DIVERSITY AND BIOGEOGRAPHY OF FUNGI WITHIN THE HOT SPRINGS OF SODA LAKES IN KENYA

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Diversity and Biogeography of Fungi within the Hot Springs of Soda Lakes in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my family. My dear parents; Mrs. Wilfrida Tero and the late Ernest Tero Lihanda, dear husband Fred Manoa Salano, children; Debbie Elabonga, Becken Joseck and Gullit Nelson Salano. Without your encouragement and support this journey would have been long and tough. You put your heart in all that I did, supported, facilitated, encouraged and prayed for me.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA   Analysis of variance

DNA   Deoxyribonucleic Acid

GPS   Global Positioning System

HSD   Honest Significant Difference

ITS   Internal Transcribed Spacer

MEA   Malt extract agar

NCBI   National Centre for Biotechnology Information

NMDS   Non Metric Dimensional Scaling

OTU's   Operational Taxonomic Units

PDA   Potato dextrose agar

PCR   Polymerase Chain Reaction

PGA   Potato glucose agar

QIIME   Quantitative Insights into Microbial Ecology

RDA   Redundancy analysis

SDA   Sabourand dextrose agar

SDS   Sodium Dodecyl Sulphate

SSU   Small Sub Unit

UCLUST   Universal Clustering

UPGMA   Unweighted Pair Group Method with Arithmetic Mean

ABSTRACT

The East African Rift valley soda lakes are sodium carbonate (Na_2CO_3)-dominated extreme environments of active volcanoes and differ from other soda lakes due to the fact that the surrounding hot springs supply water to the lake depressions. Study of microbial diversity of these soda lakes and hot springs are of great interest among microbiologists owing to their immense biotechnological applications. Hot springs harbor diverse groups of micro-organisms like fungi that have developed mechanisms to thrive at wide temperature ranges, according to their optimal growth requirements. Both culture-dependent and independent methods have been used to study the diversity of fungi from hot springs of soda lakes in Kenya. The main objective of this study was to use both culture dependent and culture independent methods to study the diversity and biogeography of fungal communities within hot springs of four selected soda lakes (Lake Bogoria, Elmenteita, Magadi and Little Magadi) in Kenya. Water, wet sediment and microbial mat samples were collected in triplicates from the hot springs of the four soda lakes. Culture independent method employed the use of 454 pyro sequencing to sequence amplicons of Internal Transcribed Spacer (ITS) gene region of the total community DNA in order to explore the fungal community composition in twenty four samples. Sequences were analyzed using QIIME pipeline Version 1.8.0, while hierarchical clustering, non-metric dimensional scaling (NMDS) and diversity indices were carried out using the R programming language version 3.1.3 and Vegan Community Ecology Package version 2.5.2. For culture-dependent approach, microbial mats and wet sediments were collected from three sampling points along the flow of the hot springs while water samples were collected from the mouth of the hot spring. Samples from the hot spring were isolated on four different media at pH 10 and at a temperature of 30 °C. From the culture independent approach, a total of 139,023 quality sequence reads were obtained from which, 2,179 operational taxonomic units (OTUs) were realized at 3% genetic distance. Three known phyla (*Ascomycota* [83.3%], *Basidiomycota* [15.8%], *Glomeromycota* [0.02%]) were identified. Richness, abundance and taxonomic analyses identified *Agaricomycetes* as the most abundant and diverse class within *Basidiomycota*. Sequences matching with *Ascomycota* had high affinities with seven known classes *Dothideomycetes* (91.7%) and *Eurotiomycetes* (70.8%) being the most abundant and diverse classes. The most abundant OTUs showed the highest sequence similarity to *Cladosporium* sp. (83.08%), *Cladosporium cladosporioides* (17.90%), *Pleosporales* sp. (86.07%), *Aureobasidium pullulans* (79.88 %) and *Aspergillus oryzae* (35.02%). From the culture dependent method, sixty one (61) fungal isolates were identified using morphological, physiological and molecular characters. Out of this, seventeen isolates were recovered from the hot spring on the shores of Lake

Bogoria while twenty two isolates were from Lake Magadi and the same number from Lake Elmenteita. All the fungal isolates grew at pH ranging from 5 - 10, temperature range of 25 - 35 °C and sodium chloride range of 5 - 30 %. Substrate utilization of the sixty one fungal isolates revealed different types of enzymes (Amylases, proteases, pectinases, lipases, xylanases and esterases) as evidenced by the clearing zones. The percentage of isolates with enzymatic activity were; Esterase (85 %), amylases and xylanases (74 %), protease (66 %), lipase (59 %), while none showed production of cellulase enzyme. Analysis of partial sequences using Blastn showed that about 11.7 % and 29.1 % of the isolates from the hot spring on the shores of Lake Bogoria were affiliated with members belonging to the genera *Penicillium* and *Aspergillus,* respectively. A total of 12 % of the isolates belonged to the genera *Alternaria* and *Fusarium* while another 41 % of the isolates clustered closely with uncultured fungus. Fungal endophyte comprised 6 %. All the isolates from the hot spring at Lake Elmenteita were from the Phylum *Ascomycota* with 31.8 % of its members affiliated to the genus *Aspergillus* and 22.7 % were closer to the genus *Alternaria* while only 4.5 % were closely related to *Debaryomyces hansenii,* a yeast of the class *Saccharomycetes*. Contrary to the hot spring on the shores of Lake Bogoria, only 13.6 % of the isolates from Lake Elmenteita were affiliated with members belonging to the genus *Penicillium.* Blastn results from the hot spring on the shores of Lake Magadi showed 50 % of the isolates were closely clustered with members of the genus *Aspergillus.* An equal number of isolates representing 13.6 % were affiliated with members belonging to the genera *Penicillium* and *Neurospora.* Apart from isolate MM7, *Parmastomyces transmutans,* which belonged to the phylum *Basidiomycota,* the rest of the isolates from the hot spring at Lake Magadi were closely related to members of the Phylum *Ascomycota.* Although both culture dependent and independent methods did not reveal the true diversity of the fungal community, the isolates recovered were a representation of thermophilic, alkaliphilic and halophilic fungal taxa found in soda lake environments. These results suggest the ability of fungi to adapt to extremes of alkalinity, temperature and salinity. The isolates also have the potential to produce useful enzymes which could be exploited for future biotechnological applications.

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Soda lakes are stable and extreme alkaline environments that are found in arid and semi-arid areas of tropical and subtropical deserts of North America, continental Asia and East African Rift Valley (Grant & Sorokin, 2011). Most of the soda lakes and pans are shallow with extreme physical and chemical conditions, special biogeochemical cycling and unique communities (Boros *et al.,* 2017). In Kenya, the soda lakes (Bogoria, Magadi, Little Magadi and Elmenteita) found in the East African Rift Valley represent the major type of naturally occurring highly alkaline environments. They are characterized by high concentrations of carbonate salts, especially sodium carbonate, related salt complexes and are depleted of Mg^{2+} and Ca^{2+} because of the insolubility of carbonates under alkaline conditions (Grant 2006; Schagerl and Renaut, 2016). The carbonate provides buffering capacity to the lake waters (Renaut *et al.,* 2013). Many soda lakes also contain high concentrations of sodium chloride and other dissolved salts, making them saline or hyper saline lakes (Grant & Jones, 2016). These hyper saline and highly alkaline soda lakes are considered some of the most extreme aquatic environments on earth. It has been proposed that the formation of alkalinity in the soda lake environment requires a combination of geographical, topographical and climatic conditions: firstly, the presence of geological conditions which favour the formation of alkaline drainage waters; secondly, suitable topography which restricts surface outflow from the drainage basin; and thirdly, climatic conditions conducive to evaporative concentration (Behr & Röhricht, 2000; Boros *et al.,* 2017).

Environmental conditions such as temperature, pH and salinity are extremely high or low in these habitats (Renaut & Gierlowski-Kordesch, 2010). Owing to their hostile nature they are often remote from the main centers of human activity and perhaps for this

reason they have not been extensively studied. The best studied regions are the lakes and solonchaks of the Central Asian (Siberia) steppes and the Rift Valley of Eastern Africa (Grant *et al*., 2004; Baumgarte, 2003). Soda lakes are also very productive presumably as a consequence of relatively high surface temperatures (30 - 45 °C), high light intensities, accessibility of phosphates, and unlimited reserves of hydrogen carbonate (HCO_3) for photosynthesis. This high productivity, $>10\,g\,cm^{-2}$ per day (Grant and Sorokin, 2011), is the driving force behind all biological processes occurring in soda lakes (Sorokin *et al*., 2015).

