

**FUNGAL SPORE AIR POLLUTION: SEASONAL CONCENTRATION,
DIVERSITY AND ANTIFUNGAL RESISTANCE IN NAIROBI CITY
COUNTY, KENYA**

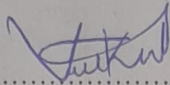
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156/CTY/PT/20840/2021**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD
OF THE DEGREE OF MASTER OF SCIENCE IN MEDICAL
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OF KENYATTA UNIVERSITY**

NOVEMBER, 2025

DECLARATION

This research thesis is my original work and has not been presented elsewhere for a degree award.

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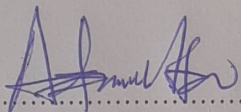
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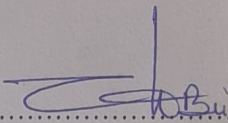
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ABBREVIATIONS AND ACRONYMS

AFR	Antifungal Resistance
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
CaCl₂	Calcium chloride
CDC	Center for Disease Control
CFU	Colony forming units
CLSI	Clinical and Laboratory Standard Institute
D	Dry season
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
IgE	Immunoglobulin E
KH₂PO₄	Potassium dihydrogen phosphate
MgSO₄	Magnesium sulphate
MIC	Minimum Inhibitory Concentration
NACOSTI	National Commission for Science, Technology and Innovation
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PM₁₀	Particulate matter of fewer than 10 micrometres
SDA	Sabouraud dextrose agar
SDS	Sodium dodecyl sulfate
SERU	Scientific ethics and review unit
SPSS	Statistical Package for Social Sciences
US	United States of America
W	Wet season
WHO	World Health Organization

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ABSTRACT

Poor air quality is associated with cardiovascular and respiratory diseases including, allergies, obstructive lung diseases, cancer, and even reduced life expectancy. According to WHO data, approximately 92% of the global population is exposed to air pollution, causing up to 6.5 million mortalities. Understanding and subsequent management of microbial air pollution have been a main focus for research. Several studies have reported various effects of Particulate matter (PM₁₀) on human health. However, limited information is available on the effects of airborne fungi on human health. Studies focusing on evaluating fungal air quality in urban environments to identify biological health risks and provide necessary information for control are limited. This work aims to determine fungal diversity and concentrations in the atmospheric air of Nairobi, Kenya, towards determining fungal air quality. Non-randomized sampling design was adopted, where sampling sites were categorized into 4 zones: (1) Traffic zones, i.e., roundabouts and major roads; (2) Commercial zones; (3) Recreation zones; Waste dumping zones. A total of 384 samples were collected; out of these, 192 samples were collected during the dry season (January to March), remaining 192 samples were sampled during wet seasons (June to July). Temperature, humidity, and wind speed data were recorded using the Accuweather mobile Application. The identified fungal isolates were enumerated using the Omelyansky formula (2013). Culture-dependent techniques were adopted for fungal identification and characterization. Moreover, their potential pathogenicity was tested by subjecting the isolates to temperature tolerance, hemolytic, and protease tests. Antifungal susceptibility tests were performed using the CLSI M38-A2 broth microdilution method against 3 azole antifungals; [fluconazole (FCZ), voriconazole (VCZ), and itraconazole (ITZ)], which are commonly dispensed antifungals. The relationship between the fungal concentration and meteorological parameters was analyzed using multiple regression and Pearson correlation. The wet season had the highest number of fungal spores (5318.88 CFU m⁻³) compared to the dry season (1929.58 CFU m⁻³). Consequently, we identified 502 isolates across two seasons; 16 genera and 38 species comprising *Ascomycota* 426(89.31%), *Basidiomycota* 21(4.40%), *Deuteromycota* 17(3.56%), *Muromycota* 7(1.47%) and *Zycomycota* 6(1.26%). The most isolated fungal species included *Candida* (17.13%), followed by *Penicillium* 66(13.15%), *Fusarium* 62(12.35%), *Aspergillus* 61(12.15%), and, *Cladosporium* 60(11.95%). Temperature, humidity, and windspeed significantly affected airborne fungal concentration ($p=.000$), ($p=.0280$), and ($p=.000$), respectively. Pearson correlation analysis showed that temperature negatively correlated with the fungal concentration significantly ($p=.000$). Humidity had significant positive correlation with fungal concentration ($p=.001$) while the wind speed negatively correlated with the fungal concentration significantly ($p=.000$). A total of 33/58(56.89%) fungal displayed growth at 37°C. Extracellular proteases production was evident in 15/58(25.86%) isolates. After hemolysis, 12(20.69%) isolates were capable of beta (complete) hemolysis, 39(67.24%) showed alpha (partial) hemolysis and 7(12.07%) displayed gamma or no hemolysis. Among the characterized isolates, most of the airborne fungal isolates tested were susceptible to voriconazole and itraconazole. However, resistance against fluconazole was observed among 4/18 (22.22%) of all the isolates tested. Therefore, our findings provide insightful information about the concentration of airborne fungi in relation to human activities, location in relation to weather patterns in Nairobi. It highlights the significance of fungal spore pollution antifungal resistance and their significance in the burden of respiratory conditions and climate change.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Fungi are ubiquitous and constitute a crucial part of airborne microflora that have also been widely distributed in soil, water, and decaying vegetation (Ursula *et al.*, 2008; Chakrabarti *et al.*, 2012; Nageen *et al.*, 2021). The atmospheric air is an environment that is in contact with all living populations on land, and the degree of its contamination markedly affects the health and well-being of the population (Donderski *et al.*, 2005; Yan *et al.*, 2016; Stewart *et al.*, 2021).

Fungal diversity in the world is estimated to be 1.5 million species, of which only about 100,000 have been described (Esparza *et al.*, 2016; Ndwiga *et al.*, 2016). The airborne fungi have been documented to cause severe health complications in humans and animals (Fang *et al.*, 2005). Outdoor air is significant as it determines the levels of fungal spores in indoor air, ultimately causing fungal infections (Shams-Ghahfarokhi *et al.*, 2014; Yamamoto *et al.*, 2015). In recent decades, the global population has primarily transitioned from a rural life to urban life, with over half of this population currently predominant in the urban environment; this has been projected to rise to 70% by 2050 (Flies *et al.*, 2020).

City life provides individuals with many opportunities, ranging from easy access to public amenities and utilities to readily available platforms for diversity in culture, business, and human interaction. As a result of densely populated urban cities, the entire ecosystem is substantially modified, resulting in negative impacts on its inhabitants. Air pollution in megacities of developing nations is a public health problem with increasing importance due to the adverse health effects on humans, animals, and plants (Shelton *et al.*, 2002; Fernstrom and Goldblatt, 2013; Be *et al.*, 2015; Baxi *et al.*, 2017;

Matos *et al.*, 2020). Notably, microorganisms (fungi, viruses, and bacteria) from environmental sources may disperse over wide distances by air currents and ultimately inhaled, ingested, or come into contact with individuals without prior contact with the infectious source (Baxi *et al.*, 2017). Information on the effects of microbial exposure in the urban environment on human health is limited.

The significance of airborne fungal contaminants can dramatically increase the health hazards triggered by the fungal spores or their metabolites (Burge, 2001; Baxi *et al.*, 2017). Besides the risk for invasive infections, fungal spores potentially induce allergenic and toxigenic conditions, (Simon-Nobbe *et al.*, 2008; Rodrigues and Albuquerque, 2018). Fungal spores exhibit substantial amounts of fungal toxic secondary metabolites, including mycotoxins which are toxic when inhaled from airborne dust, contact or biological aerosols (Araujo and Cabral, 2010). Similarly, fungal volatile organic compounds concentrated outdoors affect human health, causing lethargy, headache, and irritation of the eyes and mucous membranes of the nose and throat (Swanson, 2001; Araujo and Cabral, 2010).

Fungi; *Aspergillus*, *Candida*, *Penicillium* *Blastomyces*, *Coccidioides*, *Cryptococcus*, and *Histoplasma* species, are well-documented, potentially life-threatening airborne contaminants upon inhalation or ingestion (Hardin *et al.*, 2003; Vonberg and Gastmeier, 2006; Jalili *et al.*, 2021). Similarly, healthy individuals continuously working in polluted environments and school going children have displayed hypersensitivity reactions, including rhinitis, sinusitis, wheezing, or asthma upon fungal exposure (Tang, 2009; Liu *et al.*, 2014). Fungi and their spores are generally more resilient than viruses and bacteria, withstanding significant stresses resulting from dehydration, rehydration, and UV radiation (Karra and Katsivela, 2007). Few studies have been conducted to determine the specific characteristics of airborne microorganisms in urban

atmosphere. Therefore, there is a need for more research to understand the role of fungal spore diversity and its potential impact on human health. The purpose of this study was to determine fungal air pollution in Nairobi County in relation to fungal diversity, concentration, pathogenicity, and resistance to azole antifungals.

1.2 Statement of the Problem

Global disease burden is directly or indirectly linked to air pollution. Realizing that many respiratory diseases are caused by fungi from outdoor environments is the initial step in tackling them. The WHO estimates that over 92% of the global population is exposed to polluted air with microbial aerosols containing allergenic microorganisms, including fungi that cause respiratory diseases (Kirby, 2016; Matos *et al.*, 2020). This is largely attributed to rapid urbanization. An International study of asthma and allergies conducted in several centers globally discovered an increased prevalence of asthma in Africa, with Nairobi and Kenya accounting for 18%. Due to the increasing incidence of asthma and concern over exposure to fungi and their metabolites, research on airborne fungi is attracting interest (Burge *et al.*, 2000). Exposure to fungal metabolites can potentially triggers adverse effects, including gastrointestinal, dermatologic, respiratory, and allergy reactions (Sharma *et al.*, 2017). While acknowledging research efforts toward combatting fungal infections, investigations on airborne fungal diversity in urbanized ecosystems are largely underestimated (Nagai *et al.*, 2003).

In contrast with microbes inhabiting soil and aquatic habitats, airborne microbial communities have not been adequately characterized (Be *et al.*, 2015). Furthermore, antifungal resistance is an emerging clinical concern across the globe. Several common fungal species have developed azole resistance, including *Aspergillus fumigatus* and, recently, *A. flavus*, causing aspergillosis (Duong *et al.*, 2020). Thus, there is an urgent need to intensify isolation and characterization of airborne fungal species in highly

vibrant cities to understand the diversity of pathogenic or allergenic fungi and their capacity to influence human health (Burge, 2001). As the largest cosmopolitan city in Kenya and the most robust socioeconomic destination in East Africa, Nairobi has a population of approximately 5.1 million (The World Bank, 2022). The city houses one of the largest informal settlements on the continent. In this regard, it is important to investigate airborne fungal diversity, concentration, and potential resistant fungal strains. The findings will add to the stock of knowledge on fungal spore pollutants, Antifungal Resistance (AFR) status, influence of weather/climate change monitoring and public health threat.

1.3 Justification

Humans are mostly on the receiving end of negative effects of components in the air. The diversity of fungal spores in the air is vast (Frö Hlich-Nowoisky *et al.*, 2009). Fungi can be isolated in all habitats, and their proliferation is determined by temperature, geographical location, moisture, wind substrate type, and human activities (Burge *et al.*, 2000; Sharma *et al.*, 2017; Jalili *et al.*, 2021; Nageen *et al.*, 2021). Approximately 1 billion people suffer from acute or chronic respiratory infections globally, out of which 5.8 million succumb, and 50% of these deaths occur in sub-Saharan Africa (Jamison, 2006; Seidu *et al.*, 2019). Besides, Nairobi account for up to 18% of all reported asthma and allergy cases in Africa (Erhabor, 2021; Menezes *et al.*, 2004). Nevertheless, limited information is available on the diversity of airborne fungi in urban settings and their health implications (Be *et al.*, 2015). Airborne fungi are responsible for respiratory infections such as allergic rhinitis, asthma, and inflammation, and some have also been associated with lung and liver cancers (Hu *et al.*, 1997; Tham *et al.*, 2019; Nageen *et al.*, 2021). This has caused significant health and economic burden on global population, specifically the developing world.

Recent research has confirmed the relationship between fungal exposure to *Alternaria*, *Aspergillus*, *Penicillium*, and *Trichoderma* spp and patients with asthma and allergic rhinitis (Horner *et al.*, 1995; Yamamoto *et al.*, 2015). This has consequently prompted intense studies on the presence and types of fungal spores in various environments. However, the ideal burden of fungal infections is unknown largely due to the absence of systematic diagnosis and data collection (Guto *et al.*, 2016). In this regard, there is a renewed push for understanding airborne microbial communities, particularly fungi, due to their potential impact on human and ecosystem health in major cities of the world (Matos *et al.*, 2020; Korneykova *et al.*, 2021).

Although certain genera of fungi studied in the atmosphere have been investigated, research on airborne fungi specific to cities and high-octane economic regions is limited (Menezes *et al.*, 2004; Be *et al.*, 2015). Research targeting airborne fungi in an urbanized ecosystem is necessary to provide sustainable measures that reduce fungal allergens associated with allergies, asthma, and other major diseases (Menezes *et al.*, 2004; Zhai *et al.*, 2018; Moelling and Broecker, 2020). The present study explored the diversity and distribution of airborne fungi in Nairobi. Also, we elucidated their potential pathogenicity and resistance to azole antifungals. The findings contribute to formulating of policies that help manage public health.

1.4 Hypothesis

- (i) There is no significant difference in airborne fungi concentration in selected sites in Nairobi, Kenya.
- (ii) There is no significant impact of the environmental factors on the concentration of airborne fungi.

(iii) There is no existent of circulating azole-resistant fungal isolates in urban sites of Nairobi

1.5 Objectives

1.5.1 General Objective

To determine the fungal spore air pollution: concentration, diversity and drug resistance in Nairobi city county, Kenya.

1.5.2 Specific Objectives

- i) To determine the levels of airborne fungal diversity in Nairobi, Kenya.
- ii) To determine the impact of environmental factors on the concentration of airborne fungi in Nairobi, Kenya.
- iii) To determine the antifungal drug resistance from fungal isolates recovered from the urban sites in Nairobi County.

1.6 Significance of the Study

Due to the limited information on fungal air pollution and its impact on human health, this study aims to bridge that gap by identifying airborne fungal concentrations in Nairobi and determining their potential pathogenicity and resistance to azole. The findings of this work help in providing valuable data for formulating effective interventions for reducing urban fungal air pollution. This will further provide strategies geared toward combatting respiratory diseases of fungal origin.

CHAPTER TWO

LITERATURE REVIEW

2.1 Atmospheric Microbial Diversity in Urban Habitats

The atmosphere is both the source and sink of microorganisms serving as a platform where the microbes hover around different reservoirs in urban areas, even in distant ecosystems (Bowers *et al.*, 2011). For instance, African and Caribbean dust has been shown to move bacteria and fungi to oceans (Alberti, 2014). Microorganisms are abundant and diverse residents of the atmosphere representing the fundamental component and exerting both positive and negative effects on the health of the population and the general ecosystem (Bowers *et al.*, 2013; Alberti, 2014; Stewart *et al.*, 2021). Nonetheless, there is a limited understanding of the diversity and composition of the environmental microorganisms (Bowers *et al.*, 2013). From a health standpoint, city aerobiomes, in contrast with their rural counterparts, have been linked to human infections, including asthma and allergies. This could be potentially attributed to the microbial diversity and abundance of rural air compared to urban environments (Flies *et al.*, 2020). However, limited studies have explored the relationship between microbiomes from rural-urban atmospheric and human health (Madeline, 2021).

