ISOLATION AND IDENTIFICATION OF AIRBORNE MICRO-FLORAIN MICROBIOLOGY LABORATORY LECTURE ROOMS

PRESENTED BY

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HND/23/SLT/FT/0995

BEING A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY (ENVIRONMENTAL BIOLOGY UNIT) INSTITUTE OF APPLIED SCIENCES (IAS) KWARA STATE POLYTECHNIC, ILORIN

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN SCIENCE LABORATORY TECHNOLOGY

SUPERVISED BY:

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2024/2025 SESSION

APPROVAL PAGE

This is to certify that this project work, "**Isolation and Identification of Air Borne Micro-florain Microbiology Laboratory and Lecture Rooms"**, was written by ***Student's Name*** with Registration number ***Your Matric / Registration Number*** and has been read and approved for the award of **(Degree, ND, or HND)** in the department of ***(Your Department)***, ***Your School (SIAS, SBMT, SHSS)***, ***Institution Address.***

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Project Supervisor's Name **DATE**
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Your HOD's Name **DATE**
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CERTIFICATION

This is to certify that the research work carried out by **ABDULRAHMAN FATIMOH ASABI** with matriculation number **HND/23/SLT/FT/0995**in Institute of Applied Science (IAS), Department of Science Laboratory Technology, Kwara State Polytechnic, Ilorin has met the requirement for the award of Higher National Diploma (HND).

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DEDICATION

This project work is dedicated to family of Mr. And Mrs. Abdultahman

ACKNOWLEDGEMENT

My gratitude goes to Almighty Allah, the highest, the omnipotent and the omniscience for his guidelines, his grace and unending mercy that was evident in my life and for making me to complete my HND program successfully. My profound gratitude goes to my project supervisor **Mr. Yusuf RT** for guiding and seeing me through the successful completion of my project. His action is worthy of emulation are highly appreciated may Almighty God continue to assist him and his source of joy throughout his life.

Also, I sincerely appreciate the kindness of the head of our unit (**HOU**) **Mr. Opeyemi A.A** for his brotherly support, I pray that may Almighty Allah increase your knowledge and bless your family. I will be indebted and ungrateful if I refuse to appreciate the effort of my wonderful father **late Mr. Abdulrahman** you show me way to success with your love, support words of encouragement. May your gentle soul continue to rest in perfect peace and also, I won't be ungrateful to my beloved and wonderful mother **Mrs. Abdulrahman** for your love,support,care,word of encouragement and most especially your prayers. I pray you live long to eat the fruit of your labor in good health.

My gratitude goes to my siblings, you are a great and wonderful sibling. for their endless care, support both financially, physically I am indeed very grateful. May Almighty Allah bless you all and grant your secret prayers.

I won't be ungrateful to those who stood by me throughout my HND emotionally, financially, physically. A special thanks to my coordinator and other lecturers and staffs in biology department.

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ABSTRACT

Microflora contamination in laboratories and hospitals is becoming a serious problem worldwide, and the characterization of such contaminants offers hope for the treatment of some infections acquired in hospitals and laboratories (LAI). Microflora contamination in benches, floors, media and equipment can be affected by temperature, humidity, nutrient media in laboratories and media storage conditions and microflora sources must be determined, contaminants must be isolated and identified when standard microbiological manipulations are performed.The aim of the study is to determine the Isolation and identification of air Micro-flora in Microbiology Laboratory. In achieving this aim, the following specific objectives were laid out as follows to: determine the sources of microflora contaminants in microbiological laboratories in Nigeria, identify bacterial and fungal contaminants in biosafety laboratories selected based on morphological and biochemical properties and Genetic determination Identity of persistent Nigeria bacteria in laboratory sites after disinfection with sodium hypochlorite. The isolation of pure cultures was performed on the basis of morphological differences, using the shape of the colony, elevation, pigmentation and size to distinguish bacterial and fungal contaminants.The results showed that the laboratory sites examined were contaminated with different microbes, Macroscopic and microscopic observations of fungi confirmed the presence of Cladosporium sp, Penicillium sp, Aspergillus sp and Alternaria sp on tables, door handles, preparation rooms, gloves and biosafety cabinets, and the persistent bacteria identified were Shigella sp., Pseudomonas aeruginosa, Corynebacteria sp., Bacillus sp. and Staphylococci aureus.The contaminants were similar to the standard strains, but there was a significant difference in contamination in the three selected laboratories (analysis of variance (ANOVA P = 0.00)). The size of the PCR product was 996 bp and the RFLP patterns of the bacteria were concluded that despite the disinfection with sodium hypochlorite, bacterial and fungal contaminants remain on laboratory surfaces and equipment and, therefore, they should increase the concentration or change the disinfectant.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Gases, dust particles, water vapour and air contain microorganisms. There are vegetable cells and spores of bacteria, fungi and algae, viruses and protozoa cysts. Since air is often exposed to sunlight, it has a higher temperature and less moisture if not protected from desiccation. Most of these microbial forms will die. Air serves as transport or dispersal medium for microorganism they occur in relatively small number in air when compared with soil or water. The Micro-flora of air can be studied under two headings outdoor and indoor Micro-flora.

As a prelude to other parts of this study, this chapter will discuss the background upon which this study was initiated, the statement of problems that led to this study, the Aim and Objectives of the study. Others are Significance of the study, Scope of work, Limitation of the study and Definition of technical terms.

1.2 Background of Study

Air does not have an indigenous and flora, though a number of micro-organism are present in the air. Air is not a natural environment for microorganisms as it doesn’t contain enough moisture and nutrients to support their growth and reproduction. Quite a number of sources have been studied in this connection and almost all of them have been found to be responsible for the air microflora. One of the most common sources of air microflora is the soil. Soil microorganisms when distributed by the wind blow librated into the air and remain suspended therefore along period of time. Man made actions like digging or ploughing the soil may be release soil born microbes into the air.

Outdoor Micro-flora: The air in the atmosphere, which is found outside the buildings, is referred to as outside air. The dominant microflora of outside air are fungi. The two common genera of fungi are cladosporiul and sporobolomyces, besides this two general, under general found in air are Aspergillus, Alternaria, Phytophthora and Erysiphe. The outdoor air also contains besidispores, ascopres of yeast, fragments of mycelium and canidia of molds. Among the bacterial genera Bacillus and clostridium, sarcina, mirococcus, corynebacterium and Achromobacter are widely found in the outside air, the number and kind of microorganism may very from place to place, depending upon the human population densities.

Indoor Micro-flora: The air found inside the building is referred to as indoor air. The commonest genera of fungi in indoor air are penicillium, Aspergillus, the Commonest genera of bacteria found in indoor air are Staphylococci, Bacillus and Clostridium. In case of occupants being infected, the composition shows slight variations with latitude and to a lesser extent with attitude. The ozone owes its existence in the atmosphere to photosynthesis from oxygen under the influence of solar ultraviolet radiations. (Dr. Shiva, 2009).

