PHYSICOCHEMICAL PROPERTIES AND ISOLATION OF FUNGI FROM RESERVOIR WATER IN BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY

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CERTIFICATION

We certify that this project work was carried out by **Favour Osamagbe Asemota** in partial fulfilment of the requirement for the award of Bachelor of Science (B.Sc.) degree in Microbiology.

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Date

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(Head of Department)

Date

DEDICATION

This project is dedicated to the Almighty GOD, My Late Dad, my ever supportive Mum, to my siblings and to Yemi, for being nothing but a blessing to me all way round.

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ABSTRACT

Water is a vital resource for all known living organisms, as there is no life without it. The Earth's surface is 71% water and fresh water comprises 3% of the total amount water on the Earth's surface. The aim of the present study was to determine the physicochemical parameters and isolation of Fungi from reservoir Water in different communities in Benin City. Water samples were collected from Adolor, BDPA, Ekosodin, Isihor, Osasogie, Uniben and Uwasota all in Benin City, Edo State. The samples were then taken to the laboratory and cultured on potato dextrose agar using the pour plate technique. The pure culture of fungal isolates were identified phenotypically. The isolates antifungal susceptibility profiles were determined

The results for physiochemical analysis of the reservoir water sample showed that the pH values were within the range of 7.59 – 6.63, electrical Conductivity ranged from 13 - 159 µS/cm. The temperature range gotten from this study is 25.3 – 29.4 ^{o}C. Total suspended solid varied from 0.59 – 0.98 g/l. Biochemical oxygen demand ranged from 0.02 – 0.07. The alkalinity ranged from 0.12 to 0.5 g/l. Manganese was present in the ranged from 0.01 to 0.15 mg/l. The total fungal count was shown a ranged of 0.025×10^3 - 2.3×10^3 cfu/ml. The highest fungal count was found in Ekosodin, having a value of 2.3×10^3 cfu/ml while the lowest was from Osasogie, value of 0.025×10^3 cfu/ml. The identified fungal isolates from the various samples of water were *Penicilium chrysogenum, Yeast, Rhizopus stolonifera, Aspergillus flavus* and *Aspergilus niger.* The isolate with the lowest percentage distribution was *Penicilium chrysogenum*, asides *Penicilium chrysogenum* the rest of the isolates were found in all the sampled locations. The pathogenicity result showed all isolates were positive to Gamma Haemolysis and Lipase production. All isolates showed susceptibility to Nystatin but showed varying resistance to Vericonazole with penicillin showing the highest

susceptibility. Recent discoveries on fungi requiring special attention include the presence of opportunistic and emerging pathogens in water sources.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Water is a vital resource for all known living organisms, as there is no life without it (Shiklomonov *et al.,* 2000). The Earth's surface is 71% water and fresh water comprises 3% of the total amount water on the Earth's surface (Hamid, 2006). This small percentage of fresh water plays an essential role in human life. There are many sources of water pollution, but the most notable source is microbial contamination. Sewage or untreated wastewater releases a large amount of dissolved organic material, suspended materials, and harmful microbiological organisms (Bhat *et al.,* 2010).

Fungi are one of the most important biological sources of water contamination. These fungi may originate from the soil. Several studies have been conducted to detect the presence of fungi in water. Muhsin *et al.* (1989) isolated several fungi from well water, including *Alternaria* sp., *Aspergillus* sp., *Trichoderma viride*, *Penicillium* sp., *Fusarium oxysporum*, and *Cladosporium* sp. Moreover, Rajanaika *et al.* isolated Pythium, *Allomyces*, *Rhizopus*, *Achyla*, *Alternaria*, *Aspergillus*, *Saprolegnia*, *Chaetominm*, *Cladosporium*, *Fusarium*, *Trichoderma*, and *Penicillium* from the Tunga River in India. Kraume and Awad found 60 species of fungi belonging to 27 genera in wastewater samples, including 8.8% Geotrichum, 75% Penicillium, 65.7% yeasts, and 55.5% Trichoderma (Awad and Kraume, 2010).

Fungi are ubiquitous, heterotrophic organisms present in oceans, fresh water and drinking water. They can be divided based on the ability to colonize different environments into three groups: as mesophilic fungi, generalists and specialists (Gostincar *et al.,* 2011). Mesophilic species inhabit niches with moderate physicochemical parameters, while generalists grow under changing life conditions, but with growth optimum under moderate conditions.

Specialists inhabit extreme habitats and are unable to grow under moderate conditions (Gostincar *et al.,* 2011). Ecologically, fungi are saprophytes, degrading organic matter, with some species acting also as parasites or symbionts (Wurzbacher *et al.,* 2011). Due to their diverse life cycle, ability to form large hyphal networks and produce spores, or growing as single yeast-cells, they maximize nutrients uptake and can survive under various life conditions (Wurzbacher *et al.,* 2011). In the last 30 years, the presence of a high variety of fungi was reported from European water, including surface, ground and tap water intended for human consumption (Oliveira *et al.,* 2013).

Fungi growing in biofilms inside taps and in tap water affect the taste and odour, interfering with the chlorination process, due to the release of a large scale of products known as secondary metabolites. These may be very diverse and specific for different fungal species (Heinrichs *et al.,* 2013). While the role of secondary metabolites in the ecology of fungi is to defend their habitat, and suppress the growth of competitors (Biedunkiewicz *et al.,* 2014), some of them are toxic to animals, and may present a risk for human health in higher concentrations or under prolonged time of exposure (Schültze *et al.,* 2010). Not only secondary metabolites, but also fungal cell wall components and the fungal load itself may contribute to the emergence of allergies and other opportunistic and systemic infections, mainly in immunocompromised individuals (DeHoog *et al.,* 2014). Although in the last few decades fungi are becoming frequently recognized as causative agents of respiratory, mucosal, rhinocerebral, cutaneous and subcutaneous infections (DeHoog *et al.,* 2014).

1.2 STATEMENT OF THE PROBLEM

Fungi in water can be a serious problem including freshwater and marine fungi. Some of these fungi can cause health problems, such as infections and allergies. Also, problems associated with water quality, may be blockages of pipes ,corrosion, changes in taste and smell of the water (Paz *et al*., 2010).

Water quality is of paramount importance in ensuring the availability of safe and clean drinking water for human consumption and various domestic, industrial, and agricultural uses. In the context of reservoir water, which often serves as a vital source of freshwater supply, the physicochemical parameters and the presence of fungi are critical factors that directly impact water quality.

Reservoirs are susceptible to various contaminants, both natural and anthropogenic, that can compromise water quality. Understanding the physicochemical parameters of reservoir water, such as pH, turbidity, total dissolved solids (TDS), electrical conductivity (EC), and levels of specifications (e.g., nitrate, phosphate), is essential for assessing the suitability of the water for various purposes, including drinking water supply, irrigation, and recreational activities.

Also the presence of fungi in reservoir water is a significant concern due to the potential health risks they pose to humans. Certain fungal species can produce mycotoxins and allergenic compounds that, when ingested or inhaled, can lead to adverse health effects, including mycotoxicoses and respiratory allergies. Understanding the diversity and prevalence of fungi in reservoir water is crucial for assessing public health risks and implementing appropriate water treatment measures. Reservoir water quality can also vary seasonally due to factors such as temperature, rainfall, and runoff. Research is needed to investigate how physicochemical parameters and fungal populations change over different seasons and whether these variations have implications for water treatment and management strategies.

Human activities in the catchment area of reservoirs, including agriculture, urbanization, and industrial discharges, can introduce pollutants into the water body. It is essential to assess how these activities influence the physicochemical parameters and fungal contamination in reservoir water and to identify potential sources of pollution.

Reservoir water often requires treatment to meet regulatory standards for safe drinking water. Understanding the extent of fungal contamination and its potential resistance to common water treatment processes, such as chlorination and filtration, is vital for optimizing treatment protocols and ensuring the provision of safe and potable water.

Fungal contamination in reservoir water can also have ecological consequences, potentially affecting aquatic life and ecosystem health. Investigating the ecological interactions between fungi and other microorganisms in the water is important for assessing ecosystem resilience and stability.

Finally, Compliance with national and international water quality standards is crucial to safeguard public health. Research is needed to determine if reservoir water quality, including physicochemical parameters and fungal contamination, meets the established regulatory requirements and to recommend mitigation measures if necessary.

This research project aims to address these problems by conducting a comprehensive analysis of the physicochemical parameters of reservoir water and the isolation, identification, and characterization of fungi present in these water sources. The findings will contribute to improved water quality management, public health protection, and the development of effective water treatment strategies for reservoirs.

1.3 JUSTIFICATION OF THE STUDY

In Nigeria water borne diseases are one of the main problems in rural and urban communities. These diseases are as a result of bacterial, fungal or other microbial infection of water. Unfortunately, most water screening methods in Nigeria are focused on the occurrence and significance of bacteria with little attention to other microorganisms such as fungi (Okpako *et al*., 2009). It is on this note that I decided to investigate the prevalence and significance of fungi in reservoir water in Benin City, Nigeria.

1.4 AIM AND OBJECTIVES

The aim of the study was to determine the physicochemical parameters, identify pathogenic and other contaminating fungi in water samples collected from wells used by students for drinking and other domestic purposes in the University of Benin and communities around the institution.

The specific objectives of the study were to:

1. determine the physicochemical parameters of the well water samples
2. isolate and enumerate fungal contaminants of well water
3. determine the anti-biogram of the fungal isolates
4. determine the pathogenicity of the fungal isolates

CHAPTER TWO

LITERATURE REVIEW

2.1 Fungi

A fungus is a eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are heterotrophs and, like animals, obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) and are called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes, saprobes). Some fungi infect a living host, but kill host cells in order to obtain their nutrients; these are called necrotrophs (Hawksworth, 2001).

Fungi were once considered to be primitive members of the plant kingdom, just slightly more advanced than bacteria. We now know that fungi are not primitive at all. In fact, recent taxonomic treatments such as the Tree of Life Project show that fungi and animals both belong to the group Opisthokonta (Blackwell, 2011). Fungi may not be our next of kin, but they are more closely related to animals than they are to plants. We also recognize that organisms traditionally studied as "fungi" belong to three very different unrelated groups: the true fungi in Kingdom Fungi (Eumycota), the Oomycetes, and the slime molds.

2.2 Species of Fungi

No one knows for sure how many species of fungi there are on our planet at this point in time, but what is known is that at least 99,000 species of fungi have been described, and new species are described at the rate of approximately 1200 per year (Kirk *et al.*, 2008; Blackwell, 2011). A conservative estimate of the total number of fungal species thought to exist is 1. 5 million (Hawksworth, 2001). To come up with this figure, Hawksworth estimated the known numbers of plant and fungal species from countries in which both plants and fungi have been well-studied Great Britain and Ireland, in this case and determined there were six fungal species for every native plant species. The total number of plant species worldwide is approximately 250,000, and if the ratio of fungi to plants in Great Britain is typical of what occurs elsewhere, there should be at least 1.5 million species of fungi (Hawksworth, 2001).

If 1. 5 million fungal species are a reasonable estimate, the vast majority of all extant fungi are yet to be named. Assuming a relatively constant rate at which new species are described, it will take more than 1100 years to catalog and describe all remaining fungi. However, many of these fungi are likely to become extinct before they are ever discovered given current rates of habitat and host loss. For example, up to 2% of tropical forests are destroyed globally each year (Purvis and Hector 2000). These habitats are exceedingly rich in fungal species (Hawksworth and Rossman, 1997). For example, 15-25% of fungi collected in short-term studies in the tropics are new species (Kirk *et al.,* 2008). Callan and Carris, (2004) estimated that an 110,000 ha neotropical forest, such as in Costa Rica, could contain over 81,000 different species of plant parasitic fungi almost as many as all the known species of fungi! Consider that this estimate was based only on plant parasitic fungi, and did not take into account other ecological groups of fungi such as saprotrophs.