These environments are home to various groups of microorganisms that have adapted to these specific conditions. They have been found to support a dense and different groups of heterotrophs, organotrophs, halophiles, alkaliphiles, and alkalitolerant representatives of most important major microbial phyla (Mwirichia, 2011). Many of the microorganisms so far characterized from soda lakes have relatives in salt lakes except that they are all alkaliphilic or at least highly alkali-tolerant (Kambura *et al.,* 2012). There is a distinct difference in microbial community composition between the hypersaline, alkaline lakes such as parts of Lake Magadi with salinity approaching saturation or higher, compared with the more dilute lakes like Nakuru (Wani *et al.,* 2006; Grant *et al.,* 2004).

1.2 Formation of soda lakes

Soda lakes are formed in depressions where ground water rich in carbon dioxide, but poor in magnesium and calcium, leaches sodium from sodium-rich rocks. The absence of dissolved divalent cations (Mg^{2+} and Ca^{2+}) is crucial to avoid carbonate precipitation. Due to increased evaporation rates, carbonate salts become more concentrated leading to the formation of natural sodium carbonate/bicarbonate-buffered systems with elevated pH values (9.5 - 11) and salt concentrations of up to maximum levels (Boros & Kolpakova, 2018). Insoluble calcium and magnesium carbonates are removed from the solution through precipitation and this allows the more soluble carbonates of sodium and

potassium to build up (Baumgarte, 2003). The topography allows concentration of these salts in shallow depressions forming a closed drainage basin with a high marginal relief, with adequate rainfall to sustain streams entering the basin to produce a pool of water (Grant *et al.,* 1990). Generally, there's little or no out flow and water loss is by evaporation. In arid zones with high rates of evaporation exceeding inflow, salts accumulate (Jones *et al.*, 1994; Baumgarte, 2003). Geographical, geological and climatic conditions lead to the formation of alkaline saline deposits in soda lakes (Boros & Kolpakova, 2018). The main geochemical conditions of their formation include leaching of rock material, rich in sodium but low in cacium and magnesium ions. Areas with dry and warm climate coupled with CO_2-saturated waters, facilitate evaporative concentration of the brines in natural depressions (Eugster, 1970; Jones *et al.*, 1977). Therefore geochemical impacts determine the ions flowing into the system. Climatic influence controls the amount of water entering the system as rainfall or surface runoff and the amount leaving by evaporation, hence favoring saline lake formation.

1.3 Soda lakes in Africa

Soda lakes are found in many arid zones across the world, such as the the Wadi El Natrun lakes in Egypt, Lake Basaka, Abijatta and Shala in Ethiopia and lake Magadi, Bogoria, Elmenteita and Little Magadi in the Kenyan Rift Valley (Table 1.0). Soda lakes are the most biologically productive non-marine aquatic environments known (Leboulanger *et al.*, 2017) whose microbial composition has been well studied (Lanzén *et al.*, 2013). Much of the knowledge of saline lakes in Africa has come from studies of their chemistry, biodiversity and the magnitude of their primary production (Jones *et al.*, 1994). It is well understood that the diversity of microorganisms in saline lakes is low but the production of the few species that dominate each trophic level is high (Duckworth *et al.*, 1996). The best studied lakes are those of the East African Rift Valley which have been documented for many decades. Two of these lakes namely Lake Shala (LS) and Lake Magadi (LM) are located in Ethiopia and Kenya respectively and are the most studied (Jones & Grant, 2000). The East African Rift Valley lakes are situated in an environment of active

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volcanism and differ from other soda lakes as surrounding hot springs supply water to the lake depressions, whereas others are supplied by the leaching of rainfall through the surface into the lake basins (Odada *et al.,* 2003).

Table 1.1: pH and salinity levels in selected soda lakes in Africa and Asia

Continent	Name of lake	Country	pH	Salinity %
	Wadi El Natrun lakes	Egypt	9.5	5
	Lake Arenguadi (Green Lake)	Ethiopia	9.5-9.9	0.25
	Lake Basaka	Ethiopia	9.6	0.3
	Lake Shala	Ethiopia	9.8	1.8
	Lake Chitu	Ethiopia	10.3	5.8
	Lake Abijatta	Ethiopia	9.9	3.4
	Lake Magadi	Kenya	10	>10
	Lake Bogoria	Kenya	10.5	35
	Lake Turkana	Kenya	8.5-9.2	0.25
Africa	Lake Logipi	Kenya	9.5-10.5	2-5
	Lake Natron	Tanzania	9-10.5	>10
	Lake Eyasi	Tanzania	9.3	0.5
	Rombou Lake	Chad	10.2	2
	Lake Van	Turkey	9.7-9.8	2.3
	Khyagar Lake	India	9.5	0.6
	Sambhar Salt Lake	India	9.5	7
	Namucuo Lake	China	9.4	0.2
	Lonar Lake	India	9.5-10.5	1
	Qinghai Lake	China	9.3	2.2
	Pangong Salt Lake	India and China	9.4	0.9
Asia				

Adapted from: Lanzen *et al.,* 2013

1.4 Distribution of soda lakes in Kenya

Soda lakes and soda deserts are the most stable naturally occurring alkaline environments on earth with pH values of 10 and above and are usually characterized by large amounts of soda (sodium carbonate, or complexes of this salt). The best-studied area with soda lakes is the Kenyan - Tanzanian Rift Valley (Jones & Grant, 2000; Deocampo & Renaut, 2016). The Great Rift Valley that runs through East Africa is an arid tropical zone where tectonic activity has created a series of shallow depressions.

These shallow depressions are often closed basins with no obvious outflow where ground water and streams flowing from the surrounding highlands on the margins of the Rift Valley collect to form semi-permanent standing bodies of water. In these zones high rates of evaporation exceed inflow and salts accumulate by evaporative concentration (Deocampo & Renaut, 2016).

Kenya is endowed with many soda lakes forming part of the East African Rift Valley system and includes lakes Bogoria, Nakuru, Elementeita and Magadi. Total salinity vary with season and ranges from around 5% (w/v) total salts in the more dilute lakes such as Lake Elmenteita that is made up mainly from roughly equal amounts of $NaCl$ and Na_2CO_3 at a pH of about 11, to saturated lakes like Lake Magadi at >30 % (w/v) total salts, again dominated by $NaCl$ and Na_2CO_3 at pH values approaching 12 (Warren, 2006).

Wildlife and microbial diversity are analogous amongst all these lakes (Gierlowski-Kordesch *et al*., 2004). Lake Magadi is the most studied of the alkaline East African soda lakes (Warren, 2006) and is one of the most alkaline habitats on the Earth; pH values for this lake reach 10–12 (Grant, 2006). The lake has no permanent rivers entering its basin and solutes are supplied mainly by a series of alkaline springs with temperatures as high as 86°C. The springs feed lagoons that support a colony of fish, the *Tilapia graham* species (*Oreochromis alcalicus*), which can thrive at a temperature of 39°C and pH of 10.5 (Grant & Sorokin, 2011; Kavembe *et al.,* 2016).