There is no microbes are native to the atmosphere rather they represent allochthonous populations' transperted from aquatic and terrestrial habits into the atmosphere. Microbe of air within 300 – 1,000 or more feet of the Earth's surface are the organisms of soil that have become attached to fragments of dried leaves, strain or dust particles, being blown away by the wind. Species vary greatly in their sensitivity to a given value of relative humidity, temperature and radiation exposure.

More microbes are found in air over land masses than far at sea. Spores of fungi especially Alterneria, Cladosporium, Penicillium and Aspergillus are more numberous than other forms

over sea within about 400 miles of land in both polar and tropical air masses at all altitudes up to about 10,000 feet.

Microbes found in air over populated land areas below altitude of 500 feet in clear weather include spores of Becillus and Clostridium ascos-pores of yeasts, fragments of mycelium and spores of molds and streptomycetaceae, pollen pootozoan cysts, algae, micrococcus, corynebacterium etc.

In the dust and air of schools and hospital wards or the rooms of persons suffering from infectious disease, microbe such as tubercle bacilli, streptococci, pneumococci and staphylocci have been demonstrated. These respiratory bacteria are dispersed in air in the droplets of saliva and mucus produced by coughing, sneezing, talking and laughing.

Viruses of respiratory tract and some enteric are also transmitted from the objects contaminated with infectious secretions that after drying become infectiou dust. Droplet are usually formed by sneezing, coughing and talking. Each droplet consists of saliva and mucus and each may contain thousands of microbes. It has been estimated that the number of bacteria in a single sneeze may be between 10,000 and 100,000. Small droplets in a warm dry atmosphere are dry before they reach the floor and thus quickly become droplet nuclei.

Many plant pathogens are also transported from one field to another through air and the spread of many fungal diseases of plants can be predicted by measuring the concentration of airborne fungal spores. Human bacterial pathogen which cause important airborne disease such as diphtheria, meningitis, pneumonia, tuberculosis, and whcoping cough are described in the chapter “Bacterial Disease man”.

Therefore, in Microbiology Laboratory where the research was carried out, the activities that was conducted is to know theIsolation and identification of air Micro-flora.

1.3 Statement of Problems

Microorganisms are discharged into the air as infectious droplets, droplet nuclei and dust. Droplets are usually generated by sneezing, coughing or talking, which involve saliva and mucus while droplet nuclei are formed when small liquid droplets evaporate. Dusts are released into the air during activities such as sweeping, tillage of the soil, movement of heavy vehicles, blasting of stone and air turbulence (Mende et al., 2021). Air currents may also bring microorganisms from plant, animal and other solid surfaces into the air (Olaitan et al., 2006). Microorganisms suspended in air are only rarely found in Free State (Seino et al., 2005).

Air current influence the time which either the microorganisms or the particles laden with microorganisms remain suspended in the air. In still air, the particles tend to settle down. Air current is important in the dispersal of microorganisms as it carries them over a long distance. Air current produces turbulence which causes vertical distribution of air flora. No microbes are indigenous to the atmosphere rather they represent allochthonous populations transported from aquatic and terrestrial habitats into the atmosphere (Arora et al., 2012).

1.4 Aim and Objectives of Study

The aim of the study is to determine the Isolation and identification of air Micro-florain Microbiology Laboratory and Lecture Rooms. In achieving this aim, the following specific objectives were laid out as follows:

1. To determine sources of microflora contaminants in microbiology laboratory and Lecture Rooms in Kwara State polytechnic in Nigeria.
2. To isolate and identify the bacterial and fungal contaminants in microbiology laboratory And Lecture rooms based on morphological and biochemical characteristics.

3. To evaluate the genetic identity of persistent bacteria in laboratory and Lecture rooms after disinfection with sodium hypochlorite.
4. To determine the number species and occurrence of bacteria and fungi in the air of Microbiology Laboratory and Lecture Rooms
5. To provide recommendation on ways of minimizing contamination of the air in Microbiology Laboratory and Lecture rooms

1.5 Research Questions

The study came up with research questions so as to be able to ascertain the above stated objectives. The specific research questions for the study are stated below as follows:

- What are the sources of microflora contaminants in microflora laboratories in Nigeria?
- Are the bacterial and fungal contaminants encountered in microbiology laboratories different morphologically?
- Are the persistent bacteria to hypochlorite based disinfection genotypically similar to standard bacteria strains?

1.6 Research Hypothesis

In order to pursue the objective of this study, the following generalized statements have been designed to guide and aids in obtaining the result for the experiment to be conducted. For this work, the null hypothesis will be represented with H0 while the alternative hypothesis will be represented with hypothesis H1.

- **H0:** There is no significant bacterial and fungal contamination encountered in microbiology laboratories in Nigeria

- **H1:** There is a significant bacterial and fungal contamination encountered in microbiology laboratories in Nigeria

1.7 Significance of Study

The information from this study will help to contain the Laboratory Acquired Infections) LAI associated with microbial contaminations in microbiology laboratories. The research findings will also assist personnel in laboratories to be careful when performing standard manipulations of microbiological specimens in cell cultures. This will help in reducing the costs associated with the application of the technology in laboratories. The information will form a basis of training the personnel on capacity development in monitoring and evaluation of microflora contaminants in laboratories. Then effecting or upgrading policy on Standard Operating Procedures (SOPs) will be developed in the laboratories.

1.8 Scope of Study

The study focuses on the Isolation and identification of air Micro-florain Microbiology Laboratory and Lecture Rooms in Kwara State Polytechnic ,Ilorin.

1.9 Limitations of the study

During the course of this study, many things militated against its completion, some of which are:

1. **Time Constraint:** The time frame given to accomplish this project was very short due to school academic calendar and it was carried out under pressure which made the researcher not to implement some necessary features.
2. **Research material:** availability of research material is a major setback to the scope of the study.

3. **Frequent power failure:** This made the researcher append more money on fuel to ensure sustainable power.
4. **Financial Constraint:** Insufficient fund tends to impede the efficiency of the researcher in sourcing for the relevant materials, literature or information and in the process of data collection (internet).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter focuses on the review of related literature. A literature review includes the current knowledge as well as theoretical and methodological contributions to a particular topic. It documents the state of the art with respect to the topic you are writing. It surveys the literature in the topic selected. In this research work the literature review includes the Conceptual Review, Concept of Micro-flora, Historical Review of Micro-flora, Classification of Micro-flora, Roles of Micro-flora, and Theoretical Framework.

2.2 Conceptual Review

Air quality is one of the most significant factor affecting the health and well-being of people. It has been reported that a single person inhale's an average of approximately 10 m3 of air every day (Dacarro et al., 2003). However, the air inhaled by people is abundantly loaded with microorganisms which form part of the bioaerosol (Górny, 2004). Bioaerosol is a colloidal suspension, formed by droplets and particles of solid matter in the air, whose components can contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues (Karwowska, 2005). Biological contamination of air is mostly caused by bacteria, moulds and yeasts (Flannigan, 2001; Daisey et al., 2003; Pieckova and Kunova, 2002). They can be dangerous as pathogenic living cells but they also secrete some substances harmful to human health (Gutarowska and Jakubowska, 2001).

