

**ISOLATION AND IDENTIFICATION OF AIRBORNE FUNGI IN
SHUTTLE BUS**

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CERTIFICATION

This is to certify that this project work was carried out by Favour OSAWE with the matriculation number.LSC1605543 of the Department of Microbiology, Faculty of Life sciences, University of Benin, Benin City, Nigeria.

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APPROVAL

This is to certify that this project work has been approved in partial fulfilment for the award of Bachelor of science B.Sc (HONS) degree in Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This project is dedicated to God almighty for his strength and wisdom to in me to get to the end of a journey that started four years back.

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My regards and appreciation goes first to God Almighty for his endless mercies, grace and wisdom during the period of my stay and study in University of Benin.

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CHAPTER ONE

INTRODUCTION

1.1 Background of study.

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 (WHO, 2019).

Biological air pollutants, also known as bioaerosols, include pollen, fungi, bacteria and viruses (An *et al.* 2004). Most are microorganisms that colonize the soil, water bodies, plant surfaces, rocks and buildings (Polymenakou *et al.* 2012). In addition, bacterial toxins, mycotoxins, enzymes and fragments of plant and animal tissues are present in the air (Chmiel *et al.* 2015). Bioaerosols ranging from 1.0–5.0 μm usually float in the air, while larger ones tend to settle on surfaces (Stetzenbach *et al.* 2004). The greatest implications to human health have 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 bronchioles (Chmiel *et al.* 2015). 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 (Griffin *et al.* 2003). 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 *et al.* 2004). As we know, one of the main factors influencing human health is air quality. Therefore, the presence of microorganisms particularly, those causing infectious diseases, in both ambient air and indoor environments can be particularly dangerous.

Information on the presence of microorganisms in the air remains incomplete and numerous questions still need to be answered. Hence, this study aims at enumerating and identifying the fungal isolates found in commuter busses (University of Benin Student shuttle buses) before, during and after a trip and how to determine the antifungal susceptibility pattern of the fungal isolates on selected antifungal drugs.

1.2. Aim and Objectives

The aim and objective of this research was to isolate and identify airborne fungi that are found in the University Of Benin shuttle bus.

The specific objective were to

1. To enumerate the fungal isolate found in some University Of Benin Shuttle Buses.
2. To identify the fungal isolates found in University of Benin Shuttle Buses.
3. Determine the antifungal susceptibility pattern of the fungal isolates on selected antifungal drugs.

CHAPTER TWO

LITERATURE REVIEW

2.1 DESCRIPTION OF FUNGI

Fungi(singular-fungus) can be single celled or very complex multicellular organisms. They are found in just about any habitat but most live on the land, mainly in soil or on plant material rather than in sea or fresh water. A group called the decomposers grow in the soil or on dead plant matter where they play an important role in the cycling of carbon and other elements. Some are parasites of plants causing diseases such as mildews, rusts, scabs or canker. In crops fungal diseases can lead to significant monetary loss for the farmer. A very small number of fungi cause diseases in animals. In humans these include skin diseases such as athletes' foot, ringworm and thrush.

Fungi are subdivided on the basis of their life cycles, the presence or structure of their fruiting body and the arrangement of and type of spores (reproductive or distributional cells) they produce.

The three major groups of fungi are:

1. Multicellular filamentous moulds.
2. Macroscopic filamentous fungi that form large fruiting bodies. Sometimes the group is referred to as 'mushrooms', but the mushroom is just the part of the fungus we see above ground which is also known as the fruiting body.
3. Single celled microscopic yeasts.

2.20 CLASSIFICATION OF FUNGI

The kingdom Fungi contains five major phyla that were established according to their mode of sexual reproduction or using molecular data.

The five true phyla of fungi are the Chytridiomycota (Chytrids), the Zygomycota (conjugated fungi), the Ascomycota (sac fungi), the Basidiomycota (club fungi) and the recently described Phylum Glomeromycota. The Deuteromycota is an informal group of unrelated fungi that all share a common character – they use strictly asexual reproduction.

Note: “-mycota” is used to designate a phylum while “-mycetes” formally denotes a class or is used informally to refer to all members of the phylum.

2.21 Chytridiomycota: The Chytrids

The only class in the Phylum Chytridiomycota is the Chytridiomycetes. The chytrids are the simplest and most primitive Eumycota, or true fungi. The evolutionary record shows that the first recognizable chytrids appeared during the late pre-Cambrian period, more than 500 million years ago. Like all fungi, chytrids have chitin in their cell walls, but one group of chytrids has both cellulose and chitin in the cell wall. Most chytrids are unicellular; a few form multicellular organisms and hyphae, which have no septa between cells (coenocytic). They produce gametes and diploid zoospores that swim with the help of a single flagellum.

The ecological habitat and cell structure of chytrids have much in common with protists. Chytrids usually live in aquatic environments, although some species live on land. Some species thrive as parasites on plants, insects, or amphibians, while others are saprobes. The chytrid species *Allomyces* is well characterized as an experimental organism. Its reproductive cycle includes both asexual and sexual phases. *Allomyces* produces diploid or haploid flagellated zoospores in a sporangium.

2.22 Zygomycota: The Conjugated Fungi

The zygomycetes are a relatively small group of fungi belonging to the Phylum Zygomycota. They include the familiar bread mold, *Rhizopus stolonifer*, which rapidly propagates on the surfaces of breads, fruits, and vegetables. Most species are saprobes, living off decaying organic material; a few are parasites, particularly of insects. Zygomycetes play a considerable commercial role. The metabolic products of other species of *Rhizopus* are intermediates in the synthesis of semi-synthetic steroid hormones.