On the other hand, studies have also shown that some atmospheric microbes have protective effects; for instance, early exposure to some microbes can elicit an immune function that suppresses allergies, inflammation, and asthma (Flies *et al.*, 2020). Notably, the diversity of microorganisms in an urban environmental is not constant but depends on various physiologic factors, including temperature, humidity, available source of energy, and, importantly, human activities (Burge, 2001; Jalili *et al.*, 2021; Nageen *et al.*, 2021). There are some claims that the type and composition of atmospheric microbes depend on land use; it is argued that the microorganisms

hovering above parking lots in the city are significantly distinct from those over urban parks, dumping sites, and areas with intense human activities (Madeline, 2021).

Through high-throughput sequencing, a study in Colorado, United States of America (USA), assessed the diversity of airborne microorganisms and discovered that bacteria dominated with about 70%, fungi with 21% and plants with 9% of all bioaerosols (Bowers *et al.*, 2013). Moreover, *Ascomycota* (78%) was the dominant fungal phylum, followed by *Basidiomycota* (21%). The, *Actinobacteria* (22%), *Bacteroidetes* (9.7%), *Firmicutes* (28.2%), and *Proteobacteria* (34.6%) were the dominant bacterial phyla (Bowers *et al.*, 2013). Elsewhere, a study investigated microbial diversity of urbanized aerosols in 2 US cities and reported approximately 1,800 different bacteria, including *Firmicutes*, *Acidobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Nitrospira* (Brodie *et al.*, 2007). A recent study in Tianjin, China, reported that genera *Cladosporium* spp (16%), *Alternaria* spp (7.9%), and *Aspergillus* spp (6.1%) dominated the fungal communities in the atmosphere on non-rainy days (Niu *et al.*, 2021).

Furthermore, atmospheric mycological analysis in two big cities in Moscow (temperate) and Murmansk (Subarctic), revealed that *Trichoderma* *Talaromyces* *Aspergillus* spp and *Penicillium* spp were the dominant genera of fungi, half of which were potentially pathogenic (Korneykova *et al.*, 2021). Besides, the most pathogenic fungal species, including *Paecilomyces variotii*, *Aspergillus fumigatus*, and *Aspergillus flavus*, were isolated from the atmospheric space of residential areas and subways, particularly in Moscow (Korneykova *et al.*, 2021). A study in Fortaleza city, Brazil, reported 1521 isolates of airborne fungi with *Alternaria* spp, *Aspergillus* spp, *Penicillium* spp, *Mycelia sterilia*, and *Fusarium* spp dominating all seasons (Menezes *et al.*, 2004). Furthermore, a study in Nigeria found *A. flavus* (7.93%), *A. sydowii*

(10.37%), and *A. niger* (14.47%) as the dominant fungal species (Odebode *et al.*, 2020).

In Mali, 3 genera of fungi, including *Aspergillus* spp, *Cladosporium* spp, and *Alternaria* spp, were detected from dust samples and considered cosmopolitan fungi (Kellogg *et al.*, 2004).

Among these, *Cladosporium* spp was non-pathogenic, whereas the other two genera are pathogenic, causing skin and pulmonary infections in immunocompromised individuals (Kellogg *et al.*, 2004). All these fungi, including *Aspergillus flavus*, *A. fumigatus*, and *Alternaria alternata*, were also detected in the Western Desert of Egypt (Ismail and Moharram, 2002). Polluted air poses a substantial risk to human health, causing cardiovascular and respiratory infections (Moelling and Broecker, 2020). Therefore, it is important to understand the atmospheric microbial community in an urbanized environment, specifically the fungal composition, to better combat these diseases. The present study sought to study the diversity and concentration of the airborne fungal community in selected sites of Nairobi city county, Kenya.

2.2 Fungal Air Pollution

According to WHO, 92% of the global population has been exposed to outdoor and indoor air pollution, resulting in 6.5 million annual mortalities (Kirby, 2016). Microorganisms such as fungi and bacteria make up the organic component of the polluted atmosphere (Niu *et al.*, 2021; Pollegioni *et al.*, 2022). They may also include other components, including plant and animal residues, pollen, and viruses. Bacterial and fungal communities isolated from aerosols have gained research premium due to their capacity to dramatically influence the health of humans, animals, and the atmospheric ecosystem (Be *et al.*, 2015; Burge, 2001). Fungal diversity and concentration largely depend on physiological parameters, including temperature, substrate availability, humidity, human populations, and their activities (Burge, 2001;

Jalili *et al.*, 2021; Nageen *et al.*, 2021). Unlike bacteria, fungal spores constitute the majority of the microbial population in the atmosphere. More specifically, the fungal concentration in the atmosphere is estimated to be at 25% of the total global biomass. Therefore, one of the major groups of microorganisms is isolated in occupational and urbanized settings (Baxi *et al.*, 2017). This is attributed to air being the main mode of fungal spore dispersal. Different species of fungi disperse their spores in the environment, resulting in a rich community of fungi in the atmosphere, playing a fundamental role in air pollution (Shelton *et al.*, 2002). The majority of these fungi are pathogens and allergens of humans and animals, causing asthma, rhinitis, bronchopulmonary mycoses, allergic fungal sinusitis, and hypersensitivity pneumonitis, and even death (Shelton *et al.*, 2002; Yan *et al.*, 2016; Baxi *et al.*, 2017).

Most health complications caused by airborne fungi affect the airways, causing respiratory infections. *Cladosporium* spp, *Epicoccum* spp, *Alternaria* spp, *Aspergillus* spp, *Penicillium* spp, and non-sporulating fungi are the commonly isolated outdoor fungi found that 1102 Ascomycota, 502 Basidiomycota, 24 Zygomycota, and 5 Chytridiomycota phyla dominated the Beijing atmosphere (Shelton *et al.*, 2002; Yan *et al.*, 2016; Baxi *et al.*, 2017). In the same study, *Aspergillus* spp, *Penicillium* spp, *Sporisorium* spp, *Alternaria* spp, *Cladosporium* spp, and *Fusarium* spp were the dominant genera (Yan *et al.*, 2016). Herein, we hypothesize that an urbanized atmosphere has high fungal exposure associated with respiratory diseases. The results add the knowledge that help combat the burden of respiratory infections, specifically in developing countries.

2.3 Airborne Fungi and Human Diseases

Fungal spores are ubiquitous in indoor and outdoor environments, which induce various human health complications upon exposure, resulting in enormous health and socioeconomic burdens (Yamamoto *et al.*, 2015; Baxi *et al.*, 2017; Nageen *et al.*, 2021). Many severe and less severe illnesses, including impaired lung function, increased respiratory symptoms such as shortness of breath, wheezing, chronic obstructive pulmonary, and cardiovascular disorders, have been associated with airborne fungi; this is specifically predominant in urbanized environments, where there is a predominance of increased economic activity and high density of inhabitants (Fang *et al.*, 2005; Be *et al.*, 2015; Nageen *et al.*, 2021).

So far, an estimated 150 fungal *taxa*-causing allergies have been documented (Ursula *et al.*, 2008; Yamamoto *et al.*, 2015). A cross-sectional study by Musumba *et al.* (2018) evaluated the relationship between exposure to Particulate Matters with 10 microns or less (PM₁₀) and asthma and related respiratory diseases among factory workers in Nairobi city with many factory workers exhibiting reduced lung function, which is strongly attributed to consistent exposure to PM₁₀ (Musumba *et al.*, 2018). Furthermore, a surveillance study in urban and rural areas of Kenya discovered an increased burden of respiratory infections, and there is a need for more data to effectively monitor its state (Feikin *et al.*, 2011). About 3% to 10% of the global population is suspected of having fungal allergies (Taylor *et al.*, 2015).

In recent years, research on the many health effects of airborne fungi on humans, animals, and the environment has intensified. Therefore, this has elicited intense research to identify the diversity of these allergens, resulting in the discovery of about 100 fungi genera responsible for respiratory infections, allergy, and toxicity (Fang *et al.*, 2005; Mendell *et al.*, 2011). Over 100 species of fungi have been discovered to

cause respiratory complications targeting both animals and humans. The common groups of fungi identified as causing asthma and allergic rhinitis include *Aspergillus* spp, *Alternaria* spp, *Penicillium* spp, and *Cladosporium* spp (Fang *et al.*, 2005; Liu *et al.*, 2014; Yan *et al.*, 2016; Baxi *et al.*, 2017). The allergens, specifically IgE antigens prominent in these fungal species, trigger type I hypersensitivity respiratory reactions causing asthma or rhinitis (Yamamoto *et al.*, 2015).

The airborne fungi have been found to produce various cancers causing metabolites. For instance, studies have shown that Aflatoxin B₁, a product of *Aspergillus flavus*, causes lung and liver malignancies upon ingestion or inhalation. To note, *Aspergillus niger* has been reported to cause pulmonary infections, whereas *Aspergillus fumigatus* is responsible for invasive aspergillosis (Marr *et al.*, 2002). Notably, the invasion of fungal spores and their subsequent effects on human health is dependent on their composition, that consists genera and species and their sizes. For instance, slightly larger spore particles (>10µm) are deposited in the upper respiratory system; the pharynx and nose, whereas smaller spore particles (<10µm, <5µm) penetrate deeper into the system (Fang *et al.*, 2005). In this regard, information on the diversity and concentration of airborne fungi, specifically in urbanized settings, is needed to understand their potential health hazards (Shelton *et al.*, 2002). A number of studies on outdoor mechanisms have been documented in China (Nageen *et al.*, 2021). For the first time, this study arguably explores the diversity and concentration of airborne fungi in the atmospheric environment of Nairobi, Kenya and their potential effects on human health.

2.4 Antifungal Resistance

Antifungal resistance is less recognized, yet it affects billions globally and is responsible for approximately 1.5 million deaths annually (Shelton *et al.*, 2002; Neil, 2020). Therefore, resistance to antifungal medicines is a growing concern in clinical practice, for instance, *Aspergillus fumigatus* strains that were earlier susceptible and newly isolated species of *Candida* (*Candida auris*), which are resistant to multiple antifungal drugs (Fisher *et al.*, 2018; Rhodes and Fisher, 2019; Verweij *et al.*, 2020). In contrast with 20 classes of antibiotics, only four (4) major classes of antifungals have been approved, including echinocandins, allylamines, azoles, and polyenes, which are gradually developing resistance (White *et al.*, 2021). This is a time bomb and needs urgent intervention before these antifungals become ineffective for simple infections such as aspergillosis or candidiasis in the near future.

According to a study in UK, there is an increase in the number of patients infected by with a azole's resistant fungi, hence use of limiting the use of first line antifungals to treat these infections. This impact has led to an increased risk of fatal outcome in vulnerable patients (Neil, 2020). However, this public health concern has been officially acknowledged and incorporated under the 2019 Antimicrobial resistance (AMR) among the threat list published by the CDC (CDC, 2019; Fisher *et al.*, 2022). Currently,, clinicians are battling antifungal resistance including elevated resistance to echinocandins and azoles in *Candida* species except *Candida albicans* and to azoles in *Aspergillus fumigatus*, causing aspergillosis (Nathan, 2017). For long, research attention has been channeled towards *Aspergillus fumigatus* since they are readily isolated from the environment (up to 90%) and frequently identified to cause human aspergillosis (Duong *et al.*, 2020). Moreover, pieces of evidence have described its global distribution and drug resistance, specifically its azole resistance. However,

information on the distribution of azole-resistant *Aspergillus flavus* has been inadequately featured in research (Duong *et al.*, 2020). Azole resistance has been largely reported in *Cryptococcus neoformans* from clinical samples (Bii *et al.*, 2006). However, the AFR threat is underestimated in developing countries (Macedo *et al.*, 2021). This work is significant in exploring azole-resistant airborne fungi isolated from the atmospheric spaces of Nairobi. The findings provide important information that further help in understanding the profiles of resistant fungi, an emerging global crisis of AFR.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Nairobi, the capital city of Kenya, a populous county with approximately 5.1 million people and a hub of intense economic activity (The World Bank, 2022). Nairobi lies at $1^{\circ}17'S$ and $36^{\circ}49'E$ with a subtropical highland climate and a mean maximum temperature of $24^{\circ}C$ ($75^{\circ}F$) (Kemoi *et al.*, 2018).

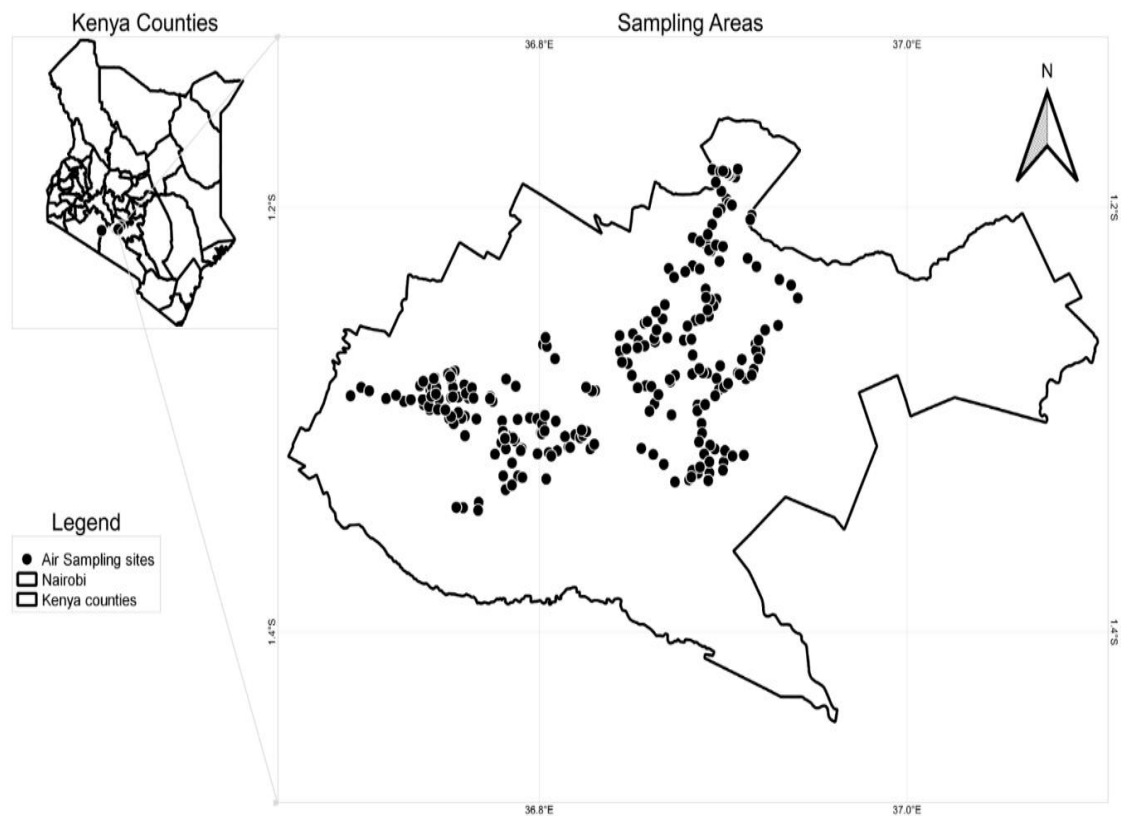


Figure 3.1: Sampling Points in Nairobi City County

(Generated by qGIS)

3.2 Study Design

A cross-sectional study design was used where air samples were collected from different sampling points at different seasons within the county. The samples were analyzed at the Mycology Division, Center for Microbiology-KEMRI. This study was

conducted during the dry season of (January to March) and wet season of (June to July), 2023.

3.3 Sampling Design

The samples were randomly selected using a stratified sampling design where sampling sites were categorized into 4 zones, including (1) Traffic zones, specifically roundabouts, and major roads; (2) Commercial zones, which included open air markets where commercial activities among traders are predominant; (3) Recreation zones, where citizens visit during leisure, including parks and gardens; (4) Waste dumping zones, which are sites where a majority of the city's waste is disposed. The samples were collected in both wet and dry seasons.

