Gram Staining**

   A drop of distilled water was placed on a clean grease free glass slide and a colony in isolates was picked with a sterilized wire loop and emulsified. The glass slide was passed over the flame three times to heat fix. The smear was flooded with crystal violet for 60seconds and rinsed with distilled water, followed by flooding it with Lugol's iodine and rinsed with distilled water after 60seconds, then decolourized with acetone and rinsed immediately with distilled water. The smear was counter stained with Safranin for 1

minute and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under microscope using X100 objective lens (Cheesbrough, 2010).

5. **Catalase test:**

   The container containing Hydrogen peroxide solution was shaken to expel the dissolved oxygen. One drop of the solution was dropped on a clean glass slide followed by the addition of a loopful 24hours old inoculum of the slide, presence of gas bubbles indicate a positive test while the absence of gas bubbles indicates negative reaction (Cheesbrough, 2010).

6. **Urease test**

   This was done as described by (Cheesbrough, 2010). Christenses urea agar was prepared by weighing 20g of plain agar ,1g of peptone,1g of glucose,0.1g of phenol red, 1.2g of disodium hydrogen orthophosphate and 5g of sodium chloride were dissolved in 100ml distilled water, heated to achieve total dissolution. The pH was adjusted to 6.8 using an electrode pH meter to give yellow colour, dispensed into universal bottles and sterilized by autoclaving at 121^0C for 15minutes. 5ml of 40% membrane sterile urea solution was aseptically introduced into the universal bottle and then allowed to solidify in slanting position. A speck of each isolate was inoculated into Christense's urea agar and incubated at 37^0C for 24hours. Liberation of red colour indicates urease positive test while initial yellow colour indicates negative test. Control test was read and recorded.

7. **Indole Test**

   A prepared 1% of tryptophan broth, dissolving 10g of peptone in 1liter of distilled water. A test tubes containing 4ml of tryptophan broth was sterilized and a growth from 18 to 24hrs. culture was inoculated into the tube aseptically and was incubated at 37^0C for 24 to 48hrs. 0.5ml of kovac's reagent was added to the broth culture. A positive result shows a pink red colouration (cherry ring red) in the reagent layer on top of medium within seconds of adding the reagent while absence of colour or the appearance of slightly yellow colour indicates negative result (Cheesbrough, 2010).

8. **Citrate test**

   This was done as described by (Cheesbrough, 2010). This detects the ability of an organism to use citrate as the sole source of carbon. Simon citrate was prepared by

weighing 2.5g of Sodium citrate, 1.5g of Ammonium phosphate, 0.2g of Magnesium sulphate, 1g of Potassium dehydrogenate phosphate and 0.1g of Bromothymol blue and dissolved in 1litre of distilled water, homogenized and dispensed in test tubes then corked with cotton wool. A speck of each isolate was inoculated into Simon citrate medium and incubated at 37^{o}C for 72hours.A positive citrate is confirmed by formation of blue colour while the initial green colour denotes negative result. Control test was read and recorded.

9. **Motility**

A straight needle was touch to a colony of a young (18- to 24-hour) culture growing on agar medium. Stab once to a depth of only 1/3 to ½ inch in the middle of the tube. The needle was kept in the same line it entered as it was removed from the semi solid medium. It was incubated at 35°-37°C and examine daily for up to 7 days. Diffuse, hazy growths that spread throughout the medium rendering it slightly opaque indicate a positive result while growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent indicate a nagetive (Cheesbrough, 2010).

10. **Sugar Fermentation Test**

This is used to detect which organism has the ability to utilize different sugars as source of carbon and energy with the production of either acid or gas or both.

The sugars used were sucrose, lactose and glucose. Peptone water broth was prepared (15g of peptone water in 1000ml of distilled water with the tested sugar) and dispensed into testtubes containing durham tubes in inverted position. Bromothymol blue indicator was added and the mixture sterilized at 121^{0}C for 1 minutes. The test organisms were inoculated into each of the test tubes and incubated for 24hours. Acid production has indicated by colour change in the medium and gas production by space produced in the durham tubes (Cheesbrough, 2010).

11. Characterization and Identification of Fungal Isolates

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation. The technique of (Cheesbrough, 2010) was also adopted for the identification of the isolated fungi using cotton blue in lactophenol cotton blue stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion

of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses.

3.0 **RESULTS AND DISCUSSION**

Microbiological quality assessment of indoor air study is one of the most vital investigations to determine the microbial indoor air pollution. The information on the indoor microbial concentrations of airborne bacteria and fungi is necessary both to estimate the health hazard and to create standards for indoor air quality control. The study of airborne microorganisms in indoor environments is also important to understand the dissemination of airborne microbes most especially the pathogenic ones (Jaffal *et al*., 1997). The type and numbers of air microbes can be used to determine the degree of cleanliness in an environment.