Zygomycetes have a thallus of coenocytic hyphae in which the nuclei are haploid when the organism is in the vegetative stage. The fungi usually reproduce asexually by producing sporangiospores. The black tips of bread mold are the swollen sporangia packed with black spores. When spores land on a suitable substrate, they germinate and produce a new mycelium. Sexual reproduction starts when conditions become unfavorable. Two opposing mating strains (type + and type -) must be in close proximity for gametangia from the hyphae to be produced and fuse, leading to karyogamy. The developing diploid zygospores have

thick coats that protect them from desiccation and other hazards. They may remain dormant until environmental conditions are favorable. When the zygospore germinates, it undergoes meiosis and produces haploid spores, which will, in turn, grow into a new organism. This form of sexual reproduction in fungi is called conjugation (although it differs markedly from conjugation in bacteria and protists), giving rise to the name “conjugated fungi.” Zygomycetes have asexual and asexual life cycles. In the sexual life cycle, plus and minus mating types conjugate to form a zygosporangium.

Sporangia grow at the end of stalks, which appear as white fuzz seen on this bread mold, *Rhizopus stolonifer*. The tips of bread mold are the spore-containing sporangia.

2.23 Ascomycota: The Sac Fungi

The majority of known fungi belong to the Phylum Ascomycota, which is characterized by the formation of an ascus (plural, asci), a sac-like structure that contains haploid ascospores. Many ascomycetes are of commercial importance. Some play a beneficial role, such as the yeasts used in baking, brewing, and wine fermentation, plus truffles and morels, which are held as gourmet delicacies. *Aspergillus oryzae* is used in the fermentation of rice to produce sake. Other ascomycetes parasitize plants and animals, including humans. For example, fungal pneumonia poses a significant threat to AIDS patients who have a compromised immune system. Ascomycetes not only infest and destroy crops directly; they also produce poisonous secondary metabolites that make crops unfit for consumption. Filamentous ascomycetes produce hyphae divided by perforated septa, allowing streaming of cytoplasm from one cell to the other. Conidia and asci, which are used respectively for asexual and sexual reproductions, are usually separated from the vegetative hyphae by blocked (non-perforated) septa.

Asexual reproduction is frequent and involves the production of conidiophores that release haploid conidiospores. Sexual reproduction starts with the development of special hyphae from either one of two types of mating strains. The “male” strain produces an antheridium and the “female” strain develops an ascogonium. At fertilization, the antheridium and the ascogonium combine in plasmogamy without nuclear fusion. Special ascogenous hyphae arise, in which pairs of nuclei migrate: one from the “male” strain and one from the “female” strain. In each ascus, two or more haploid ascospores fuse their nuclei in karyogamy. During sexual reproduction, thousands of asci fill a fruiting body called the ascocarp. The diploid

nucleus gives rise to haploid nuclei by meiosis. The ascospores are then released, germinate, and form hyphae that are disseminated in the environment and start new mycelia.

2.24 Basidiomycota: The Club Fungi

The fungi in the Phylum Basidiomycota are easily recognizable under a light microscope by their club-shaped fruiting bodies called basidia (singular, basidium), which are the swollen terminal cell of a hypha. The basidia, which are the reproductive organs of these fungi, are often contained within the familiar mushroom, commonly seen in fields after rain, on the supermarket shelves, and growing on your lawn. These mushroom-producing basidiomycetes are sometimes referred to as “gill fungi” because of the presence of gill-like structures on the underside of the cap. The “gills” are actually compacted hyphae on which the basidia are borne. This group also includes shelf fungus, which cling to the bark of trees like small shelves. In addition, the basidiomycota includes smuts and rusts, which are important plant pathogens; toadstools, and shelf fungi stacked on tree trunks. Most edible fungi belong to the Phylum Basidiomycota; however, some basidiomycetes produce deadly toxins. For example, *Cryptococcus neoformans* causes severe respiratory illness.

The fruiting bodies of a basidiomycete form a ring in a meadow, commonly called “fairy ring.” The best-known fairy ring fungus has the scientific name *Marasmius oreades*. The body of this fungus, its mycelium, is underground and grows outward in a circle. As it grows, the mycelium depletes the soil of nitrogen, causing the mycelia to grow away from the center and leading to the “fairy ring” of fruiting bodies where there is adequate soil nitrogen.

The lifecycle of basidiomycetes includes alternation of generations. Spores are generally produced through sexual reproduction, rather than asexual reproduction. The club-shaped basidium carries spores called basidiospores. In the basidium, nuclei of two different mating strains fuse (karyogamy), giving rise to a diploid zygote that then undergoes meiosis. The haploid nuclei migrate into basidiospores, which germinate and generate monokaryotic hyphae. The mycelium that results is called a primary mycelium. Mycelia of different mating strains can combine and produce a secondary mycelium that contains haploid nuclei of two different mating strains. This is the dikaryotic stage of the basidiomycetes lifecycle and it is the dominant stage. Eventually, the secondary mycelium generates a basidiocarp, which is a fruiting body that protrudes from the ground—this is what we think of as a mushroom. The basidiocarp bears the developing basidia on the gills under its cap.

2.3 Fungal Growth

Fungi can be grown in submerged cultures in several different morphological forms: suspended mycelia, clumps, or pellets (Metz and Kossen, 1977). Many studies have discussed the advantages and disadvantages of growth morphologies in terms of different products (Liao *et al.*, 2007). It has been concluded that fungal growth in pellet form is a favourable alternative which benefits most of the fungal fermentations since it not only makes repeated-batch fungal fermentation possible but also significantly improves the culture rheology which results in better mass and oxygen transfer into the biomass and lower energy consumption for aeration and agitation (Sjijdam *et al.*, 1980). Fungal morphology is a key feature in industrial metabolite production in suspended cultures (Tamerler *et al.*, 1999) stated that the pellets obtained in shake-flask cultures showed distinct layers of mycelial density with only the thin outer layer consisting of a dense mycelial network. It was shown that the process of pellet formation occurred in two steps:

- i. Aggregation of free spores to spore clusters with subsequent germination and formation of small aggregates surrounded by a loose hyphal network.
- ii. Aggregation of the primary aggregates to the final full-size pellets.