3.4 Sample Size

Since the prevalence of fungal infections caused by environmental fungi is undocumented, we used 50% as the estimated prevalence to compute the sample size, as this assumes the worst case scenario (Lettner, 2017). Therefore, the Cochran formula (Al-Hemyari, 2018) was used as follows:

$$n_o = \frac{z^2 pq}{e^2}$$

Where e is the margin of error (5%)

p is the (estimated) prevalence (50%) of the fungal infection

q is $1 - p$ (1-50%).

Therefore, $n = 1.96^2 \times 0.5 \times 0.5 / 0.05^2 = 384.16$,

which is approximately **384** samples across two seasons.

3.5 Sample Collection

The air samples were collected using culture plates during the time frame of the day between 0700hr and 1400hrs. Meanwhile, environmental factors such as temperature, humidity, and wind speed were also measured and data recorded from each sampled zone using the AccuWeather App (Turbine, 2022). Besides, GPS coordinates were also recorded for the purpose of map generation.

3.6 Fungal Isolation

An open-air plate method or settle plate method was used to sample the airborne fungi (Li *et al.*, 2010; Odebode *et al.*, 2020). Briefly, sterile Sabourauds Dextrose Agar (Oxoid) plates were opened and exposed to the air before closing the lid for 5 min. The sampling was done with a maintained minimum distance of approximately 1m from distractions. Two Petri dishes of Sabouraud dextrose agar (SDA) was supplemented with chloramphenicol antibiotic being incorporated in the media to prevent or suppress bacterial growth in each sampling. The exposed agar petri dishes were then immediately transported in a sterile cooler box to Mycology laboratory, Center for Microbiology Research – KEMRI. Incubation was done at 28°C with daily monitoring for up to 8 days while checking for any visible fungal. Similarly, two unexposed plates with media as controls were also incubated and equally monitored. To prevent any contamination, the incubators were QC bi-weekly for any possible fungal contamination.

3.7 Fungal Enumeration

Following incubation, fungal colonies were counted to determine their sample concentration expressed in CFU per cubic meter of air (CFU m⁻³). The Omelyansky formula (Bogomolova and Kirtsideli, 2009; Ljaljević-Grbić *et al.*, 2013) was used to then computed with fungal concentration expressed in CFU m⁻³ as indicated:

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

Where N represents fungal concentration.

A is the number of fungal colonies per plate.

B is the total area of the plate in cm^2 .

T is the exposure time in minutes.

3.8 Fungal Identification

The fungal colonies were identified by isolating pure colonies by sub-culturing into fresh SDA media. Morphological characteristics, including the shape, appearance, pigmentation, topography, and texture of the colonies were used in identifying the fungal isolates. Further, microscopic characteristics; sporangiospores branched septate hyphae pseudo-hyphae, conidiophores, conidia, proconidia, and arthroconidia were also used as per the standard taxonomic key for identifying different taxa (Dugan *et al.*, 2006).

3.9 Characterization of the Fungal Isolates

3.9.1 Temperature Tolerance

Pure fungal isolates were incubated using fresh Sabouraud dextrose agar (SDA) media at 37°C to examine their tolerance to human body temperature. *Aspergillus fumigatus* ATCC 204305 was used as the positive control.

3.9.2 Determination of Extracellular Protease Activity

The pure isolates were plated on skimmed milk media which encompassed 0.1g MgSO_4 , 0.05g CaCl_2 , 1.0g KH_2PO_4 , 14g agar and 5g skimmed milk in 1L of distilled water. The pH was adjusted to 4.5 based on the previous experimental protocol (Mushi *et al.*, 2018). Positive results were shown by the clearance zone around the colonies as

a result of the cleavage of the skimmed milk. Following 72 h of growth, the diameter clearance was measured and recorded. *Aspergillus niger* ATCC 20611 was used as the positive control (Nouri *et al.*, 2024).

3.9.3 Hemolytic Activity Assay

Briefly, pure fungal colony was emulsified in 1 ml of sterile normal saline. Thereafter, 10 µl of suspension was inoculated onto blood agar. The plates were then incubated at 37°C for 3 days. Hemolytic activity was detected by determining the diameter of the translucent halo around the inoculum site under transmitted light observation. *Aspergillus fumigatus* ATCC 204305 (beta hemolytic) was used as a positive control (Nayak *et al.*, 2012).

3.10 Antifungal Susceptibility Testing

Fungal isolates that were positive for temperature tolerance, hemolysis and protease tests were further subjected to antifungal sensitivity tests against 10 series of concentration of three azoles; fluconazole (FCZ), voriconazole (VCZ), and itraconazole (ITZ). Broth microdilution method was used according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2022) (Kemoi *et al.*, 2018; Clinical and Laboratory Standards Institute, 2022). Pure fungal isolates were incubated afresh in SDA media for 2-5 days until sporulation. After incubation, the colonies were aseptically harvested by scraping using a sterile cotton swab (moist in normal saline) on the sporulating plate. The cotton swab head was mixed with 5mls normal saline, vigorously shaken, and vortexed for 30s to make an inoculum. The inoculum was adjusted to match the turbidity of 0.5 McFarland standard (White *et al.*, 2021). The inoculums of the fungal isolates were mixed with the known volume of serial diluted azole antifungals and incubated for 3 days before reading the MIC. *Candida*

parapsilosis ATCC 22019 was used as positive controls, whereas non inoculated plates and sterile water was used as negative controls.

3.11 Statistical Analysis

Descriptive statistics were presented in form of means, maximum and minimum of fungal spore concentration across both seasons and specific season. Pearson correlation and multiple regression analyses were performed to analyze the relationship between the fungal spore concentration and environmental factors, that includes; temperature, humidity, and wind speed. $P < 0.05$ was considered a statistically significant value. All the statistical analyses were performed using the SPSS software version 27. Data were stored in Microsoft excel.

3.12 Ethical Consideration and Approvals

This study was approved by the Scientific and Ethics and Review Unit (SERU) of KEMRI (SERU NO. 4675) (**Appendix 2**).

CHAPTER FOUR

RESULTS

4.1 Fungal Air Pollution

This study sought to determine the levels of airborne fungi pollution in selected urban sites of Nairobi, Kenya. The fungal concentrations were determined in two seasons season, dry and wet season. The overall concentration of airborne fungi across the two seasons ranged between 180.38 to 18050.54 CFU m⁻³, with average mean, and median concentrations of 3,411.68, and 2,525.25 CFU m⁻³, respectively. The wet season had the highest number of spores averaging 5,318.88 CFU m⁻³, compared to the dry season that was 1,929.58 CFU m⁻³ (**Table 4.1**). The concentrations across all the sample sites are shown in **Appendix 2**. All the descriptive computations and storage of data were carried out in Microsoft excel.

Table 4.1: Descriptive Statistics of Fungal Concentration Across Two Seasons

	Descriptive Statistics (CFU m ⁻³)		
	Both Seasons	Dry Season	Wet Season
Mean	3411.68	1929.58	5318.88
Median	2525.25	1444.04	4332.13
Largest	18050.54	9025.27	18050.54
Smallest	180.38	180.38	180.38

KEY: The mean and median for both seasons are 3411.68 and 2527 CFU/m⁻³, respectively. For the dry season: 1929.58 and 144.04 CFU/m⁻³, respectively. For the wet season: 6716.74 and 4512.64 CFU/m⁻³, respectively.

4.2 Fungal Group Variation

A total of 502 isolates including 395 (78.69%) filamentous fungi, and 107(21.31%) yeasts were isolated across two seasons consisting of 16 genera and 38 species. They comprised *Ascomycota* 426(89.31%), *Basidiomycota* 21(4.40%), *Deuteromycota* 17(3.56%), *Muromycota* 7(1.47%), and *Zycomycota* 6(1.26%). The most isolated fungi included *Candida* spp (17.13%), followed by *Penicillium* spp 66(13.15%), *Fusarium* spp 62(12.35%), *Aspergillus* spp 61(12.15%), and, *Cladosporium* spp 60(11.95%). The

least isolated fungi included: *Aureobasidium* spp 9(1.79%), *Curvularia* spp 9(1.79%), *Geotrichum* spp 9(1.79%), *Paecilomyces* spp 7(1.39%), *Rhizopus* spp 6(1.20%), and *Mucor hemalis* 7(1.39%). The unidentified isolates accounted for 25(4.98%). The highest fungal diversity was recorded during the dry season 330(65.74%) compared to the wet season 172(34.26%) (Table 4.2).

Table 4.2: Quantitative Distribution of Fungal Genera Isolated from Various Sites in Nairobi During the Dry and Wet Seasons

Genera	Dry Season (%)	Wet season (%)	Total (%)
<i>Penicillium</i>	52(15.76)	14(8.14)	66(13.15)
<i>Aspergillus</i>	47(14.24)	14(8.14)	61(12.15)
<i>Fusarium</i>	49(14.85)	13(7.56)	62(12.35)
<i>Candida</i>	40(12.12)	46(26.74)	86(17.13)
<i>Cladosporium</i>	36(10.91)	24(13.95)	60(11.95)
<i>Rhodotourolla</i>	19(5.76)	2(1.16)	21(4.18)
<i>Alternaria</i>	14(4.24)	3(1.74)	17(3.39)
<i>Acremonium</i>	12(3.64)	12(6.98)	24(4.78)
<i>Scedosporium</i>	13(3.94)	5(2.91)	18(3.59)
<i>Cladophiallophora</i>	6(1.82)	9(5.23)	15(2.99)
<i>Aurobasidium</i>	5(1.52)	4(2.33)	9(1.79)
<i>Curvularia</i>	6(1.82)	3(1.74)	9(1.79)
<i>Geotrichum</i>	4(1.21)	5(2.91)	9(1.79)
<i>Paecilomyces</i>	5(1.52)	2(1.16)	7(1.39)
<i>Rhizopus</i>	2(0.61)	4(2.33)	6(1.20)
<i>Mucor hemalis</i>	1(0.30)	6(3.49)	7(1.39)
Unidentified	19(5.76)	6(3.49)	25(4.98)
Total	330	172	502
%	65.74%	34.26%	

4.3 Seasonal Variation of the Fungal Groups

The *Candida*, *Penicillium*, *Fusarium*, *Aspergillus*, and *Cladosporium* were the most isolated fungal groups across both seasons, accounting for 86(17.13%), 66(13.15%), 62(12.35%) 61(12.15%), and 60(11.95%), respectively (Table 4.2). In terms of each season, *Penicillium* 52(15.56%) fungal spores most isolated contaminant fungal during the dry season, whereas *Candida* 46(26.74%) yeast dominated the wet season (Table 4.2).

4.4 Spatial Distribution of the Fungal Groups

Across the two seasons, Open Markets contained the highest number of fungal spores accounting for 178 (35.46%) of all the isolates compared to that of other sampling sites like Roads 122 (24.30%), Recreational 102 (20.32%) and Dumpsites 100 (19.92%) (Table 4.3; Figures; 4.2 & 4.3).

Table 4.3: Spatial Distribution of Fungal Genera Isolates from Across Two Seasons in Selected Sites in Nairobi

FUNGAL GROUPS	ROADS	DUMPSITES	RECREATIONAL	OPEN MARKETS	TOTAL	%
<i>Penicillium</i>	14	16	12	24	66	13.15
<i>Aspergillus</i>	14	12	7	28	61	12.15
<i>Fusarium</i>	14	11	16	21	62	12.35
<i>Candida</i>	25	18	13	30	86	17.13
<i>Cladosporium</i>	14	10	19	17	60	11.95
<i>Acrimonium</i>	9	4	5	6	24	4.78
<i>Cladophiallophora</i>	6	2	5	2	15	2.99
<i>Rhodotourolla</i>	5	5	4	7	21	4.18
<i>Alternaria</i>	2	7	4	4	17	3.39
<i>Unidentified</i>	7	5	7	6	25	4.98
<i>Aurobasidium</i>	2	1	1	5	9	1.79
<i>Curvularia</i>	1	2	2	4	9	1.79
<i>Geotrichum</i>	2	1	1	5	9	1.79
<i>Paecilomyces</i>	1	1	1	4	7	1.39
<i>Rhizopus</i>	1	2	1	2	6	1.20
<i>Mucor hemalis</i>	1	1	1	4	7	1.39
<i>Scedosporium</i>	4	2	3	9	18	3.59
TOTAL	122	100	102	178	502	
%	24.30	19.92	20.32	35.46		

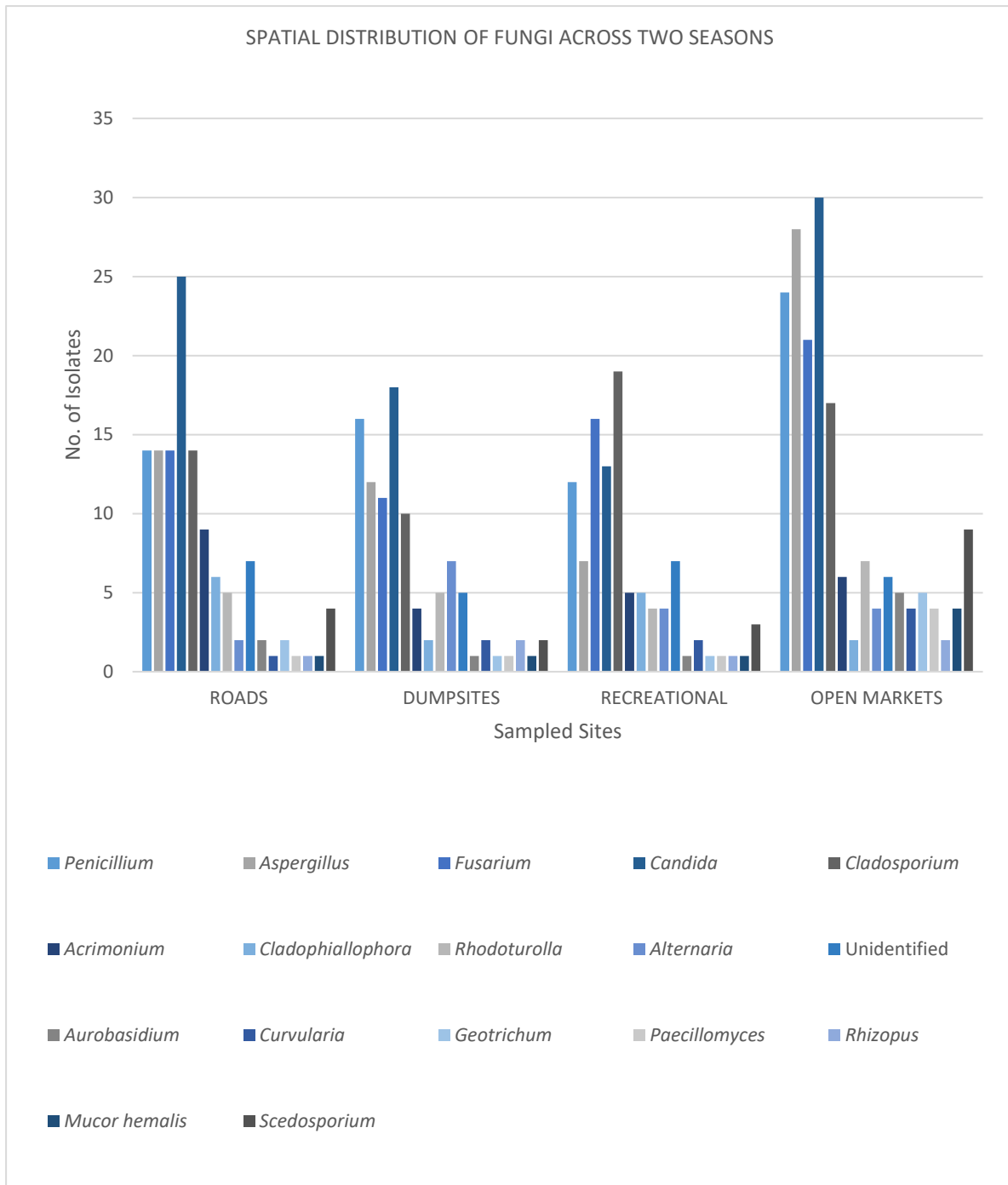


Figure 4.1: Spatial Distribution of Fungi Isolates Recovered from Nairobi During both Dry and Dry Seasons