2.3 Reproduction of Fungi

Fungi frequently reproduce by the formation of spores. A spore is a survival or dispersal unit, consisting of one or a few cells, that is capable of germinating to produce a new hypha. Unlike plant seeds, fungal spores lack an embryo, but contain food reserves needed for germination. Many fungi produce more than one type of spore as part of their life cycles. Fungal spores may be formed via an asexual process involving only mitosis (mitospores), or via a sexual process involving meiosis (meiospores). The manner in which meiospores are formed reflects the evolutionary history and thus the classification for the major groups (phyla) of fungi.

Many fungi are able to reproduce by both sexual and asexual processes. Sexual and asexual reproduction may require different sets of conditions (e. g., nutrients, temperature, light, moisture). In some fungi, two sexually compatible strains must conjugate (mate) in order for sexual reproduction to occur. The terms 'anamorph' and 'teleomorph' are used to convey the asexual and sexual reproduction morphological types, respectively, in a particular fungus. Alexopoulos *et al.* (1996), Kendrick (2000), or Webster and Weber (2007).

2.3.1 Meiospores

Examples of meiospores spores that are the products of meiosis include ascospores (Ascomycota) and basidiospores (Basidiomycota). Ascospores are formed inside a sac-like structure called an ascus. An ascus starts out as a sac of cytoplasm and nuclei, and by a process called "free cell formation" (Kirk *et al.,* 2008) a cell wall forms de novo around each nucleus and surrounding cytoplasm to form ascospores (typically eight per ascus). Ascospores vary in size, shape, color, septation, and ornamentation among taxa. Basidiospores are formed on a basidium and are typically one-celled with one or two haploid nuclei. Basidiospores vary in size, color and ornamentation depending upon the taxonomic group.

2.3.2 Mitospores

Examples of mitospores are conidia (sing. conidium), sporangiospores, and zoospores, formed by members of the phyla Ascomycota, Zygomycota, and Chytridiomycota, respectively. Another type of asexual propagule produced by fungi in several different phyla is the chlamydospore.

2.3.3 Conidia

Conidia are formed from a modified hypha or a differentiated conidiogenous cell of the fungus. Conidiogenous cells can be formed singly on hyphae, on the surface of aggregated hyphal structures, or within different types of fruiting bodies. Fruiting bodies inside which conidia are formed are pycnidia and acervuli. Sporodochia and synnemata are examples of fruiting bodies on which conidia are formed. Conidia are produced primarily by Ascomycota, although some Basidiomycota are capable of producing them as well (Kirk *et al.,* 2008).

2.3.4 Sporangiospores

Sporangiospores are asexual propagules formed inside a globose or cylindrical sporangium by a process involving cleavage of the cytoplasm. Sporangiospores are thin-walled, one-celled, hyaline or pale-colored, and are usually globose or ellipsoid in shape. One to 50,000 sporangiospores may be formed in a single sporangium. When mature, sporangiospores are released by breakdown of the sporangial wall, or the entire sporangium may be dispersed as a unit. Sporangiospores are produced by fungi in phyla Chytridiomycota and Zygomycota, as well fungal-like Oomycetes (Kirk *et al.,* 2008).

2.3.5 Zoospores

A zoospore is a microscopic, motile propagule, approx. 2 to 14 µm long and 2 to 6 µm in diameter that lacks a cell wall and is characterized by having one or more flagella. Flagella are ~ 0. 25 µm in diameter and up to 50 µm long. Zoospores are produced by one group of true Fungi (Chytridiomycota), and by fungal-like organisms in Kingdom Straminipila and some slime molds (see section "Fungal-like Organisms Studied by Plant Pathologists and Mycologists"). Two types of flagella are known—the whiplash flagellum, which is directed backward, and the tinsel flagellum, which is directed forward. The tinsel flagellum is only present in members of Kingdom Straminipila and does not occur in true fungi. The length of time zoospores are able to swim is determined by their endogenous energy reserves—zoospores cannot obtain food from external sources—and environmental conditions. Zoospores may exhibit chemotaxis— movement in response to a chemical gradient, e. g., root exudates. At the end of its motile phase, the zoospore undergoes a process called encystment in which it either sheds or retracts the flagella and produces a cell wall. The encysted zoospore, called a cyst, may germinate directly by the formation of a germ tube, or indirectly by the emergence of another zoospore (Adl *et al.,* 2005).

2.3.6 Chlamydospores

Chamydospores are survival propagules formed from an existing hyphal cell or a conidium that develops a thickened wall and cytoplasm packed with lipid reserves. The thickened cell walls may be pigmented or hyaline, and chlamydospores develop singly or in clusters, depending upon the fungus. Chlamydospores are passively dispersed, in most instances when the mycelium breaks down. Chlamydospores are formed by many different groups of fungi and are often found in aging cultures (Blackwell, 2011).

2.4 Microbial Quality of Drinking Water

Microbial quality of water is usually monitored by measuring microorganisms using indicator organisms such as *Escherichia coli* (Obi *et al*., 2008). However, over-reliance on indicator bacteria to determine the sanitary and public health safety of treated drinking water has its own challenges, including the fact that other pathogens like enteroviruses and protozoa are more resistant to disinfection than E. coli, such that a zero count of E. coli does not essentially indicate the absence of other microorganisms (Meals *et al*., 2013). Heterotrophic plate count is the only indicator method for fungi as it is used to indicate changes in microbial concentration that show entry or regrowth in treated drinking water (Zerzghi *et al*., 2010; Babič *et al*., 2017). The problem, however, is that there is no regulatory value with the heterotrophic plate count, leading to a conclusion of compliance that is defined as a 'no abnormal change' which may ultimately not indicate the presence or absence of fungi (Babič *et al*., 2017). The available methods may not be reliable to detect and/or quantify all the waterborne pathogens including fungi that are also known to resist disinfection.

The WHO has an international obligation of issuing guidelines of universal application (WHO, 2017) that also entail the issuing of directives, determination of legislation, setting of recommendations, and requires the testing and monitoring of drinking water quality (Babič *et al*. 2016). A preventative approach that only monitors the quality of treated drinking water, the 'Water Safety Plans' was endorsed by the WHO (WHO, 2017). The plan considers factors that may contribute to endangering the quality of water from the source of water to the end user (José Figueras and Borrego, 2010). While the WHO did not include fungi in the routine list of microbiological parameters used to determine the quality of treated drinking water, it has labelled fungi as nuisance organisms because of taste and odour problems (Sonigo *et al*. 2011).

Other water quality legislations include those by the United State Environmental Protection Agency, an agency of the federal government of the United States created for the purpose of protecting human health and the environment by drafting and implementing regulations based on laws passed by Congress (Kanematsu and Barry 2016). The EU Drinking Water Regulations (2014) recommend the application of water quality standards for Europe, European Union Countries and Northern Europe, and procedures for sampling frequency and methods of analysis (EU 2014). The above water quality legislative bodies also do not regulate the limits for fungi in treated drinking water, and the presence of fungi in tap water samples (Babič *et al*. 2017). The USEPA did look at the addition of microsporidia in drinking water regulations although it was later withdrawn from its 'Contaminant Candidate List' (Babič *et al*. 2017). Sweden is the only country that currently includes specific measures for the monitoring of micro-fungi in treated drinking water. The Swedish Drinking Water Guidelines specify a criterion of 100 CFU of micro-fungi per 100 mL in treated water as being fit for human consumption (Sammon *et al*., 2010).

The EU only issued a recommendation for mycotoxins to protect consumer's food and animal feeds from harmful effects of mycotoxins that became effective as legislation from the 1st October 2006 (Zain, 2011). Mycotoxin regulations in food and feeds have been established in approximately 100 countries worldwide, with only 15 of these countries from Africa, to protect the consumer from harmful effects of these mycotoxins (Wagacha and Muthomi, 2008). Ever since the 2003 FAO report which discovered that there were only aflatoxin regulations in Africa, there has been little or no progress regarding mycotoxin regulations in the continent (Misihairabgwi *et al*., 2017). Developing countries do not take into consideration mycotoxin regulations, predominantly due to lack of country-specific data of certain toxins, resources to obtain toxicological and exposure data and the analytical capacity to enforce the regulations (Wagacha and Muthomi 2008; Misihairabgwi *et al*. 2017).

2.5 Occurrence and Prevalence of Fungi in Drinking Water

Fungi are natural inhabitants of composting plants, soil and water (Korzeniewska 2011; Calvo-Polanco *et al*., 2016). Terrestrial fungi are capable of migrating from soil into fresh water systems through animals, plants and soil (Wurzbacher *et al*., 1998; Magwaza *et al*., 2017). Fungal species like Fusarium and Aspergillus species have been identified to multiply in water reservoirs where they have been implicated in waterborne infections (Kanzler *et al*., 2007). Fungi in treated drinking water was first noticed in the 1960s and 1970s due to taste and odour problems, but was little considered as it was not the main focus for the analysis (Hageskal *et al*., 2009), and also because the contributory connection between the occurrence of fungi and water quality was not yet understood (Doggett, 2000). The consumption of drinking water polluted by fungi had not been associated with serious disease casualties until studies performed in the 1980s revealed a number of cases linked to fungal contaminated treated drinking water (Hageskal *et al*., 2009). Fungal deposition in water distribution systems is attributed to spores and not hyphae growth, which raises concerns that mycotoxins' production of taste and odour problems suggest that vegetative growth occurs in situ (Doggett 2000). In the water distribution system, the biomass of microorganisms suspended in the water phase is the main site of the occurrence of fungi belonging to moulds, including pathogenic species that are harmful to humans (Grabin´skaŁoniewska *et al*., 2007).

Fungi in treated drinking water from the various investigations, shows that the most prevalent fungi are *Acremonium* sp., *Alternaria* sp., *Aureobasidium* sp., *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., *Epicoccum* sp., *Exophiala* sp., *Fusarium* sp., *Geotrichum* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium* sp., *Phialophora* sp., *Phoma* sp., *Rhizopus* sp., *Trichoderma* sp. And *Verticillium* sp. Most of the fungal genera described in the studies are dematiaceous fungi which are capable of secreting melanin or melanin-like pigment in their cell walls. This makes them thick-walled species with hydrophobic spores which give them the advantage to resist water treatment (Babič *et al*., 2017). These persistent fungi normally originate from soil, wood and decomposing plant material (Fox *et al*., 2016), which explains why they end up in raw water. Cladosporium sp., Penicillium sp., Fusarium sp., Penicillium sp., Aspergillus sp., Phoma sp., Epicoccum sp., Trichoderma sp., Acremonium sp., Exophiala sp., Alternaria sp. And Phialophora sp. are capable of producing mycotoxins and other secondary metabolites that produce toxic chemicals which impair water quality and become a threat to humans and animals (Sonigo *et al*., 2011).

2.6 Sources and Types of Fungi Found in Water

Physical openings in storage facilities, and lack of cover allow microorganisms to be introduced from the air, animals, introduction of untreated surface or groundwater, etc can be a source of Fungal infection to stored water (US EPA, 2002). Soil-borne fungi can enter underground storage systems through leaks on the ground and mains joints if the main pressure is low, or during potentially during maintenance (University of Sheffield, 2009). Airborne species can be introduced from the air in contact with stored water (Göttlich *et al*., 2002 and Gonçalves *et al*., 2006). Physical entrapment of the spores may be responsible for the introduction of hydrophobic spores in water systems (Gonçalves *et al*., 2006). Once introduced, fungal species can become established on the inner surfaces of pipes, including interaction and reaction with, sealings and coatings, and biofilms within distribution systems, or can be suspended in the water (Göttlich *et al*., 2002, Grabinska-Loniewska *et al*., 2007 and Gonçalves *et al*., 2006). Some species are found throughout water distribution networks, while others may be restricted to localised sites (Kelley *et al*., 1997). For example, Göttlich *et al*. (2002) classified *Phialophora, Exophiala* and *Acremonium* as widespread and resident, and *Verticillium* and *Phoma* as transients with restricted distribution. The presence of transient species indicates that either such species grow at localised points within the system or that the system is regularly breached, allowing frequent local contamination (Kelley *et al*., 1997). Water with long residence times in dead ends, tidal points and oversized pipes, and stored water on the consumer side, i.e. in tanks and other storage facilities, is particularly vulnerable to fungal colonisation (Paterson and Lima, 2005, Hageskal *et al*., 2007). Terminal pipe ends are favoured locations for fungal colonisation as they typically do not support sufficient concentrations of residual chlorine to kill fungi (Grabinska-Loniewska *et al*., 2007). At the consumer side, installations such as cisterns, heating tanks, taps, and shower heads can yield large numbers of fungi (in terms of Colony Forming Units (CFUs)) (Hageskal *et al*., 2007). For example, Anaissie *et al*. (2002) found that Aspergillus species were significantly more likely to be isolated in significantly greater concentrations ($p=0.001$) from cold water storage tanks than from municipal water or water from cold taps.