These lakes are characterized by steep fault escarpments, deep gorges, canyons, and craters on the rift floor, some of which have gushing geysers and hot springs. Historically, the lakes were thought to have been one continuous system called Lake Kamatian (Grant *et al.,* 1999). Reconstruction of the history of the four lakes based on dated sedimentary time-series data reveal unique hydrological, ecological and species richness trends that have fluctuated through time between alkaline and freshwater conditions (Grant *et al.,* 1999; Sorokin *et al.,* 2014).
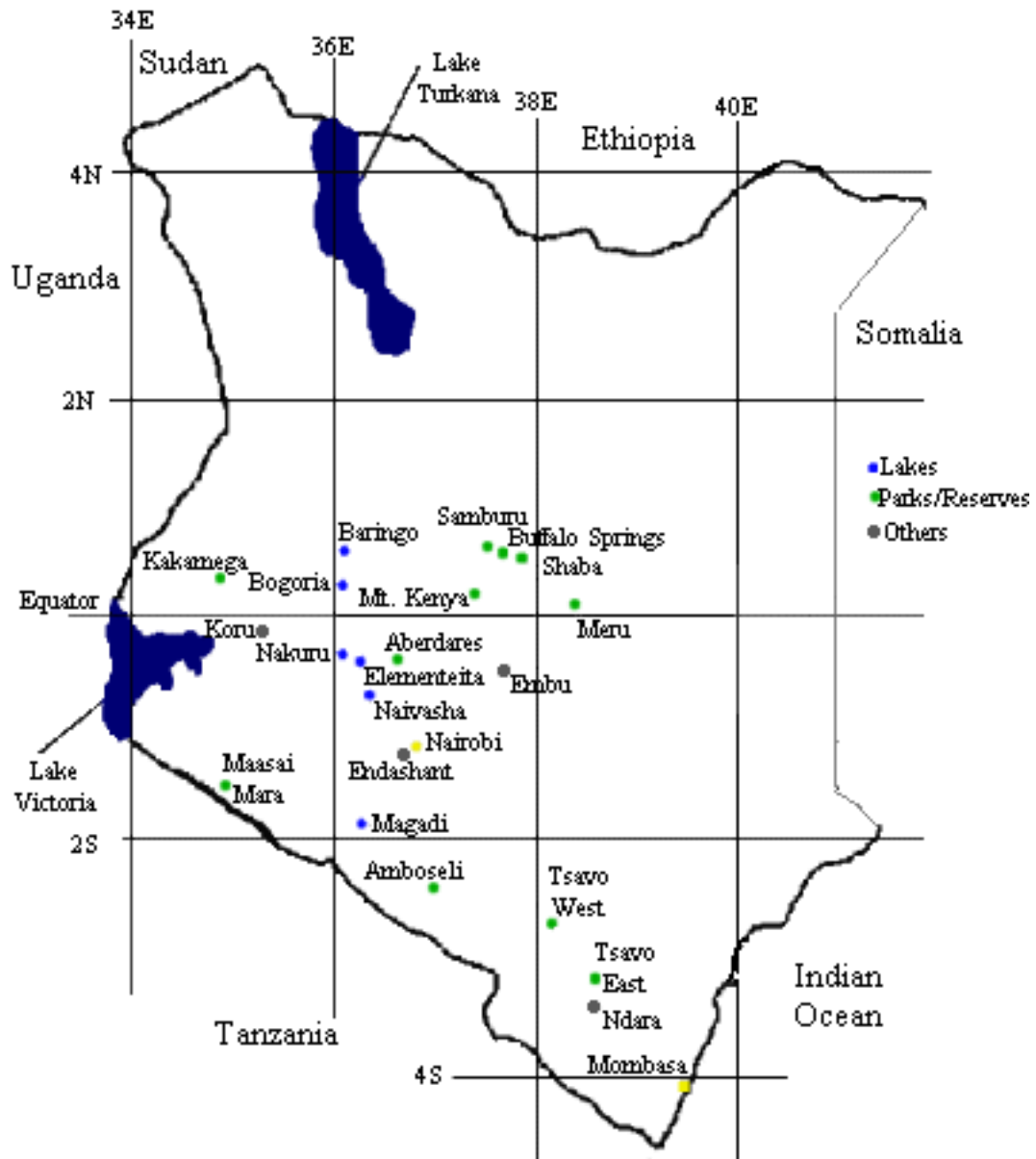
Figure 1.1: A map of Kenya showing the distribution of soda lakes (Lakes: Elmentaita, Nakuru, Bogoria and Magadi): Modified from Grant (2004).

1.5 Biogeography

Biogeography is a science that attempts to describe spatial distribution of plants and animals (biological diversity) and how these distribution change over time (Ganderton & Coker, 2005; Lomolino *et al.,* 2006). Organisms and biological communities vary in a highly regular fashion along geographic gradients of latitude, elevation, isolation and habitat area. Biogeography scientists seek to explain the reason as to why organisms live within their habitats. While biogeography has traditionally focused on macro-organisms, i.e. plants and animals, microbiologists have studied biogeographical questions for many decades and there has been a recent resurgence of interest in microbial biogeography (Green & Bohannan, 2006; Martiny *et al*., 2006; Ramette & Tiedje, 2007). This resurgence has been led, in part, by advancements in molecular tools that allow us to survey uncultivated microbes in the environment and a growing recognition that microbial taxa are the most biologically diverse taxa on earth (Chistoserdova, 2010).

At present, the study of microbial biogeography is in its initial stages. However, a wide variety of microbial taxa exhibit biogeographical patterns and since microbial communities are not homogeneous across habitat-types and within a given habitat, microbial diversity can vary between locations separated by millimeters to thousands of kilometers. Despite their importance, few studies have considered which factors generate and maintain fungal diversity and their distribution patterns (Mohamed & Martiny, 2011). As indicated by the dictum 'everything is everywhere, but the environment selects' (Fondi, 2016), environmental factors have long been considered to have a strong influence on fungal (Tedersoo *et al*., 2014; Schadt & Rosling, 2015) and bacterial biogeography (Nemergut *et al.,* 2011; Doherty *et al.,* 2017). Therefore, if microbial biogeography did not exist, there would be no spatial or temporal heterogeneity in microbial communities. Therefore Biogeography aims at explaining any spatial patterns in the diversity of microorganisms and that geographical distance does not influence the distribution of both abundant and rare microbial communities in the hot springs of the soda lakes (Lindström *et al.,* 2005).

1.6 Statement of the problem

Kenyan soda lakes are an example of relatively simple but highly productive ecosystems that can serve as models for studying microbial diversity under extreme conditions. Previous exploration of the microbial communities in soda lakes was based on use of conventional isolation methods (Jones & Grant, 1999; Baumgarte, 2003; Salano *et al*., 2011; Kambura *et al*., 2012; Sorokin *et al.,* 2014). However due to the unculturability of most microbes, very few organisms have been isolated from these extreme environments. Culture independent studies done so far have heavily focused on the diversity of prokaryotes (Sorokin *et al*., 2015; Andreote *et al.,* 2018; Rojas *et al*., 2018) and a few have tried to investigate the diversity fungal communities. (Tourova *et al.,* 2013; Kilmer *et al.,* 2014; Kambura *et al*., 2016; Paul *et al.,* 2016). These studies have provided novel insights and significant advances in research on fungal ecology. An increasing number of studies have tried to establish the linkage between microbial community composition/diversity and physicochemical conditions such as temperature, pH and water chemistry (Purcell *et al.,* 2007; Ward & Castenholz, 2002; Whitaker *et al.,* 2003). However, no comprehensive studies have been performed for comparison of fungal diversity within and across the hot springs of soda lakes in Kenya using high throughput sequencing technology and it is not clear if the relationship of microbial community composition, abundance and diversity to physicochemical conditions in these springs differs from those at other terrestrial springs. In this study a combination of both culture dependent and culture independent techniques were used to study the biogeography and diversity of fungal communities within the hot springs of four soda lakes in Kenya.

1.7 Justification

Similar to other fields in microbiology, species identification in fungi can be assessed through the use of either culture-dependent (Dickson *et al.,* 2014) or culture-independent methods (Öztürk *et al*., 2013). Culture-dependent methods are based on isolating and culturing the sample under study on media and confirmation of pure culture isolates prior

to their identification according to either morphological, biochemical or genetic characteristics.

Though cultural methods are simple and inexpensive and can be used in both quantitative and qualitative testing, they are labor-intensive and yield results after several days of repeated culture and confirmation steps.

Community-level studies (Bayer *et al.,* 2013) rely more on culture-independent methods based on the direct analysis of DNA without any culturing step. These methods (Jany & Barbier, 2008) are based on protocols where total DNA is directly extracted from the substrate. Culture independent methods (Nakamura *et al.,* 2016) typically aim at collecting DNA from the whole community. Coupled with a global analysis, these methods make it possible to study the total diversity from the bulk extract in a single step. As they are fast and potentially more exhaustive, these methods are well suited for analyzing microbial communities (Agrawal *et al.,* 2015) over time. Therefore this study involved application of both culture dependent and culture independent methods to determine the diversity and biogeography within samples collected from the hot springs of lake Bogoria, Elmenteita, Magadi and Little Magadi in Kenya.

1.8 Hypotheses

1. Temperature gradients affect the fungal community within the hot springs of Lake Bogoria, Elmenteita, Magadi and Little Magadi lakes in the Kenyan rift valley.
2. The hot springs of soda lakes in Kenya have diverse fungal communities.

1.9 Objectives

1.9.1 General objective

To study the biogeography and diversity of fungi within the hot springs of soda lakes in Kenya using both culture dependent and culture independent approach.