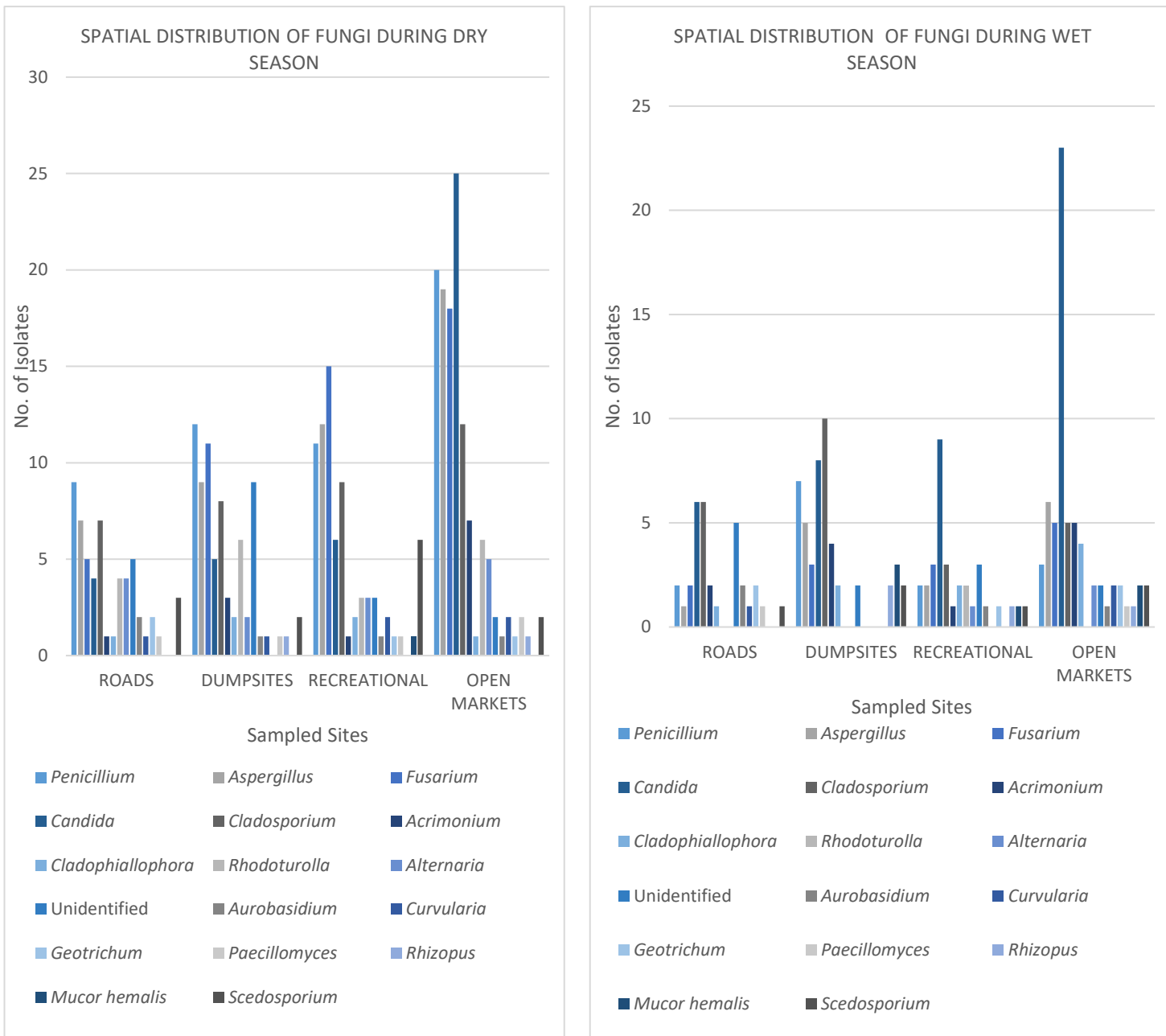


Figure 4.2: Spatial Distribution of Fungi Isolates Recovered from Nairobi During both Dry and Dry Seasons

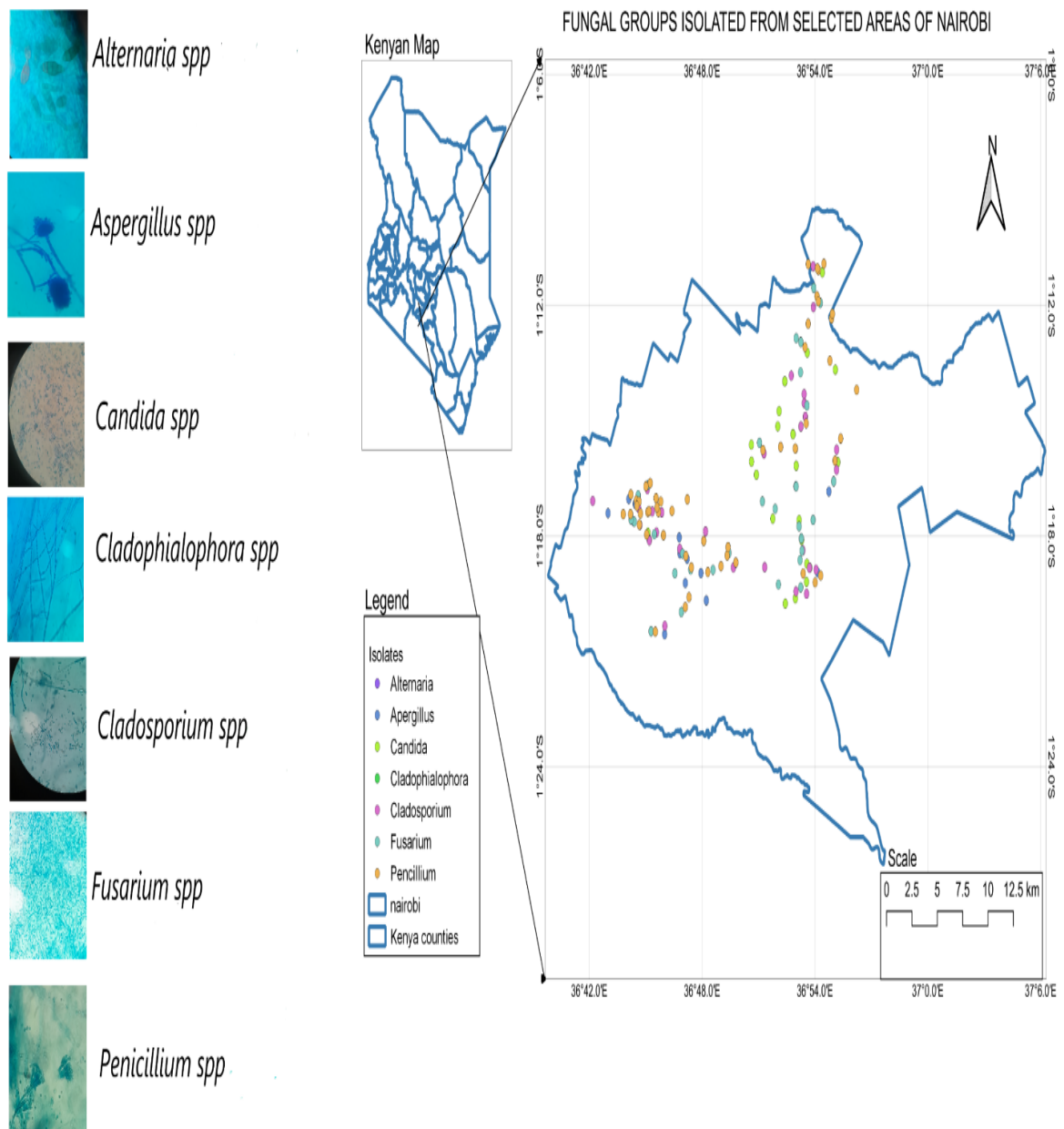


Figure 4.3: A qGIS Distribution of Major Fungal Groups Isolated across all the Sampling Sites in Nairobi City

4.5 Fungal Diversity

Macromorphological and micromorphological identification yielded the following fungal groups, the majority representing the following phyla: *Ascomycota* 426(89.31%), *Basidiomycota* 21(4.40%), *Deuteromycota* 17(3.56%), and *Muromycota* 7(1.47%) *Zycomycota* 6(1.26%) (**Figure 4.4**).

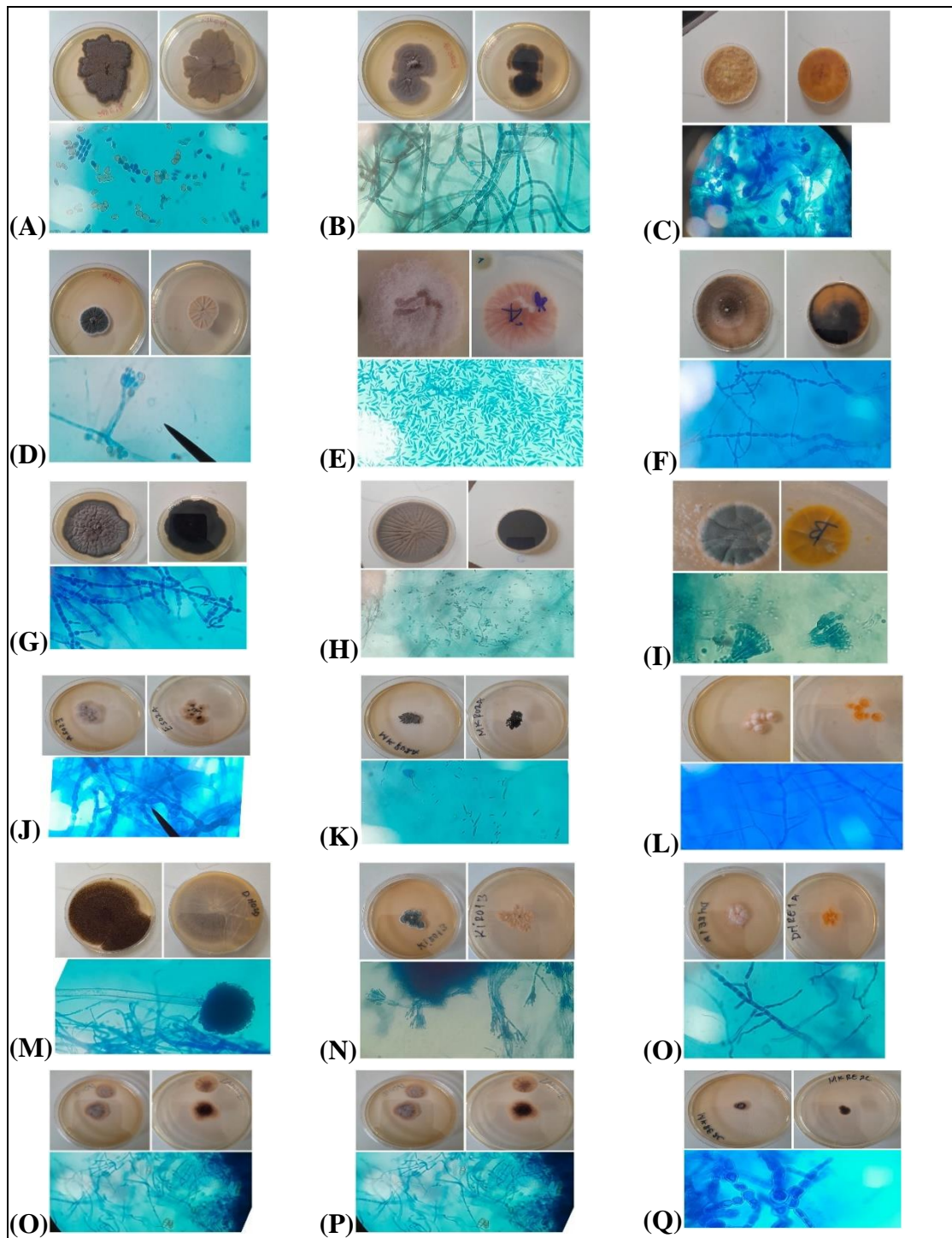
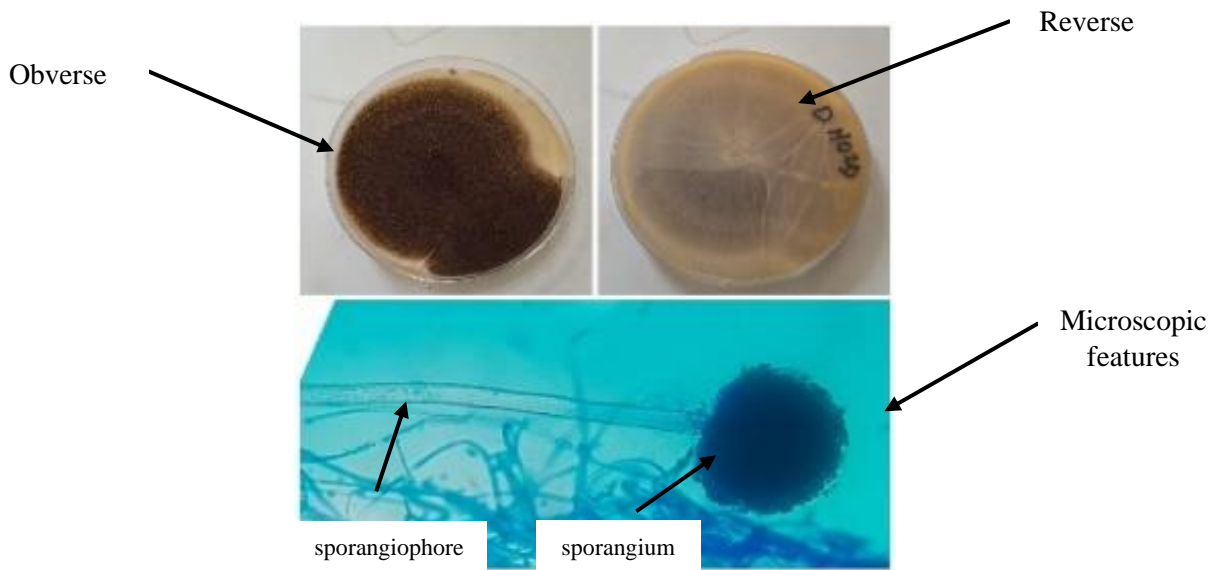


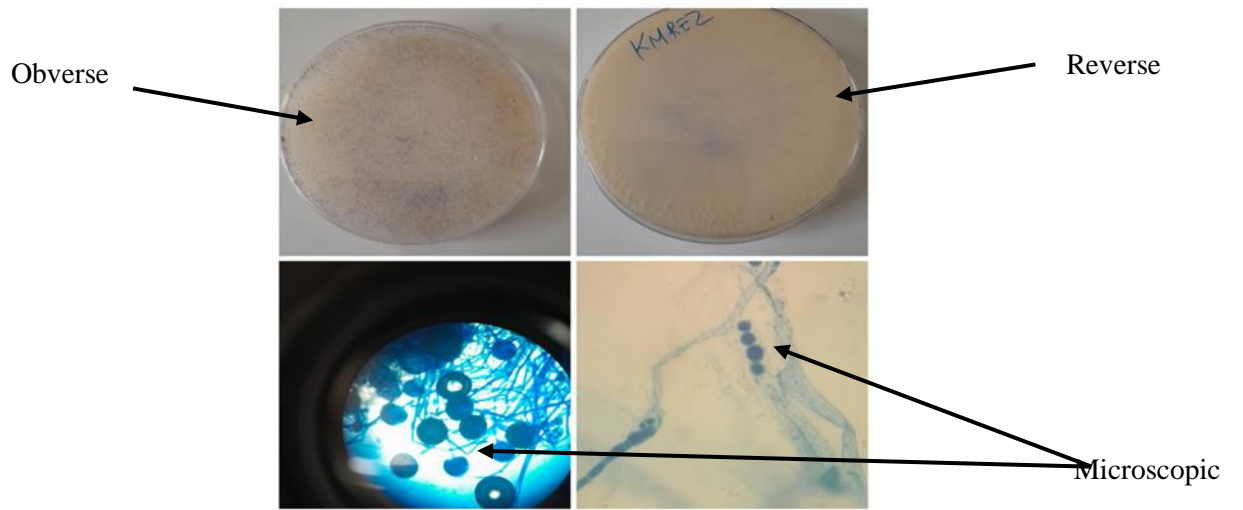
Figure 4.4: Fungal Isolates Identified Across the Two Seasons

