



Microbial Evaluation of Indoor Air of Science Laboratories in IBB University Lapai, Nigeria

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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. It becomes important to embark on microbial evaluation of indoor air of science laboratories of in Ibrahim Badamasi Babangida university Lapai. The indoor air was assessed in the morning, noon and evening using the settled plate method. The result reveals Nine (9) fungal isolates and eight (8) bacterial isolates. The bacterial isolates include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas species*, *Micrococcus luteus* and *Escherichia coli* while the fungal isolates include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium specie*, *Mucor species*, *Cephalosporium specie*, *Veticillium specie*, *Aspergillus fumigatus*, and *Microsporium gypseum*. *Staphylococcus aureus* and *Escherichia coli* were the most frequently isolated airborne bacteria while *Aspergillus niger* and *Aspergillus flavus* were the most frequently isolated fungi. The least occurred bacteria was *Pseudomonas species* in Physics laboratory. The least occurred fungi were *Veticillium specie* and *Microsporium gypseum* in biology and physics laboratory respectively. It is advisable that strict measures be put in place to check the increasing microbial loads in Laboratories.

Keywords: Indoor Air, Microorganisms, Institutions and Isolated Fungi.

INTRODUCTION

Microorganisms make up most of the earth's biomass, they are found almost in every environment, surviving in extremes of radiation, heat, pressure, salinity, cold, and darkness (Royhscid, *et al.*, 2001). The air comprises nitrogen, oxygen and carbon dioxide and also the traces of other gases, inorganic particles and particles of biological origins. Bioaerosol particles include bacteria, fungi, virus, and their spores, lichen fragments, protists (e.g. protozoa, algae and diatoms) spores and fragment of plants, pollen, small seeds as well as fecal materials. (Lacey, 2006). Airborne microorganisms carried by dust clouds can directly impact human health via pathogenesis, the exposure of sensitive individuals to cellular components and development of sensitivity (Asthma) through prolonged exposure (Griffin, *et al.*, 2007).

Atmosphere (The layer nearest to the earth) contains all major groups of microbes ranging from algae to the viruses. In addition to gases, dust particles and water vapor, air also contains microorganisms. Since air is often exposed to

sunlight, it has a higher temperature and less moisture, so most of these microbial forms will die. Air current is also important in the dispersal of microorganisms as it carries them over a long distance.

The presence of microorganisms in the atmosphere was revealed by the clever experiments of spallanzani in the middle of the 18th century (Capanna, 1999) and of Pasteur at the end of the 19th century (Pasteur, 1890). Yet, the atmosphere still presents a frontier for pioneering microbiologists. Aside from classical pursuits of aerobiology (descriptions of the abundance and diversity of microorganism in the atmosphere, of their response to the physical-chemical conditions of the atmosphere and of their dissemination), Microbes are normally found in atmosphere within 300-10,000 feet above from the land.

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Fungal spores such as *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* have been reported to be found above 4000 feet from the land of both polar and non polar air masses. Organisms found below 500 feet is mainly in over populated area, these include spores of bacillus and clostridium ascospores of yeast and fragments of mycelium, mould, streptomycetaceae, pollen, protozoan cysts, algae, micrococcus and corynebacterium. Air found in school and hospital or living places of the person suffering from infectious disease, have also been associated with tubercle bacilli, streptococci and pneumococci (Polymenakou, 2012).

Air is the simplest of all environments and it occurs in a single phase, gas. It is not a natural environment for the growth and reproduction of microorganisms. It does not contain necessary amount of moisture and utilizable form of nutrient. In addition to gasses, dust particles and water vapour, air also contains microorganisms. Microorganisms such as bacteria, fungi virus and their spores are almost always present in the air. The quality of indoor environment is not readily controlled and can place laboratory technicians at risk (Jaffal *et al.*, 1997). Microorganisms are the primary sources of indoor air contamination due to the fact that enclosed spaces can confine aerosols and allow them to build up to infectious levels (Jaffal *et al.*, 1997). Vast amount of microorganisms which can be harmful to human health is deposited in the laboratory air due to one or more factors which include; human normal flora, human activities. The sources of laboratory air micro flora could be due to many factors which include: staff normal flora, visitors, students, materials in the laboratory, also man activities like coughing, sneezing, talking, and yawning may be source of laboratory infections (Ekhaïse *et al.*, 2008). Materials such as file may also be a viable source of microorganisms in the laboratory (Burge *et al.*, 2000).