Airborne microorganisms are usually derived from various natural sources such as soil, animals, and humans (Posfai et al., 2003; Mouli et al., 2005; Fang et al., 2007). Human activities such as

sewage treatment, plants and animal rendering, fermentation processes and agricultural activities do emit microorganisms into the air (Recer et al., 2001; Adhikari et al., 2004; Gillum and Leventin, 2008). Several studies have identified human activities like talking, sneezing and coughing (Kalogeraskis et al., 2005), while other human activities such as vehicular transportation and human movements, washing in homes and business centres, flushing of toilets and sewages, sweeping of floors and roadsides can generate bioaerosols indirectly (Kalogeraskis et al., 2005; Chen and Hildermann, 2009). Since micro- organisms can lodge in/on dust particles, dust therefore is a potential source of bioaerosols.

In recent years, monitoring of the number of airborne microorganisms has gained interest due to increasing concerns on public health, the threat of bioterrorism, surface biodeterioration and spread of plant diseases (Douwes et al., 2003; Pieckova and Jesenska, 1999; Stetzenbach et al., 2004). Exposure to bio aerosols, containing airborne microorganisms and their by products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity, pneumonitis and toxic reactions (Frachia et al., 2006; Górny et al., 2002).

Exposure to outdoor air microorganisms has been associated with allergic respiratory symptoms, asthma exacerbation, asthma related death and infections (Dales et al., 2004; Peternel et al., 2004). Several findings of epidemiological research indicate that exposure to high concentration of microorganisms frequently leads to allergies, asthma, hay fever (Björnsson et al., 1995; Newson et al., 2000), pneumonia (Siersted and Gravesen, 1993), and many other health side-effects, including infections (Renn et al., 2001). In recent years, dramatic increase in the number of allergic reactions to fungal spores has been reported, and young people do constitute a large group of allergy sufferers, whose symptoms persist throughout the year (Jain, 2000). For this

reason, there is need for regular monitoring of the air in order to determine its quality as it affects the health of humans in the public as they go about their daily activities. Presently in Nigeria, attention is yet to be given to the monitoring of airborne microorganisms, whether outdoor or indoor. This study is therefore an attempt to provide some empirical data that could stimulate both outdoor and indoor bioaerosol research in Nigeria.

2.3 Concept of Micro-flora

In microbiology, collective bacteria and other microorganisms in a host are historically known as flora. Although microflora is commonly used, the term microbiota is becoming more common as microflora is a misnomer. Flora pertains to the Kingdom Plantae. Microbiota includes Archaea, Bacteria, Fungi and Protists. Microbiota with animal-like characteristics can be classified as microfauna (Wikipedia, 2021).

2.4 HistoricalReview of Micro-flora

The terms "Flora" and "Fauna" were first used by Carl Linnaeus from Sweden in the title of his 1745 work Flora Suecica and Fauna Suecica. At that time, biology was focused on macroorganisms. Later, with the advent of microscopy, the new discovered ubiquitous microorganisms were fit in this system. Then, Fauna included moving organisms (animals and protist as "micro-fauna") and Flora the organisms with apparent no movement (plants/fungi; and bacteria as "microflora"). The terms "microfauna" and "microflora" are common in old books, but recently they have been replaced by the more adequate term microbiota (Berge, 2020). Microbiota includes Archaea, Bacteria, Fungi and Protists (Wikipedia, 2021).

2.5 Classification ofMicro-flora

Microflora are grouped into two categories based on the origin of the microorganism (Hao et al., 2004).

1. **Autochthonous Flora -** Bacteria and microorganisms native to the host environment.
2. **Allochthonous Flora -** Temporary micro-organisms non-native to the host environment (Wikipedia, 2021).

2.6 Roles of Micro-flora

Microflora is a term that refers to a community of bacteria that exist on or inside the body, and possess a unique ecological relationship with the host (Natividad et al., 2015). This relationship encompasses a wide variety of microorganisms and the interactions between microbes. These interactions are often a mutualistic relationships between the host and autochthonous flora. Microflora responsible for harmful diseases are often allochthonous flora (Wikipedia, 2021).

The modern term is "Microbiome" and include microorganisms that have different roles in ecosystems or hosts, including free-living organisms, or organisms associated to hosts, such animals (including humans) or plants (Gilbertet, 2014).

2.7 Theoretical Framework

Microorganisms are found almost everywhere, and their presence in the air was demonstrated by the work of Lazzaro Splallanzani in 1768 and of Louis Pasteur at the end of the 19th century (Meraj-ul-Haque et al., 2016). However, air is not a natural medium for growth and reproduction of microorganisms, any organism, that airborne contain must have originated from a living or non living source (humans, animals, plants, food, water or soil) (Yaghoub and Elagbash, 2010). Though microorganisms are found in both indoor and outdoor environments, most of the people spend their lives indoors: in houses, industries, offices, colleges, schools, hospitals etc., where

they are exposed to many bioaerosols (biological air borne contaminants such as bacteria, viruses, fungi or their byproducts). Exposure to these airborne particles can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Naruka and Gaur 2014; Sheik et al., 2015; Yassin and Almouqat, 2010). In addition, long-term contact of people with bioaerosols can influence a person's mental power and learning ability (Naruka and Gaur, 2014). Different environmental conditions such as temperature, UV light, dryness and humidity, play a role in controlling the growth of airborne particles. Nevertheless the microbes manage to reach new hosts through the air for its survival (Sheik et al., 2015). Poor ventilation, crowded conditions and increase in number of air conditions inside building nowadays can facilitate the spreading and the survival rates of airborne particles and also can increase the chance of people at risk of airborne infections. Among dust particles present in the indoor environment, fungus which reproduce by forming spores, some bacteria especially gram positive bacteria and some viruses can survive for a long time in the air (Sheik et al., 2015; Jacob, 2016).

Despite the need to monitor bio-aerosol levels in evaluating health risks, differences between automatic techniques and passive sedimentation techniques hamper results comparison. Automated techniques, although they are efficient in quantitative analysis, are of limited use because they require heavy and noisy equipment and need a constant power supply. The passive sedimentation technique is also limited because it does not permit an adequate quantitative analysis, but it is still widely recommended in the literature for use as a microbiological alert (Abe et al., 2012). It is well known that exposure to bioaerosols can cause adverse effect on people and for this reason it is important to check the sanitary conditions of air in the place they live.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

In this chapter, the Study Area, Media Preparation, Sample Collection, Bacterial Identification, Gram Staining, Biochemical Test, Air Sampling and Microbiological Examination, Identification of Bacterial and Fungal Isolates, Characteristics of Microbiology Laboratory, Air Sampling, Suspension of Air Samples, Enumeration of Airborne Viable Counts of Bacteria (VBCs), Quantification of Airborne Viable Counts of Fungi (VFCs), Quantification of total viable microbial counts (TVMCs), Micrometeorological Measurements, and Statistical Analysiswill be discussed.

3.2 Study Area

This study was carried out at Institute Of Applied Science , Kwara State Polytechnic. The samples were collected from eight different Microbiology Laboratory, Lecture Room 29, LR 28, LR 27, LR26, LR 25, LR 24, LR 21 and Seminar Room.

3.3 Media Preparation

The media used were prepared according to the manufacturer’s instructions.