1.9.2 Specific objectives

1. To assess fungal diversity within the hot springs of lake Bogoria, Elmenteita, Magadi and Little Magadi in Kenya using 454 pyrosequencing.
2. To evaluate change on fungal diversity across a wide range of temperatures within the hot springs.
3. To isolate and characterize fungi from different temperature zones within the hot springs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbial diversity of soda lakes

Soda lakes are aquatic ecosystems that exhibit high productivity rates presumably because of the high ambient temperatures, high light intensities, availability of phosphates and unlimited access to CO_2 in these carbonate rich waters (Grant, 2004; Shvartsev *et al.,* 2014; Isupov *et al.,* 2016). Soda lakes harbor diverse and dense microbial populations (Grant & Jones, 2016; Harper *et al.,* 2016) that result in distinct coloration of the lake waters. The East African Rift valley soda lakes support a dense and diverse population of autotrophic, heterotrophic, organotrophic, halophilic, alkaliphilic and alkalitolerant representatives of major bacterial, archaeal and fungal phyla (Boros & Kolpakova, 2018; Oduor & Kotut, 2016*)*. Alkaliphilic cyanobacteria (*Spirulina, Arthrospira, Anabaenopsis, and Cyanospira*) are responsible for the generally high level of primary production and nitrogen fixation in these soda lakes (Sorokin *et al*., 2004; Antony *et al.,* 2012). These microbes have attracted considerable attention as a source of biomolecules with biotechnological potential and also for studying adaptive mechanisms to extreme environmental parameters like temperature and pH (Glaring *et al.*, 2015). Many of the microorganisms from soda lakes have relatives in salt lakes except that they are all alkaliphilic or at least highly alkali-tolerant (Sorokin *et al*., 2014).

2.2 Microbial diversity and ecology of the soda lakes in Kenya

The East African Rift Valley is the most extensive, presently active continental extension zone on earth (Dawson, 2008). It's the best-studied area where soda lakes have formed. The salinities of the soda lakes found in Kenya range from approximately 5 % total salts (w/v) to 30 % and pH values from 9 to above 11.5 (Schagerl & Renaut, 2016). Kenya's Rift Valley soda lakes include Bogoria, Nakuru, Elmenteita and Magadi. Wildlife and microbial diversity are analogous amongst all these lakes (Gierlowski-Kordesch *et al*.,

2004). Tallar and Suen (2017) described these lakes as very important natural assets with considerable aesthetic, cultural, economic, recreational, scientific, conservation and ecological values for mankind. Although, fungi prefer acidic to neutral pH range for growth, reports on alkaliphilic and halophilic fungal species has been documented from soda lakes like lake Magadi of Kenya, lake Natron of Tanzania (pH 11–12) and the Dead sea of Israel (Oren & Gunde-Cimerman, 2012; Grum-Grzhimaylo *et al.*, 2013a,b, 2016).

Although the soda lakes are widely distributed across the globe (Grant and Sorokin, 2011) only a few have been studied. Lake Magadi is among the most studied and most stable highly alkaline environment among the East African soda lakes, with a consistent pH of 10.5 to 12 (Sorokin *et al.,* 2014; Boros & Kolpakova, 2018). The alkaline lake niche limits diversity in microbial life due to high pH and high salinity (Pontefract *et al.,* 2017). Microbes living in this niche must have modified proteomes in order to survive in such an environment. The specificity for microbial life in this niche has attracted researchers to study how microbial life exists in this environment. Lake Bogoria is known for its flamingo populations and it's partially fed by hot springs. Alkaliphilic communities contain representatives of major trophic groups. Primary producers dominate the lakes (Leboulanger *et al.,* 2017).

The Kenya soda lakes remain as fragile ecosystem due to low water level through evaporation, pollution through human activities in the catchment's areas, domestic effluent and industrial waste. This could mean the microbial diversity in the lakes is changing due to human activities, which may result in loss of certain species (Wilhelm *et al.,* 2014; Boros & Kolpakova, 2018). The first comprehensive culture survey of soda lake environments that attempted phylogenetic placement of the isolates was that carried out by Duckworth *et al.* (1996), who isolated several hundred strains of aerobic, heterotrophic alkaliphilic, and haloalkaliphilic organotrophs from a range of soda lakes in the East African Rift Valley. The Kenyan soda lakes such as Bogoria and Elmenteita are also characterized by hot springs which host both hyperthermophilic and haloalkalithermophilic microorganisms. However, salinity is also an important defining

factor in the alkaline lakes. There is a distinct difference in microbial community composition between the hypersaline, alkaline lakes such as parts of Lake Magadi with salinity approaching saturation or higher, compared with the more dilute lakes like Nakuru. Several hundred strains of non-photo-trophic aerobic organotrophs have been isolated from the environs of Rift Valley soda lakes on a variety of media. About 100 of these have been examined phenotypically and chemotaxonomically in some considerable detail for the purpose of numerical taxonomy (Sorokin *et al*., 2014). Many of the strains isolated in pure culture can be assigned to existing taxa as new species or novel genera, but some isolates have no close phylogenetic ties with known microbes and appear to be separate lines of evolutionary descent perhaps peculiar to the soda lake environment.

Phototrophic eukaryotes of the diatoms belonging to the genera *Nitzchia* and *Navicula* are predominant in these ecosystems (Burganskaya *et al.,* 2018). Using traditional culture-based methods, Salano (2011) isolated and characterized fungi from Lake Magadi and reported relatively low levels of diversity for fungal communities in these extreme environment. Similar studies were carried on Lake Sonachi and Lake Elmenteita and revealed low diversity of fungi, with the majority showing similarity to terrestrial species (Ndwigah *et al*., 2015).

2.3 Fungal diversity in soda Lakes in the world

The most widely studied ecosystems are the Great Salt Lake (Utah, USA), the Dead Sea (Israel), the alkaline brines of Wadi Natrun (Egypt), and Lake Magadi in Kenya (Oren, 2002). It is noteworthy that low taxonomic biodiversity is observed in all these saline environments (Humayoun *et al.,* 2003; Glaring *et al.,* 2015) most probably due to the high salt concentrations.

Fungi are ubiquitous in most of these ecosystems where they usually colonize a diverse range of substrates. The highest diversity of fungi is found in tropical regions, mainly in tropical forests (Hawksworth, 2001). In Puerto Rico, fungal studies have concentrated on

forest and coastal ecosystems (Lodge *et al*., 2002; Cantrell *et al.,* 2004; Nieves-Rivera, 2005). However, many fungi can adapt to extreme environmental conditions of water, temperature, pH and salinity (Rampelotto, 2013).

Most of the fungi that can be found in extreme environments belong to the imperfect stage of the *Ascomycota*, which have been reported in mangroves, saline soils, marine sediments, sea water, salt marshes, and sand dunes (Hyde & Pointing *et al*., 2000). A number of fungal species and strains inhabiting natural hypersaline environments have recently been described **(**Glaring *et al.,* 2015). Studies on fungal communities populating such extreme environments have been largely conducted in the northen latitudes, in and around salterns along the Adriatic coast, in the region of Russia and the Dead Sea, the Mediterranean coast, the Red Sea coast, and the Atlantic coast, Gulf of California, Great Salt Lake, Utah and the West Coast of Indian peninsula (Gunde-Cimerman *et al.,* 2005). Imran *et al.* (2016) described fungi isolated from several natural and man-made hypersaline environments including those found in France, Namibia, Portugal, Slovenia, Spain, and Dominican Republic.

Reports on presence of filamentous fungi in the hypersaline waters of the Dead Sea (340 g $^{l-1}$ total dissolved salts) and so it is clear that it does in fact support life despite the high salinity, survival of their spores and mycelia in this hostile environment have invoked great interest (Kis-Papo *et al.,* 2003). Further, a gene responsible for High Osmolarity Glycerol (HOG) response pathway from one such Dead Sea-fungus Eurotium herbariorum has been identified for stress tolerance to freezing and thawing (Jin *et al.,* 2005). This has further led to production of a recombinant yeast *Saccharomyces cerevisiae* containing the gene HOG (Martinez *et al.,* 2012). The genetically transformed yeast proved to withstand high salinity and also extreme heat and cold. However, high salt tolerance is governed by several genes and this might be the first step towards genetic engineering of salt-tolerant agriculture crops (Kis-Papo *et al.,* 2003).

A diverse fungal fauna was recently discovered in environments with salinities ranging between 15–32%, where it was assumed that bacteria only were able to grow. These fungi were first isolated in hypersaline waters of Secovlje salterns in Slovenia (Gunde-Cimerman *et al.,* 2000) and subsequently in the salterns of La Trinitat (Ebro Delta, Catalonia, Spain) and Bonmatí (Santa Pola, Valencian Community, Spain), with the occurrence of the same dominant species. The majority of species isolated belonged to melanized meristematic and yeast-like fungi, and a few different genera of filamentous fungi were also identified (Méjanelle *et al.,* 2001). Among the isolated halotolerant and halophilic mycobiota, dematiaceous Ascomycetales of the following genera were found: *Hortaea, Phaeotheca, Trimmatostroma, Aureobasidium, Alternaria* and *Cladosporium* (Zalar *et al*., 1999). Five melanized species were isolated from La Trinitat salterns (Spain) and further enriched in cultures, as well as *S. cerevisiae* (Jean-Luc Legras, 2007) a pertinent reference for Ascomycetes. These melanized species form black, clump like colonies that consist of dividing cells in the water. This unique morphology has been interpreted as a response to multiple stress factors that helps the fungi to tolerate high temperatures and low water activity by optimizing the volume-surface ratio. (Plemenitaš & Gunde-Cimerman, 2005).