Liao *et al.* (2007) reported that a change in fungal morphology is influenced by medium composition, inoculum, pH, medium shear, additives (polymers, surfactants, and chelators), culture temperature, and medium viscosity. However, for individual strains, each factor has different importance to the growth morphologies thus much of the study on fungal pellet formation is limited to the level of the individual strain (Metz and Kossen, 1977). Peptone was demonstrated to have a positive effect on pellet formation which is represented by uniform pellets (Liao *et al.*, 2007). Metal ions are a very important factor in the metabolism of fungi. Foster and Waksman (1939) stated that the organism utilized the energy three times more efficiently when metal ions were added to the medium, which made for a relatively fast and abundant fungal growth. However, (Liao *et al.*, 2007) demonstrated that the metal ions caused the fungus to grow so fast and caused the filaments to tangle with each other and ultimately forming a clumpy morphology.

2.4 AIRBORNE FUNGI

As we know fungi are present in air in spore shape but sometimes we find hyphae in air.

Factors affecting the distribution of fungi includes:

1. Temperature
2. Humidity
3. Speed of air
4. Rains
5. Cycle of spores production.

Methods of exposing the Petri dish: Is the simplest method of obtaining spores from air which is to expose Petri dish with PDA to air for a period of time then incubate the plate at room temperature.

2.5 Presence of Airborne Fungi in an Indoor Environment:

Indoor air environments represent one of the many places of occurrence and importance with regard to the presence of fungi. Their adaptive characteristics favor their global dispersion, allowing the survival of these microorganisms in several habitat types (Nevalainen *et al.*, 2015; Coombs *et al.*, 2018). Monitoring of airborne fungi has already been recorded in the 19th century (Maddox, 1870), as well as at the beginning of the second half of the 20th century. This initial research on the topic aimed to investigate concentrations of fungi in the outside air (Morrow *et al.*, 1942; Hirst, 1952)

It is very clear today that most of the fungi present in indoor air come to these sites mainly through external air, which is an important source of biological contamination for indoor air (Lee *et al.*, 2006; Crawford *et al.*, 2015; Abassi *et al.*, 2018). Although external air contributes significantly to the composition and increase in the concentration of fungal spores in indoor air, this is not the only way indoor air contamination can occur. Domestic and everyday activities may have a considerable impact on the concentrations of spores in the air (Lehtonem *et al.*, 1993; Awad *et al.*, 2018). Manipulation of organic material (firewood and potted plants), handling of bedding, dog and cat hair, human skin, hair and nails, as well as clothing and human occupation can positively influence the spores in the indoor air (Reponen *et al.*, 1992; Pitkaranta *et al.*, 2008). The factors considered preponderant in influencing the presence and development of fungi in environments with indoor air are: humidity, temperature and nutrient availability (TANG *et al.*, 2015).

In general, the temperature and humidity values for indoor fungi growth may vary depending on the species considered. Indoor air environments usually have optimum temperature values

for fungi growth, ranging from 10-35 °C . For relative humidity, values below 75% are reported as limiting for fungal growth in buildings (Rowan *et al.*, 1999). The critical relative humidity conditions for microbiological growth in building materials were defined by Johansson and collaborators , who established that depending on the group of materials (wood, concrete and others) the fungal growth could occur in values of minimum relative humidity of 75% and maximum of 95%. Polizzi and collaborators analyzed the metabolic response of some species of fungi found in indoor air under different environmental conditions and found that temperatures of 25 °C and 30 °C, as well as values of relative humidity in the range of 97-100% were considered ideal conditions for the growth of *Penicillium* spp., *Aspergillus* spp. and *Periconia* spp. Fungal growth in building materials was observed at temperatures and relative humidity values of 5 °C to 91% RH, 10 °C in 90-95% RH, 20 °C in 86-90% RH, and 25 °C in 78 -86% RH (Nielsen *et al.*, 2004). Once present in the environment and compromising air quality, the determination of a limit value of fungal concentration that is not enough to cause health risks to exposed individuals would be extremely relevant. Nowadays, the existence of an international standard guide concerning the maximum acceptable levels and values of fungal bioaerosols related to good air quality in indoor environments has not been established.(Rao *et al.*, 1996) reviewed and compared the regulations and quantitative standards for fungi in existing indoor air environments and concluded that a better characterization of fungi sources for these sites as well as more data related to the effects of acute and chronic exposure to these pathogens could be of great assistance in the elaboration of a reliable standard document. According to the American Conference of Governmental Industrial Hygienists (ACGIH), the absence of data related to the exposure and response to concentrations of bioaerosols, as well as the non-availability of a standard collection method in analyses of fungal bioaerosols make it difficult to draw up a common standard (ACGIH, 2009). A review containing some recommendations from governmental and private organizations about concentrations of fungi in the air can be found in the review published by (Rao *et al.*, 1996). At present, there is an establishment of values of acceptable concentrations for various fungal bioaerosols whose variation depends on the country in question.