Seasons can affect the results of an indoor microbial analysis. Reponen *et al.*, 1992 reported that during winter, the indoor air counts for fungi were significantly lower than other seasons. He also reported that air borne bacteria did not exhibit clear seasonal pattern. Commonly isolated microorganisms from indoor air include *Staphylococcus sp.*,

Micrococcus sp., *Aspergillus sp.*, *Penicillium sp.*, *Cladosporium sp.*, *Rhizopus sp.* This present study aimed to investigate the types of air micro flora in some science laboratories of Ibrahim badamasi babangida university lapai.

METHODOLOGY

Microbiology laboratory, Biology laboratory, Biochemistry laboratory, Chemistry laboratory and physics laboratory of Ibrahim Badamasi Babangida University Lapai were used for the study. The samples for the study were collected from different portions in the laboratories, namely; bench tops, sinks incubators, floor and inoculating hood.

Preparation of Culture Media

The culture media used in this study include; Nutrient Agar (NA) for the enumeration and isolation of bacteria, Potato Dextrose Agar for the enumeration and isolation of fungi. All the culture media used were prepared according to the manufacturers specifications. All the media were then sterilized by autoclaving at 121°C for 15 minutes. Allowed to cool to about 45°C and dispensed into the already sterilized Petri-dishes

Air Sampling and Microbiological Examination

The samples were collected from different portions in the laboratories using the exposed prepared plate techniques following the method demonstrated by Augustowska and Dutkiewicz, 2006 and Wang *et al.*, 2015 The samples were collected three times a day i.e. in the morning, between the hours of 8:00 am and 10:00 am, in the afternoon, 12 noon -2:00pm and in the evening 4:00pm – 6:00pm. The exposed plates contain nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for fungi. They were allowed to stay for 5 -10 minutes, after which the plates were wrapped in a foil paper to avoid contaminations during transportation. The plates were transported to the laboratory for microbiological examination. The bacterial culture plates were incubated at 37°C for 24 – 48 hours and the culture plates for fungi were incubated at room temperature, 20°C - 28°C for three to five days. The total number of colony forming unit (CFU) for the bacterial and fungal air micro flora were then enumerated and converted to organism's colony forming unit per cubic meter after incubation as

demonstrated by Ekhaise and Ogboghodo, 2011.

Characterization and Identification of Isolates

Following the techniques described by Bergey's Manual of Determinative Bacteriology, the bacterial colonies were initially characterized by cultural, morphological and microscopic examination of the isolates (Gerhardt *et al.*, 1994). The pure bacterial isolates were subjected to different conventional biochemical test such as catalase, coagulase, oxidase, indole test citrate utilization and sugar fermentation for proper characterization and identification. The fungal colonies were also identified based on the colony appearance and microscopic examination of the spore and hyphae. The reproductive and vegetative structures of fungal isolates were observed through microscopic examination. A sterile inoculating needle was used to pick a portion of the colony and was placed on a microscopic clean grease-free slide on which a drop of lacto phenol had been

placed. The colony portion was then teased apart with the aid of two sterile mounted needles. A cover slip was used to cover and viewed under x10 and x40 objective of the microscope. The cellular morphology was noted and compared with the known taxa using standard mycological atlas for proper identification.