3.4 Sample Collection

Sedimentation Technique which involves the opening of plate with specific culture media was employed for this study (Sekulska, 2007). Prepared plates of nutrient agar were exposed to air for 30mins at different sites in the respective Microbiology Laboratory and Lecture Room. After sampling, all plates were immediately taken to the microbiology laboratory and Lecture rooms

and incubated at 37ºC for 24 hours for isolation of bacteria. The colonies were sub-cultured onto a new fresh medium in order to obtain pure culture.

3.5 Bacterial Identification

Identification of bacterial isolates was done using the standard procedures according to Cheesbrough (2009). Bacterial colonies were initially characterized by morphology and using staining techniques (Gram staining) and identified further by biochemical tests.

3.6 Gram Staining

Gram's staining was done to find the reactions of the bacterial isolates to Gram reagents. A smear was prepared and heat fixed. The crystal violet (primary stain) stain was flooded over the fixed culture for 60 seconds, the stain was washed with water. The iodine solutions were added onto the smear for 60 seconds, pour off and rinsed with water. A few drops of decolorizer (ethyl alcohol) was added and washed with water immediately after 5 seconds and finally safaranin (Secondary stain) was added for 60 seconds and washed, the smear was allowed to air dry. After drying the slide was mounted under microscope and observed. The stain differentiates bacterial species into two groups; Gram- positive bacteria, which take up crystal violet dye (primary stain) and are stained violet and Gram-negative, which pick up safranin (Secondary stain) are thus stained red after decolourization with alcohol.

3.7 Biochemical Test

Biochemical tests such as Catalase, Oxidase, Indole, Methyl Red test (MR), Coagulase, Voges Proskauer (VP), Citrate utilization, were carried out on the isolated bacteria according to cheesbrough (2009).

3.8 Air Sampling and Microbiological Examination

Air samples were collected from ten different locations of Microbiology Laboratory by plate sedimentation methods as employed by Stryjakowska-Sekulska et al. (2007) and Ekhaise et al. (2008). The ten locations were Main Campus, Angwan Lambu, Federal Medical Centre (FMC), Emir's Palace, Pyanku Campus, Government Residential Area (GRA), Main Market, Angwan NEPA, Dadin kowa and High Court. Petri plates containing culture media suitable for bacteria and fungi were used as sampling surfaces. Trypticase Soy Agar (TSA) supplemented with cyclohexamide (which inhibits growth of fungi) was used for the determination of total number of bacteria, while Malt Extract Agar (MEA) supplemented with chloramphenicol (which inhibits growth of bacteria) was used for the determination of the total number of fungi (Kalwasiṋska et al., 2012). Plates in triplicates for each type of culture medium were exposed to air in each of the ten locations for 30 min in order to allow air microorganisms to settle gravitationally directly on the media surfaces of the plates. Plates with TSA were incubated for 48 h at 37°C, while the plates with MEA were incubated at 30°C for 7 days (even though colonies were counted on the 3rd day). The total number of colony forming units were enumerated and expressed as colony forming units per cubic meter of air (CFU/m3) (Stryjakowska- Sekulska et al., 2007).

3.9 Identification of Bacterial and Fungal Isolates

The identification of bacterial colonies was carried out according to the standard microbiological methods as described by Holt (1994), Cheesbrough (2000) and Aneja (2003), in which the colonies were characterized using macroscopic (cultural) and microscopic (morphological) features as well as biochemical tests. The API system (bio-Mérieux, Marcy-l'Etoile, France) was also used to further confirm the identity of the bacterial species.

Identification of all fungal isolates was also carried out using standard methods based on macroscopic and microscopic features as described by Ellis (1971), Domsch et al. (1980), Singh et al. (1991), Barnett et al. (2007) and Barnett and Hunter (1999).

3.10 Characteristics of Lecture Rooms

Prior to air sampling, classroom characteristics viz. type of ventilation, number of installed fans and exhaust system, floor area of selected classroom, ceiling height, volume, furniture status, walls, cleanliness, number of students, average occupancy daily and on the day of sampling, visible appearance of microbial growth, if any, were examined and recorded during the air sampling session.

3.11 Air Sampling

Air sampling, for detection and isolation of viable counts of bacteria and fungi was carried out for a period of ten months as academic session commences from July to April. Twice in a month air sampling both inside and outside the three schools were carried out in order to characterize the IAQ.

Air sampling for presence of culturable viable counts of bacteria and fungi was conducted at respiratory height (at 1.2 meter) for 25 minutes using air samplers APM 823 (Envirotech Pvt Ltd., India), which was operating at flow rate of 28.5 liters per minute (LPM) and sucked through poly-tetra-flouro-ethylene (PTFE) membranes (47 mm diameter, 0.4 µm pore size).

3.12 Suspension of Air Samples

Each PTFE membrane was soaked in 10 ml of sterile normal saline and vigorously shaked using vortex mixture for 5 min to prepare a suspension of the trapped particles. From this suspension,

100 µl was used for spread plate culture on duplicate Petri dishes (10 cm diameter) containing respective culture media for isolation of viable counts of Bacteria and Fungi.

3.13 Enumeration of Airborne Viable Counts of Bacteria (VBCs)

Nutrient agar (NA) (Hi media laboratories) medium plates were used for enumeration and quantification of airborne VBCs. NA plates were incubated in B.O.D. incubator at 37° ± 1°c for 24 hours.

3.14 Quantification of Airborne Viable Counts of Fungi (VFCs)

Same suspension of air sample was also used for isolation of fungi. Rose Bengal Streptomycin Agar (RBA) (Hi media laboratories) medium was used for isolation of viable counts of fungi. RBA plates were incubated at 28 ± 2°C for 5 days.

Various fungal and bacterial colonies in each sample were counted and calculated by direct colony count or using colony counter and expressed as colony forming unit. Data was reported as colony forming unit (cfu) per cubic meter of air on the basis of the total air sampled on each filter membrane. Following formula was used for calculating CFUs

cfu / m3 □ No.of colonies in 0.1 ml / 0.1 ml □ 10 ml / volume of sample dair(m3)

3.15 Quantification of total viable microbial counts (TVMCs)

Quantification of total viable microorganisms in indoor and outdoor air was acquired summing the total number of viable counts of bacteria plus total number of viable counts of fungi obtained from above steps.

3.16 Micrometeorological Measurements

During the study period microclimate parameters, temperature (°C), and relative humidity (Rh%) of indoor and outdoor air were measured using Thermo hygrometer (Easl Scientific Industries

India) in order to assess the influence of meteorological variations on the concentration of viable counts of bacteria and fungi.

3.17 Statistical Analysis

Turkey test as recommended by Zar (1999) for analysis of data for multiple-comparison was used to determine the statistical significance of the concentrations of bacteria and fungi in the air sampled from the different locations of Faculty of Science SSU. Statistical Package for Social Sciences (SPSS) version 20.0 software was employed for this analysis. Percentage frequencies of occurrence of species of bacteria and fungi at the different locations were also computed according to the methods of Sampo et al. (1997).