Fungi are a heterogeneous group of organisms including true fungi (Kingdom Eumycota or Fungi), lichens (a fungus and an alga in symbiotic relationship), the true slime molds (Myxomycetes, Kingdom Protozoa), and the water molds (Oomycetes, Kingdom Chromista). At present the latter two are sometimes referred to as fungi-like organisms due to their phylogenetic differences from the true fungi. Frequently, they are still called fungi, since they

are traditionally studied by mycologists and covered in mycology. Fungi as a group inhabit a wide range of niches and environments from plants, plant debris, soils, and animals to exposed rock (some lichens), rivers and lakes (aquatic fungi), the sea (marine fungi), the North Pole, and the tropics. They have developed many different modes of obtaining nutrients. Fungi function in both beneficial and detrimental ways from a human perspective. Some fungi, such as powdery mildews and rusts, are obligate parasites of plants. Some fungi exist in symbiotic relationships with plant roots to form mycorrhizae or with algae to form lichens. Some fungi are able to break down or detoxify wastes and other pollutants. A large number of fungi survive as saprobes recycling nutrients in ecosystems, such as carbon, nitrogen, and sulfur. Unfortunately, some of these saprobes have been found to grow in the indoor environments of buildings and have led to building related complaints and illnesses. Samson estimated that 100–149 species occurs indoors, while Miller and Day found approximately 270 species recovered from the dust indoors. Li and Yang found that over 600 species were identified from samples collected from indoor environments in North America based on the information in a database of a commercial laboratory. However, the exact number still remains unknown. Likely it will exceed 600 species, as occasionally a new record is discovered from samples collected from the indoor environment. A majority of indoor fungi are anamorphic fungi. Fungi develop and release their spores into the air for dispersal. Human beings are often exposed to fungi by inhalation of airborne fungal spores, hyphal fragments, and fungal by-products, especially in indoor environments. Certain fungi have been associated with asthma and respiratory conditions.

Fungi produce a variety of secondary metabolites, including mycotoxins and some fungal volatile organic compounds (VOC's), also known as microbial VOCs (MVOCs). Mycotoxins are harmful to animals and humans and are suggested as a major possible human health risk factor in buildings with mold problems. In addition to mycotoxins, some MVOCs produced by actively growing fungi as both primary and secondary metabolites are known irritants or hazardous chemicals. They may pose a health risk to building occupants and workers handling fungal matter. This chapter reviews the existing literature on airborne fungi with an emphasis on indoor fungal growth and contamination as well as the principal human health effects of exposure to fungi, mycotoxins, and other by-products. It is important to note that bacteria (including actinomycetes), mites, insects, and other microbes may also grow in a wet, damp indoor environment.

A wealth of literature on outdoor airborne fungi can be found in reviews by Gregory, Flannigan et al., Lacey, and Levetin. It is important to note that outdoor airborne spores are

often the source of indoor fungal spora. Their impact on indoor airborne fungal populations could be immediate or delayed until they have settled and colonized an indoor environment. Sampling of airborne and indoor fungi is not covered in this chapter. For any issues or questions related to sampling of indoor and airborne fungi.

AIRBORNE FUNGAL POPULATIONS

It must be emphasized that it is to the selective advantage of fungi to release, disperse, and disseminate their spores and occasionally hyphal fragments or partial conidiophores in air from one location to others. They cannot survive and complete their life cycles by staying afloat in air for an indefinite period of time. The dispersal of fungal spores can be short or long distance. The majority disperse their spores over a short distance. Therefore, when discussing fungal contamination, identifying and locating the source of fungal colonization is often of higher importance than assessing airborne fungi data. In addition to airborne dispersal, some fungi rely on running water, insects, and animals for dispersal of their spores. A large collection of literature on assessing indoor fungal populations has been accumulated. The majority of the literature was based on air sampling data. These include hospitals and health care facilities, residential dwellings, schools, and office buildings. The focus of hospital sampling was often on *Aspergillus* species, including *A. fumigatus*, an opportunistic human pathogen. General fungal populations were identified in nonhospital sampling.

A comprehensive assessment of fungal contamination in the indoor environment should include consideration of factors such as outdoor air, air conditioning, heating and ventilation systems, ventilation mode, heating, occupant density, ventilation rate, moisture (including water damage, high relative humidity [RH] in the air, and dampness), maintenance, on-site inspection, air sampling, surface and source sampling, sample analysis, risk analysis, and finally remedial actions. Unfortunately, the majority of investigations fail to follow the approach of comprehensive assessment, often due to insufficient strategy, labor, time, and funds as well as understanding of environments and ecosystems indoors.

An alternate approach to identifying indoor fungal contamination is to focus on inspection and surface/source sampling.

Factors Affecting Airborne Fungal Populations

Three important factors that directly affect airborne fungal populations are the availability of food/substrates and free water for fungal growth and the methods of spore dispersal.

Other physical, chemical, and biological parameters affecting fungal growth, and subsequent airborne fungal populations, can be found in recent references.

Substrates (Including Water Activity).

Fungi are achlorophyllous and heterotrophic, take up nutrients by absorption from substrates, and require simple sugars, carbohydrates, and other organics, such as vitamins, amino acids, and essential mineral elements to survive.

In the natural environment, fungi have developed a number of ways to obtain these nutrients, such as necrotrophic, symbiotic, and saprotrophic relationships.

Humans share food, living space, environments, and resources with fungi. We utilize fungi to produce bread; mantou (steamed bun); cheese; edible mushrooms; alcoholic beverages; and useful by-products, such as antibiotics, enzymes, and organic acids. Some fungi cause food spoilage or make food toxic to humans. *Botrytis cinerea* is a well-known pathogenic fungus causing gray mold disease on grapevines, strawberries, and many other fruits and produce. Species of *Penicillium* and *Aspergillus* often cause spoilage in foodstuffs and make them inconsumable to animals and humans. On the other hand, *Penicillium camemberti* and *P. roqueforti* are used in cheese production. Fungi are also known to cause wood stains, wood decay, biodeterioration, and biodegradation of polymers, carpet, plaster, drywalls, wallpaper, paints and organic coatings, fuels and lubricants, leather, fabric products, paper, and wood products. These references underscore the very likelihood that fungi can and will grow in artificial environments. Consequently, controlling nutrient sources to limit fungal proliferation/growth is practically impossible.