A study of coastal mycology in Puerto Rico was performed by Nieves-Rivera (2005) including many aspects of fungi in marine environments. Another study of arenicolous filamentous fungi in the Mayaguez Bay shoreline (Ruiz- Suarez, 2004) reported the presence of *Aspergillus, Cladosporium, Dreschlera, Fusarium, Geotricum, Penicillium, Trichoderma, Mucor* and *Rhizopus*. The genus *Aspergillus* represented the 80% of total fungal abundance. The data suggested that salinity concentration may regulate the abundance of fungi in the shoreline.

Salt marshes, a moderate salt environment, are another habitat for halotolerant fungi (Sarah *et al*., 2013). Fungi have an important role in the decomposition of salt marsh grasses. Halophytes from salt marshes in Europe were examined for the presence of mycorrhizal fungi (Hildebrandt *et al.,* 2001). Large quantities of spores were found in

saline soil; 80% of them were identified as *Glomus geosporum*. Also, they have found that several plants from the salt marsh were mycorrhizal. Another study suggests that these fungi confer salt tolerance to plants based on the occurrence of *G. geosporum* and *G. caledonium* spores in salt marshes (Landwehr *et al.,* 2002). This suggestion was supported later by Carvalho *et al.* (2004). They indicated that fungi in salt marsh plants may have the potential to confer salt tolerance to them and may influence the distribution of this vegetation. This study was conducted in a salt marsh from Portugal and showed that fungal adaptation is one of the possible reasons for the existence of mycorrhizal fungi in salt marshes (Carvalho *et al.,* 2004).

The first record for filamentous fungi in Dead Sea by Buchalo *et al.,* (1998) reported a novel species, *Gymnascella marismortui* (Ascomycota). This fungus was shown to be an obligate halophile that grew optimally in a range of 10-30% of Dead Sea water. Moreover, they isolated *Ulocladium chlamydosporum* and *Penicilium westlingii* with salt tolerance in the range of 3 to 15% NaCl at 26 °C. In another study, *Aspergillus versicolor*, *Chaetomium globosum, Eurotium herbariorum, E. amstelodami and E. rubrum* were isolated from Dead Sea waters and together with *Gymnascella marismortui* were tested for survival of spores and mycelia in Dead Sea water for prolonged time (Kis-Papo *et al*., 2001; 2003a). After the first record of fungi, Kis-Papo *et al.,* (2003b) performed a study of genomic diversity of *A. versicolor* and their results suggested that genomic diversity was positively correlated with stress. The species *Trichosporon mucoides*, *Rhodotorula larynges,* a *Candida glabrata*-like strain and a *Candida atmosphaerica*-like strain were also isolated from these waters. *Candida glabrata* were not known for their halotolerance (Butinar *et al.,* 2005b). *Candida atmosphaerica* was found associated with another extreme environment, the deep-sea hydrothermal systems of the Mid-Atlantic Rift (Gadanho & Sampaio, 2005).

The Mono Lake in California, an alkaline, hypersaline and closed basin, was the site of a study performed by Steiman *et al.* (2004). This lake is compared with the Dead Sea due to its high salinity. Those conditions permit the mineral formation named tufa. Steiman *et*

al., (2004) isolated many species of the genera *Aspergillus, Achaetomium, Acremonium, Alternaria, Chaetomium, Cunninghamella, Ulocladium, Embellisia, Fusarium, Phoma, Sporothrix, Penicillium, Papulaspora, Geotricum, Curvularia, Mucor, Phialophora, Phaeoramularia, Sporormiella and Thelebolus.* Interestingly, in this study they were not able to isolate fungi from water using culture methods. They suggested that the salinity, and most important, the pH (very alkaline), were not favorable for fungal growth (Steiman *et al.,* 2004).

2.4 Influence of Salinity, pH and Temperature on Fungal diversity

Fungi are sensitive to any change in their medium of growth (Deacon *et al*., 2006). Extremely hyper saline environments usually display low fungal abundance and diversity salinity as they induce stress on the fungi. It's only the xerotolerant and/or osmotolerant species, *Wallemia* and *Phaeotheca* that can exist under such conditions (Butinar *et al.*, 2005a, 2005b). Stress induced by salinization can lead to either decline in numbers of fungal species or changes in their diversity (Varnam & Evans 2000).

pH is a key factor in determining the fungal community composition in any ecosystem. It may directly affect fungal community composition by imposing a physical constraint on fungal survival and growth. Some fungal taxa may be unable to grow if the soil pH falls outside a certain range. Fungi generally grow well in a wide range of pH conditions (Dinah & Akira, 2017), some grow well in neutral to slightly alkaline conditions (Yamanaka, 2003) while others do well in acidic conditions.

Temperature is an important factor in regulating fungal activities and shaping the microbial community of an area.

2.5 Fungi in Biotechnology

Fungal biotechnology or 'mycotechnology' has advanced considerably in the last decades. Terrestrial fungi are used in the production of various extracellular enzymes, organic

acids, antibiotics and anti-cholesterolemic statins (Pointing & Hyde, 2001). Enzymes from extremophiles, extremoenzymes, have a great economical potential in agricultural, chemical and pharmaceutical processes. They are used in biological processes by increasing specificity and catalytic activity, and are stable at extreme incubation conditions (Chadha & Patel, 2008). Fukumori *et al.* (1985) reported the use of an alkaline protease to decompose the gelatinous coating of X-ray films, from which silver was recovered by Fujiwara and coworkers. Ligninolytic enzymes from members of basidiomycetes have a potential in several industrial and biotechnological processes within a wide variety of organic and inorganic substrate specificities (Couto & Herrera, 2006). Such applications include the detoxification of industrial effluent (Nilsson, *et al.*, 2006; Zhao, 2007; Butt *et al.,* 2001) mostly from textile and petrochemical industries and removing the phenolic compounds from the beer and wine in the food industry (Minussi *et al.*, 2002). In addition, their capacity to remove xenobiotic substances and produce polymeric products makes them a useful tool for bioremediation purposes (Pointing, 2001).

Lignin-degrading fungi or their enzymes have the ability to degrade highly toxic organic compounds such as dioxins and polychlorinated biphenyls (PCB's), and could have an important role to play in the remediation of contaminated soils and the disposal of chemical wastes. Deguchi *et al.* (1997) indicated that lignin-degrading fungi can degrade synthetic textile polymers such as nylon previously thought to be non-biodegradable.

The development of Quorn myco-protein (Choi *et al.,* 2004) from a filamentous fungus by Rank Hovis McDougall PLC and ICI (now Zeneca) is a recent innovation in food technology. The culture employed, was a strain of *Fusarium graminearum*, isolated from a field in Marlow, Buckinghamshire. Probably the most thoroughly tested food ever to appear on supermarket shelves, annual sales of Quorn are now in excess of £15 million in the UK.

The use of biological control agents rather than toxic chemicals for the control of pests has intensified over years (Butt *et al.,* 2001). Considerable research has been devoted to the possibility of using fungi to control fungal pathogens (mycofungicides), insects (mycoinsecticides) and weeds (mycoherbicides), a trend that will be enhanced by developments in the production of genetically engineered strains of fungi (Jackson *et al*., 2000). Several species of fungi have now been commercially formulated as mycoinsecticides. Fungal biocontrol agents are useful in greenhouses because target pathogens are confined and environmental parameters are relatively constant (Shah & Pell, 2003).

Fungi are used in the production of primary metabolites like ethanol, citric acid, gluconic acid, amino acids, vitamins, nucleotides and polysaccharides and secondary metabolites like antibiotics such as penicillin, cephalosporins, fusidic acid and griseofulvin (Namikoshi *et. al.*, 2002). Cyclosporins were first isolated from *Tolypocladium inflatum* in 1976 as antifungal compounds and later showed to possess immunosuppressive activity. Cyclosporin A is currently the most widely used drug for preventing rejection of human organ transplants (Borel, 2002).