Table 3.1: Bacterial concentration in the outdoor air environment of different locations

Location	Mean (cfu/m^3)	Standard deviation
Microbiology Laboratory	$4.2x10^1$	$\pm$ 1.13
LR 29	$4.5x10^1$	$\pm$ 0.83
LR 28	$4.2x10^1$	$\pm$ 1.13
LR 27	$7.6x10^1$	$\pm$ 2.27
LR 26	$5.4x10^1$	$\pm$ 0.07
LR 25	$3.0x10^1$	$\pm$ 2.33
LR 24	$1.0x10^2$	$\pm$ 4.67
LR 23	$6.0x10^1$	$\pm$ 0.67
LR 21	$4.2x10^1$	$\pm$ 1.13
Seminar Room	$4.2x10^1$	+ 1.13

GRA, Government Reserved Area

Table 3.2: Fungal concentration of the outdoor air environment of the different locations in Microbiology Laboratory

Location	Mean (CFU/m3)	Standard Deviaton
Microbiology Laboratory	$1.2x10^1$	±2.06
	$1.2x10^1$	±2.06
LR 29	$1.7\ x10^1$	±1.56
LR 28	$3.9\ x10^1$	±0.64
LR 27	$1.5\ x10^1$	±1.76
LR 26	$4.8\ x10^1$	±1.54
LR 24	$7.0\ x10^1$	±3.74
LR 23	5.0 x101	±1.74
LR 21	2.6 x101	±0.66
Seminar Room	3.7 x101	±0.44

**GRA, Government Reserved Area*

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Analysis of Micro-flora in Microbiology Laboratory

The present study was conducted to isolate and identify airborne bacteria in some selected Microbiology Laboratorys within the Federal University Dutse. A total of sixty four samples were collected during the course of this study out of which fifty six (87.5 %) were positive while eight (12.5%) showed negative bacterial growth (Table 4.5). Four bacterial genera were identified from the sampling sites as shown in Table 4.6. Overall, 124 bacteria were isolated comprising of Staphylococcus aureus, Streptococcus spp, E. coli and Bacillus subtilis (Table 4.7). Staphylococcus aureus has the highest percentage occurrence of 51% followed by E. coli (25%) and Streptococcus species 21% while Bacillus subtilis recorded the least 3% (Table 4.7). These pathogens could be linked with several infections such as gastrointestinal tract, respiratory tract, urinary tract and skin disorders.

Table 4.5: Number and percentage of positive bacterial samples

S/N	Sample Areas	No. of Samples	No. of Positive Samples	Percentage (%)
1	Microbiology Laboratory	8	8	100%
2	Lecture Room 29 (LR 29)	8	6	75.0%
3	LR 28	8	8	100%
4	LR 27	8	8	100%
5	LR 26	8	5	62.5%
6	LR 25	8	7	87.5%
7	LR 24	8	6	75.0%
8	Seminar Room	8	8	100%
	Total	64	56	87.5%

Table 4.6: Isolated Bacteria at various Microbiology Laboratory and Lecture rooms

Sample areas	Isolated organisms
Microbiology Laboratory	Staphylococcus aureus, Streptococcus spp, E. coli
Lecture Room 29 (LR29)	Staphylococcus aureus, Streptococcus spp, E. coli, Bacillus spp
LR 28	Staphylococcus aureus, Streptococcus spp, E. coli
LR 27	Staphylococcus aureus, Streptococcus spp, E. coli, Bacillus spp
LR 26	Staphylococcus aureus, Streptococcus spp, E. coli
LR 25	Staphylococcus aureus, Streptococcus spp, E. coli
LR 24	Staphylococcus aureus, Streptococcus spp, E. coli, Bacillus spp
Seminar Room	Staphylococcus aureus, Streptococcus spp, E. coli, Bacillus spp

Table 4.7: Percentage frequency of occurrences of the isolated bacteria

Organisms	Frequency of occurrence	Percentage (%) of Occurrences
Staphylococcus aureus	63	51
Streptococcus spp	26	21
E. coli	31	25
Bacillus	4	3
Total	124	100

Staphylococcus aureus belong to normal flora of the human skin and nose, it is likely that these organism may be originated from the nose and skin flora of the students and staff of our Polytechnic. However, this higher incidence of Staphylococcus aureus obtained from this study correlate with several and similar findings of the studies conducted by several researchers. A study conducted by Yaghoub and Elagbash (2010) at Omdurman and El-Rhibat hospital Sudan

found that Staphylococcus aureus was the predominant air bacteria isolated from these hospitals. This study also support the finding of Sheik et al. (2015), in which the occurrence was reported to be 38% in a research conducted to detect the airborne microorganism from a college in Saudi Arabia. This result is also inconformity with the result obtained by Badri et al. (2016), who reported Staphylococcus aureus as the highest bacteria isolated from their study. In the present study Staphylococcus aureus was the dominant isolated organism and this bacterium is a common causative agent of various human diseases, it is responsible for many gastrointestinal tract infections, respiratory tract infections and skin disorders (Yaghoub and Elagbash, 2010). Another pathogen E. coli (25%) which was also isolated is of medical concern. It is one of the most commonly examined Gram-negative bacteria in microbiology. Though it is well known that E. coli inhabits the human bowel as part of normal microbiota, some strains are capable of causing a significant intestinal/diarrheal and extraintestinal infections (Alteri and Mobley, 2012). E. coli is a leading cause of urinary tract infections and intra abdominal infections in which the extent of the disease can range from cystitis to life threatening sepsis (Ejrnaes. 2011). It is well known that E. coli is the most common etiologic agent of urinary tract infections (Alós, 2005). Uropathogenic E. coli (UPEC) infections occur in otherwise healthy individuals and account for more than 90% of uncomplicated urinary tract infections (Warren, 1996). Also the isolation of Streptococcus species 21% is of great concern due to the fact that thisbacteria are responsible for many cases of meningitis, endocarditis, bacterial pneumonia and necrotizing facilities. The reasons for high percentage frequency of occurrence of bacteria in this study could be due to low minimal usage of disinfection procedures against airborne pathogens, more number of students attending lecture classes and low degree of hygiene practices.

4.2 Results

Table 4.3: Distribution of bacterial species isolated from the outdoor air environment of the different locations in Microbiology Laboratory.

Bacterial Isolate	Location*										Occurrence
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**(%)**
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	100
Streptococcus pyogenes	+	+	+	+	+	+	+	+	+	+	100
Escherichia coli	+	+	+	+	+	-	+	+	+	+	90
Bacillus sp.	+	+	+	+	+	+	+	+	+	+	100
Enterobacter aerogenes	-	-	-	-	-	-	+	+	+	+	40
Shigella sp.	-	-	-	-	-	+	+	+	+	+	50

**Locations: A = Main Campus; B = Angwan Lambu; C = Federal Medical Center; D = Emir's Palace; E = Pyanku Campus; F = Government Reserve Area; G = Main Market; H = Angwan NEPA; I = Dadin Kowa; J = High Court Area.*

Table 4.4: Distribution of fungal species isolated from the outdoor air environment of the different locations of Institute Of Applied science Kwara State Polytechnic Microbiology Laboratory and Various lecture rooms.