RESULTS

Microbial Load in Study Areas

The presence of microbial air contaminants in the five different laboratories studied in Ibrahim Badamasi Babangida University Lapai varied from laboratory to laboratory. The concentration and availability of airborne microorganisms (bacteria and fungi) varied with time of investigations. The presence of airborne bacterial in the laboratories was recorded high in the evening compared to morning and afternoon time of studies. All through the study, Microbiology and Biology laboratories were observed to record high bacterial and fungal count (see Figure1).

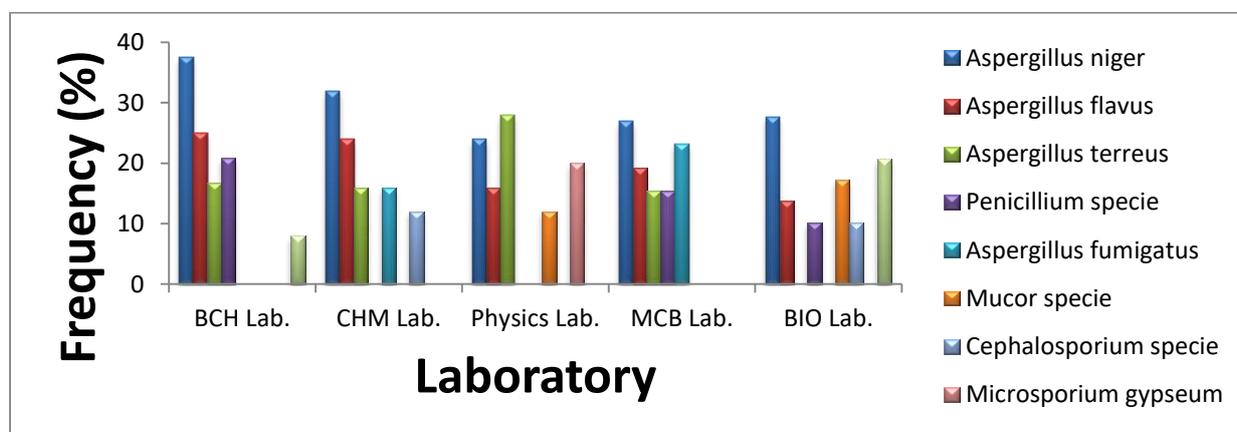


Figure 1: Percentage Occurrence of Fungi Isolates Present In the Studied Laboratories

Keys: BCH: Biochemistry, CHM: Chemistry, MCB: Microbiology, BIO: Biology, Lab.: Laboratory

DESCRIPTION OF FUNGAL ISOLATES

The characteristics of the fungal isolates as obtained through both colonial and microscopic examination are given below in Table 1

Table 2: Cultural and Microscopic Characteristics of Fungal Isolates from The Laboratory Air Samples

Colour	Texture	Growth	Hyphae	Spore type	Features	Probable organisms
Black	Granular	Abundant	Septate	Conidia appear in chains on sterigmata	Vesicled and unbranched conidiophores	<i>Aspergillus niger</i>
Bright Green	Granular	Abundant	Septate	Conidia appear in chains on sterigmata	Vesicled and unbranched conidiophores	<i>Aspergillus flavus</i>
Brownish grey	Loose	Abundant	Septate	Sporangiospore	Presence of columella	<i>Mucor species</i>
Green	Suede	Sparse	Septate	Conidias	Phalides were observed. Conidia were seen, vesicle absent.	<i>Penicillium species</i>
Blue Green			Septate	conidias	Conidiospore short, smooth walled with a conical shape terminal vesicles	<i>Aspergillus fumigates</i>
Brown	Granular		Septate	Conidia appear in chains	Vesicled and unbranched conidiophores	<i>Aspergillus terreus</i>
Grey white		Abundant	Non-Septate		Conidia: unicellular and later head-shaped at the end of conidiophores.	<i>Cephalosporium species</i>
Yellowish brown			Septate	Microconidia and macroconidia	Microconidia appers rough and oral pear shape. Macroconida: rough walled fusiform.	<i>Mirosporium gypseum</i>
Green			Septate	Conidia unicellular	Conidiophores: septate borne vertically on the hyphae with whisky-like ramification.	<i>Verticillium specie</i>

Bacteria Isolates

Eight (8) bacteria isolates were identified in the course of this study after culturing and characterization. The bacteria include: *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis* and *Pseudomonas specie*. Figure 2

shows the occurrence of bacteria isolates in Microbiology laboratory, Biology laboratory, Biochemistry laboratory, Chemistry laboratory, and Physics laboratory respectively. Table 2 shows the characteristics of the bacteria isolates as were seen in their respective colonies on nutrient agar and also shows their biochemical characteristics and their action on different sugars.