2.7 Abiotic and Anthropogenic Factors Influencing Ecology of Fungal Taxa in Water Systems

a. Raw Water Source

Studies that included analyses of both groundwater-derived and surface water-derived drinking water found that isolation of fungi was more likely from surface water-derived drinking water (Hageskal *et al.*, 2006). For example, Hageskal *et al.* (2006) found that a greater proportion of surface water- derived drinking water samples were positive for fungi than groundwater-derived samples. However, there was not a great difference in the total mean number of CFUs obtained from all samples of surface water-derived water taken by Hageskal *et al.* (2007), compared to all samples of groundwater-derived water (9.5 CFU 100 ml-1 and 8.4 CFU 100 ml-1 respectively). There was one anomalous data point in the groundwater sample – sampling of one shower head produced 100 CFU 100 ml-1, which increased the total number of CFUs found in groundwater-derived water samples. In a study of untreated source water, Pereira *et al.* (2009) found significantly higher mean levels of fungi in surface and spring water (1750 CFU 100 ml-1 and 1025 CFU 100 ml-1 respectively) than in groundwater (66 CFU 100 ml-1).

The source of the raw water affects the total number of CFUs found due to biotic and abiotic differences between surface and groundwater. Surface waters tend to contain larger amounts of organic matter, which both provide nutrients and a substrate for fungal growth. Differences in acidity and calcium content may also account for some of the variation – studies in Norway and Portugal found that surface water is slightly more acidic with a lower calcium content (Hageskal *et al.*, 2007 and Pereira *et al.*, 2009). Furthermore, groundwater has lower levels of turbidity and total organic carbon compared to spring and surface water (Pereira *et al.*, 2009). It could be expected that seasonal variation in the detection frequency of fungi is more prominent in surface-water derived water supplies, given the greater exposure that surface water has to climatic influences compared to groundwater. However, this hypothesis was not supported by the results of the study conducted by Hageskal *et al.* (2007) which looked at the frequency of positive samples by season.

b. Water Temperature

Temperature is an important influence on fungal counts, as it affects survival, growth rate and ability to reproduce. Species differ in their particular temperature requirements (see Table 3-4 for examples). For example, filamentous fungi were found by Gonçalves *et al*. (2006) to be particularly prevalent during the winter when temperatures are colder. In Norway, fungi were 14 times more likely to be isolated from cold tap water than from hot tap water, although this depended on the precise temperatures considered (Hageskal *et al*., 2006).

Studies of fungi in other environments such as soil and the laboratory have also observed that fungi can grow at low temperatures (Pietkainen *et al*., 2005), even as low as -20°C. Furthermore, Pietkainen *et al*. (2005) noted that soil fungi are better adapted to cold environments than bacteria, in terms of having a higher growth rate at lower temperatures. This would therefore result in a change in the composition of microbial communities to favour fungi.

Biofilm formation, an important location of fungal colonisation, is affected by water temperature (Lund and Ormerod, 1995). The highest rates of biofilm formation in water distribution systems have been observed to be at water temperatures of 15-25°C (Donlan *et al*., 1994). Once established, the water temperature influences the microbial composition of the biofilm (Rogers *et al*., 1994) as different temperatures will favour different species. For example, the biofilms that formed at 20°C were dominated by bacteria with 96% of microbes being Pseudomonas, with several protozoa also being present. At 40°C, 50°C and 60°C, Aspergillus spp. were a key component of the climax community, along with several bacterial species but no protozoa.

c. Nutrient Concentration

Heterotrophic organisms such as fungi require nutrients for survival and growth, including assimilable organic carbon (AOC), phosphorus and ammonium. Such nutrients tend to concentrate at the solid-liquid interface, and can become trapped in biofilms at this interface. The level of nutrients often regulates the rate and extent of biofilm growth. Indeed, some countries such as the Netherlands prefer controlling AOC over disinfection for limiting biofilm growth. Phosphorus and ammonium concentrations may be limiting for microbial growth. Higher concentrations may facilitate the recovery of microbes that have been stressed by disinfectants (US EPA, 2002). Such studies have focused on bacteria when investigating the influence of nutrients on biofilm development, and further research is needed to determine the effect on fungi in biofilms. The overall influence of nutrient concentration on fungal establishment in water distribution systems is likely to be different from that for bacteria, given that fungi are able to grow in environments that appear to be nutrient free (Kinsey *et al*., 2003). Competition for nutrients between bacteria and fungi in culture is thought to occur (Gonçalves *et al*., 2006), but the extent to which such competition influences ecology of biofilms in water distribution systems is not known.

2.8 Biotic Factors Influencing Ecology of Fungal Taxa in Water Systems

a. Interactions with Bacteria

Understanding the interactions between bacteria and fungi is important in order to determine if bacterial content, a commonly measured parameter of drinking water, can be used as an indicator of fungal content (Gonçalves *et al*., 2006). If the absence of a correlation is common across distribution systems, it can mean that there is the potential for bacteriologically safe water to contain potentially pathogenic fungi.

Different studies have found different relationships between fungi and bacteria. These differences could arise from the different species compositions isolated from water systems, differences in methodologies, or different biological mechanisms affecting the relationship. For example, the interactions between fungi and biofilm-bacteria may explain the positive relationships (Jefferson, 2004). Fungi are often secondary colonisers of pre-established bacterial biofilms (Paterson and Lima, 2005 and Kinsey *et al.*, 2003).

The different ecological requirements of the two organisms can theoretically lead to commensal relationships, in which one benefits while the other is unaffected (Jefferson, 2004). This theory suggests that negative correlations between fungi and bacteria in biofilms are unlikely. Furthermore, it has been demonstrated that fungi colonise pre-established bacterial biofilms, again indicating a positive relationship should be expected (Doggett, 2000). Negative relationships observed may be related to the culturing process, where bacteria and fungi are in direct competition for resources (Gonçalves *et al.*, 2006).

These findings illustrate that correlations with bacteria depend on whether filamentous fungi or yeasts are being considered, and which bacteria are being assessed. Whether the remaining variation in findings between studies is due to differences in the specific composition of species, or to differences in methodology (such as the amount of time samples are cultured to allow for slow-growing fungi) is unclear. Therefore, there is a need for further research to investigate the different correlations between fungi and bacteria, and what factors influence such associations. This will allow it to be determined whether, and in which circumstances, bacterial contamination of drinking water indicates fungal contamination.

If bacteria and fungi inhabit the same location specific interactions have been observed. For example, culturing marine bacteria and fungi together has led to the production of novel compounds that are not produced by either species separately in laboratory conditions (Shank and Kolter, 2009). Fungi-bacteria interactions can also inhibit secondary metabolite production.

2.9 Water Treatment and Disinfection

Under the Water Quality Regulations water must not contain any microorganism, parasite, or other substance at a concentration or value which would constitute a potential danger to human health. This can be achieved through disinfection, which is defined in the Regulations as being 'a process of water treatment to remove or render harmless to human health every pathogenic micro-organism that would otherwise be present in the water'. This involves a number of processes carried out in a water treatment plant as well as maintaining a residual disinfection throughout the water distribution system to inactivate microorganisms introduced after the treatment plant. Aflatoxins or other fungal toxins may be degraded by physical, chemical, or biological methods (Dimitrokallis *et al.*, 2008).

Ionisation of water with silver and copper, a well-recognised method of controlling Legionella in hospital water supplies, has resulted in a significantly lower prevalence of fungi compared to non-ionised water in hospital distribution systems. However, as the effectiveness of this method has only been investigated by one study, further research is needed to confirm the finding (Pedro-Bodet *et al.*, 2007). Furthermore, it is not used as a method of treating public drinking supplies.

Chemical disinfectants are frequently also used as the last process in a water treatment plant and to maintain a residual concentration throughout the distribution system. Residual concentrations are needed to inactivate fungi that enter the system after the treatment plant and those which are initially only partially inactivated and thus can recover later in the system. The efficacy of chemical disinfectants against fungi is variable between species (Kinsey *et al.*, 2003).

Efficacy of chlorine is the most dependent on temperature - inactivation of spores occurs less frequently at lower temperatures. The exposure time to free chlorine that is needed to inactivate fungi is longer than for other chemical disinfectants, particularly ozone and chlorine dioxide (Paterson and Lima, 2005). Spores are more resistant than hyphal cells, with some being extremely chlorine-resistant (Kelley *et al.*, 1997). Such spores could thus allow the establishment of fungi in the water system even if treatment processes have removed the vegetative cells. Once fungi are established in the system, it can be difficult to maintain sufficient concentrations (i.e. of 0.4 to 0.5 mg l-1) (Rosenzweig *et al.*, 1983) of free chlorine to prevent colonisation and biofilm formation (Grabinska-Loniewska *et al.*, 2007). This is because the chlorine demand of fungi is high (Kelley *et al.*, 1997). Chlorine demand can also be affected by other microbes in the system and the material from which the pipes are made (Kelley *et al.*, 1997). It has been suggested that initial free chlorine concentrations of approximately 1 mg l-1 are sufficient for spore inactivation and to provide sufficient residual chlorine in the system to assist in prevention of new growth (Kelley *et al.*, 1997; Kinsey *et al.*, 2003) and development of biofilms (Momba *et al.*, 2000). However, concentrations of free chlorine are not always as high as 1 mg/l at UK treatment works and are likely to be much lower in distribution systems (0.3 mg l-1). Therefore, inactivation and prevention of regrowth within the UK's water distribution system is likely to be lower than suggested by these studies.

Chlorine dioxide and ozone have been found to be the most effective in studies by Kelley *et al.* (2001). However, chlorine dioxide is not widely used in the UK and ozone is not used in the UK to provide a residual disinfectant in the distribution system. Ozone has a lifetime of less than one hour in water due to its rapid decomposition. In most cases, i.e. apart from very short distribution systems, it does not remain long enough to provide a disinfectant residual throughout the distribution system. Therefore, it does not have an effect on biofilms and fungi present in the system after treatment.

Where water is treated with ozone it is usually replaced by chlorine or chlorine dioxide as a final step in order to maintain a disinfectant residual (Camel and Bermond, 1998). Chloramines are another common choice of disinfectant. There are three types: monochloramine, dichloramine and nitrogen trichloride. Monochloramine is most commonly used as the other two negatively affect the taste and odour of the water (Chung *et al*., 2006). Monochloramine is more stable than chlorine, chlorine dioxide and ozone, and therefore may be more effective in the long-term, due to its greater persistence in distribution systems (Kelley *et al*., 2001). Monochloramine is a stronger fungicide than other chloramines (Arnitz *et al*., 2009).

Combinations of a number of removal and inactivation processes are likely to be the most effective. For example, in a Polish study, two different combinations of treatment processes were used successfully to remove all species but *A. fumigatus* and *A. niger*. The first treatment process involved filtration and aeration, including sand filters and sand filters with activated carbon, and disinfection with chlorine and chlorine dioxide. The second included chemical coagulation using aluminium sulphate, silica and pulverised carbon; alkalisation with lime; fast filtration with sand; and disinfection with chlorine and chlorine dioxide (Grabinska-Loniewska, 2007).