Bacterial Isolate	Location*										Occurrence
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**(%)**
Aspergillus flavus	-	+	-	+	+	-	+	+	+	+	70
Aspergillus niger	+	+	+	+	-	+	+	+	+	+	100
Aspergillus fumigatus	-	+	+	-	+	-	+	+	-	+	60
Penicillium sp.	-	+	+	+	-	+	+	+	+	+	80
Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+	100

Absidia corymbifera	-	-	-	+	-	-	+	+	-	-	30

**Locations: A = Microbiology Laboratory; B = Lecture room 29; C = Lecture room 28; D = Lecture room 30; E = Lecture room 31; F = Seminar Room; G = Lecture room 27; H = Lecture room 26; I = Lecture room 25; J =Lecture room 24*

4.3 Presentation and Analysis of Data

The data collected from the respondents were analyzed in tabular form with simple percentage for easy understanding.

A total of 50 (fifty) questionnaires were distributed and 50 questionnaires were returned.

The Socio-demographic Characteristics of the Respondents

This section deals with the description of the characteristics of all the respondents (50) involved in the study by randomly selection of respondents from the study area. The characteristics of respondents include age, gender, and educational state.

RESEARCH QUESTION 1: What are the sources of microflora contaminants in microflora laboratories and Lecture rooms In Kwara State Polytechnic ?

TABLE 4.1: Response of the respondent

Response		**Frequency**	**Percent**	**Cumulative Percent**
Valid	Filled	31	62.00%	62.00%
	Unfilled	19	38.00%	100.00%
	Total	50	100.00%	

From the above table it shows that 62.0% of the respondents filled the section while 38.0% of the respondents didn’t respond.

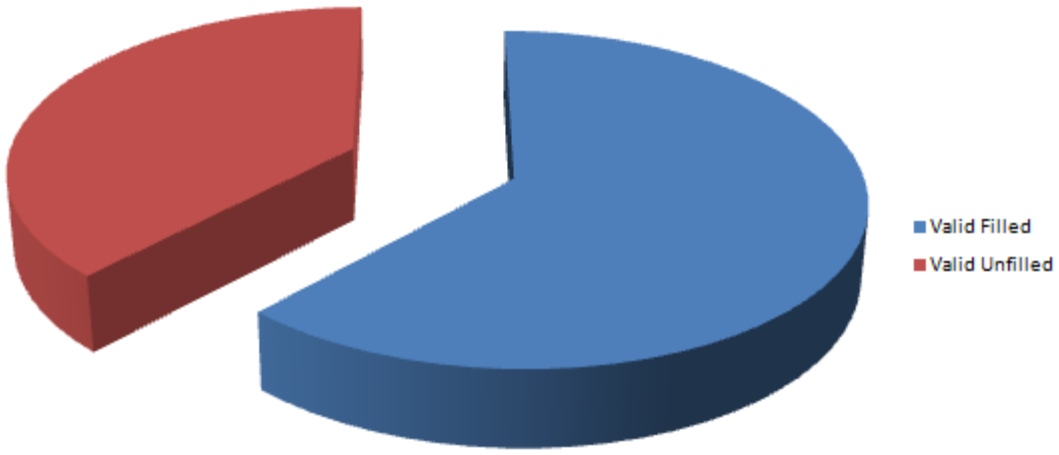

Figure 4.1: Response of the respondent

RESEARCH QUESTION 2: Are the bacterial and fungal contaminants encountered in microbiology laboratories and Lecture rooms different morphologically?

TABLE 4.2: Response of the respondent

Response		**Frequency**	**Percent**	**Cumulative Percent**
Valid	Yes	31	62.00%	62.00%
	No	19	38.00%	100.00%
	Total	50	100.00%	

From the above table it shows that 62.00% of the respondents responded yes while 38.00% of the respondents marked no.

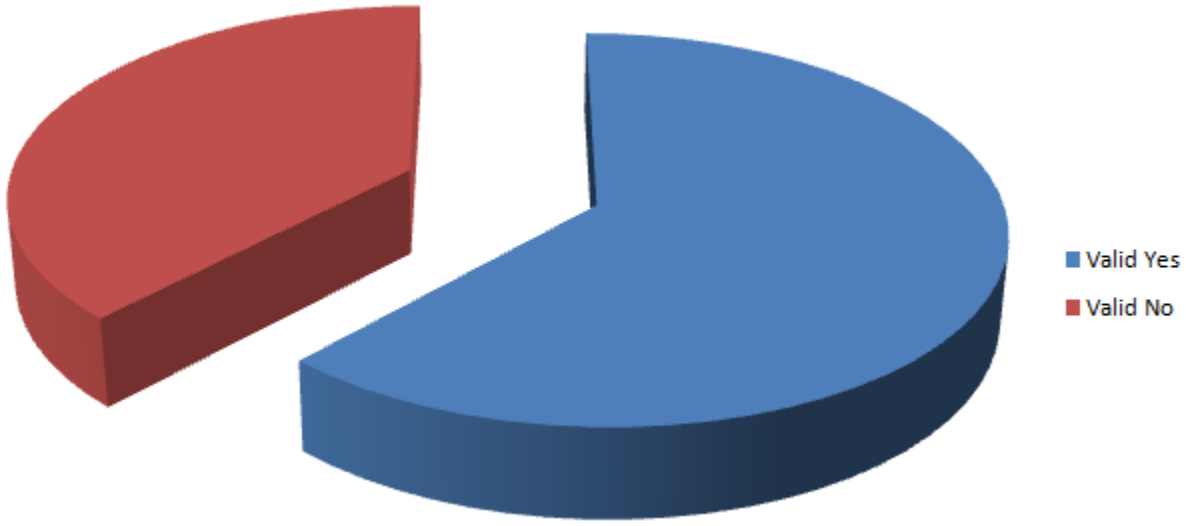

Figure 4.2: Response of the respondent

RESEARCH QUESTION 3: Are the persistent bacteria to hypochlorite based disinfection genotypically similar to standard bacteria strains?

TABLE 4.3: Response of the respondent

Response		Frequency	Percent	Cumulative Percent
Valid	Yes	22	44.00%	44.00%
	No	28	56.00%	100.00%
	Total	50	100.00%	

From the above table it shows that 44.0% of the respondents responded yes while 56.00% of the respondents marked no.