The use of fungi in bioremediation is based largely on the versatile enzymic abilities of wood-rotting fungi, *Phanerachaete chrysosporium* (Pointing, 2001). These fungi produce ligninases which degrade complex aromatic polymers like those present in wood. Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy-metal ions (Gadd, 2010). Strains of *Penicillium*, *Rhizopus* and *Aspergillus* (Say *et al*., 2004; Ahmad *et al*., 2005) have been used in the removal of such metal ions.

2.6 Microbial communities in Hot springs

Soda lakes are extreme environments that are characterized by steep fault escarpments, deep gorges, canyons and craters on the rift floor, some of which have gushing geysers

and hot springs. Such environments are unique sites to study how organisms interact with each other and their physical environment and they serve as analogues that reflect early earth or extraterrestrial settings (Greenwood *et al*., 2002). Hot springs are considered extreme environments yet they contain a diverse array of microorganisms that are capable of surviving and functioning under such conditions (Rothschild & Mancinelli, 2001). Geothermal springs are unique sites for extremophilic microorganisms.

The microbial communities of hot springs have been extensively studied in certain areas such as Yellowstone National Park (Blank *et al.*, 2002; Meyer-Dombard *et al.*, 2005). Such studies (Siering *et al.*, 2006; Mathur *et al.*, 2007) focused on abiotic factors and their effects on microbial communities. These studies revealed surprising bacterial and archaeal diversity and microorganisms that were unknown, and those that had not been cultivated (Blank *et al.*, 2002; Meyer-Dombard *et al.*, 2005). These extreme environments are inhabited by extremophiles that are so well-adapted that they readily grow and multiply (Rothschild & Mancinelli, 2001). Extremophiles inhabiting hot springs are considered to be the closest living descendants of the earliest life forms on Earth (Olsen *et al*., 1994; Woese *et al*., 1990). Therefore, these springs provide insights into the origin and evolution of life.

2.7 Culture dependent and culture independent methods for studying fungal diversity

Soda lakes in the East African Rift valley are some of the best characterized soda lakes in the world and several microorganisms have been described from them (Grant & Sorokin, 2011) using both culture dependent and culture independent techniques. Culture-based approaches involve the isolation of fungi from samples using culture media. Such methods are useful in understanding the physiological potential of isolated organisms. Though culture dependent approach does not provide comprehensive information on the composition of microbial communities it is still used as a compliment to culture independent methods. Culture independent methods rely on the use of DNA based

molecular community analysis to give a description of the diversity of a particular sample from the environment (Jany & Barbier, 2008).

Whereas culture dependent studies are keen on isolating novel organisms and exploring their properties, the culture independent methods offer a more comprehensive assessment of microbial diversity (Tringe *et al*., 2008). Several culture-independent studies have investigated microbial communities in hot springs, such as those in Tengchong, China and Iceland (Hou *et al*., 2013).

Earlier culture independent studies that were done on soda lakes in Kenya relied on the analysis of clone libraries of PCR amplified ribosomal deoxyribonucleic acid (rDNA). This did not represent an accurate picture of the diversity within a given community due to low speed and coverage of a cloning and Sanger-sequencing based approach. These approaches gave a lower number of amplicon sequences compared to the millions of sequences generated by high throughput sequencing technologies such as Illumina Sequencing (Pawlowski *et al*., 2014) and 454 pyro sequencing. In the recent years, 454 pyrosequencing has emerged as an efficient alternative approach to traditional Sanger sequencing and is widely used in both *de novo* whole-genome sequencing and metagenomics (Margulies *et al.*, 2005). It has since been followed by other platforms, like Illumina/Solexa and Applied Biosystems sequencing by Oligonucleotide Ligation and Detection (ABI/SOLiD) which have made high-throughput sequencing an affordable tool for many new organisms and applications. Generally high-throughput sequencing is a promising method, as it provides enough sequencing depth to cover the complex microbial communities (Shendure & Ji, 2008). It has been applied to analyze among others microbial communities in marine water (Qian *et al.,* 2010), soil (Roesch *et al*., 2007; Lauber *et al*., 2009), human hand surfaces (Fierer *et al.,* 2008) and human distal intestine (Claesson *et al*., 2009).

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Research authorization

Permission to conduct research on the four soda lakes was granted by the National Commission for Science, Technology and Innovation (NACOSTI). All other necessary documents to access and collect samples from the soda lake were obtained from the National Environment and Management Authority (NEMA) and the Kenya Wildlife Service (KWS).

3.2 Study sites

Samples were collected from four hot springs of soda lakes in Kenya namely Lake Bogoria, Lake Elmenteita, Lake Magadi and Little Magadi (*Nasikie Eng'ida*) all located in the Kenyan rift valley.

Lake Bogoria, (geographical coordinates 0°13′ 33″ N, 36° 05′ 41″ E) lies at an altitude of 1,000 metres above sea level. The lake waters are alkaline (pH 10.5) and saline (up to 100 g/l total dissolved salts). The shoreline of the lake is fringed by geysers and hot springs confirming the volcanic origin of this harsh landscape (Cioni *et al.,* 1992; Renaut & Owen, 2005). Ten (10) geysers that erupt up to 5 m high were observed at least in four locations around the lake (Tiercelin & Vincens, 1987). There are however other minor perennial springs that discharge fresh water into the lake (North Lewis, 1998). The more than 200 alkaline hot springs and geysers found on the shores supplement water recharge for the lake (Harper *et al.,* 2003). Though hyper-saline, the lake is highly productive with abundant cyanobacteria that feed the flamingos.

Lake Elmenteita is situated 0º 27'S, 36° 15'E on the floor of the Kenyan Rift Valley at 1,776m above sea level in central part of Kenya some 20 km south-east of Nakuru town

and has no direct outlet (Melack, 1988). At its southern end, it is fed by the Kariandusi hot springs and two small streams, the Mereroni and Kariandusi, flowing from the eastern plateau. The present size of Lake Elmenteita is roughly 20 km^2 and the depths rarely exceed 1.0 m. The water temperatures in the lake ranges between 30 °C and 40 °C, the alkalinity is high and the pH is above 9 with a high concentration of carbonates, chlorides and sulphates (Mwaura, 1999). Lake Magadi, 1° 52′S 36° 16′E is a saline, alkaline lake, approximately 100 square kilometers in size, lying in a catchment of faulted volcanic rocks, north of Tanzania's Lake Natron (Behr, 2002). During the dry season, it is 80% covered by soda and is well known for its wading birds, including flamingos. The hot springs (up to 86°C) discharge saline alkaline waters into the lake and their compositions and temperatures have remained constant for over 50 years (Eugster, 1970). Individual springs differ greatly in concentration, temperature and carbonate-bicarbonate ratio (Jones, Eugster & Rettig, 1977). Little Magadi (*Nasikie eng'ida*) 1° 45' 00" S, 36° 17' 00" E is located about 40 km south of the main Lake Magadi (**Figure 3.1**).

3.3 Measurements of physicochemical parameters at sampling sites

A Garmin eTrex 20, a hand-held GPS receiver with multi-constellation ability assuring an absolute positioning accuracy of 3 meters, was used to sample the points. The GPS was set up to use the local datum approved by the Survey of Kenya, which is Arc 1960. To accurately show the sampled hot springs to scale, maps were constructed in ArcGIS 10.3 using the GPS coordinates captured from the lake during fieldwork. The layers for towns, rivers, lakes and roads were added from ArcGIS Online database to enrich the thematic maps, as shown in **Figure 3.1.** The right cartographic standards were adopted to ensure adequate visualization and scale for the two maps. During sampling, the temperature, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) were measured on site using Electrical Chemical Analyzer (Jenway - 3405), whereas the pH was measured with a portable pH-meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5-10). Temperature was recorded at three distinctive points along the flow of the hot spring and