2.10 Public Health Implications of Mycotoxigenic Fungi in Treated Drinking Water

The presence of fungi in treated drinking water and its health impacts were not taken seriously until cases caused by fungal contaminated water were reported in Finland and Sweden during the 1980s and 1990s (Dufour *et al.* 2003; Boe-Hansen *et al.* 2003). Waterborne filamentous fungi are known to act as pathogens or allergens that have adverse impacts on human health, and mostly on immune-compromised patients (Oliveira *et al*., 2013). Transmission of pathogenic microorganisms by drinking water has continued to be a major cause of water-related illnesses, as confirmed by the frequencies of outbreaks reported around the world (WHO 2008b). Fungal infections are a challenge to cure as fungal cells are eukaryotic, just like human cells (Yamaguchi *et al*., 2007). Pathogenic fungi are believed to have caused hostile infections that have contributed to high mortality rates (Arvanitidou *et al*., 1999: Khan *et al*. 2010; Mayer *et al*., 2013; Tsui *et al*. 2016; Pal 2017). Fungal infections were quite low from the late 1950s and early 1960s, yet over the past two decades, fungal infections have dramatically increased as they are easily diagnosed (Khan *et al*., 2010). Most of the fungi that were identified in Table 1 are dematiaceous fungi responsible for causing a number of cutaneous and subcutaneous infections including invasive and contagious infections (Pfaller and Diekema 2004). A significant proportion of waterborne illnesses related to fungi are likely to go undetected by the communicable disease surveillance and reporting systems. The possible health impacts caused by fungi in treated water are still not well documented, although protective measures are recommended for people who are at high risk (Hageskal *et al*. 2012), especially the increasing population of patients having an impaired immune system, as their immune effector cells become compromised allowing fungi to colonise and attack the human tissues, leading to more complications (Oliveira *et al*. 2013). Fungi have been implicated in a number of diseases causing allergies, respiratory illness, cutaneous infection and life-threatening meningitis (Pfaller and Diekema 2004; Sulaiman *et al*., 2014). *Alternaria* sp., *Cladosporium* sp., *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. have been linked to allergies and respiratory illness (Korzeniewska, 2011; Máiz *et al*. 2018). Cryptococcus and Candida cause meningitis (Black and Baden 2007), with the Candida species responsible for cutaneous infections (Khan *et al*., 2010; Volk, 2013). Taste and odour problems in water are caused by *Aspergillus* sp., *Acremonium* sp., *Phialophora* sp. and *Penicillium* sp. (Hageskal *et al*., 2012; Sonigo *et al*., 2011). Fungi such as *Rhizopus, Fusarium, Alternaria*, *Aspergillus* and *Penicillium* produce mycotoxins that are harmful to public health as these mycotoxins are carcinogenic and have the ability to impair the immune system (Bhat *et al.* 2010; Sonigo *et al.* 2011; Magwaza *et al.* 2017). Mycotoxins of great concern for public health include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids (Zain, 2011). The types of infections caused by mycotoxigenic fungi depend on the type of mycotoxin, the concentration and length of exposure; as well as age, health, and sex of the exposed individual (Bennett and Klich, 2003). Mycotoxins found in water may be extremely diluted and may not be of major concern, but their concentrations may increase resulting in hazardous levels to human health, particularly when water is stored in reservoirs for longer periods (Siqueira, 2011). The absence of toxigenic fungi in treated drinking water may not provide assurance that the water is free of mycotoxins, as mycotoxins may persevere long after the fungi has died (Pitt *et al.*, 2000). Mycotoxins have serious and chronic effects on humans and animals, as many of them are believed to be carcinogenic, cytotoxic, mutagenic and may lead to immunosuppressive complexes (Arroyo-Manzanares *et al.*, 2013). Although now there are reports regarding advances in antifungal therapy, it is worth noting that the number of cases of infection and antifungal resistance are also alarmingly high, and the control of antifungal disease does not indicate any possibilities of being achieved soon (Araj *et al.*, 2013; Meirelles *et al.*, 2017; Rodrigues *et al.*, 2017; Pellon *et al.* 2018). Treated drinking water quality without pathogenic microorganisms including fungi is very critical for the health of all humans and mostly those with immunodeficiency conditions. Table 2 shows the toxic effects of some of the mycotoxins, producing genera and health effects.

The consumption of fungi-contaminated drinking water has, as far as is known, not caused acute disease, at least in immuno-competent individuals (Hageskal *et al.*, 2009). However, there is a risk of superficial or localised infection in healthy individuals and more severe and invasive infection in immuno-compromised patients. Some species also have the potential to cause allergic reaction and disease. Furthermore, the health effects of fungal secondary metabolites should be the object of further research since some are toxic and others are thought to have caused taste and odour problems in tap water. Studies that directly assess whether fungi in drinking water are responsible for fungal infections and allergies are few. Therefore, while it is known that fungal species have been isolated from drinking water and that some fungal species cause the disease, the extent to which the two are linked is not well known.

The four principal pathways by which people can be exposed to fungi in drinking water are:

- ingestion– drinking contaminated water directly;
- inhalation of aerosolised spores while showering or in the sauna;
- skin contact with contaminated water, such as while showering or bathing; and
- introduction through mucous membranes, such as the skin, eyes and oral cavity, while showering or bathing.

Aerosolisation of spores or fragments of hyphae from water has been particularly investigated as a pathway of exposure. Skin contact with fungi in water while bathing can be a source of allergic skin irritation

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

Benin City is the capital and largest city of Edo state, southern Nigeria. Benin City is situated on a branch of the Benin River and lies along the main highways from Lagos to the eastern states. The location has a natural ground water reservoir (Ezomo and Aiyohunyin, 2012; Ikhile, 2016).

3.2. Collection of Reservoir Water Samples

Reservoir Water samples were collected from Adolor, BDPA, Ekosodin, Isihor, Osasogie, Uniben and Uwasota all in Benin City, Edo State. One (1) litre was collected from each location in total. The samples were collected in sterile 1 litre containers and were transported to the laboratory then stored at 4 °C until used.

3.3 Physico-chemical properties of Reservoir Water Samples

- **Temperature (°C)**

The temperatures were determined Using electrometric method. Hama Temperature Meter was used for the reading, 50ml of water sample was poured into 100ml plastic beaker, the meter was powered-on and the probe of the meter was inserted into the sample for the reading. This was determined electrometrically using a calibrated Hanna pH Meter.

- **Electrical Conductivity and Total Dissolved Solid (TDS)**

These were determined using Hanna instrument (3-in-1) for EC, TDS and Salinity. The meter probe was dipped into the sample and left for about 3 minutes for equilibration before the reading was recorded. Electrical conductivity was reported in µS/cm while TDS and salinity were reported in mg.

- **Dissolved Oxygen (DO) Determination**

Dissolved oxygen was determined using Winkler method (Titrimetric) A standard DO bottle was used. The bottle was filled with water sample making sure that bubbles were not trapped, 2ml of MgSO4 5H2O was added and 2ml of alkaline-iodide solution was also added. It was thoroughly mixed by rotating and inverting the bottle several times. The precipitate was allowed to settle, then 1ml of sulphuric acid was added, and gently mixed. Then, 100ml of the sample solution was measured into the conical flask and 2ml of starch indicator was added, and titrated against 0.0125N of NaS203.5H20. The end point was carefully observed and recorded by colour change from straw yellow to colourless. Thus, it was calculated as:

DO (mg) = 8.0x Vol of titrant x 0.0125 x 1000

Vol of sample taken

- **Turbidity (NTU)**

This was determined using HACH colorimeter. The meter was switched on and allowed to warm-up for 30 minutes. The instrument was calibrated with known standards. Then the water sample was poured into the empty turbidity bottle, and inserted into the chamber of the instrument, and set the meter to read. The meter was allowed to stabilize, and record of the value was taken from the displayed.

- **Heavy Metals**

The instrument was calibrated using calibration blank and five series of working standard solutions of each metal to be analyzed. The water samples were determined for the concentrations of heavy metals (Cu, Pb, Zn, Cd and Mg) using flame atomic absorption spectrophotometer (FAAS, Model: AA-320N, Shanghai, China). Final concentrations of the metals in the water samples were calculated using the following formula (Uwah *et al.,* 2021)

Concentration (mg/ml) = Concentration(mg/L)×VWConcentration

(mg/ml)=Concentration(mg/L)×VW

where *V* = Final volume (50 ml) of solution, and *M* = Initial weight (0.5 g) of sample measured

Using a Flame Atomic Absorption Spectrophotometer (FAAS) involves a series of steps to measure the concentration of specific elements in a sample. FAAS is a widely used analytical technique in chemistry, environmental science, and other fields for trace metal analysis. Here's a detailed explanation of how to use a Flame AAS:

Safety Precautions:

Before starting, ensure that you are following all safety protocols, including wearing appropriate personal protective equipment, working in a well-ventilated area, and being aware of the chemicals you are handling.

Instrument Setup:

1. Instrument Warm-Up:

- Turn on the Flame AAS and allow it to warm up. The warm-up time can vary depending on the instrument but typically takes around 15-30 minutes.
- Check and set the flame type (usually air-acetylene or air-acetylene-nitrous oxide) depending on the elements you are analyzing.

2. Optical System Alignment:

- Verify that the optical system is properly aligned by using a reference solution of known concentration.
- Ensure that the burner head and nebulizer are correctly aligned and the flame is stable.

Sample Preparation:

3. Sample Collection and Preparation:

- Collect your sample and ensure it is properly prepared. Depending on your application, you may need to digest or dissolve solid samples.

- Filter the sample to remove any particulate matter that could clog the nebulizer.

4. Dilution (if necessary):

- If your sample has a high concentration of the element you're analyzing, dilute it with a suitable solvent to bring it into the linear range of the instrument.

Calibration:

5. Standard Solutions:

- Prepare a series of standard solutions of known concentrations for the element you are analyzing. These standards will be used to create a calibration curve.

- Typically, you'll want at least three standards with concentrations covering the expected range of your samples.

6. Calibration Curve:

- Inject the standard solutions into the AAS one by one, and record the absorbance values at the characteristic wavelength for the element.

- Plot a calibration curve using the absorbance values against the known concentrations of the standards.

Analysis:

7. Sample Analysis:

- Inject a small volume of your sample into the AAS. The sample is typically introduced through a nebulizer and mixed with the fuel and oxidant gases before entering the flame.

- The AAS will measure the absorbance of the element at its characteristic wavelength in the flame.

Data Analysis:

8. Concentration Calculation:

- Using the calibration curve, determine the concentration of the element in your sample by comparing the sample's absorbance to the curve.

- Many FAAS instruments have software that automates this calculation.

- **Carbonate/ Bicarbonate and Total Alkalinity (mg/l)**

Titrimetric method was used by measuring 100ml of water sample into titration flask, and then 2-drops of phenolphthalein indicator was added, and titrated with 5 N H_2SO, until pink colour just disappeared. The volume of acid consumed was recorded. To the same solution, 2-3 drops of methyl orange indicator was added, and titrated further until colour changes from yellow to red. The additional volume of acid consumed was recorded. It was calculated as:

Bicarbonate alkalinity = (B-A) x 50 x 1000 mg/ 50 x 100

Bicarbonate ion Conc (HCO_2) = Bicarbonate alkalinity x 1.22 (mg/l)

- **Salinity as Chloride (Cl)**

100ml of water sample was quantitatively measured into a 250-ml of conical flask, followed by the addition of 1ml of K2CrO4 indicator, and titrated with 0.014N AgNO3. The mixture was fitrated from yellow to reddish colour, the colour changes from yellow to reddish brown at the end-point was observed and recorded. Thus calculated as

Chloride (Cl-) 35.5 x Cb x Vb x 1000

Vol of sample

Where Cb, = Concentration of AgNO, (Normality)

Vb = Volume of AgNO, (Consumed)

- **Total Hardness**

50ml of water sample was quantitatively measured into 150ml capacity conical flask, added were 2ml of buffer solution and 2-drops of Eriochrome Black T indicator after which the mixture was titrated with 0.01N EDTA from wine colour to blue end-point. Total hardness was calculated as follows:

Total Hardness as CaCO3, mg/l = ml of (EDTA) (0.01) 50 x 1000

Vol of sample (ml)

- **Calcium Hardness**

50ml of sample was measured into 150 ml conical flask followed by addition of 1ml of 8. O N KOH. 4-drops of calcium indicator, and titrated with 0.01N EDTA from wine colour to blue end-point. The calcium hardness was calculated as follows:

Ca as CaCO, (mg/l) = (ml of EDTA) (0.01) 50 x 1000

Vol of Sample

- **Magnesium Hardness**

Mg as CaCO, = Total Hardness - Calcium Hardness (mg/l)

- **Sulphate (mg/l)**

This was determined by Turbidimetric Method. A filtered quantity of sample was measured into conical flask and made up to 100ml with distilled water.5ml of conditioning reagent was added and stirred. 0.5g of barium chloride crystal was then added and stirred again. after one minute the absorbance was red at 420nm.