One of the critical factors affecting indoor fungal growth is water. There are a number of ways to measure water availability in materials. Water or moisture content of a material is expressed as a percentage of the oven dry weight. However, water content does not suggest the actual availability of free water in the material to fungi. A better measurement of water availability to fungi is water activity. Water activity is numerically equal to equilibrium relative humidity (ERH) expressed as a decimal. If a sample of substrate is held at constant temperature in a sealed enclosure until the water in the sample reaches equilibrium with the water vapor in the enclosure, then $a_w = \text{ERH}/100$. Another expression is: $a_w = \frac{1}{4} \frac{\text{vapor pressure of water in substrate}}{\text{vapor pressure of pure water}}$. A detailed discussion of water activity of fungi in food and materials is presented by Gravesen *et al.*, Hocking and

Miscamble, Li and Yang, Smith and Onion, Troller and Scott, Adan *et al.*, and Huinink and Adan.

Many common indoor fungi are hydrophilic and require a_w near 1 for growth. Some xerophilic fungi, however, have optimal water activity ranging from 0.65 to 0.90. Both mesophilic and xerophilic fungi can be found in the indoor environment.

It should be noted that a very short period of peak humidity, even below saturation RH, may lead to fungal growth. Adan *et al.* showed that it took only 69 h for *Penicillium chrysogenum* Thom to develop from spore germination to sporulation and 73 h to develop mycelial mass on pure gypsum at 21°C and 97% RH observed with cryo-SEM.

Fungal Spore Discharge Mechanisms

Fungal spores are released by two basic mechanisms: active spore discharge and passive spore release. Concentrations of certain airborne fungal spores have been known to peak during certain hours of the day or night. This periodicity is related to spore discharge mechanisms and environmental factors in nature. Details of these two mechanisms and environmental factors affecting spore release are presented by Lacey and Levetin.

Fungi with active spore discharge include such common airborne fungi as *Sporobolomyces*, *Epicoccum*, *Nigrospora*, and some smut-like yeasts. Many ascospores and basidiospores also have active discharge mechanisms. *Sporobolomyces* and some basidiospores are usually most abundant at night or in the predawn hours. Their spore release requires the absorption of moisture to build-up release pressure or forming a droplet at the hilar area. Dry spore fungi, such as *Aspergillus*, *Penicillium*, and *Cladosporium*, are often hydrophobic. They become airborne by passive force, such as air movement or rain droplets. *Cladosporium* usually dominates the airborne spore population during the day. Its spores stay airborne owing to the buoyancy of warmer air.

Some fungi, such as puffballs (*Calvatia*, *Lycoperdon*, *Scleroderma*) of basidiomycetes, are able to produce a huge number of spores and release their spores in “spore clouds or puffs” when affected by raindrops, humans, or small animals. The spore clouds may persist for a period of time until air mixing and dilution disperse them. Results of air sampling can be greatly affected by whether the spore cloud has dispersed.

Many fungi that are frequently detected indoors and outdoors produce spores in a slimy mass. These include such common indoor contaminants as *Acremonium* (although some species of *Acremonium* produce dry spores), *Aureobasidium*, *Fusarium*, *Phoma*, *Stachybotrys*, *Trichoderma*, and yeasts.

Slimy spores may be released into the air when they become dry, disturbed, or attached to other particles. Their dissemination is often assisted by insects, mites, small animals, or water. Because slimy spores do not become airborne easily, their detection indoors should be considered significant. Any detection of *Stachybotrys* in air samples should be taken indoors.

Airborne Diseases

Airborne diseases refer to the grouping term for those human diseases that are caused by organisms that can be transmitted by the air an important environmental aspect for global public health. The mucous membrane of the respiratory system is a specific type of a 'gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO_2 reacts with water that is present in the respiratory system, creating H_2SO_4 , which irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases.

Bioaerosols may, among other things, carry microbes that penetrate organs via the respiratory system. After settling, microbes from the air may find their way onto the skin or, carried by hands, get into the digestive system (from there, carried by blood, to other systems, e.g. the nervous system). Fungi that cause skin infections, intestinal bacteria that cause digestive system diseases or nervous system attacking enteroviruses are all examples of the above.

Opinions on the importance of airborne infection have swung over the centuries from extremes of belief to extremes of disbelief. Galen, (1943) in the second century, is credited with the magnificent aphorism: “When many sicken and die at once, we must look to a single common cause, the air we breathe,” and Chapin, after an extensive survey of available evidence in 1910, concluded: “Without denying the possibility of such (airborne) infection, it may be fairly affirmed that there is no evidence that it is an appreciable factor in the

maintenance of most of our common contagious diseases.” Chapin made a grudging exception in the case of tuberculosis: “It is assumed that tuberculosis, as it occurs in human beings, is usually an air-borne disease, and there is more reason for such an assumption concerning this than concerning most diseases.” Tuberculosis remains the most characteristic and well documented infection which is airborne from man to man, but epidemiologic studies point strongly toward airborne transmission of many other infections, particularly those caused by viruses in the respiratory tract.

The sources of human airborne pathogens are the respiratory secretions of infected people. These secretions harbour large numbers of organisms and contaminate the environment in several ways. The patient’s infecting organisms can often be cultured from skin and bedclothes, so the possibility exists of transfer to a susceptible person by direct contact or fomites. The patient also contaminates the air by coughing, sneezing, spitting, singing and even talking. The size of the droplets expelled into the air varies over a wide range. The larger droplets contain more pathogenic organisms than the smaller ones, but settle out of the air quickly. They can impinge on the skin of a recipient at close range and can be deposited in the upper respiratory tract if inhaled. Transmission by the larger respiratory droplets is an extension of the concept of direct contact.

The importance of the smallest respiratory droplets in the transmission of infection has been emphasized. These droplets evaporate instantly when expelled into the air. The dried residues which remain, called droplet nuclei, are so small (1 to 3 μm) that their tendency to settle is negligible. They go wherever the air goes. They disperse throughout the air of a room and are carried on air currents throughout a building. Transmission of infection from man to man by droplet nuclei is an indoor phenomenon, being limited to confined atmospheres in which the concentration of infectious particles can reach levels which constitute a hazard to susceptible people sharing the same air supply. The organisms in droplet nuclei remain airborne and

potentially infectious until they die, are vented to the outdoors or are killed. When inhaled, they are deposited both in the upper respiratory tract and in the lungs.