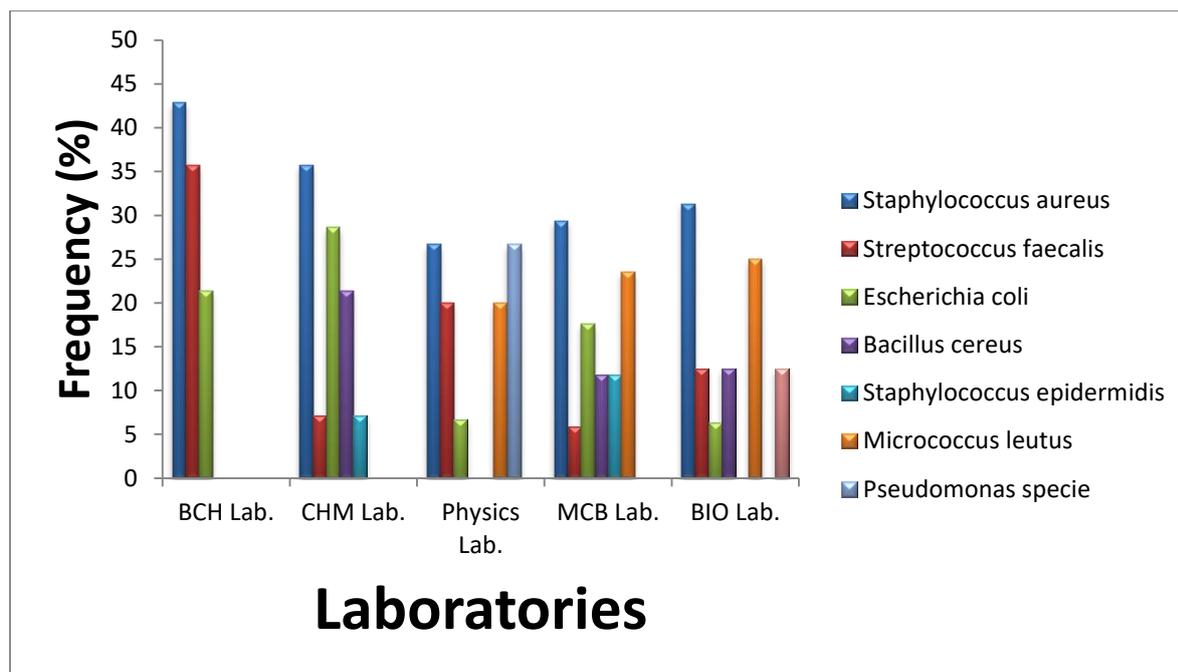


Figure 2: Percentage Occurrence of Bacteria Isolates Present in the Studied Laboratories

Keys: BCH: Biochemistry, CHM: Chemistry, MCB: Microbiology, BIO: Biology, Lab.: Laboratory

DESCRIPTION OF BACTERIAL ISOLATES

The characteristics of the bacterial isolates as obtained through both colonial and microscopic examination are given below in Table 2

Table 2: Microscopic and Biochemical Characteristics of bacteria isolates obtained from the laboratory air sample