- **Nitrate (mg/1)**

50.0ml of filtered water sample was measured into an evaporating dish and evaporated to dryness, after cooling, 1ml phenoidisulphonic acid was added. the content of the evaporating dish was transferred into 50ml volumetric flask with 25- 35ml of distilled water. 4ml of ammonium hydroxide was added to develop the colour and diluted to a volume with distilled water. The blank was also carried out. The nitrate content in the sample was measured at 410 nm using the UV vis spectrophotometer.

- **Phosphate (PO) APHA 425C**

Measure 40ml of the sample, add 5ml of Antimony Molybdate to the solution, followed by2ml ascorbic acid. The blank solution is subjected to the same treatment as the sample. After about 10-20 mins, measure the absorbance both sample and blank solution with UV-vis spectrophotometer at a wavelength of 880nm

- **Total Suspended Solid (TSS)**

Dry a clean dish of suitable size at 103-105˚C in an oven until constant weight is achieved. Cool to room temperature in a desiccator. Note the weight, pipette after mixing thoroughly 100ml of the samples accurately into a dish and evaporate to dryness on a steam bath. Wipe the outside of the dish and dry the residue in an oven for about 1 hour at 103-105°C Transfer quickly the dish to a desiccator, cool to room temperature and weigh. Retum the dish to the oven, dry further for 10-20 minutes, reweigh after cooling to room temperature Repeat until the weight of the dish plus residue is constant to within 0.05mg, Substract the weight of the dish to obtain the weight of the total solids

- **Chemical Oxygen Demand (COD)**

Round bottom flask, reflux condenser and cork were washed with 20% H_2SO_4. A portion of the sample was taken and mixed together for 2 minutes. A known volume of the digested sample (5 mL) was placed into the round bottom flask, 1.5mL of potassium dichromate solution was added, 0.5 g mercury sulphate was added and 3.5mL sulphuric acid reagent was carefully run down the inside of the tube. The tube was tightly capped, inverted to be mixed several times and afterwards placed in black digester, preheated to 150oC and reflux for 2 hours

After cooling to room temperature, the tubes were placed in test tube rack, transferred the digested sample quantitatively into an acid washed conical flask and added 1 to 2 drops of ferroin indicator. Titration was done using 0.05M Ferrous Ammonium Sulfate (FAS) from a blue-green color to a reddish-brown color and a blank containing the reagent, and a volume of distilled water equal to that of the sample.

Calculation

COD as mg O_2/L = (A-B) x M x 16000

÷mL of sample

where A= mL of FAS used for blank

B= mL of FAS used for sample

M= morality of FAS

3.4 Sterilization

All glass ware was washed with disinfectants and rinsed thoroughly in distilled water; air dried and sterilized using a hot air oven, the inoculating loop was sterilized in flame until it was red hot. Media preparations were sterilized by autoclaving at 121^{o}C for 15 minutes. The work bench and immediate environment were sterilized be wiping with disinfectants and absolute alcohol and flaming Bunsen flame before microbial analysis started.

3.5 Media Preparation

3.5.1 Potato Dextrose Agar Preparation

Potato Dextrose Agar was used for the general isolation of Fungi present in the water samples. It was prepared by dissolving 23.0g of Potato Dextrose Agar in 1000ml (1L) of distilled water. The media was also sterilized in an autoclave at a temperature of 121°C for 15 minutes. The sterile medium was allowed to cool before dispensing into appropriate sterile Petri dishes.

3.5.2 Preparation of Tryptone Soy Agar

It was prepared by dissolving 45.0g of TSA in 1000ml (1L) of distilled water. The media was also sterilized in an autoclave at a temperature of 121°C for 15 minutes. The sterile medium was allowed to cool before dispensing into appropriate sterile Petri dishes.

3.6 Serial Dilution and Pour plate techniques

In the laboratory, 1ml each of water samples was dissolved in 9ml of sterile water respectively, which served as stock cultures under aseptic conditions the water in these test tubes was autoclave at a temperature of 121°C for 15 minutes which served as diluents after cooling. Using a sterile pipette, 1 ml of the stock water sample (Ekosodin), was inoculated into the first test tube which gave a dilution factor of 10^{-1}. Pour plates of each of the serial dilution. Counts (cfu/ml) of microorganism contaminants of the water samples were obtained by plating out 1ml of each of the of the 10-fold dilution on duplicate plates, using Potato

Dextrose Agar for fungi. The plates were incubated for 3–7 days at 35°C. Fungi growing on the agar plates were purified by single spore and hyphal-tip technique.

3.7 Enumeration and isolation of total fungal count

An aliquot of 1 ml was inoculated using the pour plating technique. Appropriate media was used for fungal. Potato dextrose agar (supplemented with chloramphenicol) for fungi. Plates were cultured at 28±2ºC for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below as bescibed by Willey *et al*. (2008).

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum} \qquad (1)$$

3.8 Isolation and Characterization of Fungal from Samples

Colony forming unit per ml (CFU/ml) of fungal isolates were counted and pure cultures were identified. Identification was based on cultural and morphological characteristics of isolates grown on Potato Dextrose Agar with reference to the manual of Barnett and Hunter (1972). Visual observation of the agar cultures was made periodically and the fungus and surrounding agar, especially of the underside, surface texture and the presence of macroscopic structures were noted. Further observations were made on slide mounts The mounts were made both in distilled water and lactophenol cotton blue.

A dissecting needle was used to remove some portions of the fungus and placed on two separate slides, one with a drop of water and the other with the lactophenol cotton blue. The fungal fragments were then teased until thinly spread after which a cover slide was placed on top and observed under light microscope with X10 and X40 objectives.

3.8.1 Spore Staining

For fungal identification, a smear of the test organisms was made on slides containing trypan blue in lactophenol and then covered with cover slips. Fungi having spores, stained blue when viewed under the microscope.

3.9 Antibiotics Susceptibility Test

The antibiotic sensitivity testing will be carried out to dermine the susceptibility of the isolated fungi to various antifungal agents. The test was carried out using the disc diffusion method as described by Bauer *et al.,* (1966) on Mueller-Hinton agar and was interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (2002). The antibiotics used for isolates are Nystatin and Vericonazole.

3.10 Test for Pathogenicity

3.10.1 Test for protease production

Protease test is a test that measures the activity of protease enzymes. Proteases are enzymes that break down proteins into smaller polypeptides or single amino acids (López-Otín and Bond, 2008). Samples were inoculated on Trypton soy agar prepared by dissolution of 40g of TSA in 1000ml of distilled water, after which it is incubated at 28± 20C (Uyar et al., 2011).10% Tween 80 was added as a supplement to the media . The presence of a clear zone around the colonies shows a positive result of protease production

3.10.2 Test for Haemolysin production

Haemolysin production was detected using the method described by Martinez-Martinez *et al.*, (1999). All the fungal isolates were grown on 5% sheep blood agar at 37^0C for 24hr. The presence of a clear zone around the colonies was taken as positive for haemolysin production.

3.10.3 Test for Lipase

The isolated fungi were screened for the production of lipase using spirit blue agar containing lipase reagent as Marshall (1992) method. The lipolytic activities of the isolates were then compared by measuring the width of the areas of clearing or area of deep colour around the colonies.

3.11 Statistical analysis

The data were analysed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined.

CHAPTER FOUR

RESULTS

Table 4.1 shows The physiochemical analysis of the reservoir water sample. The pH found in this study falls within the range of 7.59 – 6.63, electrical Conductivity exhibited differences across the different locations, ranged from 13 to 159 µS/cm. The temperature range gotten from this study is 25.3 – 29.4^{0}C. Total suspended solid varied from 0.59 – 0.98 g/l. Biochemical oxygen demand ranged significantly among the sampled reservoir water from 0.02 – 0.07. The alkalinity ranged from 0.12 to 0.5 g/l. Manganese was present in the reservoir water and the concentration ranged from 0.01 to 0.15 mg/l.

Table 4.2 shows the total fungal count of the water samples, from the samples the count ranged from 0.025 x 10^{3}cfu/ml to 2.3 x 10^{3}cfu/ml. The highest fungal count was found in Ekosodin, having a value of 2.3 x 10^{3}cfu/ml while the lowest fungal count was from Osasogie, having a count value of 0.025 x 10^{3}cfu/ml.

Table 4.3 shows the cultural and morphological characteristics of fungi isolated from the reservoir water. The fungal community isolated from the various samples of water were *Penicilium chrysogenum, Yeast, Rhizopus stolonifera, Aspergillus flavus and Aspergilus niger.*

Figure 4.1 shows the percentage distribution of all five isolates across the locations sampled, the percentage distribution ranged from 71.4% to 100%. The isolate with the lowest percentage distribution was *Penicilium chrysogenum*, asides *Penicilium chrysogenum* the rest of the isolates were found in all the sampled locations.

As shown in Table 4.4, the fungal isolates were resistance to Vericonazole. There were variations of isolates resistances to Nystatin. The isolates *Penicilium chrysogenum, Yeast, Aspergillus flavus and Aspergilus niger* were susceptible to Nystatin, except *rhizopus* *stolonifer* which showed resistance. *Penicilium chrysogenum* showed the highest zone of inhibition with a value of 27 mm.

The pathogenicity test of the isolates is shown in Table 4.5. The isolates *Yeast, Rhizopus stolonifera, Aspergillus flavus and Aspergilus niger* showed their ability to produce lipase and gamma heamolytic activity on blood agar, except *Penicilium chrysogenum* that was positive to protease production.

Table 4.1: The physiochemical analysis of reservoir water samples from different locations in Benin City.

Parameter	RWA	RWB	RWE	RWI	RWO	RWUn	RWUw	**WHO**
pH	7.59	6.71	6.66	6.33	6.72	6.18	6.69	6.5-8.5
Tempt. (ºC)	25.3	28.3	25.3	26.3	29.4	28.3	26.3	< 35
EC (µS/cm)	46	37	48	33	159	26	13	1000
Turb. (NTU)	0.63	0.27	1.83	1.11	0.9	0.74	0.21	5
TSS (mg/ml)	0.78	0.98	0.9	0.59	0.65	0.72	0.69	< 10
Alkalinity	0.21	0.41	0.43	0.40	0.50	0.24	0.12	<50
Hardness (mg/ml)	1.99	2.15	2.67	2.95	1.05	2.5	2.57	100-500
Phosphate (mg/L)	0.12	0.56	1.84	1.99	0.09	0.1	0.1	5
Nitrate (mg/L)	0.67	0.95	1.05	1.5	0.57	0.66	0.54	40-50
Sulphate (mg/L)	0.75	0.82	0.91	0.79	1	0.12	0.13	60
BOD(mg/ml)	0.02	0.01	0.02	0.03	0.07	0.02	0.03	10
COD(mg/ml)	0.41	0.56	0.5	0.26	0.31	0.36	0.34	10
Copper (mg/ml)	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.02
Lead (mg/ml)	0	0	0	0	0	0	0	0.01
Zinc (mg/ml)	0.03	0.03	0.02	0.06	0.28	0.02	0.02	0.2
Cadmium (mg/ml)	0	0	0	0	0	0	0	0.02
Manganese (mg/ml)	0.02	0.09	0.11	0.09	0.15	0.01	0.08	0.2

Key:
RWA=Reservoir Water Adolor
RWB=Reservoir Water Bdpa
RWE=Reservoir Water Ekosodin
RWI=Reservoir Water Isihor
RWO=Reservoir Water Osasogie
RWUN=Reservoir Water UNIBEN
RWUW=Reservoir Water Uwasota
WHO= World Health Organisation

Table 4.2: Total Fungal Count (cfu/ml x 10^3) of water samples.