The precise mechanism of transmission of respiratory tract infections in man is of great practical importance since control measures may be specific for a single mechanism. For example, droplet nuclei can be controlled by techniques of air disinfection which do not affect larger respiratory droplets (Riley *et al.* 1962).

The Survival of Microorganisms in Air

The survival of microorganisms in air depends on physicochemical and climatic characteristics (such as humidity, gases, and temperature) and on their ability to form spores. A general classification of survival power in the aerosol state consists of two main groups:

- (1) Hardy microbes, fungi, spore forming bacteria, and encysted protozoa
- (2) Tender pathogens, which commonly spread among individuals in close proximity, including inhaled pathogens.

Health impacts of air pollution

Road vehicle emissions are one of the most important sources of human exposure to air pollution. Air pollution concentrations on roads are relatively high, and commuters face unavoidable exposure during commuting as they are near the source of emission. The deleterious effects of traffic-related atmospheric air pollution on health have been documented in many studies worldwide. Emissions from road traffic result in a complex mixture of harmful air pollutants. In many areas, vehicle emissions have become the dominant source of air pollutants, including carbon monoxide (CO), carbon dioxide (CO₂), volatile organic compounds (VOCs), hydrocarbons (HCs), nitrogen oxides (NO_x), and particulate matter of aerodynamic diameter 2.5 μm (PM_{2.5}) (Zhang and Batterman, 2013).

These toxic particles easily penetrate human airways. Inhaled PM_{2.5} can reach the lung alveoli and induce local and systemic responses in the body, impacting cardiovascular and respiratory function (Brook *et al.* 2010). Thus, traffic-related air pollution has been implicated in a range of illnesses related to respiratory diseases and cardiovascular complications. From a public health perspective, the health effects of air pollution are both chronic (long term) and acute (short term). Breathing in high quantities of exhaust fumes can cause short-term irritation to the respiratory tract within a few minutes of exposure. Short-term inhalation of air pollutants may exacerbate ongoing irritation such as cough, mucous buildup, and inflamed airways. Although the acute, short-term effect is of the least concern to the general public, over time, long-term exposure eventually merges with the chronic effect. Of concern, prolonged exposure over many years places tremendous stress on the body and can be detrimental to human health.

Air pollution is now the world's largest environmental health risk (Cheng, 2018). In 2015, the WHO released a report stating that at least one in eight deaths worldwide is caused by air pollution. On a global scale, air pollution accounts for an estimated 9% of deaths due to lung cancer, 17% due to chronic obstructive pulmonary disease, more than 30% due to ischemic heart disease and stroke, and 9% due to respiratory infections (WHO, 2014). It is estimated that approximately 80% of the world population lives in environments with pollution levels exceeding the air quality guideline (AQG) established by the WHO (Van Donkelaar *et al.* 2010). It is also a well-established fact that air pollution shortens life expectancy. The high level of particles in air pollution is related to lung cancer risk, cardiovascular disease, and mortality. In many countries in Asia, concentrations of ambient air pollutants exceed levels associated with increased risk of acute and chronic health problems. In particular, studies have shown that exposure to air pollution in traffic has been associated with chronic health effects, particularly cardiovascular and respiratory diseases (Adar *et al.* 2008; Peters *et al.*

2004). Much evidence has also been found showing the high prevalence of respiratory disease symptoms and asthma exacerbation among those who reside near high-traffic roads (Shima *et al.* 2003; Brauer *et al.* 2002).

On the Asian continent, China, as one of the fastest developing countries, is battling the health impact associated with air pollution. Annual average PM_{2.5} concentrations in Chinese megacities exceed the WHO's guideline of 10 µg/m³, and the corresponding black carbon (BC) concentrations are approximately 5 µg/m³. The primary sources of air pollution in China are industrial output, coal and biomass combustion, and traffic. Nevertheless, a recent report noted that emissions from heavy urban traffic are the main contributors to urban air pollution in China (Ji *et al.* 2016). The 2010 Global Burden of Disease reported that exposure to air pollutants is the fourth leading health risk factor for Chinese people (Yang *et al.* 2010). Likewise, air pollution in China is also associated with elevated rates of mortality whereby an estimated 350,000 to 500,000 premature deaths were reported to be linked to air pollution (Chen *et al.* 2013). Similarly, in Jakarta, one of the most polluted cities in the world, air pollution was largely associated with motor vehicles, particularly emissions largely created by diesel vehicles (Resosudarmo and Napitupulu, 2004).

Physical and psychological health impacts on public transport commuters

From June to October 2016, a total of 800 public transport commuters in urban cities in the heart of Selangor, Malaysia, were interviewed face to face. The commuters were approached at the Light Rail Transit train stations along the Kelana Jaya and Ampang lines in the state of Selangor, Malaysia. In the interview, self-reported adverse health effects (both physical health, 15 items, and psychological health, 7 items) associated with exposure to atmospheric air pollutants during the daily commute were queried. Self-reported control measures used by participants to mitigate their exposure to atmospheric air pollutants were also assessed.

The majority (56.5%) of respondents were aged 30 years and below (mean age, 30.8 years; standard deviation, 6.6; age range, 18–54 years). The ethnic and gender distribution of the study participants closely matched that of the general Malaysian population. A total of 41% of the study respondents spent more than 1 hour commuting by public transport daily. When the respondents were queried on adverse physical health effects they experienced from exposure to air pollution during their daily commute, as shown in Figure 2, the highest percentage reported physical fatigue or weakness (35.5%), followed by coughing (23.6%). A considerable proportion reported headache (16.1%), light-headedness (14.6%), and breathing difficulties (14.5%). Air pollution has a wide range of effects on human health. This study evidenced the experience of various physical health impacts commonly associated with exposure to atmospheric air pollutants by daily public transport users.