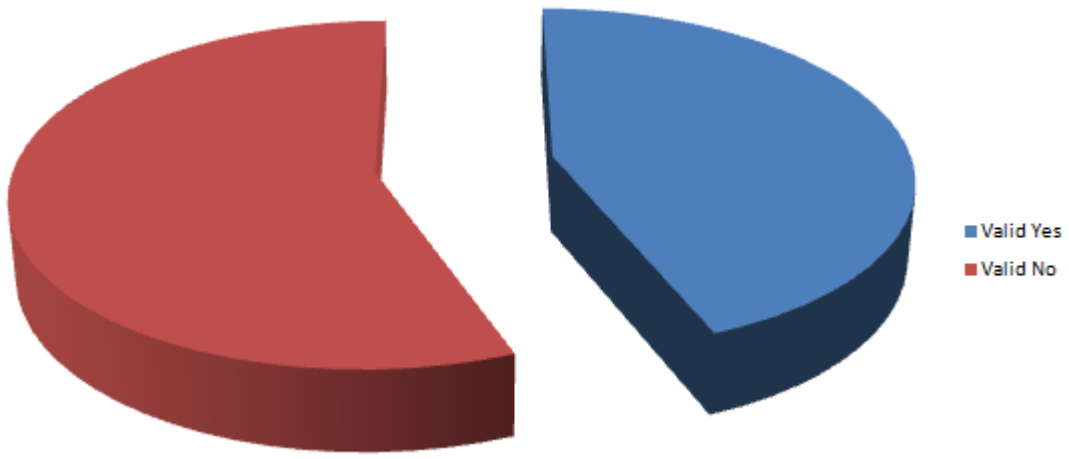

Figure 4.3: Response of the respondent

4.4 Test of Hypothesis 1

Hypothesis One

H0; There is no significant bacterial and fungal contamination encountered in microbiology laboratories and Lecture rooms in Kwara State Polytechnic

H1; There is a significant bacterial and fungal contamination encountered in microbiology laboratories and Lecture rooms in Kwara State Polytechnic

Table I: There is no significant bacterial and fungal contamination encountered in microbiology laboratories and Lecture rooms in Kwara State Polytechnic

Response	Observed N	Expected N	Residual
Yes	27	16.6666667	10.33333333
No	13	16.6666667	-3.66666667
Undecided	10	16.6666667	-6.66666667
Total	**50**		

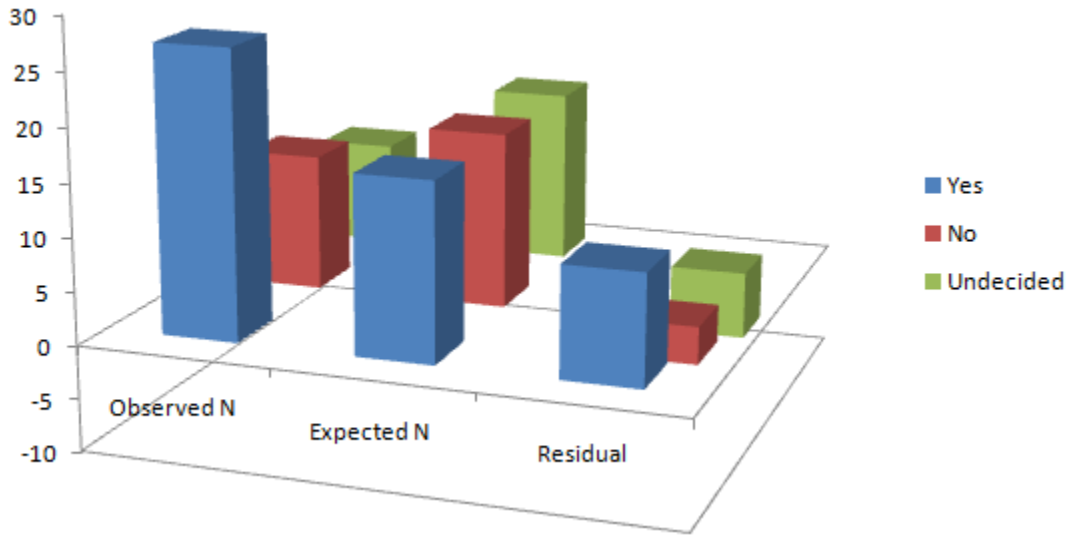

Figure I: There is no significant bacterial and fungal contamination encountered in microbiology laboratories in Nigeria

Test Statistics

	There is no significant bacterial and fungal contamination encountered in

	microbiology laboratories and Lecture room
Chi-Square	28.211[a]
Df	2
Asymp. Sig.	.000

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 16.7.

Decision rule:

The researcher therefore rejects the null hypothesis which states that there is no significant bacterial and fungal contamination encountered in microbiology laboratories in Nigeria, as the calculated value of 28.211 is greater than the critical value of 5.99.

Therefore the alternate hypothesis is accepted which states thatthere is a significant bacterial and fungal contamination encountered in microbiology laboratories in Nigeria.

4.5 Discussion of Findings

The results of the bacterial concentrations in the outdoor air environment of different locations of Microbiology Laboratory are shown in Table 3.1. The bacterial concentrations in the different locations ranged from 2.8 x 103 to 6.4 x 103 CFU/m3. The highest bacterial concentration of 6.4 x 103 CFU/m3 was recorded at the Main Market, followed by 6.2 x 103 CFU/m3 and 5.7 x 103 CFU/m3 recorded for Emir's Palace and Angwan NEPA, respectively. The lowest con-centration of 2.8 x 103 CFU/m3 was recorded at Government bacteria and fungi isolated are presented in Tables 4.3 and 4.4, respectively. Six bacterial species were isolated at varying

frequencies of occurrence. The bacterial species with their respective frequencies of occurrence were Staphylococcus aureus (100%), Streptococcus pyogenes (100%), Escherichia coli (90%), Bacillus spp. (100%), Enterobacter aerogenes (40%) and Shigella spp. (50%).

Nine species of fungi belonging to seven genera were isolated with respective percentage frequencies of 100% (Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer), 80% (Penicillium spp.), 60% (Aspergillus fumigatus, Candida albicans) and 30% (Mucor spp., Absida corymbifera, Alternaria alternata), respectively.

Epidemiological studies have shown that a large number of people around the world are exposed to biological agents (Daisey et al., 2003; Dales et al., 2004; Golofit-Szymczak and Gorny, 2010). Unfortunately, there is no official reference limit for the microbiological quality of air in human environments, whether indoor or outdoor. The lack of quantitative health-based guideline values or thresholds for the acceptable levels of microbial contamination in the air may be due to a lack of dose-response relationship for most of the air microbiological agents (Golofit-Szymezak and Górny, 2010). Several investigators in this area had highlighted that source data on concentrations of biological agents in the air environmentsare still insufficient (Adhikar et al., 2004; Mouli et al., 2005; Golofit-Szymczak and Gorny, 2010; Kalwasińska et al., 2012). This notwithstanding, the qualitative and quan- titative information on the composition and concentrations of microorganisms in the air environment of human habitations at any point in time would help in alerting the public of possible health risk that may be encountered by vulnerable individuals.

Several researchers in this area had earlier reported that exposure to high concentrations of microorganisms in the air frequently lead to allergies, asthma (Björnsson et al., 1995; Newson et

al., 2000), pueumonia (Siersted and Gravesen, 1993), and other health side-effects. In addition to public health advantage, routine monitoring of air quality can serve as a means of military surveillance for the detection of any possible biological threat of bioterrorism (Douwes et al., 2003).