Sample location	**Fungal count (CFU x10^3)**
RWA	0.06
RWB	1
RWE	2.3
RWI	0.26
RWO	0.025
RWUN	0.285
RWUW	0.19

Key:

RWA=Reservoir Water Adolor

RWB=Reservoir Water Bdpa

RWE=Reservoir Water Ekosodin

RWI=Reservoir Water Isihor

RWO=Reservoir Water Osasogie

RWUN=Reservoir Water Uniben

RWUW=Reservoir Water Uwasota

Characteristics of isolates				Microscopy		
Isolates	**growth form**	**colour**	**Reverse colour**	**Hyphae**	**Spores**	**Identity**
1	Velvety to woolly texture on PDA	Blueish-green with white border	Dark green	Septate	Simple, Long and Erect Conidiophores arising from the mycelium. Bearing conidia produced	*Penicilium chrysogenum*
2	Ovoid shaped budding yeast	Cream	Yellow	septate	Small round oval structures with distinct appearance. They have thick protective outer layer.	*Yeast*
3	Woolly network of hyphae with root like rhizoids	Light grey	grey	Non-septate	Sporiangiospore rises vertically from mycelium bearing sporangia	*Rhizopus stolonifer*
4	Widely spread and floccose in green texture	Yellowish	yellow	Septate	Thick walled coloured conidiospores with glucose vesicles bearing sterigmata over its entire surface	*Aspergillus flavus*
5	Velvety to flaky on PDA at 28^0C	Black	Brown	Septate Upright	Conidiospores were long simple and terminating at the globos vesicle	*Aspergillus niger*

Table 4.3: Cultural and Morphological Characteristics of Fungal Isolates from Reservoir Samples.

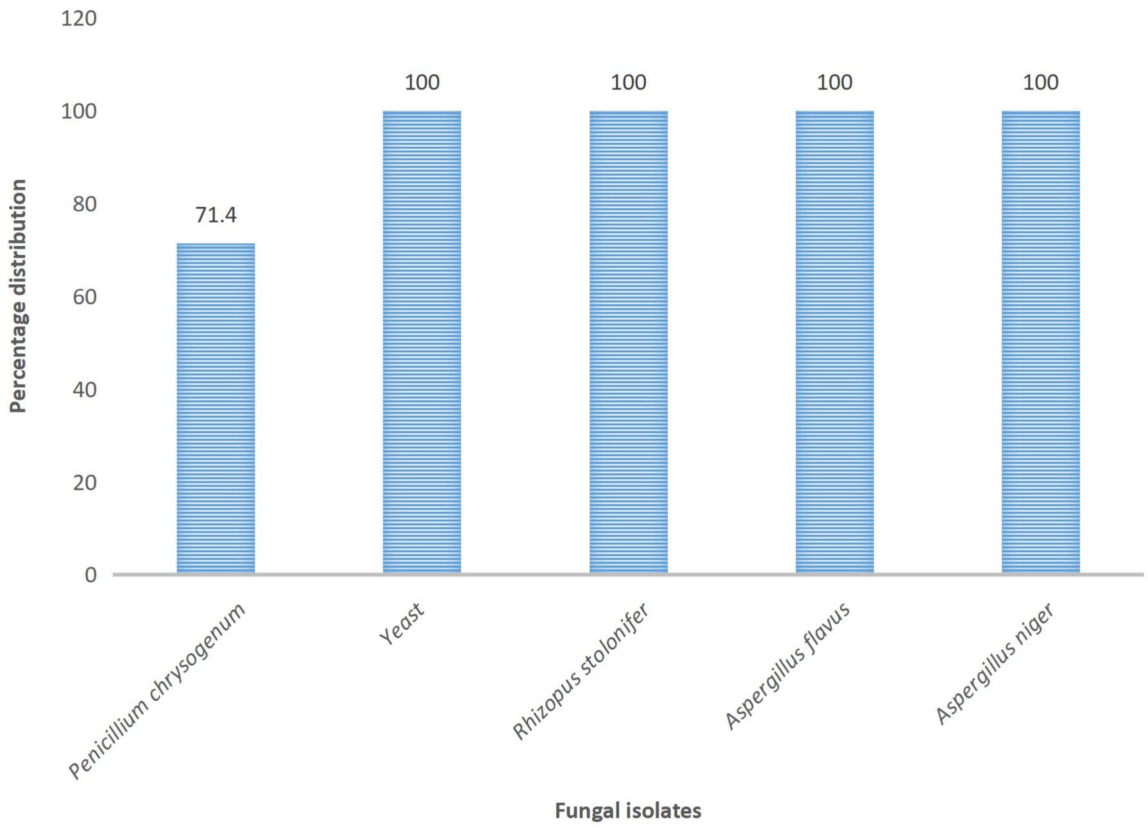

Figure 4.1: Percentage distribution of the fungal isolates in the water samples

Table 4.4: Antibiotics susceptibility of Fungal isolates from reservoir water.

Isolate	Zone of inhibition (mm)	
	Nysiatin	Vericonazole
Penicilium chrysogenum	S (27)	R (0)
Yeast	S (18)	R (0)
Rhizopus stolonifer	R (10)	R (0)
Aspergilus flavus	S (22)	R (0)
Aspergilus niger	S (22)	R (0)

Key

0 – 11 mm = Resistant (R)

11- 12mm = intermediate (I)

13 - > = Susceptible (S)

Table 4.5: Pathogenicity test of the isolates from reservoir water samples of different locations in Benin City.

Isolates	**Lipase**	**Haemolysin**	**Protease**
Penicilium chrysogenum	+	ϒ	-
Yeast	+	ϒ	+
Rhizopus stolonifer	+	ϒ	+
Aspergilus flavus	+	ϒ	+
Aspergilus niger	+	ϒ	+

KEY

+ = Positive

\- = Negative

CHAPTER FIVE

DISCUSSION

The results from this study showed that reservoir water has high fungal contaminants. Ground water is probably the most common source of fungal contamination especially for underground reservoirs. Due to high adaptability at a physiological level, fungi may colonise environments with extreme growth conditions, one of them being also oligotrophic water systems (Bärlocher *et al.,* 2008).

Table 4.1 shows the physiochemical analysis of the reservoir water sample. The ideal pH for good water is 6.5 - 9.5, while an acceptable range is 5.5 - 10.0, as indicated by Stone and Thomforde, (2003). The pH found in this study falls within the range of 7.59 – 6.63, as presented in Table 1. This finding aligns with the conclusions of Stone and Thomforde (2003), the results from this study falls also within the WHO limit of 6.5 to 8.5. The temperature from this study. Electrical Conductivity exhibited differences across different locations, spanning from 0.13 to 15.6 mS/cm (Table 4.1). In contrast, the findings of Ehiagbonare *et al.* in 2009 displayed elevated conductivity values for natural water in Okada, contradicting the current study's outcomes on conductivity the fish pond water source was noted as polluted. The WHO limit for 1000ms/cm. Acidic conditions may hinder the growth of beneficial bacteria involved in the nitrogen cycle, affecting ammonia conversion. Alkaline conditions can harm acid-loving microorganisms the alkalinity of water sampled in this study was within the range of 0.12 – 1.83. The WHO limit for alkalinity is < 50.

The temperature range gotten from this study is 25.3 – 29.4^{0}C. The WHO limit for temperature is < 35^{0}C. Total dissolved solid varied from 0.59 – 0.98 g/l. The highest value being 0.98 g/l and the least value being 0.59 mg/l. Ground water contamination and contamination with soil have been reported to increase total dissolved solids (Ogbeibu and Victor, 1989; Ogbeibu and Edutie, 2006). This may have been responsible for the variation from location to location of the reservoir water in this study. Chemical dissolved oxygen varied from reservoir to reservoir (Table 4.1). The result of this investigation corroborate with the results of Das et al. (1990) who had a similar result like those obtained from this investigation. COD indirectly affects microorganisms by indicating the presence of organic pollutants. High COD levels suggest the presence of organic compounds that microorganisms can potentially break down. However, excessively high COD may indicate the presence of toxic substances or a high organic load that can overwhelm microbial populations. The COD in this study ranged from 0.31 to 0.56 this is within the WHO limit of 10.

Biochemical oxygen demand varied significantly among the sampled reservoir water 0.02 – 0.07. The highest value was 0.07 and least was 0.02 mg/l. These are all below FEPA standard (Federal Environmental Protection Agency of Nigeria, 1991). The FEPA limit is 30 mg/l and the WHO limit is 10mg/l. This directly tells that the reservoir water is not polluted and may have no negative impact on human health if consumed. However, permissible limit as reported by APHA (1992) is 4 mg/l. This highest value from this study is significantly lower than this bench mark. Accumulation of low BOD results in organisms being stressed, suffocated and death (APHA, 1992). Elevated BOD levels can lead to an increase in the activity of decomposer microorganisms as they work to break down the excess organic material. However, this can deplete dissolved oxygen levels, potentially harming fish and other aerobic organisms.

The alkalinity ranged from 0.12 to 0.5 g/l. The alkalinity of unpolluted pond was reported by Shastree *et al.* (1991) to be 1.71 – 2.35 g/l. This is at variance with results of this study as lower values were obtained. The WHO limit for alkalinity is < 50.

Nitrate is an essential nutrient but also a good indicator of contamination from natural and human activities. Levels above 45 mg/l are considered harmful to aquatic organisms. The nitrates were ranging from 0.54 mg/L to 0.95 mg/L in the present findings. The average nitrates were found to be extremely low as compared to the tolerable limits in all the reservoir. When NO^3 level is above 40 mg/L, it leads to “methaemoglobinaemia” also called blue -baby disease Kiran et al. (2010). Microbes involved in nitrogen cycling (*nitrosomonas, nitrobacter*) can thrive in the presence of nitrates, helping to convert toxic ammonia into less harmful nitrate.

Water hardness refers to the concentration of calcium and magnesium. As calcium and magnesium bond with carbonates and bicarbonates, alkalinity and water hardness are closely interrelated and produce similar measured levels. The hardness of water is not a pollution parameter but indicates water quality. Waters are often categorized according to degrees of hardness as follows: 0 – 75 mg/L = soft 75 – 150 mg/L= moderately hard 150 – 300 mg/L= hard and Above 300 mg/L= very hard. In the present investigation, total hardness level varied from 1.05 to 1.99 mg/L and included under soft category Kiran *et al.* (2010). The water softness maybe attributed to the rainy season as this study was conducted during the rainy seasons.

Manganese was present in the reservoir and the concentration ranged from 0.01 to 0.15 mg/l. Trivery and Khatavker (1986) reported Manganese concentration which ranged from 7.32 to 18 mgl, these are higher values than the result of this study. Also Desia (1982) reported higher value of 70 mg/l. The two are not supportive of the result of this study. Certain microorganisms, like calcium-dependent diatoms, can benefit from higher hardness levels. However, extremely hard water may lead to mineral deposits, which can affect water quality. The WHO limit for Manganese is 0.2 and the findings from this study falls within this range.

Copper and Zinc ranged from 0.01 - 0.02(mg/l) for copper and 0.02 - 0.28 (mg/l) for zinc. There was no significant difference in the level of concentration between the two. These are heavy metals. This range of values was lower than those of Kuz'mina and Ushakova (2007) which was 10.6 (mg/l). The presence of heavy metals significantly decreased hemoglobinlytic proteinase activities (Kuz'mina and Ushakova, 2007). Cadmium and Lead were detected in small quantities for the samples used in this study. Excessive concentrations of heavy metals can disrupt microbial communities by inhibiting enzymatic activities and metabolic processes. Certain microorganisms may be more tolerant, while others can be severely impacted or killed by metal toxicity. WHO limit for Zn and Cu is 0.2 and the findings from this study were in within this limit. Lead and Cadmium were not found in the water samples used in this study.