This research shows the type of bacteria and fungi isolated from microbiology laboratory in Nnamdi Azikiwe University, Awka as shown in Table 1: The plate count of the bacterial and fungal colonies from the indoor air samples and Table 2: The morphological characteristics of the bacteria isolates. They were characterized based on shape, elevation, color, margin, surface, and transparency. The isolates are all opaque and possess smooth surfaces. Color varied as some are white and some are creamy, some colonies are flat and some are also raised. The shape was also observed as most were circular and some were irregular. The margin also revealed that most of the isolates were entire and some were undulated.

The total bacteria isolated from the laboratories are six (6) in number which includes; *Staphylococcus aureus, Bacillus* sp., *Pseudomonas aeruginosa, Escherichia coli, Streptococcus* sp. and *Proteus* sp. In this study, the most frequently isolated bacteria were *Staphylococcus aureus* and *Bacillus* sp. (Fig. 1). These airborne micro floras obtained are similar to those obtained by Ekhaise *et al*., (2010), who reported the isolation of bacterial isolate, which includes *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Bacillus* sp., *Proteus mirabilis and Streptococcus* sp. with *Staphylococcus aureus* being the most prevalent bacterial isolate isolated from indoor air.

Staphylococcus aureus is reported as a cause of infections of the skin, deeper tissue and organs (Lateef, 2003). The presence of these isolated bacteria in the atmosphere could be traced to a

variety of factors. These include the student normal flora, students and staff cloths, visitors and materials. Staffs and students' activities such as sneezing, coughing, talking, yawning e.t.c (Ekhaise *et al*., 2008).

Bacillus specie are widely distributed in the environment, the primary habitat is the soil. This bacteria causes food poisoning, localized infections related to trauma, deep seated soft tissue infections, and systemic infections (e.g. meningitis, endocarditis e.t.c) (Barri *et al.,* 1994). *Staphylococcus aureus* causes numerous infections at various sites of the body, some of which include: skin infections, infections of surgical and trauma wounds, food poisoning and gastro intestinal tract infections may be caused by consuming food contaminated with *Staphylococcus aureus*. Infections are also associated with intravascular devices (prosthetic heart valves, shunts, etc.) but also commonly occur in prosthetic joints, catheters, and large wounds.

As shown in Table 3: Microscopic and Biochemical tests for the identification of the bacterial isolates and also the probable organisms. The isolates displayed different reactions to gram stain as some were gram positive and some were gram negative. Their various reactions to different biochemical test was also displayed on this table. It also showed the probable bacteria isolates and they include *Staphylococcus aureus, Bacillus* sp., *Pseudomonas aeruginosa, Escherichia coli, Streptococcus* sp. and *Proteus* sp.

Table 4: Sugar Fermentation Test of the Bacterial Isolates. Their ability to ferment sugar to produce acid and gas was shown on this table.

The fungi isolated from the laboratories include: *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp. *Aspergillus* and *Penicillium* sp. were the most frequently isolated fungal isolates in this study (Fig. 2). Although, *Aspergillus* may be tolerable for healthy individuals, it may be dangerous for high-risk individuals. The spores readily invade the airways and could lead to Aspergillosis in immune compromised hosts (Gangneux, 2004). Table 5: Microscopic and Biochemical tests for the identification of the fungal isolates and the probable fungal organisms. Their various reactions to reagents are shown on this table. The table also showed the probable fungal isolates and they include; *Fusarium* species, *Aspergillus* sp., *Rhizopus* sp. and *Penicillum* sp.

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 dry atmosphere and high temperature in dry seasons influence the movement of airborne microbial

particles and the support evidences for the concentration of fungal species within the period. All the isolated bacteria as well as the fungi with exception of *Penicillium specie* are potential pathogens that are capable of causing disease (Adeniran *et al.*, 2003).

Molds are ubiquitous in the biosphere, and mold spores are a common component of household and workplace dust. When mold spores are present in abnormally high qualities, they can present especially hazardous health risks to humans, including allergic reactions or poisoning by mycotoxins, (Dallongeville *et al.,* 2015) or causing fungal infection (mycosis). A fungal ball in the lungs is eventually created by *Aspergillus niger* after it infects a person's lungs and begins to grow. The health effects of *Aspergillus niger* include hearing problems and even hearing loss. Also in Fig 2: The pie chart for percentage occurrence of the bacterial isolates and Fig 3: The pie chart for percentage occurrence of the fungal isolates were presented.