S/N	Cultural Morphology	Gram Reaction	Shape	Catalase	Oxidase	Coagulase	Citrate	Indole	Maltase	Sugar Test				Probable organism
Nutrient agar										Lactose	Sucrose	Glucose	fructose	
1	Whitish waxy opaque growth	+	Rod	+	-	-	-	-	-	-	A	A+/-	A	<i>Bacillus cereus</i>
2	Regular scattered spreading flat colony	+	Cocci	+	-	-	-	-	-	-	A/G	-	A	<i>Micrococcus luteus</i>
3	Yellow non-mucoid spherical colony	+	Cocci	+	-	+	-	-	-	G	A	A	A	<i>Staphylococcus aureus</i>
4	Whitish spherical waxy opaque growth	+	Rod	+	-	+	-	-	-	-	A	A+/-	A	<i>Bacillus subtilis</i>
5	White non-mucoid discrete colony	+	Cocci	-	-	-	-	-	-		A/G	A/G		<i>Streptococcus faecalis</i>
6	Creamy circular rough opaque growth	-	Rod	+	-	-	-	+	-	A/G	A/G	A/G	A/G	<i>Escherichia coli</i>
7	Yellowish non-mucoid round colony	+	Cocci	-	-	-	-	-	-	-	-	-	-	<i>Staphylococcus epidermidis</i>
8	Creamy circular smooth translucent growth	-	Rod	+	+	-	+	-	+	-	A	A	+	<i>Pseudomonas species</i>

A: Acid Produced, AG: Acid Gas Produced, -: Negative, +: Positive

DISCUSSION

The study of airborne microorganisms in indoor environments is 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 different laboratories in Ibrahim Badamasi Babangida University, Lapai. The total bacteria isolated from the laboratories are eight (8) in number which include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas species*, *Micrococcus luteus* and *Escherichia coli*.

In this study, the most frequently isolated bacteria were *Staphylococcus aureus*, *Streptococcus faecalis* and *Bacillus species*. These airborne microfloras 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 species*, *Proteus mirabilis* and *Streptococcus species* 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 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 such as flowers. Staffs and student's activities such as sneezing, coughing, talking, yawning *e.t.c* (Jaffal *et al.*, 1997; Alberti *et al.*, 2001; Ekhaise *et al.*, 2008). The variation in air contaminants at different time of the day is supported by the findings of Marshal *et al.*, (2008) who reported that variations of climatic condition such as distinct wet and dry seasons, may selectively favor certain physiological type. The fungi isolated from the laboratories include: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium specioie*, *Mucor species*, *Cephalosporium specie*, *Veticillium specie*, *Aspergillus fumigatus*, and *Microsporium gypseum*. *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus* were the most frequently isolated fungal isolates in this study. 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).

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). *Micrococcus luteus* is the causative agent in cases of intracranial abscesses, and pneumonia, septic arthritis, endocarditis, and meningitis (Bannerman and Peacock 2007). They can be found on the skin of human and soil which could be the source of their presence in the air. *Bacillus specie* are widely distributed in the environment; the primary habitat is the soil. This bacterium 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). *Streptococcus specie* causes diseases such as pharyngitis, respiratory infections, bacterial pneumonia, *e.t.c*. However, many *Streptococci species* are not pathogenic, and form part of the commensal human micro biota of the mouth, skin, intestine and upper respiratory tract (Patterson 1996). *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*. Septicemia and endocarditis are also diseases associated with *Staphylococcus epidermidis*. Infections are also associated with intravascular devices (prosthetic heart valves, shunts, *etc.*) but also commonly occur in prosthetic joints, catheters, and large wounds.

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; Weinhold, 2007) 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.

Aspergillus flavus produces the carcinogenic mycotoxins, aflatoxin which often contaminates food such as nuts. *Aspergillus fumigatus* causes infection in humans more often than any other *Aspergillus specie* (Croft, 2016 and Geiser 2009). People who handle or who are exposed extensively to *Aspergillus fumigatus* often develop a hypersensitivity to it so that they develop severe allergic reactions to the mold.

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. Observations made during sampling rounds found out that cabinets, working surfaces and top of equipments were not free from visible dust. This was of concern as it could lead to an increase in microbial contaminants. 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

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