Table 4.2 shows then total fungal count of the water samples from the samples the count ranged from 0.06 x 10^3cfu/ml to 2.3 x 10^3cfu/ml.

Table 4.3 shows the cultural and morphological characteristics of fungi isolated from the reservoir water. The fungal community isolated from the various samples of water were *Penicilium notalatum, Yeast, Rhizopus stolonifera, Aspergillus flavus* and *Aspergilus niger*. These isolates are similar to that gotten from Muhsin *et al*. (1989) who isolated several fungi from well water, including *Alternaria alternate Aspergillus sp., Trichoderma viride, Penicillium sp., Fusarium oxysporum, and Cladosporium sp.* Moreover, Rajanaika *et al.* (2009) also isolated *Pythium, Allomyces, Rhizopus, Achyla, Alternaria, Aspergillus, Saprolegnia, Chaetominm, Cladosporium, Fusarium, Trichoderma,* and *Penicillium* from the Tunga River in India.

Kraume and Awad, (2007) in their study found 60 species of fungi belonging to 27 genera in wastewater samples, including 8.8% *Geotrichum*, 75% *Penicillium*, 65.7% yeasts, and 55.5% *Trichoderma*.

The reason for the presence of *Aspergillus* sp, Yeast, and *Rhizopus* during the study period may be attributed to the fact that these fungi are soil fungi that may have contaminated the water from the ground since these reservoirs are mostly dug underground. This is supported by several studies showing the presence of soil fungi in water sources (Bettucci and Roquebert, 1995). The reason for the abundant presence of *Aspergillus* may be attributed to the fact that this fungus is able to produce large numbers of asexual breeding units, it is able to survive in different environments, and it secretes enzymes that enable it to benefit from different food sources (Flannigan and Sellars, 1977). *Rhizopus* is a widespread fungus that may exist in water. Candida, which is a genus of pathogenic fungi, was also isolated, indicating the serious public health impact of water from these wells if it was used for drinking or domestic purposes. All the isolates in this study were distributed across all the samples analysed.

Figure 4.1 shows the percentage distribution of all five isolates across the locations sampled, the percentage distribution ranged from 71.4% to 100% asides *Penicilium chrysogenum* the rest of the isolates were found in all the sampled locations. The frequency of fungi isolation from this study is similar to a study by Suliman, (2022) that found that the frequency of isolation to be equal between different water samples to be between (99.96–100%), with a fungal isolation rate of 6.247–6.25%.

Table 4.5 shows the pathogenicity of the isolates, all isolates showed Gamma Haemolysis and were all positive to Lipase, all the isolates but *Penicilium chrysogenum* were positive for protease production. All isolates showed susceptibility to Nystatin but showed varying resistance to Vericonazole with penicillin showing the highest susceptibility.

5.1 Conclusion

Recent discoveries on fungi requiring special attention include the presence of opportunistic and emerging pathogens in water sources. Many environmental species (particularly of the genus Aspergillus) recently display resistance to azoles, being the target of many studies as a serious health risk. In addition, many water-borne fungi showed resistance to the usual water disinfection procedures, allowing them to enter water distribution systems; where they form mixed biofilm communities with bacteria, algae and protozoa. Biofilms increase ability to survive heat- and chlorination-shocks. Consequently, fungal presence in tap water distribution systems leads to the enrichment of the sturdiest fungi tolerating 37 ◦C, in certain water-related indoor environments (e.g., dishwashers, washing machines, bathrooms and showers). Enrichment of fungi in indoor environments may affect human health via direct exposure, such as inhaling of aerosols, contact or through drinking; and indirectly by exposure to contaminated surfaces, dishes or clothes. Thus, the present knowledge of ecology and pathogenesis of fungal contaminants in water reveals the need to measure and regulate their presence in drinking water at least in the environment with high numbers of immunocompromised people.

5.1 Recommendation

Ensuring reservoir water safety is crucial for public health and environmental preservation. It is important that a multi-faceted approach which includes regular testing, water treatment and source protection measures are taken in other to keep these water sources free from contaminants.

REFERENCES

Akhionbare, W. N., Ukwoma, F. P. O. and Akhionbare, S. M. O. (2010). Environmental impact analysis of a water supply project - (The case of Ikpoba River Dam Waterworks, Benin City, Nigeria). *Journal of Applied Science Research* **6**(12): 2081 – 2084.

Arnitz, R., Nagi, M. and Gottardi, W. (2009). Microbicidal activity of monochloramine and chloramines T compared. *Journal of Hospital Infection* **73** (2): 164-170.

Arroyo-Manzanares, N., Huertas-Pérez, J. F., Gámiz-Gracia, L., Arvanitidou, M., Kanellou, K., Constantinides, T. C., Awad M. F and Kraume, M. (2010). The occurrence of fungi in activated sludge from membrane bioreactors. *International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering* **71**: 561-564.

Babič, M. N., Gunde-Cimerman, N., Vargha, M., Tischner, Z., Magyar, D., Veríssimo, C., Sabino, R., Viegas, C., Meyer, W. and Brandão, J. Fungal contaminants in drinking water regulation: A tale of ecology, exposure, purification and clinical relevance. *International Journal of Environmental Research and Public Health* **14** (6): 636.

Babič, M. N., Zalar, P., Ženko, B., Džeroski, S. and Gunde-cimerman, N. (2020). Yeasts and yeast-like fungi in tap water and groundwater, and their transmission to household appliances. *Fungal Ecology* **20**: 30–39.

Bärlocher, F., Seena, S., Wilson, K. P., Williams, D. D. (2008). Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshwater* **53**: 368–379.

Bennett, J.W. and Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*. **16** (3): 497–516.

Bettucci, L., and Roquebert, L. (1995). Studies on Microfungi from Tropical Rain Forest Litter and Soil: *A Preliminary Study Nova Hedwigia*. **61**(12):111-118.

Bhat, R., Rai, R. V. and Karim, A. A. (2010). Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety* **9** (1): 57–81.

Biedunkiewicz, A., Kowalska, K., Schulz, Ł., Stojek, K., Dynowska, M., Ejdys, E., Sucharzewska, E. and Kubiak, D. (2014). Mycological monitoring of selected aquatic ecosystems in the context of epidemiological hazards drinking water. *Annal Parasitology* **60**: 191–198.

Black, K., E. and Baden, L., R. (2007). Fungal infections of the CNS treatment strategies for the immunocompromised patient. *CNS Drugs* **21**(4): 293–318.

Boe-Hansen, R., Martiny, A. C., Arvin, E. and Albrechtsen, H.J. (2003). Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions. *Water Science and Technology* **47** (5): 91–97.

Calvo-Polanco, M., Sánchez-Castro, I., Cantos, M., García, J. L., Azcón, R., Ruiz-Lozano, J. M., Beuzón, C. R. and Aroca, R. (2019). Effects of different arbuscular mycorrhizal fungal backgrounds and soils on olive plants growth and water relation properties under well-watered and drought conditions. *Plant Cell and Environment* **39** (11): 2498–2514.

Chung, S., Oliphant, K., Vibien, P. and Zhang, J. (2006). An examination of the relative impact of common potable water disinfectants (chlorine, chloramines and chlorine dioxide) on plastic piping system components. *PPXIII Washington D.C.* 456 – 521pp

De Bruin, A., Ibelings, B., Kagami, M., Mooij, W.M. and van Donk, E. (2008). Adaptation of the fungal parasite *Zygorhizidium planktonicum* during 200 generations of growth on homogeneous and heterogeneous populations of its host, the diatom *Asterionella formosa*. *Eukaryote Microbiology*. **55**: 69–74.

DEFRA (Department for Environment, Food and Rural Affairs). (2011) *A Review of Fungi in Drinking Water and the Implications for Human Health*. BIO Intelligence Service: Paris, France, pp 107.

DeHoog, G.S., Guarro, J., Gené, J.and Figueras, M. J. (2014). *Atlas of Clinical Fungi Electronics*. **51**: 23–34.

Dimitrokallis, V., Meimaroglou, D.M. and Markaki, P. (2008). Study of the ochratoxin A effect on *Aspergillus parasiticus* growth and aflatoxin B1 production. *Food Chemistry and Toxicology* **46**:2435–2439

Doggett, M. S. (2000). Characterization of fungal biofilms within a municipal water distribution system. *Applied and Environmental Microbiology* **66** (3): 11–14.

Dufour, A., Snozzi, M., Koster, W., Bartram, J., Ronchi, E. and Fewtrell, L. (2003). Assessing Microbial Safety of Drinking for Fungi **3** (11): 1–30.

Ezomo, F. O. and Aiyohuyin, E. O. (2012). Shallow Aquifer in Isihor Village of Edo State, Nigeria. *International Journal of Science and Technology*: **1**(8): 12-21.

Flannigan, B. and Sellars, P.N. (1977). Amylase, Beta Glucosidase and Beta Xylosidase Activity of Thermotolerant and Thermophilic Fungi Isolated from Barley. *Trans Br Mycology Society* **69**(2):316-327.

Fox, A.R., Houser, K. H., Morris, W. R. and Walton, R. C. Dematiaceous fungal endophthalmitis: report of a case and review of the literature. *Journal of Ophthalmic Inflammation and Infection* **6**(1): 43.

Gonçalves, A. B., Paterson, R.M. and Lima, N. (2006). Survey and significance of filamentous fungi from tap water. *International Journal of Hygiene and Environmental Health* **209** (3): 257–264.

Gostincˇar, G.M. and Gunde-Cimerman, N. (2011). Evolution of fungal pathogens in domestic environments. *Fungal Biology,* **115**: 1008–1018.

Göttlich, E., van der Lubbe,W., Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., Flemming, H.-C. and de Hoog, S. Fungal flora in groundwater-derived public drinking water. *International Journal of Hygiene and Environmental Health*. **205**: 269–279.

Grabinska-Loniewska, A., Konillowicz-Kowalska, T., Wardzynska, G. and Boryn, K. (2007). Occurrence of fungi in water distribution system. *Polish Journal of Environmental Studies* **16** (4): 539-547.

Hageskal, G., Tryland, I., Liltved, H. and Skaar, I. (2012). No simple solution to waterborne fungi: various responses to water disinfection methods. *Water Science and Technology* **12** (2): 220–226.

Hamid, R. H. (2009). *An environmental study of Oomycetes in the Tigris River and their pathogenicity of some fish*. Master thesis, College of Science, Iraq. pp 236

Heinrichs, G., Hübner, I., Schmidt, K. C., deHoog, G. S. and Haase, G. (2013). Analysis of black fungal biofilms occurring at domestic water taps (II): Potential routes of entry. *Mycopathologia*, **175**: 399–412.

Ikhile, C. I. (2016). Geomorphology and hydrology of the Benin Region, Edo State, Nigeria. *International Journal of Geosciences* **1**: 144 - 157.

José Figueras, M. and Borrego, J. J. New perspectives in monitoring drinking water microbial quality. *International Journal of Environmental Research and Public Health* **7**(12): 4179–4202.

Kanematsu, H. and Barry, D.M. (eds) *World Health Organization's standards from the viewpoint of health risks. In: Corrosion Control and Surface Finishing*. Springer Verlag, Tokyo, Japan, pp. 79–88.

Kraume, M. and Awad M.F (2010). The occurrence of fungi in activated sludge from MBRs World Academy of Science. *Engineering and Technology*. **71**:561-564.

Kauffmann-Lacroix, C., Costa, D. and Imbert, C. (2016). Fungi, water supply and biofilms. In *Fungal Biofilms and Related Infections*, 1st ed.; Imbert, C., Ed.; Springer International Publishing: Cham, Switzerland, Volume 3, pp. 49–61.