Data resulting from this study revealed that the concentrations of bacteria in Microbiology Laboratory ranged from2.8 x 103 - 6.4 x 103 CFU/m3and that of fungi ranged from4.71 x 102 - 4.60 x 103CFU/m3. However, the concentrations for both bacteria and fungi have been shown to vary ($P<0.05$) in the different locations of the Microbiology Laboratory. The concentrations of bacteria at all the locations exceeded the recommended limit (103CFU/m3) suggested by National Institute of Occupational Safety and Health (NIOSH). The American Conference of Governmental Industrial Hygienists (ACGIH) had suggested 500 CFU/m3 for culturable bacteria (Kalogerakis et al., 2005). Górny and Dutkiewiez (2002) earlier presented to WHO Expert Meeting in Berlin, a proposed Residential Limit Values of 250 CFU/m3 for bacterial concentrations.

Considering all available threshold limits for bacterial concentrations in the air environments, it is clear that the outdoor air of Faculty of Science SSU Microbiology Laboratory is heavily loaded with bacteria. The Main Market that had the highest bacterial concentration followed by the Emir's Palace is among the busiest locations in the metropolis in terms of human and vehicular movements. The high bacterial concentration recorded in these two locations is not surprising, and this agrees with reports by several researchers (Kalogeraskis et al., 2005; Chen and Hildermann, 2009). Similarly, the concentrations of fungi in most of the locations exceeded

the recommended proposal of 103 CFU/m3 as threshold limits for fungal concentrations in the air (Górny and Dutkiewiez, 2002).

The qualitative analysis of the microbial flora provides additional information on airborne microorganisms in the outdoor air of Microbiology Laboratory and lecture room. In this study, six species of bacteria, S. aureus, S. pyogenes, E. coli, Bacillus spp., E. aerogenes and Shigella spp., and nine species fungi belonging to six genera, which included A. flavus, A. niger, A. fumigatus, Penicillium spp., R. stolonifer, A. corymbifera, A.alternata, Mucor spp. and C. albicans were isolated from the outdoor air environment of Microbiology Laboratory. Some of these bacteria and fungi have been shown to be amongst the most common bacterial and fungal species isolated from the air (Burge and Hoyer, 1990).

From this study, S. aureus, S. pyogenes, Bacillus spp. and E. coli are the most prevalent bacterial species isolated. S. aureus is known to be carried in the naso- pharynx, throat, skin, cuts, boils, nails, and such can easily contribute to the microflora in the Microbiology Laboratory and Lecture roomswhich is always busy with activities involving in most cases human and vehicular movements. Bacillus spp. is spore-forming soil bacteria and the most persistent in the atmosphere (Shaffer and Lighthart, 1994). S. pyogenes are often found as commensals in the upper respiratory tract of human (Cheesbrough, 2000). If host defenses are weakened or a new highly virulent strain is introduced it can lead to acute suppuration infections (Brooks et al., 2001). E. coli is an enteric coliform which is a normal resident flora of the large intestines of mammals including humans and are used as indicators of pollution of feacal origin (Willey et al., 2008). The high prevalence of E. coli in the air of Microbiology Laboratory suggests a very low personal and environmental hygiene practice in this town.

A. niger and Penicillium spp. are the most predominant species isolated. Maktkovic et al. (2007) in their study reported Aspergillus spp. and Penicillium spp. as the predominant genera of organisms isolated from the air, while Ekhaise et al. (2008) reported Aspergillus species as the most common genus of fungi in the air environment. Aspergillus and other species of fungi have been implicated as pathogenic in causing several mycotic infections. The relatively high concentrations of fungi in the air environment of Microbiology Laboratory and Lecture rooms may pose only little health hazard to healthy individuals, but would pose serious danger and special risk to immunosuppressed persons and other severely immunocompromised individuals (Flannigan et al., 1994). Fungal spores from species of Penicillium have been implicated with allergies and elicit asthma in vulnerable individuals (Flannigan et al., 1991).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Introduction

It is important to ascertain that the objective of this study was on the Isolation and identification of air Micro-florain Microbiology Laboratory and Lecture Rooms in Kwara State Polytechnic. In the preceding chapter, the relevant data collected for this study were presented, critically analyzed and appropriate interpretation given. In this chapter, certain recommendations made which in the opinion of the researcher will be of benefits in addressing the Isolation and identification of air Micro-florain Microbiology Laboratory.

5.2 Summary

Air current influence the time which either the microorganisms or the particles laden with microorganisms remain suspended in the air. Microflora is a term that refers to a community of bacteria that exist on or inside the body, and possess a unique ecological relationship with the host. This relationship encompasses a wide variety of microorganisms and the interactions between microbes. These interactions are often a mutualistic relationships between the host and autochthonous flora. Microflora responsible for harmful diseases are often allochthonous flora (Wikipedia, 2021).

The study was carried out to determine the Isolation and identification of air Micro-florain Microbiology Laboratory and Lecture rooms in kwara state Polytechnic, Ilorin. In achieving this aim, the following specific objectives were laid out to determine sources of microflora contaminants in selected microflora laboratories and lecture roomsin Nigeriaand isolate and identify the bacterial and fungal contaminants in microbiology laboratories based on morphological and biochemical characteristics.

Data were collected from the primary source which questionnaire was used as an instrument of data collection while secondary data were sources from textbooks, journals, newspapers and the internet were employed.The information from this study will help to contain the Laboratory Acquired Infections) LAI associated with microbial contaminations in microbiology laboratories and Lecture Rooms.

The research findings will also assist personnel in laboratories to be careful when performing standard manipulations of microbiological specimens in cell cultures. This will help in reducing the costs associated with the application of the technology in laboratories. The information will form a basis of training the personnel on capacity development in monitoring and evaluation of microflora contaminants in laboratories and lecture Rooms. Then effecting or upgrading policy on Standard Operating Procedures (SOPs) will be developed in the laboratories.

5.3 Conclusion

The importance of evaluating the quality of the air humans breathe whether indoor or outdoor, especially in the urban areas where there is high vehicular traffic and human activities involving rapid movements cannot be over-emphasized. The number and type of airborne microorganisms can also be used to determine the degree of cleanliness as a means of determining the source of human discomfort and certain airborne microbial infections.

In conclusion, the isolation and identification of air microflora in the microbiology laboratory and Lecture rooms revealed the presence of diverse microorganisms, including bacteria and fungi, commonly found in the air. These microorganisms are introduced into the laboratory environment from various sources such as human activities, ventilation systems, and outdoor air. The findings highlight the importance of maintaining proper hygiene and air quality control in

laboratory settings to minimize potential contamination risks. Regular monitoring of air microflora can help ensure a safer working environment, reducing the risk of interference in microbiological experiments and the spread of airborne pathogens. The study also underscores the significance of understanding the types of microorganisms present in the air, which can have implications for both laboratory safety and public health.

5.4 Recommendation

Based on the findings, the following recommendations are hereby suggested that:

1. Kwara State Polytechnic Lecture Room should implement regular air monitoring to identify and control the presence of airborne microorganisms.
2. Air filtration systems should be installed and maintained to reduce microbial contamination.
3. Laboratory personnel should adhere to strict hygiene protocols, including the use of personal protective equipment and regular cleaning of workspaces.
4. The laboratory environment should also be assessed for proper ventilation to minimize the influx of outdoor contaminants.
5. In addition, standard operating procedures should be updated to include periodic microbial assessments to ensure continued air quality control, thereby reducing the risk of contamination in experiments.

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