CHAPTER THREE

MATERIALS AND METHOD

3.0 Study Design

University of Benin shuttle bus, is located inside University Of Benin City, Edo state in South Nigeria (latitude 6°20.022'N 5°36.009'E / 6.333700°N 5.600150°E).

It is among the universities owned by the Federal Government of Nigeria and was founded in 1970.

3.1 Sample collection site/Study Design:

Air samples were collected in duplicate from the University of Benin shuttle buses. Sampling was done 10 mins before movement (where the passengers were not seated), 10 mins during movement (where the passengers were seated) and 10 mins after movement (when the passengers were already out) in the shuttle bus going to ring road and new benin in the morning and evening for 2 days, after which these samples were taken to the laboratory for incubation for 24hr at ambient temperature (Sekulska, 2007).

3.2 Location of Laboratory Work

The samples were analysed in microbiology laboratory, in the Department of microbiology, University of Benin.

3.3 Preparation of Media Used

The media used for this study include: Potato Dextrose Agar (PDA) for isolation of fungal species. The media was prepared aseptically following the manufacturer's instructions, sterilized at 12 °C, 15Psi for 15 minutes. The media was poured into petri dishes observing strict precautions to avoid contamination and therefore stored in an apparently sterile canister for further use.

3.3.1 Description of Media Used.

➤ **Potato Dextrose Agar (PDA):** is a general purpose medium for the cultivation of fungi, yeasts and molds that can be supplemented with antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products and testing cosmetics. PDA can be used for growing clinically significant yeast and molds. The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.

➤ **3.4. Isolation of fungi:**

➤ Serial dilution plate method was used for the isolation process of plant pathogenic fungi according to the method described by (Waksman, 1994). Sample dilutions were made by suspending 1g of each sample in 10ml of sterile water. Dilutions of 10^{-3} and 10^{-5} were used to isolate fungi in order to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Potato dextrose agar media and was performed in duplicates. Chloramphenicol (250mg) was added to the medium to prevent bacterial growth before pouring into the Petri dishes. The plates were then incubated at $28 \pm 2^\circ\text{C}$ for 7 days.

➤ **3.5. Identification of fungi**

The fungi isolated were identified using the morphological characteristics of the colony and microscopic examination according to the method described by (Barnett and Hunter, 1972). The colony length which includes the length and width of the colonies, the presence or absence of mycelium, the colour, wrinkles, furrows and any other pigment and the morphological characters were evaluated (Diba *et al.*, 2007).

➤ **3.5.2. Morphological characterization**

The morphological characterization of fungal colony on the various media inoculated were observed for their shape, name of colony, nature of hyphae and spore type.

CHAPTER FOUR

RESULTS

The fungal quality of indoor air of commuter busses (University of Benin shuttle bus) was evaluated in this study and the results are as follows;

Table 4.1 shows the mean difference in fungal count of air sample of University of Benin shuttle bus before, during and after a trip at morning, afternoon and evening. The result showed that there was no significant difference in fungal count of morning and afternoon samples irrespective of whether it was before or during the trip. However afternoon and evening however showed significant difference.

Figure 4.1 shows the mean fungal count of air samples from University of Benin shuttle buses. The result revealed that the highest fungal count of $6.25 \times 10^3 \text{cfu/m}^3$ was observed in a afternoon sample after the trip of the second day, closely followed by $5.66 \times 10^3 \text{cfu/m}^3$ observed after trip in the evening of the second day while the least fungal count ($8.6 \times 10^2 \text{cfu/m}^3$) was observed in the evening of the first day before the trip.

Table 4.2 shows the cultural and morphological characteristics of fungi isolates. The result revealed that were present in air samples.

Table 4.3 shows the distribution pattern of fungi isolates. The result showed that both were present before, during and after the trip as well as being present at morning, afternoon and evening.

Figure 4.2 shows the percentage occurrence of fungi isolates. The result showed that was most prevalent with a percentage of , closely followed by with a percentage of . The least occurrent was with percentage of .

Table 4.3 shows us the antifungal susceptibility to voriconazole.

Table 4.1: Mean difference in fungal count of University of Benin commuter shuttle buses

$\times 10^3 \text{cfu/m}^3$

	DAY 1 (RING ROAD)			DAY 2 (NEW BENIN)		
	Before	During	After	Before	During	After
Morning	1.53±0.17aA	1.53±0.06aB	2.28±0.22aC	2.16±0.28aA	2.48±0.39aB	5.07±0.50aC
Afternoon	2.55±0.28bA	3.03±0.28aB	3.81±0.50bC	4.99±0.39aA	5.66±0.44bB	6.25±0.94bC
Evening	0.86±0.00bA	1.06±0.17bA	1.41±0.11cB	3.03±0.61bA	3.50±1.17cB	5.11±0.78cC