(A). *Aureobasidium pullulans*; (B). *Cladophialophora carroni*; (C). *Ulocladium chartarum*; (D). *Penicillium chrysogenum*; (E). *Fusarium oxysporum*; (F). *Cladophialophora batiana*; (G). *Alternaria* spp; (H). *Cladosporium* spp; (I). *Penicillium citrinum*; (J). *Aureobasidium pullulans*; (K). *Cladosporium cladosporoides*; (L). *Acrimonium strictum*; (M). *Aspergillus niger*; (N). *Penicillium* spp; (O). *Acrimonium* spp; (P). *Curvularia australiensis*; (Q). *Curvularia lunata*

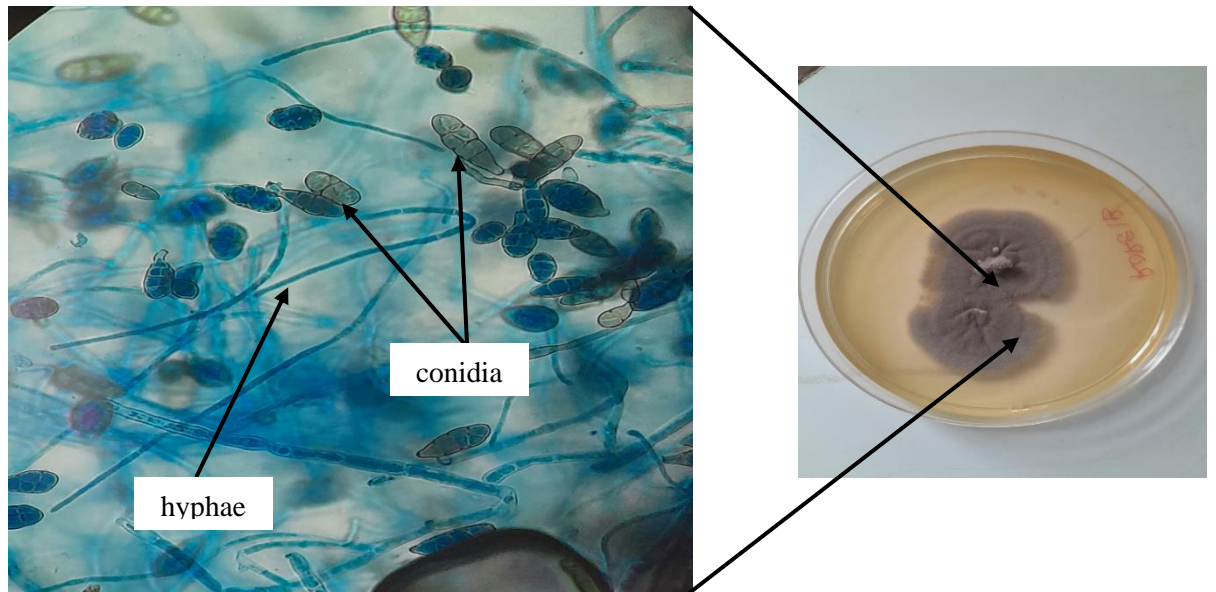
A: *Aspergillus niger*



B: *Mucor hemalis*



C: *Alternaria alternata*



D: *Fusarium* spp

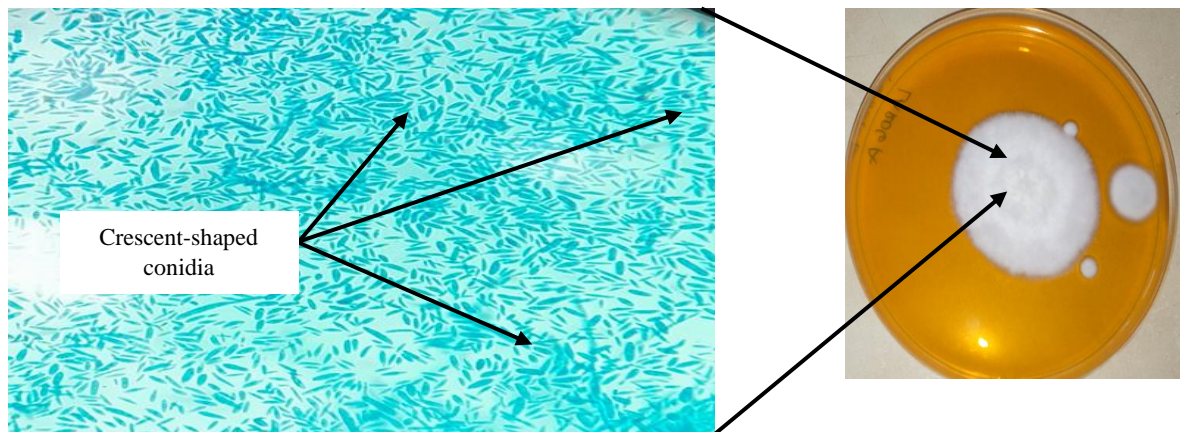


Figure 4.5: Macroscopic and Microscopic Images of the Identified Fungal Isolates

(A) *Aspergillus niger*; (B) *Mucor hemalis*; (C) *Alternaria alternata*; (D) *Fusarium* spp.

4.6 Impact of Physical and Environmental Effects on Airborne Fungal Concentration

Multiple regression analysis was conducted to determine the effect of temperature, humidity, and wind speed on airborne fungal concentration. The levels of temperature, humidity, and wind speed across all the sampled sites are shown in **Appendix 2**.

Overall, the results showed that the utility of the model was significant. The utility of the model was fit $F(3,291) = 14.527$, $R^2 = .130$, $p = .00$ (**Appendix Table 1B**). Moreover, multicollinearity among the variables was not a concern as the tolerance and VIF levels were within acceptable limits (**see Appendix Table 1B**). Across the two seasons, temperature, humidity, and windspeed significantly affected airborne fungal concentration ($\beta = -.358$, $t = -4.175$, $p = .000$), $\beta = -.199$, $t = -2.208$, $p = .0280$), and ($\beta = -.230$, $t = -3.691$, $p = .000$), respectively (**Appendix Table 1C**).

4.7 Correlation Analysis

Across the two seasons, Pearson correlation analysis showed that temperature negatively correlated with the fungal concentration ($r = -.293$, $p = .000$), humidity positively correlated significantly with the fungal concentration ($r = .187$, $p = .001$), and wind speed negatively correlated significantly with the fungal concentration ($r = -.272$, $P = .000$) (**Appendix Table 1D**).

4.8 Characterization of Fungal Isolates

A total of 58 isolates, wet season (32) and dry season (26) were further subjected to physical chemical characteristics; temperature tolerance, extracellular protease and hemolytic assays. The selection of the isolates was based on the season, frequency of isolation and the unique features of the isolates from the rest (**Appendix Table 1A**).

4.8.1 Temperature Tolerance

Pure fungal isolates were incubated using fresh Sabouraud dextrose agar (SDA) media at 37°C to examine tolerance to human body temperature. *Aspergillus fumigatus* ATCC204305 was used as the positive control. Out of the 58 isolates, 25 isolates survived the high temperature of 37°C whereas 33 didn't (Figure 4.7; Appendix Table 1A).

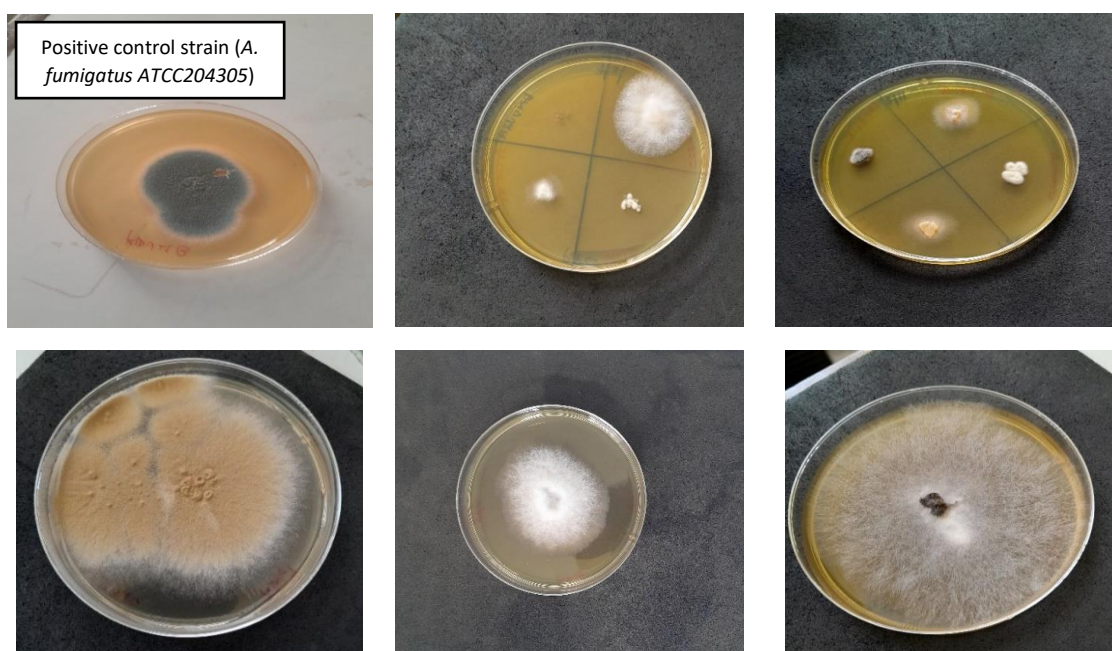


Figure 4.6: Fungal Isolates that Tolerated High Temperatures (37°C)

4.8.2 Extracellular Protease Activity

The isolates were plated on skimmed milk media which encompassed 0.1g MgSO₄, 0.05g CaCl₂, 1.0g KH₂PO₄, 14g agar and 5g skimmed milk in 1L of distilled water. The pH was be adjusted to 4.5 based on the previous experimental protocol (Mushi *et al.*, 2018). Positive results were shown by the clearance zone around the colonies as a result of the cleavage of the skimmed milk (Figure 8). *Aspergillus niger* ATCC20611 was used as the positive control. Following 72 h of growth, the diameter clearance was measured and recorded. Consequently, a total of 15 isolates out of 58 displayed protease

activities. Out of 58 samples tested, 15 fungal samples were positive, whereas 43 were negative (**Appendix Table 1A**).

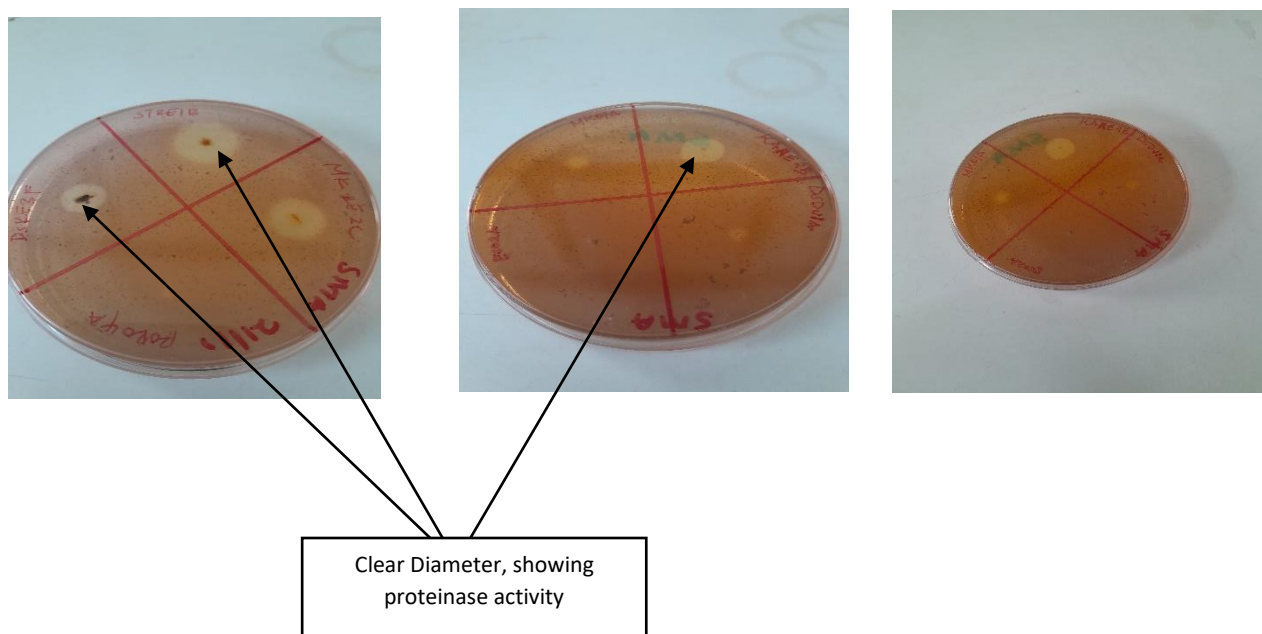


Figure 4.7: Fungal Isolates that Showed Protease Activity Shown by the Clearance Zone Around the Colonies

4.8.1 Hemolytic Activity

For hemolytic assay, a fungal colony was emulsified in 1 ml of sterile normal saline. Thereafter, 10 μ l of suspension was inoculated onto blood agar. The plates were then incubated at 37°C for 3 days. Hemolytic activity was detected by determining the diameter of the translucent halo around the inoculum site under transmitted light observation and characterizing them into beta, alpha and gamma hemolysis. *Staphylococcus aureus* ATCC23235 (beta hemolytic) was used as a positive control. Among the 58 isolates, 12 of them were beta hemolytic, 39 were alpha hemolytic and 7 were gamma hemolytic (**Appendix Table 1A**).