From the current study, indoor air contains pathogenic and non-pathogenic microorganisms. Therefore, good hygiene should be maintained in the laboratory to reduce the prevalence of these microorganisms.

Table 1: Plate count of the bacterial and fungal isolates from the indoor air samples

Samples	Bacteria (10^4 cfu/m^3)	Fungi (10^2 cfu/m^3)
A	45	23
B	55	29
C	37	19

Table 2: Morphological characteristics of the bacterial isolates from the indoor air Samples

Isolate	Shape	Color	Elevation	Margin	Surface	Transparency
1	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
2	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
3	Circular	White	Raised	Entire	Rough	Opaque

4	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
5	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
6	Irregular	Creamy	Flat	Undulated	Smooth	Opaque

Table 3: Microscopic and Biochemical tests for the identification of the bacterial isolates

Isolates	Gram stain	Rod/Cocci	Mot	Cat	Urease	Ind	Cit	Probable organism
1	-	Rods	+	+	-	-	-	*Pseudomonas aeruginosa*
2	-	Rods	+	+	+	-	+	*Proteus* sp.
3	+	Rods	+	+	-	-	+	*Bacillus* sp.
4	-	Rods	+	+	-	+	-	*Escherichia coli*
5	+	Cocci	-	+	+	-	+	*Staphylococcus aureus*
6	+	Cocci	-	-	-	-	-	*Streptococcus* sp.

Key:
Mot = Motility
Cat = Catalase
Ind = Indole
Cit = Citrate
+ = Positive
- = Negative

Table 4: Sugar Fermentation Test of the Bacterial Isolates

Isolates	Fructose	Sucrose	Galactose	Maltose	Lactose	Glucose	Probable organism
1	AG	AG	AG	A	A	AG	*Pseudomonas aeruginosa*
2	A	A	A	A	A	AG	*Proteus* sp.
3	A	A	A	A	A	AG	*Bacillus* sp.
4	-	AG	-	-	AG	AG	*Escherichia coli*
5	AG	AG	AG	A	A	AG	*Staphylococcus aureus*
6	AG	A	A	A	A	AG	*Streptococcus* sp.

Key:

A = Acid

G = Gas

- = Negative

Table 5: Morphology and biochemical test of the fungal isolates

Isolates	Colour	Appearance	Colour Undrerside	Microscopic Appearance	Probable Organisms
1	Bluish/green	Powdery	White	Septate, hyaline, conidia-singled-celled and round with smooth walls in chains.	*Penicillium* sp.
2	brown	Powdery	White	Broad hyphae, scarcely septate, round sp.	*Rhizopus* sp.
3	Green brown	Dry	White and brown	Conidiophores end with a sac-like structure. Phialides are attached to this sac-like structures and conidia are attached to philiades in chains.	*Aspergillus* sp.
4	Orange	Wooly	Orange	Septate, hyaline. Philliades are ling, cylindrical and branched. Microconidia; single-celled macroconidia-curved foot cell at the base	*Fusarium* sp.