these temperature assigned to all the sample types used in this study.
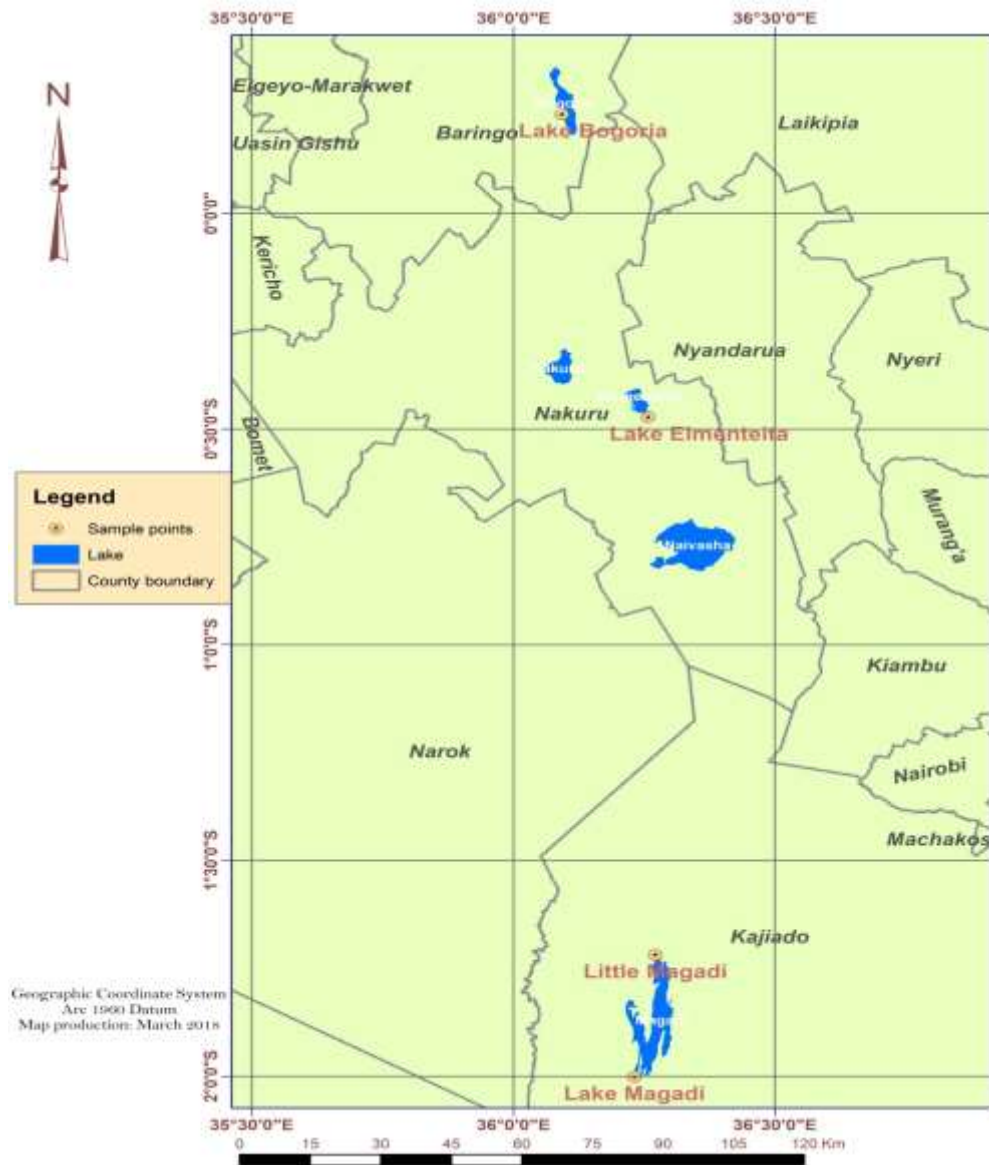
Figure 3.1: Map showing the spatial context of the study area and locations of the sampled points of the hot springs.

3.4 Sample Collection

Sampling was done in the four soda lakes found in the Kenyan rift valley from 8th to 11th July 2014. Water samples were collected from the mouth of the hot springs (Main Lake Magadi at 43.8 ℃, pH 8.8, Little Magadi at 81.9 ℃, pH 8.6, Lake Bogoria at 84.6 ℃, pH 9.0 and Lake Elmenteita at 45 ℃, pH 8.7) in triplicates using one litre sterile containers. Wet sediments and microbial mats were collected from the ground of the rivulets of the hot springs in triplicates using 500g sterile jam jars at three distinct points that differed in temperature (Main Lake Magadi 43.9 ℃, 41 ℃, 37.9 ℃, Little Magadi 81.9 ℃, 76.3 ℃, 67.9 ℃, Lake Bogoria 84.6 ℃, 77.7 ℃, 54 ℃, Lake Elmenteita 45 ℃, 44.7 ℃, 33.8 ℃). The samples were labeled and transported on ice in cool boxes to the laboratory at Jomo Kenyatta University of Agriculture and Technology and were stored at -80 ℃.

3.5 Preparation of culture media

Fungi were isolated from the microbial mats, sediments and water samples using Potato dextrose agar (PDA), Malt extract agar (MEA), Potato glucose agar (PGA) and Sabourand dextrose agar (SDA) as culture media. Water from the lake was used to prepare culture medium; 50 g of malt extract agar (65 g of SDA and 39 g for PDA and PGA) were separately weighed and dissolved in one litre of the lake water (Salfinger & Tortorello, 2015). The mixture was then heated with frequent agitation to boiling to completely dissolve the medium. It was sterilized by autoclaving at 121 ℃ for 15 minutes and later cooled to 45-50 ℃. In order to restrict any bacterial growth, the media were supplemented with chloramphenicol (200 mg/l) and mixed well before dispensing on petri dishes (Jorgensen *et al.,* 2015).

3.6 Isolation of fungi from sediments and mats

One gram of microbial mats and sediments were weighed and separately suspended in 9 ml of sterile distilled water and vortexed thoroughly. From this stock solution of 10 ml, serial dilutions were performed to 10^{-7}. Aliquots of 100 μl (0.1ml) from the highest

dilutions were drawn separately and plated on sterile agar medium aseptically and spread using a glass rod. The plates in triplicates were incubated at 30 °C until visible colonies sporulated. Pure cultures were sub-cultured into fresh agar plates (Dipal & Pandey, 2012). Each isolate was preserved on agar slants at 4 °C in a refrigerator in universal bottles for downstream studies.

3.7 Morphological characterization of fungal isolates

Colony morphologies of the isolates were described using standard microbiological criteria, with special emphasis on colour, shape, size, elevation, form and pigmentations on both obverse and reverse side. Preliminary characterization by simple staining (using lacto phenol cotton blue dye) of each of the pure isolates was done and observed under a light microscope at ×100 (Keast *et al.,* 1984) in order to differentiate the isolates based on their morphology (Cappuccino & Sherman, 2002).

3.8 Physiological characterization of fungal isolates

3.8.1 Determination of optimum temperature for growth

To determine the ability of the isolates to grow over a range of temperatures, potato glucose agar (PGA) medium at pH 8.0 was prepared, sterilized and dispensed in sterile petri dishes. Each isolate was inoculated and incubated at different temperature levels (25 °C, 30 °C, 35 °C, 40 °C and 45 °C) for 7 days (Nazina *et al.,* 2001). Two un-inoculated plates for each temperature were used as controls. Experiments were done in triplicates and the growth of isolates was observed and noted by measuring the diameter of the colony in millimeters.

3.8.2 Determination of optimum pH for growth

In order to determine the optimum pH requirements for the growth of the isolates, four batches of potato glucose agar (PGA) media were prepared and pH was adjusted to 5.0,

7.0, 8.5 and 10.0 using 1 M HCl and 1 M NaOH solutions, respectively. The media were then autoclaved and dispensed in petri dishes. Each medium was inoculated with the isolates in triplicates and incubated at 30 °C, which was the maximum growth temperature for the isolates for a period of 7 days. The growth (diameter) was measured in millimeters. For each pH, two uninoculated plates were used as controls.

3.8.3 Growth on different media

This experiment was to determine the effect of media on the cultivation of the fungal isolates. Four types of media, namely malt extract agar (MEA), Sabourand Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Potato Glucose Agar (PGA) were used. Plates with the four types of media were seperately inoculated in triplicates with six mm agar disc fungal inoculum and incubated at 30 °C for a period of 7 days. The diameter of the fungal colonies were measured in millimeters after two days for a period of 14 days.

3.8.4 Determination of optimum salt concentration for growth

Four batches of potato glucose agar (PGA) medium were prepared in 1 litre of distilled water and each supplemented with 5 %, 10 %, 20 % and 30 % sodium chloride concentration. This was to determine the ability of the isolates to grow at different sodium chloride concentration. Three sets of replicates for each concentration were conducted. These were incubated at 30 $^{\circ}$C then checked for growth after 7 days by measuring the diameter of the colony in millimeters.

3.9 Enzymatic characterization of fungal isolates

3.9.1 Determination of amylase activity

The ability to degrade starch was used as a test for production of amylolytic enzymes as described by Hankin and Anagnostakis (1975). The isolates were inoculated on nutrient agar (NA) with 0.2 % of soluble starch (g l^{-1}), pH 8.0 in triplicates. After 5 days of

incubation, the cultures were flooded with iodine solution (1 % w/v). A yellow zone around a colony in an otherwise blue medium indicated amylolytic activity while negative isolates indicated a blue black colour all over the plate (Castro *et al.,* 1993).