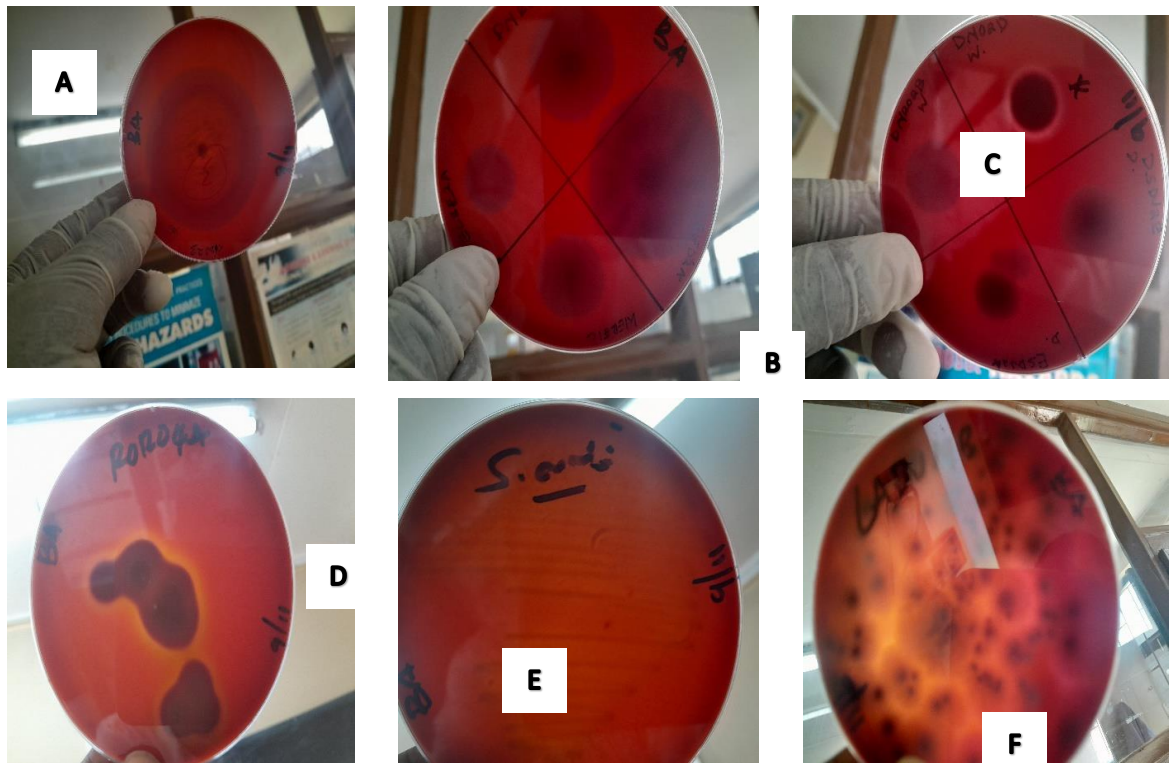


Figure 4.8: Fungal Isolates that Showed Hemolysis on Blood Agar: A, B - Alpha Hemolysis; C, D & F - Beta Hemolysis; E - Positive Control (*Staphylococcus aureus*)

4.9 Antifungal Susceptibility Test Results

A total of 18 fungal isolates that showed positive results in the initial tests were selected for antifungal sensitivity tests against ten concentration series of three azoles;

fluconazole (FCZ), voriconazole (VCZ), and itraconazole (ITZ) based on CLSI M38-A2 broth microdilution method. From the analysis, most of the fungal isolates six of the isolates were susceptible to voriconazole and itraconazole while four were resistant to fluconazole (Table 4.4).

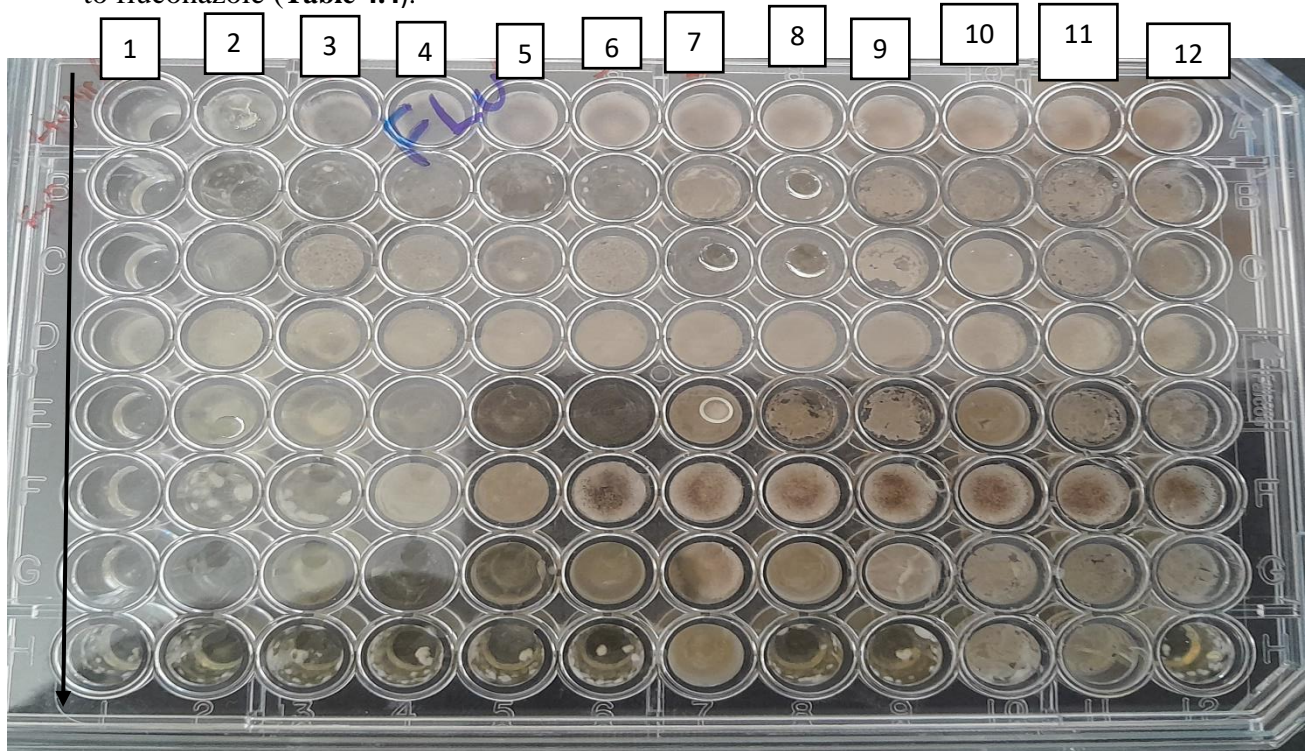


Figure 4.9: Micro Titer Plate Showing Antifungal Sensitivity Tests

1 - negative control; 12 - positive control; 2-10 Antifungal dilution from the highest to the lowest. The arrow shows the isolates.

Antifungal susceptibility testing of the isolates against fluconazole (FCZ), voriconazole (VCZ), and itraconazole (ITZ) showed varying sensitivity patterns. Resistance was identified with fluconazole, where four isolates (LADU19E, KADU2B, KIDU2A, and KIDU2B) exhibited high MIC values ($16 \mu\text{g/L}$) and were regarded as resistant. The other isolates displayed MICs between 0.0325 and $8 \mu\text{g/L}$ and were considered sensitive. In contrast, all isolates were consistently sensitive to voriconazole and itraconazole, with low MIC values (0.0156 – $4 \mu\text{g/L}$), indicating effective antifungal activity. These results indicate a reduced reliability of fluconazole as a standalone

treatment due to emerging resistance, whereas voriconazole and itraconazole continue to demonstrate high effectiveness against the tested isolates.

Table 4. 4: Summary of Antifungal Sensitivity Tests

Isolate	ANTIFUNGAL SENSITIVITY TEST					
	MIC Concentration ($\mu\text{g/L}$)		MIC Concentration ($\mu\text{g/L}$)		MIC Concentration ($\mu\text{g/L}$)	
	FCZ	Interpretation	VCZ	Interpretation	ITZ	Interpretation
LADU19E	16	R	0.03125	S	4	S
ECRO1C	0.0625	S	0.25	S	8	S
LARO4E	0.125	S	0.0156	S	1	S
KADU2B	16	R	0.015625	S	0.015625	S
ECRE2A	0.0625	S	1	S	4	S
DNO2D	4	S	0.25	S	4	S
WERE1A	0.5	S	4	S	2	S
STRE1B	0.5	S	2	S	0.0325	S
ECRE1A	8	S	0.015625	S	0.015625	S
KIDU2A	16	R	0.015625	S	0.015625	S
DSRE3F	4	S	0.015625	S	0.015625	S
ECRE1A	1	S	0.015625	S	0.015625	S
RORO4A	1	S	0.015625	S	0.015625	S
RUDU1B	1	S	0.015625	S	0.015625	S
ESO2A	0.0325	S	0.015625	S	0.015625	S
EERE1C	0.0325	S	0.015625	S	0.015625	S
KARE2B	0.5	S	0.015625	S	0.015625	S
KIDU2B	16	R	0.015625	S	0.015625	S

Key: Resistant isolates were confirmed if MIC value was above the CLSI resistance clinical breakpoint (Voriconazole > 1, Itraconazole >1, Fluconazole >4 $\mu\text{g/L}$).

Abbreviations: VCZ = voriconazole, ITC = itraconazole, FCZ = Fluconazole. S = Susceptible; R = Resistant

CHAPTER FIVE

DISCUSSION CONCLUSIONS AND RECOMMENDATIONS

5.1 Variation in Fungal Spore Concentration

This is the first study in Kenya on outdoor distribution and diversity of culturable airborne fungi across dry and wet seasons. Informed by diverse human activities, we sampled four strategic sites including; open markets, roads, recreational sites, and waste dumping sites. A total of 504 fungi were isolated across the two seasons, which included 16 genera and 38 species. Several studies across different cities have reported varying numbers of fungal diversity. In Tianjin City China, 1,015 fungal strains belonging to 175 species and 82 genera were reported (Nageen *et al.*, 2021). Moreover, Hangzhou, a famous city in southeast China reported 352 fungi from different sampling sites and across seasons distributed among 21 genera and 85 species (Fang *et al.*, 2019). In Lagos and Oyo States of Nigeria, 44 fungal species and strains were isolated with *Aspergillus*, *Penicillium*, and *Fusarium* being the dominant fungal groups (Odebode *et al.*, 2020).

In the present study, the fungal concentration ranged from 180.38 to 18050.54 CFU m⁻³ across 302 sampling sites in both seasons with an average concentration of 3411.68 CFU m⁻³. Numerous studies have reported varying concentrations of airborne fungi. China reported an average concentration of 625 CFU m⁻³ in Tianjin City (Nageen *et al.*, 2021). In Styria, a southeastern state of Austria, the median concentration of xerophilic fungal spores in the urban region was 2.0×10^3 CFU m⁻³ and that for *Cladosporium* was 1.7×10^3 CFU m⁻³ (Haas *et al.*, 2023). Another study on airborne fungal communities along pedestrian bridges found fungal concentrations ranging from 10 to 180 CFU m⁻³ (Al-Shaarani *et al.*, 2023). These variations in spore concentrations can be explained by the diverse weather patterns in different cities. For instance, the

meteorological factors in Nairobi, Kenya are not similar to that in Johannesburg, South Africa. Moreover, anthropogenic activities have a substantial influence on the overall fungal spore concentration, at a particular time.

5.2 Seasonal Variation in Fungal Spore Concentration

In the current study, the wet season had the highest average concentration (5318.88 CFU m⁻³) compared to the dry season (1929.58 CFU m⁻³). This is consistent with the findings reported in other previous studies. For instance, seasonal variation of airborne fungal spores in Mérida, Mexico revealed that the concentrations were high in the wet season (1653 and 1432 CFU m⁻³) unlike that in the dry season (1326 and 1145 CFU m⁻³) (Ponce-Caballero *et al.*, 2013). Moreover, a study in Lagos, Nigeria revealed that the concentrations were significantly higher during the wet/rainy season compared to the dry season (Odebode *et al.*, 2020). Several other studies across the globe have confirmed that indeed concentration of the fungal spore during the wet season is higher than that in the dry season (dry (Christopher, 2020; Valeria *et al.*, 2012; Woo *et al.*, 2018). Unlike in dry season, fungal spores spread rapidly and voluminously during the wet season because high humidity allows the fungal sporulation and germination hence more spores (Kuka *et al.*, 2022; Patel *et al.*, 2018; Talley *et al.*, 2002). Besides, abundant nutrient and water availability boost fungal spore sporulation during the wet season. On the other hand, the lack of water during the dry season limits the ability of fungi to thrive. This also explains the high diversity (330/502) and low abundance (1929.58CFUs) during the dry season, as well as high diversity (330/502) and low abundance (1929.58CFUs) during the wet season. Therefore, there is likelihood of higher fungal spore allergies and infections in the wet than in dry season. Nevertheless, this needs to be supported by epidemiological studies.

5.3 Airborne Fungal Diversity and their Spatial Variation

Similar to many previous studies, most of the strains isolated in this study belonged to the phylum Ascomycota 426(89.31%), followed by Basidiomycota 21(4.40%), Deuteromycota 17(3.56%), Muromycota 7(1.47%), and Zycomycota 6(1.26%) (A and A, 2019; Al-Shaarani *et al.*, 2023; Fang *et al.*, 2019; Haas *et al.*, 2023; Odebode *et al.*, 2020; Patel *et al.*, 2018; Ponce-Caballero *et al.*, 2013; Yan *et al.*, 2016). Ascomycota are often the owing to their diverse ecological role, mutualistic relationships with other microbial communities, their survival in diverse environments (soil, plants, animals; aquatic & terrestrial habitats) as well as their capacity for reproduce via both sexual and asexual reproduction. By contrast, other studies have found Basidiomycota as the predominant phylum (Fröhlich-Nowoisky *et al.*, 2009; Womack *et al.*, 2015).

Herein, *Candida* 86(17.13%) was the most isolated species with 16.83%, followed by *Penicillium* spp 66(13.15%), *Fusarium* spp 62(12.35%), *Aspergillus* spp 61(12.15%), and, *Cladosporium* spp 60(11.95%). *Candida* is the most renown normal flora whose growth is promoted by most and warm environments. In addition, this versatile yeast can form biofilms, enabling its survival in stress-filled environment, including adapting to climate change. *Aureobasidium* spp 9(1.79%), *Curvularia* spp 9(1.79%), *Geotrichum* spp 9(1.79%), *Paecilomyces* spp 7(1.39%), *Rhizopus* spp 6(1.20%), and *Mucor hemalis* 7(1.39%) were the least isolated strains. The unidentified isolates accounted for 25(4.98%). Furthermore, some studies have found *Cladosporium* spp to be the most dominant fungal group (Haas *et al.*, 2023; Patel *et al.*, 2018; Ponce-Caballero *et al.*, 2013). This study isolated and identified common airborne fungi reported in many previous studies including *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., and *Fusarium* spp. (Al-Shaarani *et al.*, 2023; Jalili *et al.*, 2021; Li *et al.*, 2010; Menezes *et*

al., 2004; Ponce-Caballero *et al.*, 2013; Shams-Ghahfarokhi *et al.*, 2014; Yan *et al.*, 2016).

The highest number of strains was recorded during the dry season 330(65.74%) compared to that during the wet season 172(34.26%). Across the two seasons, Open Markets harbored the highest number of the strains, accounting for 178(35.46%) of all fungi isolated followed by Roads 122(24.30%) Recreational 102(20.32%), and Dumpsites 100(19.92%). *Penicillium* spp. was the most commonly isolated fungi across the seasons and sampling sites. This is consistent with various findings suggesting that postharvest fungi in cereals make their way to the markets and colonize the atmosphere around them (Bot, 2012; Habib *et al.*, 2021; Niu *et al.*, 2018). Moreover, post-harvest rot in fruits, such as citrus fruits and vegetables is known to contribute to the fungal counts and diversity (Bot, 2012; Habib *et al.*, 2021; Luciano-rosario *et al.*, 2020; Niu *et al.*, 2018; Wu *et al.*, 2017).

A study in Namibia on processed food contamination at the Open Markets isolated *Aspergillus* species. (Nawases *et al.*, 2018). Besides, all the other foods in the Open Market were contaminated with *Aspergillus parasiticus* except for ground nuts (Nawases *et al.*, 2018). Thus, the findings of the present study indicate that Ascomycota phylum of fungi, including *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp are the most circulating group in the atmosphere. Open markets have the lion's share of all the stains compared to other sampling sites.