Kelley, J., Paterson, R., Kinsey, G., Pitchers, R. and Rossmoore, H. (1997). Identification, significance and control of fungi in water distribution systems. *Proceedings of the Water Technology Conference*, Denver, CO, USA, pp. 9–12

Khan, M. S., Ahmad, I., Aqil, F., Owais, M., Shahid, M. and Musarrat, J. (2010). Virulence and pathogenicity of fungal pathogens with special reference to Candida albicans. In: *Combating Fungal Infections: Problems and Remedy* (I. Ahmad, M. Owais, M. Shahid and F. Aqil, eds). Springer- Verlag, Berlin, Heidelberg, Germany, pp. 21–45.

Kinsey, G., Paterson, R. and Kelley, J. (2003). *Filamentous fungi in water systems. In: Handbook of Water and Wastewater Microbiology* (N. J. Horan, ed.). Academic Press, London, pp. 239

Korzeniewska, E. (2011). Emission of bacteria and fungi in the air from wastewater treatment plants – a review. *Bioscience Reports* **1** (3): 393–407.

López-Otín, C. and Bond, J. S (2008). Proteases: multifunctional enzymes in life and disease. *The Journal of Biological Chemistry* **283**(45): 30433–30437.

Magwaza, N., Nxumalo, E. N., Mamba, B. B. and Msagati, T. A. M. (2017). The occurrence and diversity of waterborne fungi in African aquatic systems: their impact on water quality and human health. *International Journal of Environmental Research and Public Health* **14**(6): 546.

Magwaza, N., Nxumalo, E. N., Mamba, B. B. and Msagati, T. A. M. The occurrence and diversity of waterborne fungi in African aquatic systems: their impact on water quality and human health. *International Journal of Environmental Research and Public Health.* **14** (6), 546.

Máiz, L., Nieto, R., Cantón, R., de la Pedrosa, E. G. G. and Martinez-García, M. Á.(2018) Fungi in bronchiectasis: a concise review. *International Journal of Molecular Sciences* **19**(1): 1–13.

Meals, D. W., Harcum, J. B. and Dressing, S. A. Monitoring for Microbial Pathogens and Indicators. National Nonpoint Source Monitoring Program Tech Notes 9, Tetra Tech, Fairfax, VA, USA pp. 239 – 245

Medeiros, A.O., Pascoal, C., Graça, M.A.S. (2009). Diversity and activity of aquatic fungi under low oxygen conditions. *Fresh Biology* **54**, 142–149

Mefeters, G.A and Singh A. (1991). Effect of aquatic environmental stress on enteric bacterial pathogens. *Journal of Applied Bacteriology* **20**:115-120.

Misihairabgwi, J. M., Ezekiel, C. N., Sulyok, M., Shephard, G. S. and Krska, R. Mycotoxin contamination of foods in Southern Africa: a 10-year review (2007–2016). *Critical Reviews in Food Science and Nutrition* **1549** (7852): 1–13.

Muhsin, T.M., Zwain, K.H., Lafta, A.H. and Lafta, A.H. (1989). A Study on the fungal populations of ground waters in Basrah, Iraq. *Pol Arch Hydrobiology* **36**(3):312-322.

Novak Babiˇc, M., Zalar, P., Ženko, B., Džeroski, S. and Gunde-Cimerman, N. (2016). Yeasts and yeast-like fungi in tap water and groundwater, and their transmission to household appliances. *Fungal Ecology* **20**: 30–39.

Obi, C. L., Igumbor, J. O., Momba, M. N. B. and Samie, A. Interplay of factors involving chlorine dose, turbidity flow capacity and pH on microbial quality of drinking water in small water treatment plants. *Water SA* **34** (5): 565–572.

Okpako, E. C., Osuagwu, A. N., Duke, A. E. and Ntui, V. O. (2009). Prevalence and significance of fungi in sachet and borehole drinking water in Calabar, Nigeria. *African Journal of Microbiology Research* **3**(2): 56-61.

Oliveira, B. R., Crespo Barreto, M. T., San Romao, M. V., Benoliel, M. J., Samson, R. A. and Pereira, V. J. New insights concerning the occurrence of fungi in water sources and their potential pathogenicity. *Water Research* **47**: 6338–6347.

Paterson, R. R. M. and Lima, N. (2005) Fungal Growth and Mycotoxin Production in Drinking Water. IBB – Biological Engineering Centre, University of Minho, Braga, Portugal.

Paterson, R. R. M. and Lima, N. Fungal Growth and Mycotoxin Production in Drinking Water. IBB – Biological Engineering Centre, University of Minho, Braga, *Portugal. Pathogenicity Mechanisms. Virulence* **4** (2): 119–128.

Paz, Z., Komon-Zelazowska, M., Druzhinina, I. S., Aveskamp, M. M., Shnaiderman, A., Aluma, Y., Carmeli, S., Ilan, M. and Yarden, O. (2010). Diversity and potential antifungal properties of fungi associated with a Mediterranean sponge. *Fungal Diversity*. **42**(1): 17–26.

Pedro-Botet, M.L., Sanchez, I., Sabria, M., Sopena, N., Mateu, L., Garcia-Nunez, M. and Rey-Joly, C. (2007). Impact of copper and silver ionization on fungal colonization of the water supply in health care centers: Implications for immunocompromised patients. *Clinical Infection Diseases*. **45**: 84–86

Pellon, A., Ramirez-Garcia, A., Buldain, I., Antoran, A., Souto, L. M., Rementeria, A. and Hernando, F. L. (2018) Pathobiology of lomentospora prolificans: could this species serve as a model of primary antifungal resistance? *International Journal of Antimicrobial Agents* **51**: 10–15.

Pereira, V. J., Fernandes, D., Carvalho, G., Benoliel, M. J., San Romao, M. V. and Barreto-Crespo, M. T. Assessment of the presence and dynamics of fungi in drinking water sources using cultural and molecular methods. *Water Research* **44**: 4850–4859.

Pfaller, M. A. and Diekema, D. J. (2004). Rare and emerging opportunistic fungal pathogens: concern for resistance beyond Candida albicans and Aspergillus mini review. *Journal of Clinical Microbiology* **42**(10): 4419–4431.

Pitt, J. I., Bacilico, J. C., Abarca, M. L. and Lopez, C. (2000). Mycotoxins and toxigenic fungi. *Medical Mycology* **38** (1): 41–46.

Rajanaika, P.D., Hoskeri, J. and Krishna, V. (2009). Diversity of Aquatic Fungi in Relation to Environmental conditions in Tunga River (South India). *Diversity of Aquatic Fungi.***1**(6):54-62.

Rodrigues, C. F., Rodrigues, M. E., Silva, S., Henriques, M. Saada, A. and Ibrahim, M.N. Environmental pollution and the role of microorganisms, positive and negative. *Arab Thought House* **20**:24 - 28.

Sammon, N. B., Harrower, K. M., Fabbro, L. D. and Reed, R. H. Three potential sources of microfungi in a treated municipal water supply system in sub-tropical Australia. *International Journal of Environmental Research and Public Health* **8** (3):713–732.

Schültze, N., Lehmann, I., Bönisch, U., Simon, J.C. and Polte, T. (2010). Exposure to mycotoxins increases the allergic immune response in a murine asthma model. *America Journal Respiration Critical Care Med*ical **181**:1188–1199.

Shiklomonov, I.A. (2000). Appraisal and Assessment of World Water Resources. *Water International* **25**(1):11-32.

Sonigo, P., De Toni, A. and Reilly, A. Review of Fungi in Drinking Water and the Implications for Human Health. Final report WD 0906. *Bio Intelligence Service*. **45:** 23 - 65.

Sulaiman, I., Jacobs, E., Simpson, S. and Kerdahi, K. (2014). Molecular identification of isolated fungi from unopened containers of Greek yogurt by DNA sequencing of internal transcribed spacer region. *Pathogens* **3**(3): 499–509.

Topkaya, B. (1998). Water resources in the middle east: Forthcoming problems and solntions for sustainable development of the region report. Akdeniz University Faculty of engineering: Antalya, Turkey pp. 18.

Volk, T. J. (2013). Fungi. *Encyclopedia of Biodiversity* **3**: 624–640.

Wagacha, J. M. and Muthomi, J. W. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology* **124** (1): 1–12.

Wurzbacher, C., Kerr, J. and Grossart, H. P. Aquatic fungi. In: The Dynamical Processes of Biodiversity – Case Studies of Evolution and Spatial Distribution (O. Grillo and G. Venora, eds). InTech, Rjeka, Croatia, pp. 227–258.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society* **15** (2): 129–144.

Zerzghi, H., Gerba, C. P. and Pepper, I. L. Long-term effects of land application of Class B biosolids on the soil microbial populations, pathogens, and activity. *Journal of Environment Quality* **39**(1): 402–408.

APPENDIX A

CULTURE MEDIA

GRAM STAINING AND BIOCHEMICAL REAGENTS

STAIN AND REAGENT

Gram stain

The Gram stain was prepared using two stains (crystal violet and safranin or carbol fuchsin), Gram's iodine, and a decolorizing agent (ethyl alcohol).

A. Gram crystal violet

Solution A

Crystal violet - 2.0 g

Dissolved in ethanol (95%) - 20.0 ml

Solution B

Ammonium oxalate - 0.8 g

Distilled water - 80.0 ml

Gram iodine

Iodine (crystalline) - 1.0 g

Potassium - 2.0 g

Distilled water - 300.0 ml

3.0g of medium was dissolved in 300.0 ml of distilled water.

It is very important to note that; crystalline iodine, potassium and distilled water were combined to produce iodine solution and that Gram's iodine solution was stored in a dark bottle and protected from light so that it does not degrade.

Decolorizer

95 % ethyl alcohol was used.

Gram safranin

Safranin-O (certified) - 0.25 g

Ethyl alcohol (95 %) - 100.0 ml

Working solution:

Safranin stock solution – 10.0ml

Distilled water – 90.0 ml

BIOCHEMICAL REAGENTS

Indole medium

Peptone – 20.0 g

Sodium chloride – 5.0 g

Distilled water – 1000 ml

pH – 7.4

25.0 g of indole medium was dissolved in 1000 ml of distilled water and autoclaved for 15 min at 121 ºC and dispensed aseptically into sterile test tubes.

Oxidase reagent (Kovac’s oxidase)

Amul-alcohol – 15.0 ml

p-dimethyl-aminobenaldehyde – 0.5 ml

Concentrated HCl – 50ml

Small quantity of Kovac’s reagent was prepared by dissolving the aldehyde into alcohol and adding the acid slowly and then kept inside the refrigerator.

Catalase test

3% Hydrogen peroxide

APPENDIX II

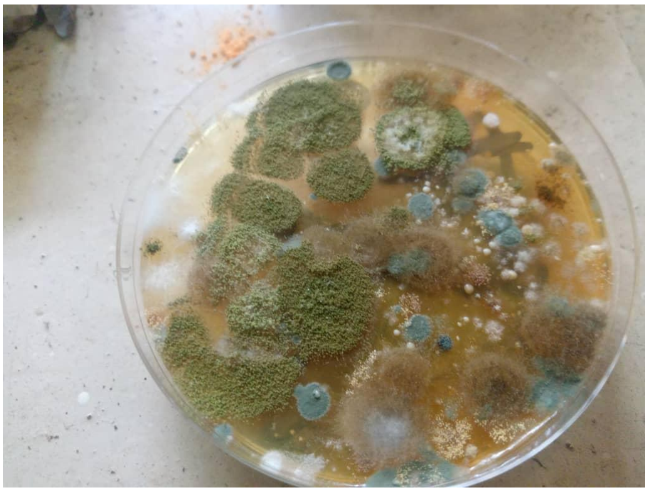

Plate 1 Plate 2

Plate 1 and 2 shows fungal growth of the reservoir water samples after 5 days of incubation.

Plate 3 Plate 4

Plates 3 and 4: Colony forming units of reservoir water samples from Isihor and Uwasota

Plate 4 Plate 5

Plates 4 and 5: Colony forming units of reservoir water samples from Bdpa and Osasogie