Key: Superscript abc

Superscript \overrightarrow{ABC}

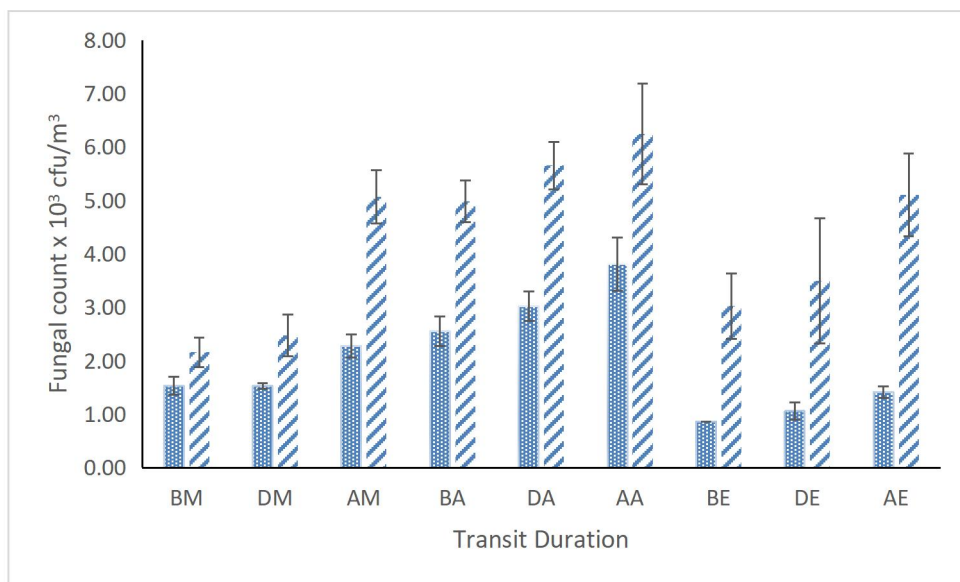


Figure 4.1: Total fungal count of University of Benin shuttle bus air

Key:

BM: Before transition in the Morning when there was no passenger in the bus

DM: During transition in the Morning when passengers were in the bus

AM: After transition in the Morning when the passengers were out of the bus

BA: Before transition in the Afternoon when there was no passenger in the bus

DA: During transition in the Afternoon when passengers were in the bus

AA: After transition in the Afternoon when the passengers were out of the bus

BE: Before transition in the Evening when there was no passenger in the bus

DE: During transition in the Evening when passengers were in the bus

AE: After transition in the Evening when the passengers were out of the bus

Table 4.2: Cultural and morphological characteristics of fungal isolates

Morphological characteristics
Blue green septate
Black cottony, reverse is yellow
Yellowish cottony
Pink septate tree like
Greenish
Dark wall thick wall
Darkly coloured, waxy, spherical
Pale green cottony
Whitty waxy reverse brownish

TABLE 4.3 ANTIFUNGAL SUSCEPTIBILITY TEST

ISOLATES	VOR	NS
	-	1(1)
	10(10)	-
	6(6)	-
	-	-
	-	4(4)
	7(7)	-
	10(10)	-
	5(5)	-
	-	-

KEY:

VOR: VORICONAZOLE

NS: NON SUSCEPTIBLE

CHAPTER FIVE

DISCUSSION

The fungal quality assessment of indoor air study is one of the most vital investigations to determine the microbial indoor air pollution. The information on indoor microbial concentrations of airborne fungi is necessary to estimate both the health hazard and to create standards for indoor air quality control. The fungal quality of indoor air of commuter busses (University of Benin shuttle bus) was evaluated in this study. The results obtained indicate that there was significant difference in the number of fungal in University of Benin shuttle bus indoor air. The levels of fungi was found to be related to the population density as well as to the human activities and traffic in the studied zones. This conclusion is true when the crowded zones (During the trip) were compared to the zone of low or no human activity . Human activities seem to be the main generator of outdoor bioaerosols as indicated by many reports (Menteşe *et al.*, 2009; Ostro, 2004). Different human activities can contribute in generating or increasing the bioaerosol levels. These include shedding of skin cells, talking, coughing, and sneezing. Sneezing is one of the most vigorous mechanisms of spreading airborne microorganisms by generating as many as two millions of droplets per sneeze (Krishna, 2004). The present study tends to enumerate and isolate the fungal isolates found in some University of Benin shuttle buses and also to determine the antifungal susceptibility pattern of the fungal isolate on selected antifungal drugs.

As shown in Figure 4.1, the mean fungal count of air samples from University of Benin shuttle buses. The result revealed that the highest fungal count of $6.25 \times 10^3 \text{cfu/m}^3$ was observed in an afternoon sample after the trip of the second day, closely followed by $5.66 \times 10^3 \text{cfu/m}^3$ observed after trip in the evening of the second day while the least fungal count ($8.6 \times 10^2 \text{cfu/m}^3$) was observed in the evening of the first day before the trip.

The Exposure to bio-aerosols has become a significant public health concern. Currently, there is not a precise legislation in Nigeria on the microbiological quality of the air. In this context, the European Community Commission (ECC) (1993) which has proposed five different categories to evaluate the level of microbial contamination in the indoor air of non-industrial environments is adopted for comparison in this current study. From Table 2, it can be deduced that bacteria had a minimum value (785cfu/m³), maximum value (2422cfu/m³). Consequently, going by the ECC standard and grouping, the indoor air of the shuttle buses as a whole, shows a high level of microbial contamination as contamination levels of bacteria recorded as the range falls above the recommended microbial counts (<500cfu/m³).

Statistically, there was no significant difference in fungal count of morning samples irrespective of whether it was before, during or after the trip. Afternoon and evening however showed significant difference at 95% confidence level ($p < 0.05$).

Based on cultural, morphological and biochemical characterization of the fungal isolates were identified as fungi contaminants of indoor air of shuttle buses. This is similar with Two previous studies on fungal flora in air samples in Benghazi and Shahat in Libya conducted in 2010 reported higher concentrations of fungal colonies in the air conditioner in all locations, with *Penicillium* and *Rhizopus* in outdoor samples of Benghazi (Hamady, 2010), *Cladosporium cladosporioides* in indoor samples of Shahat (Ibrahim, 2010) predominating the isolated colonies.

The distribution pattern of fungi isolates showed that both and were present before, during and after the trip as well as being present at morning, afternoon and evening.

The percentage occurrence of fungi isolates shown in figure 4.2 revealed that was most prevalent with a percentage of 24%, closely followed by with a percentage of 20%. The least occurrent was with percentage of 7%.

CONCLUSION

In Conclusion, all buses sampled were heavily contaminated with fungi as they were above ECC limit of less than 500cfu/m³. Thus, attention must be given to control the factors which favors the survival and presence of fungi in the air to safeguard the health of commuters.