5.4 Meteorological Parameters and Fungal Concentration

The concentration and diversity of airborne fungi depend on numerous factors, including geographical location, human activities, climate, and season. Airborne fungi concentration can also be influenced by meteorological parameters including

temperature, rainfall, wind speed, and humidity (Kilic *et al.*, 2020; Nageen *et al.*, 2021). In the present study, Pearson correlation analysis indicated a significant negative correlation between temperature and fungal concentration ($r = -.293$, $p = .000$). However, humidity was positively correlated with the fungal concentration significantly ($r = .187$, $p = .001$). There was a significant negative correlation between wind speed and fungal concentration ($r = -.272$, $P = .000$).

These results indicate that these meteorological parameters play a key role in determining fungal concentration. Temperature demonstrated a significantly negative correlation with environmental fungal concentration, in line with findings reported by other studies (Nageen *et al.*, 2023; Ponce-Caballero *et al.*, 2013). This is because high temperatures and the resulting dry conditions hinder the growth of fungi. Humid environments favor the proliferation of fungi, hence increasing fungal concentration significantly. The wind speed has a significant negative correlation with fungal concentration. High windspeed disperse and dilute the concentration of fungal spores at a particular site, hence reducing the overall fungal load (Grinn-Gofroń and Bosiacka, 2015). This explains the negative correlation between windspeed and fungal concentration. Furthermore, multiple regression analysis revealed that temperature, humidity, and windspeed significantly affected airborne fungal concentration ($p = .000$), ($p = .0280$), and ($p = .000$), respectively. All three weather variables had a significant impact on the overall fungal concentration during the dry season compared to the wet season. This is in agreement with the findings reported by other studies (Kallawicha *et al.*, 2017; Pathak, 2012). Alongside anthropogenic activities, temperature, humidity and windspeed play a critical role in the overall fungal spore concentration.

5.5 Characteristics of the Isolated Airborne Fungi

5.5.1 Temperature Tolerance

Temperature or thermal tolerance is the ability of an organism to grow and thrive at relatively high temperatures (Robert *et al.*, 2015). The fungi kingdom encompasses the most thermotolerant organisms; however, the subject of temperature is dependent on the specific environment in question. One major advantage mammal has over fungi is the ability to thrive at high temperatures of 37°C. This high temperature is one of the major barriers of fungal infection (Smith *et al.*, 2023). Nonetheless, recent studies have reported the increasingly emergence of environmental fungi able to survive mammalian body temperature unlike in decades prior. In the present study, 33/58(56.89%) fungal isolates could grow at 37°C. Since these are largely environmental isolates, their ability to thrive at these temperatures is attributed to thermal selection pressures caused by erratic and dynamic weather patterns that have pushed these microbes to adjustment. This potentially points to the discovery of these environmental isolates in clinical environments. However, this needs validation through epidemiological research.

5.5.2 Production of Extracellular Protease Enzyme

Production of extracellular proteinases by saprophytic fungi are majorly responsible for cell nutrient provision. However, pathogenic fungi use these enzymes to invade the host during infection (Hogan *et al.*, 1996; R. *et al.*, 2003). These proteinases among other hydrolytic enzymes for instance digest cell barriers enabling adhesion, successful penetration, survival and dissemination within the host (Satala *et al.*, 2023). Proteases are fungal arsenal in overcoming host defense barriers such as epithelium, basement membrane, among others promoting the spread of fungi. Therefore, these proteases have been strongly considered as being bifunctional or multifunctional (Satala *et al.*, 2023). Herein, extracellular proteases production was investigated from the isolates and

15/58(25.86%) were positive, among which all were thermotolerant, implying that they could be virulent and capable of invasive fungal infection in the host.

5.5.3 Production of Hemolysins

Furthermore, the capacity of the isolates to produce hemolysins that lyses erythrocytes was evaluated using blood agar media supplemented with chloramphenicol antibiotic to inhibit potential bacterial contamination during incubation (Nayak *et al.*, 2012; Perini *et al.*, 2019). We examined the degree of hemolysis, and demonstrated that 12(20.69%) isolates were capable of beta (complete) hemolysis, 39(67.24%) showed alpha (partial) hemolysis and 7(12.07%) displayed gamma or no hemolysis. Hemolysins lyse blood cells releasing iron, which is an important component for microbes especially during infection process (Aktas and Yigit, 2015; Nayak *et al.*, 2012). One major limitation of this work is that we did not consider the uncultivable fungi, and this should be a focus in future research using advanced molecular techniques approach.

5.6 Antifungal Resistance

Azole antifungals are the standard treatment for most fungal infections and has resulted in significant overall survival among patients (Kemoi *et al.*, 2018; Resendiz-Sharpe *et al.*, 2019, 2021). Nevertheless, azole resistance has recently evolved as a substantial clinical challenge in managing mycoses (Resendiz-Sharpe *et al.*, 2019). In the present study, most of the airborne fungal isolates tested were susceptible to voriconazole and itraconazole. However, resistance against fluconazole was observed among 4 (22.22%) of all the isolates tested. Across developing nations, studies have continually documented azole resistance through culture dependent techniques from various sources, including soil samples, hospitals and its environs, compost as well as flower beds (van der Linden *et al.*, 2015; Moin *et al.*, 2020; Amona *et al.*, 2022; G. *et al.*, 2022). A recent study in Africa reported azole resistance as follows: environmental

(17.1%) and clinical (1.3%) (Kemoi *et al.*, 2018; Amona *et al.*, 2022). Scientists have suggested that the environmental-associated resistance is the major route of infections in patients diagnosed with fungal infections (Resendiz-Sharpe *et al.*, 2021). Therefore, a successful and optimal approach in managing azole-resistant infections involves a wholesome and systematic surveillance of environmental isolates. Our findings provide link on antifungal resistance between clinical settings and the environmental settings (Tsitsopoulou *et al.*, 2018). As a limitation of this study, future studies should focus on the unculturable airborne microbial communities in relation to respiratory conditions and seasonal variations in temperature, humidity, and wind speed. In addition, an epidemiological study should be considered to establish a correlation between the environmental isolates and clinical isolates. A comparison of rural and urban settings will be critical.

5.7 Conclusions

- i) Wet Season had increased spore concentration in the air, with open markets exhibiting the highest overall fungal concentration.
- ii) The most isolated fungi were *Candida*, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Fusarium* species. The airborne fungal isolates exhibited the ability to produce proteases, hemolysins and ability to tolerate high temperature to their adaptability to high changing environmental conditions.
- iii) There is a significant influence of meteorological factors (temperature, speed, and windspeed) on fungal concentration.
- iv) Fluconazole resistance was observed in certain isolates with voriconazole and itraconazole exhibiting high efficacy.

5.8 Recommendations

- i) An evident of significant fungal spore concentration in these environmental fungi, indicating fungal outdoor air quality.
- ii) There is a need for a routine antifungal susceptibility with reduction in use of fluconazole with consideration of voriconazole and itraconazole as better alternative.
- iii) There is an evident potential in these environmental fungi to evolve into pathogens.
- iv) There is need to implement periodical monitoring of the urban fungal spores in high-risk areas such as open markets, especially during the wet season when concentrations peak.

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Supplementary Table 1 Characteristics of fungal isolates and statistical computation

A summary showing characteristics of fungal isolates during both seasons (Wet - 32, Dry – 26)

Sample Code	Isolate	Season(W/D)	Temperature tolerance (37°C) (pos./neg.)	Extracellular Protease test		Hemolysis Test
				pos/neg	Diameter	
STRE1B		W	pos	pos	0.4/1.7	gamma
DNO2D	<i>Aspergillus niger</i>	W	pos	neg	NA	beta
KIDU21B	<i>Aspergillus fumigatus</i>	D	pos	neg	NA	alpha
ECO2A	<i>Fusarium/Aspergillus</i>	W	pos	neg	NA	alpha
DNRO2B	<i>Aspergillus flavus</i>	W	pos	neg	NA	alpha
MKRE2C		D	pos	pos	0.2/1.4	alpha
LADU19B	<i>Aspergillus fumigatus</i>	D	pos	neg	NA	alpha
RORO4A	<i>Rhinocladiella atroviveus</i>	D	pos	neg	NA	beta
RUREIC	<i>Scedosporium prolificans</i>	D	neg	neg	NA	alpha
LARO4E	<i>Aureobasidium pullulans</i>	D	neg	neg	NA	alpha
ECRO1C	<i>Aspergillus fumigatus</i>	D	neg	neg	NA	alpha
DSDU5C	<i>Alternaria alternata</i>	D	neg	neg	NA	alpha
ESDU2A		D	neg	neg	NA	alpha
DSRE3F	<i>Rhinocladiella atroviveus</i>	D	neg	pos	0.1/1.0	alpha
KIO9B	<i>Aspergillus terreus</i>	D	neg	neg	NA	alpha
LARO4E		W	pos	pos	0.3/0.9	alpha
LADU20A		W	neg	neg	NA	alpha
KIDU1B	<i>Cladophialophora batiana</i>	W	neg	neg	NA	beta
DSRO1A	<i>Chrysosporium</i>	W	neg	neg	NA	gamma
ROO2ROB		W	neg	neg	NA	alpha
ECO2B	<i>Cladosporium cladosporoides</i>	W	pos	neg	NA	beta
LAO8A		W	pos	neg	NA	gamma
EERE1A	<i>Scedosporium prolificans</i>	D	neg	neg	NA	alpha
ENRO2A	<i>Mucor hiemalis</i>	D	pos	neg	NA	alpha
KIDU23D		D	pos	neg	NA	beta

ROO1A	<i>Penicillium spp</i>	D	neg	neg	NA	gamma
KIDU22B		D	pos	neg	NA	beta
RUDU1B	<i>Geotrichum spp</i>	D	neg	pos	0.1/0.5	alpha
KADU2A		D	pos	neg	NA	alpha
EERE1C		D	neg	pos	0.3/0.6	alpha
RORO1A	<i>White fungus Aspergillus</i>	D	pos	neg	NA	alpha
ROO6A	<i>niger Penicillium</i>	D	pos	neg	NA	alpha
RORO3A	<i>accuminatum</i>	D	pos	neg	NA	alpha
DSDU2E		D	pos	neg	NA	alpha
DSRE1B	<i>Cladophialopho ra carronii</i>	D	neg	neg	NA	alpha
LARE15B		D	neg	neg	NA	gamma
DSRO5B	<i>Geotrichum candidum</i>	D	pos	neg	NA	alpha
KARE2A		W	pos	neg	NA	alpha
ESO2A		W	neg	neg	NA	beta
MKO1A		W	neg	pos	0.1/0.6	alpha
DSDU1A		W	neg	neg	NA	beta
KARE2B		W	neg	pos	0.2/1.2	alpha
STRE1A		W	neg	neg	NA	alpha
WERE1A		W	neg	pos	0.2/0.5	alpha
DSO1A		W	neg	neg	NA	alpha
EEDU2A		W	neg	pos	0./0.6	alpha
ECRE2A		W	neg	pos	0.5/0.5	alpha
ECRE2A		W	neg	pos	0.2/0.6	beta
LARE2A		W	neg	neg	NA	alpha
EERO2B		W	neg	pos	0.6/0.8	alpha
EWRO2C		W	neg	neg	NA	alpha
DNRO2A		W	neg	pos	0.2/0.5	alpha
DNDU2A		W	neg	neg	NA	alpha
LAO1B		W	neg	neg	NA	gamma
KIDU2B		W	pos	neg	NA	beta
KMO2C		W	pos	neg	NA	gamma
KIDU2A		W	pos	neg	NA	beta
ECRE1A		W	pos	neg	NA	beta
TOTALS			Wet - 32	Positive - 33	Positive - 15	Beta -12
			Dry - 26	Negative -25	Negative - 43	Gamma -7
						Alpha -39

Supplementary Table 2 Model summary showing its fitness

The utility of the model was fit $F(3,291) = 14.527$, $R^2 = .130$, $p = .00$.

Model Summary^b

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	Change Statistics			Sig. F Change	Durbin-Watson
						F Change	df1	df2		
1	.361 ^a	.130	.121	2932.32440	.130	14.527	3	291	.000	1.221

a. Predictors: (Constant), Windspeed (m/s), Temperature (°C), Humidity (%)

b. Dependent Variable: Fungal concentration (CFU m⁻³)

ANOVA^a

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	374724094.077	3	124908031.359	14.527	.000 ^b
	Residual	2502171181.336	291	8598526.396		
	Total	2876895275.412	294			

a. Dependent Variable: Fungal concentration (CFU m⁻³)

b. Predictors: (Constant), Windspeed (m/s), Temperature (°C), Humidity (%)

Supplementary Table 3 Multiple Regression coefficients

The tolerance and VIF levels were within acceptable limits. Across the two seasons, temperature, humidity, and windspeed significantly affected airborne fungal concentration ($\beta = -.358$, $t = -4.175$, $p=.000$), $\beta = -.199$, $t = -2.208$, $p=.028$), and ($\beta = -.230$, $t = -3.691$, $p=.000$), respectively.

Model	Coefficients ^a								
	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Collinearity Statistics	
	B	Std. Error				Beta	Lower Bound	Upper Bound	Tolerance
1 (Constant)	14287.472	2597.618		5.500	.000	9174.970	19399.973		
Temperature (°C)	-.27875	66.765	-.358	-4.175	.000	-410.161	147.354	.407	2.457
Humidity (%)	-40.533	18.357	-.199	-2.208	.028	-76.662	-4.404	.367	2.723
Windspeed (m/s)	-.60176	163.035	-.230	-3.691	.000	-922.638	280.882	.768	1.302

a. Dependent Variable: Fungal concentration (CFU m⁻³)

Supplementary Table 4 Correlation analysis across two seasons

Across the two seasons, temperature negatively correlated with the fungal concentration significantly ($r = -.293$, $P = .000$), humidity positively correlated with the fungal concentration significantly ($r = .187$, $P = .001$), and wind speed negatively correlated with the fungal concentration significantly ($r = -.272$, $P = .000$).

		Correlations			Fungal concentration (CFU m ⁻³)
		Temperature (°C)	Humidity (%)	Windspeed (m/s)	
Temperature (°C)	Pearson Correlation	1	-.770**	.385**	-.293**
	Sig. (2-tailed)		.000	.000	.000
	N	295	295	295	295
Humidity (%)	Pearson Correlation	-.770**	1	-.481**	.187**
	Sig. (2-tailed)	.000		.000	.001
	N	295	295	295	295
Windspeed (m/s)	Pearson Correlation	.385**	-.481**	1	-.272**
	Sig. (2-tailed)	.000	.000		.000
	N	295	295	295	295
Fungal concentration (CFU m ⁻³)	Pearson Correlation	-.293**	.187**	-.272**	1
	Sig. (2-tailed)	.000	.001	.000	
	N	295	295	295	295

** . Correlation is significant at the 0.01 level (2-tailed).

Supplementary Table 5 Sample sites with meteorological measurements and fungal concentrations

The initial in the sample sites code are the last one or/and two letter just before the numeral: RO: means Roads and Roundabouts; O: means Open Markets; DU: means Dumping Sites; RE: means Recreational sites.