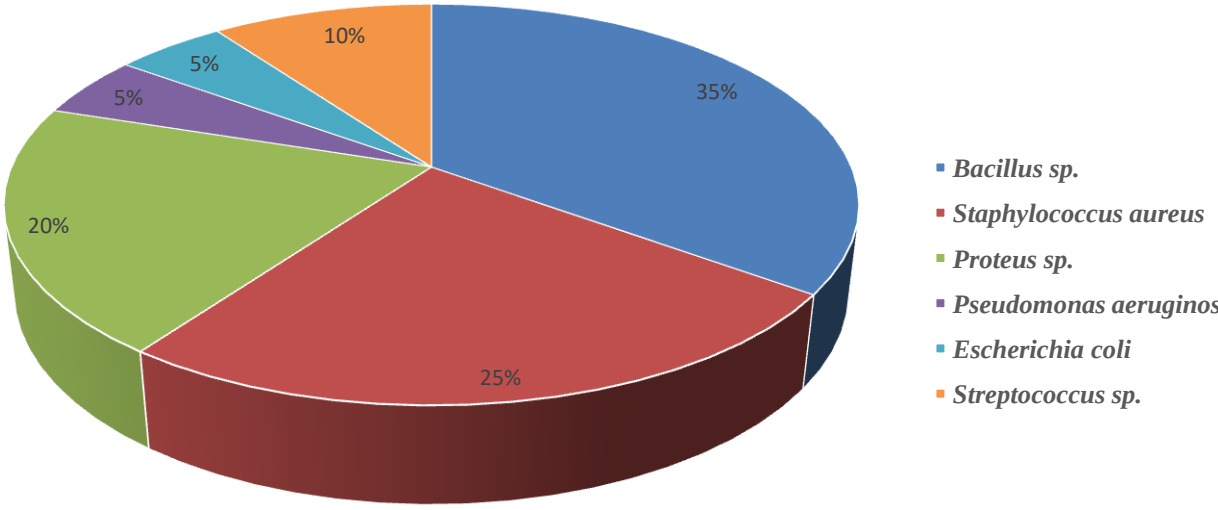

Figure 1. Percentage occurrence of the bacterial isolates

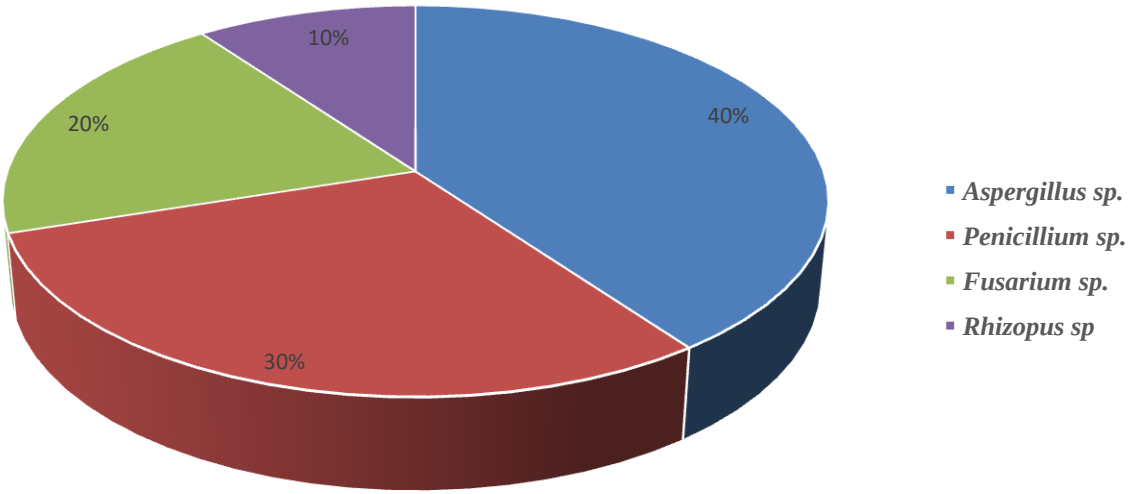

Figure 2. Percentage occurrence of the fungal isolates

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CONCLUSION

In conclusion, the results generated in this study clearly suggest that regardless of season, indoor environment allows aerosols buildup which could potentially lead to infections and contamination of equipment and test leading to false result. Population of students in a laboratory at a time may also increase the proliferation of airborne contaminants. Proliferation of airborne contaminants in the laboratories could also be influenced by poor and deficient hygiene conditions, also a low degree of cleanliness and minimal disinfection procedure used against airborne contaminants. Improper activities of the staff and students such as working without taking precautions could also contribute to the air contaminants. It is advisable that strict measures should be put in place to check the increasing microbial loads in the laboratories and all activities in the laboratory must be performed with precautions and according to standard.

References

Leach JM, Braithwaite PA, Lee SE, Bouch CJ, Hunt DV, Rogers CD. Measuring urban sustainability and liveability performance: the City Analysis Methodology. International Journal of Complexity in Applied Science and Technology. 2016;1(1):86-106.

Mølhave A, McDonnell J, Atman CJ. Seeing and hearing design: Elucidating the design process–A workshop at Central Saint Martins College of Art and Design. InTechnical Report CELT-11-03 2011.

Zain ME. Impact of mycotoxins on humans and animals. Journal of Saudi chemical society. 2011 Apr 1;15(2):129-44.

Horner AJ, Bisby JA, Bush D, Lin WJ, Burgess N. Evidence for holistic episodic recollection via hippocampal pattern completion. Nature communications. 2015 Jul 2;6(1):1-1.

Douglas KB. Sexuality and the Black church: A womanist perspective. Orbis Books; 2018 Sep 26.

Griffin P, Care E, editors. Assessment and teaching of 21st century skills: Methods and approach. Springer; 2014 Oct 21.

Taylor M. Partial differential equations II: Qualitative studies of linear equations. Springer Science & Business Media; 2013 Apr 17.

Chakravarty P, Kovar B. Engineering case report: evaluation of five antifungal agents used in remediation practices against six common indoor fungal species. Journal of occupational and environmental hygiene. 2013 Jan 1;10(1):D11-6.

Morey RD, Rouder JN. Bayes factor approaches for testing interval null hypotheses. Psychological methods. 2011 Dec;16(4):406.