Sample Site (code)	Temperature (°C)	Humidity (%)	Windspeed (m/s)	Fungal concentration (CFU m ⁻³)
DNRO1	24	55	5.4	1623.38
DNRO2	27	49	5.4	1623.38
DNRO3	27	45	5.4	1984.13
DNRO4	28	36	5.8	1443.00
DNRO5	30	33	5.8	1082.25

DNRO6	30	31	5.8	360.75
DNO1	22	62	5.0	2164.50
DNO2	25	58	5.0	2525.25
DNO3	24	55	5.4	3246.75
DNO4	27	45	5.4	2705.63
DNO5	29	41	5.4	1262.63
DNO6	30	33	5.8	901.88
DNRE1	27	49	5.4	1443.00
DNRE2	27	45	5.4	2344.88
DNRE3	29	41	5.4	1082.25
DNRE4	28	36	5.8	541.13
DNRE5	28	36	5.8	1082.25
DNRE6	30	31	5.8	721.50
DNDU1	24	55	5.4	1082.25
DNDU2	27	49	5.4	2705.63
DNDU4	28	36	5.8	1623.38
DNDU5	30	31	5.8	541.13
DSRO1	21	64	5.0	1623.38
DSRO2	23	57	5.4	1803.75
DSRO3	27	51	5.4	901.88
DSRO5	27	43	5.8	1082.25
DSRO6	28	39	6.3	1262.63
DSO1	24	58	5.0	2705.63
DSO2	23	57	5.4	1623.38
DSO3	27	51	5.4	1082.25
DSO5	27	43	5.8	541.13
DSO6	28	39	6.3	901.88
DSRE1	21	64	5.0	1082.25
DSRE3	23	57	5.4	2705.63
DSRE5	29	36	6.3	541.13
DSDU1	21	64	5.0	1262.63
DSDU2	21	64	5.0	4509.38
DSDU3	27	43	5.8	2886.00
DSDU4	28	39	6.3	721.50

DSDU5	28	39	6.3	2525.25
DSDU6	29	36	6.3	1443.00
LARO1	20	65	2.7	360.75
LARO2	20	65	2.7	721.50
LARO4	22	52	3.1	2164.50
LARO6	26	43	3.1	1082.25
LAO7	20	60	3.6	721.50
LAO8	22	52	4.0	360.75
LAO9	24	47	3.6	2525.25
LAO10	24	47	3.6	541.13
LAO11	23	49	4.0	180.38
LARE12	18	72	2.7	721.50
LARE13	20	65	2.7	360.75
LARE14	23	53	3.6	721.50
LARE15	23	49	3.6	360.75
LARE17	25	41	4.0	360.75
LADU18	18	72	2.7	1443.00
LADU19	20	60	3.6	901.88
LADU20	20	60	3.6	180.38
LADU21	26	43	3.1	541.13
KIRO1	17	69	4.0	1623.38
KIRO2	19	75	2.7	180.38
KIRO3	21	60	4.0	1082.25
KIRO4	21	60	4.0	1803.75
KIRO5	25	56	4.0	180.38
KIRO6	26	29	5.8	721.50
KIO7	18	74	3.6	1803.75
KIO8	18	74	3.6	1443.00
KIO9	24	44	4.9	721.50
KIO10	23	46	3.6	901.88
KIO11	27	29	5.8	180.38
KIO12	28	31	7.6	2525.25
KIRE13	19	75	2.7	180.38
KIRE14	25	56	4.0	180.38

KIRE15	22	49	4.0	180.38
KIRE16	26	44	4.9	901.88
KIRE18	27	46	3.6	2344.88
KIDU19	22	66	2.7	2525.25
KIDU20	22	66	2.7	721.50
KIDU21	22	64	3.6	360.75
KIDU22	21	64	3.6	2164.50
KIDU23	20	63	4.0	1803.75
KIDU24	27	29	5.8	360.75
RORO1	17	91	3.6	1985.56
RORO2	17	95	2.7	180.51
RORO3	19	81	3.1	1805.05
RORO4	19	80	7.6	2888.09
RORO5	22	72	4.5	1444.04
RORO6	22	70	4.5	1444.04
ROO1	18	87	4.2	541.52
ROO2	18	82	5.0	2707.58
ROO3	18	81	5.0	2527.08
ROO4	19	80	4.7	1083.03
ROO5	22	71	4.5	3429.60
ROO6	22	64	6.1	5415.16
RORE1	18	81	5.0	541.52
RORE3	19	81	4.7	722.02
RORE4	21	72	5.3	902.53
RORE5	21	72	5.3	1444.04
RORE6	21	72	5.3	1263.54
RODU1	17	91	3.9	722.02
RODU2	19	81	4.7	541.52
RODU3	19	80	4.7	722.02
RODU4	21	81	5.3	361.01
RODU5	22	64	5.3	1263.54
KARO1	12	96	3.6	1624.55
KARO2	18	93	3.1	722.02
KAO1	19	93	3.1	1624.55

KAO2	18	93	3.1	9025.27
KARE1	11	100	3.3	180.51
KARE2	21	87	3.1	722.02
KADU1	13	93	3.3	1805.05
KADU2	19	93	3.1	3429.60
RURO1	19	87	2.5	5054.15
RURO2	20	82	3.1	2166.06
RUO1	22	80	1.9	361.01
RURE1	20	82	1.9	3068.59
RURE2	20	82	3.1	2166.06
RUDU1	20	82	3.1	2166.06
RUDU2	20	82	3.1	3790.61
ESRO1	27	56	4.7	2707.58
ESRO2	27	56	4.7	1444.04
ESO1	23	72	3.6	3790.61
ESO2	27	56	4.7	1444.04
ESRE1	23	72	3.6	3068.59
ESRE2	26	56	4.2	1624.55
ESDU1	23	72	3.6	1263.54
ESDU2	25	63	4.7	1624.55
ENRO1	25	73	1.7	8483.75
ENRO2	25	73	1.7	4512.64
ENO1	25	73	1.7	2707.58
ENO2	23	72	3.9	2166.06
ENRE1	25	73	1.7	1083.03
ENRE2	31	63	1.7	541.52
ENDU1	31	63	1.7	3971.12
ECRO1	19	88	4.2	7761.73
ECRO2	23	68	4.7	3068.59
ECO1	19	88	4.2	2707.58
ECO2	21	72	4.7	9025.27
ECRE1	24	68	4.7	9025.27
ECRE2	23	68	4.7	1624.55
ECDU1	20	88	4.2	3790.61

ECDU2	23	68	4.7	1083.03
EERO1	29	50	3.6	1083.03
EERO2	30	49	3.1	4693.14
EEO1	27	50	3.6	7581.23
EEO2	30	47	3.1	1624.55
EERE1	27	46	3.6	541.52
EERE2	26	46	5.6	2888.09
EEDU2	28	48	3.9	3068.59
EWRO1	24	64	3.6	1623.38
EWRO2	29	50	3.6	2344.88
EWO1	27	62	3.6	2886.00
EWO2	25	56	4.7	2344.88
EWRE1	26	53	3.6	2164.50
EWRE2	29	50	3.6	3246.75
EWDU1	27	62	3.6	1984.13
EWDU2	25	56	4.7	180.38
STRO1	20	93	1.7	4873.65
STO2	20	93	1.7	4151.62
MTRO1	17	93	2.5	5776.17
MTRO2	19	93	2.5	3971.12
MTO1	18	93	2.5	2346.57
MTO2	18	87	3.1	902.53
MTRE1	19	87	2.5	1805.05
MTDU1	18	93	2.5	2707.58
MTDU2	18	87	3.1	722.02
DNRO1	22	52	1.7	12445.89
DNRO2	22	52	1.7	60064.94
DNO1	24	53	3.6	23268.40
DNO2	22	49	3.9	5952.38
DNRE1	22	52	1.7	4689.75
DNRE2	21	52	3.6	7756.13
DNDU1	25	52	3.9	11544.01
DNDU2	22	52	1.7	11363.64
DSRO1	20	58	3.1	7034.63

DSRO2	20	57	3.6	6132.76
DSO1	20	57	3.6	2525.25
DSO2	20	57	3.6	6854.26
DSRE1	25	52	3.9	3066.38
DSRE2	21	52	3.6	2525.25
DSDU1	20	58	3.1	6313.13
DSDU2	20	57	3.6	4329.00
LARO1	17	88	2.2	2344.88
LARO2	20	79	2.2	1082.25
LAO1	21	78	2.2	2344.88
LAO2	23	71	2.8	6493.51
LARE1	17	88	2.2	3787.88
LARE2	21	78	2.2	6673.88
LADU1	23	71	2.2	2705.63
LADU2	23	71	2.2	4870.13
KIRO1	23	71	2.8	2705.63
KIRO2	21	52	3.1	3246.75
KIO1	19	65	2.8	3427.13
KIO2	19	65	2.8	6673.88
KIRE1	18	64	3.1	180.38
KIRE2	20	58	3.1	180.38
KIDU1	23	71	2.8	5230.88
KIDU2	23	64	3.1	30303.03
RORO1	21	65	2.2	18050.54
RORO2	24	45	2.2	6859.21
ROO1	22	65	6.4	9025.27
ROO2	21	67	6.4	20036.10
RORE1	19	69	4.2	4512.64
RORE2	23	55	5	7039.71
RODU1	18	87	2.2	9386.28
RODU2	19	87	2.5	11371.84
KARO1	17	81	3.1	4151.62
KARO2	18	75	3.1	4512.64
KAO1	18	74	3.1	5776.17


KAO2	19	72	3.3	6498.19
KARE1	20	70	3.3	2527.08
KARE2	17	79	2.2	1985.56
KADU1	18	75	2.5	3790.61
KADU2	17	77	2.2	4873.65
RURO1	19	66	3.3	9747.29
RURO2	20	68	3.6	7761.73
RUO1	19	65	3.6	15162.45
RUO2	20	64	3.3	11732.85
RURE1	18	74	3.1	3790.61
RURE2	18	69	2.8	1263.54
RUDU1	20	64	3.6	2888.09
RUDU2	21	63	3.3	3249.10
ESRO1	18	72	2.5	2888.09
ESRO2	18	72	2.5	2346.57
ESO1	19	66	2.5	2166.06
ESO2	18	72	2.5	5054.15
ESRE1	18	72	2.5	5776.17
ESRE2	20	63	1.7	5776.17
ESDU1	20	65	2.5	2166.06
ESDU2	20	63	1.7	4512.64
ENRO1	16	77	4.2	4512.64
ENRO2	17	77	4.2	5054.15
ENO1	16	77	4.2	7220.22
ENO2	17	85	3.9	2166.06
ENRE1	18	74	4.2	8483.75
ENRE2	17	77	4.2	4332.13
ENDU1	15	82	3.6	12996.39
ENDU2	17	77	4.2	5595.67
ECRO1	16	77	4.2	3790.61
ECRO2	17	77	3.1	2527.08
ECO1	18	77	3.1	2346.57
ECO2	17	77	3.1	5234.66
ECRE1	18	70	4.2	3068.59

ECRE2	17	77	3.1	9205.78
ECDU1	17	77	4.2	3249.10
ECDU2	18	77	5.3	4151.62
EERO1	18	77	5.3	3429.60
EERO2	17	77	5.3	2888.09
EEO1	17	77	5.3	2527.08
EEO2	20	72	3.6	7039.71
EERE1	17	72	3.6	3610.11
EERE2	18	72	3.6	6859.21
EEDU1	19	68	5.2	5415.16
EEDU2	16	77	5.2	3610.11
EWRO1	15	84	2	4148.63
EWRO2	15	87	2.5	3787.88
EWO1	16	77	2	3607.50
EWO2	16	77	2	5772.01
EWRE1	15	87	2.5	13528.14
EWRE2	16	87	2.5	12806.64
EWDU1	17	75	3.1	20021.65
EWDU2	16	87	2.5	13528.14
MKRO1	16	72	4.7	3971.12
MKRO2	15	72	4.7	5234.66
MKO1	15	75	4.7	5595.67
MKO2	17	67	4.2	5234.66
MKRE1	15	82	4.7	1985.56
MKRE2	16	72	4.7	1985.56
MKDU1	16	72	4.7	43321.30
MKDU2	16	72	4.7	30324.91
KMRO1	18	67	4.2	2166.06
KMRO2	17	67	4.2	2527.08
KMO1	17	67	4.2	3610.11
KMO2	18	63	4.2	4332.13
KMRE1	16	63	4.2	15342.96
KMRE2	16	63	4.2	1083.03
KMDU1	17	67	4.2	3068.59

KMDU2	19	62	4.2	3429.60
STRO1	20	68	3.7	5234.66
STRO2	19	69	3.5	5415.16
STO1	18	71	3.6	4512.64
STO2	20	69	3.5	5956.68
STRE1	19	70	3.8	3971.12
STRE2	20	69	3.6	7581.23
STDU1	19	66	3.5	3790.61
STDU2	20	71	3.4	6317.69
MTRO1	18	52	3.6	13357.40
MTRO2	19	53	3.1	3068.59
MTO1	21	54	3.3	3249.10
MTO2	22	52	3.6	3429.60
MTRE1	18	52	3.6	13537.91
MTRE2	20	55	3.1	6678.70
MTDU1	17	55	3.1	2527.08
MTDU2	18	57	3.9	2888.09
WERO1	18	67	3.6	7581.23
WERO2	20	66	3.9	7039.71
WEO1	20	64	3.6	1444.04
WEO2	21	64	3.3	1444.04
WERE1	20	64	3.6	1985.56
WERE2	19	63	3.1	2346.57
WEDU1	20	64	3.6	1444.04
WEDU2	19	66	3.9	4151.62

APPENDICES

Appendix I: Research Approval Letter



KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke P.O. Box 43844, 00100
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Internal Memo

FROM: Executive Dean, Graduate School **DATE:** 4th June 2024

TO: Mr. Kipchumba Kiprop Vincent **REF:** 156/CTY/PT/20840/2021
C/O Department of Biochemistry, Microbiology and Biotechnology

SUBJECT: APPROVAL OF RESEARCH PROPOSAL
=====

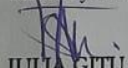
We acknowledge receipt of your Research Proposal after fulfilling recommendations raised by the Graduate School Board of 14th June 2023.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Also, please ensure that you publish article(s) from your thesis before submitting it to Graduate School for examination as per the Commission for University Education and Kenyatta University guidelines.

Thank you.


JULIA GITU
FOR: EXECUTIVE DEAN, GRADUATE SCHOOL

cc. Chairman, Department of Biochemistry, Microbiology and Biotechnology

Supervisors:

1. Dr. Anthony K. Nyamache
C/O Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University
2. Dr. Christine Bii, KEMRI
C/O Biochemistry, Microbiology and Biotechnology
Kenyatta University

JG/ren

Appendix II: KEMRI License



In Search of Better Health

KENYA MEDICAL RESEARCH INSTITUTE

OFFICE OF THE DIRECTOR RESEARCH & DEVELOPMENT

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KEMRI/RD/22

October 02, 2024

**TO: VINCENT KIPROP KIPCHUMBA,
PRINCIPAL INVESTIGATOR.**

**THROUGH: THE DEPUTY DIRECTOR, CMR,
NAIROBI.**

Dear Sir,

RE: PROTOCOL NO. KEMRI/SERU/CMR/P0222/4675 (REQUEST FOR ANNUAL RENEWAL AND DEVIATION): FUNGAL SPORE POLLUTION: CONCENTRATION, AND DIVERSITY IN SELECTED AREAS IN NAIROBI

Thank you for the continuing review reports for the period **July 03, 2023 to May 22, 2024**.

The Expedited Review Team noted that a protocol deviation was submitted due to the late submission of request for continuation. The measures taken to preclude future occurrence was deemed unsatisfactory.

After careful consideration, the Expedited Review Team finds that more information is necessary before a final decision on the study can be reached:

1. CRR Submission Form
 - a. Under the progress summary (part 5), kindly provide summarized information on the major activities of the project within the reporting period. E.g number of participants recruited, follow-ups done, lab work done or as stated in part 4.
 - b. In the CRR form (part 4) Please write the objectives as approved by KEMRI SERU
 - c. Provide a separate report for the period June 05, 2024 to September 10, 2024
2. Protocol deviation
 - a. Please note that the reason for failure to renew (waiting for funding) is not a sufficient reason for failure to seek renewal and is not related to the renewal of the study. The study can still be renewed in the absence of funding.
 - b. Measures taken to preclude future occurrence is not satisfactory. Kindly highlight measures that will help you not to forget renewing the study like calendar reminders e.t.c

Please address the issues raised and submit the revised application to the SERU Secretariat via seru@kemri.go.ke by **November 02, 2024** for further action.

Yours faithfully,

**ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT**

In Search of Better Health