3.9.2 Determination of esterase activity

The medium used was as described by Sierra (1957). It contained (g l^{-1}): 10.0 g peptone, 5.0 g NaCl, 0.1 g CaCl$_2$ 2H$_2$O, 18.0 g agar at pH 8.0. To the sterilized culture media, sterilized Tween 80 was added in a final concentration of 1 % (v/v). This medium was inoculated with the isolates in triplicates and incubated at 30 $^\circ$C. The presence of a precipitation of calcium crystals around the colonies showed the presence of esterase enzyme activity.

3.9.3 Determination of lipase activity

Lipase activity was measured following the method of Sierra (1957). However, Tween 80 was substituted by Tween 20. The experiment was set up in triplicates and positive isolates for lipase were indicated by a precipitation of calcium crystals around the colonies

3.9.4 Determination of protease activity

For the determination of protease activity, the isolates were cultured on a media containing g/l 8.0 g Nutrient Broth, 1.0 g glucose, 18.0 g agar and the pH was adjusted to 8.0 (Vieira, 1999). After autoclaving the media, 15.0 ml of autoclaved skimmed milk, was added. The microorganisms were inoculated in triplicates and incubated at 30 $^\circ$C for 7 days, after which 2.0 ml of 0.1M HCl was added to the plates. Positive isolates exhibited a clearing zone, indicative of proteolysis (Cappuccino & Sherman, 2002).

3.9.5 Determination of cellulase activity

The medium (7.0 g KH$_2$PO$_4$, 2.0 g K$_2$HPO$_4$, 0.1 g MgSO$_4$.7H$_2$0, 1.0 g (NH$_4$)$_2$SO$_4$, 0.6 g yeast extract, 10 g microcrystalline cellulose and 15 g agar per liter) of Stamford *et al.*

(1998) was used. The plates were inoculated in triplicate and incubated at 30 $^{\circ}$C for 7 days. After incubation, the plates were stained with 0.1 % (w/v) Congo red dye and the diameter of each cleared zone was recorded. Appearance of a clear halo around the fungal growth indicated cellulose activity.

3.9.6 Determination of xylanase activity

The medium (5.0 g Birch wood xylan, 5.0 g Peptone, 5.0 g Yeast extract, 1.0 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$ and 20.0 g agar per litre) as described by Nakamura *et al.* (1993) was used. The inoculated plates were incubated at 30 $^{\circ}$C for 7 days. The plates were then flooded with 0.1 % (w/v) Congo red dye and left for 30 minutes before they were washed with 1M NaCl solution. Positive xylanolytic isolates were detected based on the clear zones of hydrolysis of xylan.

3.10 Molecular characterization

3.10.1 Total DNA extraction

Total community DNA was extracted from each sample (sediments and microbial mats) in triplicate using phenol chloroform as described by Sambrook *et al.* (1989) with a few modifications. Water for DNA extraction was filtered through a 0.22µM Whatman filter papers using a water pump (model Sartorius 16824) and DNA was extracted from the filtered papers.

The sediments and microbial mats obtained from the ground rivulets (**described in section 3.4**) were thawed from -80 ºC. Sediment samples (0.4 g) and 0.4 g of microbial mat samples were weighed into separate sterile eppendorf tubes. 500 µl of solution A (50 mM Tris pH 8.5, 50 mM EDTA pH 8.0 and 25% sucrose solution) was added to each tube and mixed by gently inverting several times and centrifuged at 13000 revolutions per minute for one minute to remove salts and exopolysaccharides from sediment samples. The supernatant was discarded and the sample re-suspended in 200 µl of solution A. To

this were added 5 µl of Lysozyme (20 mg/ml) and 5 µl of RNAse A (20 mg/ml), gently mixed and incubated at 37 °C for one hour. Following incubation, 600 µl of solution B (10 mM Tris pH 8.5, 5 mM EDTA pH 8.0 and 1% SDS) was added and contents were mixed by inverting the eppendorf several times. 10 µl of Proteinase K (20 mg/ml) was added, mixed gently and incubated at 50 °C for 1 hour.

DNA was extracted in three replicates by adding equal volumes of phenol: chloroform, mixed by inverting several times and centrifuged for 15 minutes at 13000 revolutions per minute. This step was repeated once and a second extraction was done with equal volume of Chloroform: Isoamyl alcohol (24:1) mixed by inverting several times and then centrifuged for 15 minutes at 13000 revolutions per minute. The aqueous phase was carefully transferred into a new tube. This step was repeated once to remove all the phenol from the sample and DNA precipitation was done overnight at -80 °C, by adding an equal volume of isopropanol and 0.1 volumes of 3M NaCl. DNA pellets were washed twice by use of 70 % Ethanol, air dried at room temperatures for 20 minutes and stored at -20 °C.

3.10.2 PCR Amplification and 454 pyrosequencing

Polymerase chain reaction (PCR) amplification of the fungal internal transcribed spacer (ITS) rDNA genes from genomic DNA was performed using ITS1 (5′TCCGTAGGTGAACCTTGCGG3′) and ITS4 (5′TCCTCCGCTTATTGATATGC 3′) primers with barcodes according to White *et al*. (1990). Amplification proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial heating at 94 °C for 3 minutes, followed by 28 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 40 seconds and extension at 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. PCR products were purified using the TaKaRa Agarose Gel DNA Purification kit and qualified on 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples from each sample type were pooled together separately in equal proportions based on their DNA concentrations. Pooled samples were purified using Agencourt

Ampure beads (Agencourt Bioscience Corporation, MA, USA). The pooled and purified PCR products were used to prepare DNA library by following pyrosequencing protocol (Martin Kreutz *et al.,* 2013). Sequencing was performed at Molecular Research DNA, MR DNA (www.mrdnalab.com, Shallowater, TX, USA) utilizing Roche 454 FLX titanium instruments and reagents following the manufacturer's guidelines**.**

3.10.3 Pyrosequencing Data analysis

Sequences were depleted of barcodes and primers using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) developed at the service provider's laboratory. Low quality sequences were identified by denoising and filtered out of the dataset (Reeder and Knight, 2010). Sequences that were < 200 base pairs after phred20-based quality trimming, sequences with ambiguous base calls and those with homopolymer runs exceeding 6bp were eliminated. Sequences were then analyzed by a script optimized for high-throughput data to identify possible chimeras in the sequence files, and all definite chimeras were depleted (Gontcharova *et al*., 2010). *De novo* Operational Taxonomic Unit (OTU) clustering was done with standard UCLUST method using the default settings as implemented in QIIME pipeline Version 1.8.0 at 3 % genetic distance (Caporaso *et al*., 2010a). Taxonomy was assigned to each OTU using nucleotide Basic local Alignment Tool (BLASTn) against SILVA SSU Reference 119 database at default e-value threshold of 0.01 in QIIME (Quast *et al*., 2013).

3.11 Statistical analysis

Alpha diversity indices (Shannon, Simpson, richness, observed species and Evenness) in each sample were calculated using Vegan Community Ecological package version 2.5.2 in R software version 3.1.3 (R Development Core Team, 2012; Zhao *et al*., 2014). Community and environmental distances were compared using Analysis of similarity (ANOSIM) test, based upon Bray-Curtis distance measurements with 999 permutations. Significance was determined at 95 % confidence interval (p = 0.05). Non Metric

Dimensional Scaling (NMDS) and hierarchical clustering were carried out using the R programming language (DeLong *et al*., 2006) and the Vegan package (Oksanen *et al*., 2012). The comparative analysis of total microbial diversity of various sample types was estimated using UPGMA clustering (Coman *et al*., 2015). To support OTU-based analysis, taxonomic groups were derived from the number of reads assigned to each taxon at all ranks from domain to genus using the taxa_summary.txt output from QIIME pipeline Version 1.8.0.

CHAPTER FOUR

DIVERSITY AND DISTRIBUTION OF FUNGAL COMMUNITIES WITHIN THE HOT SPRINGS OF SODA LAKES IN THE KENYAN RIFT VALLEY

4.1 Introduction

Extreme environments like the hot springs, saline and/or alkaline lakes, deserts and the ocean beds are found in nature. They are believed to have harsh conditions unfit for survival for most known life forms (Satyanarayana *et al.,* 2005). Such environments are found in all continents such as North America (Great Salt Lake, Big Soda lake and Mono lake in California), Africa (the alkaline soda lakes of Egypt, Wadi El-Natrun), Asia (the Dead Sea) and the soda lakes of Antarctica (Cantrell *et al*., 2006; Grant & Sorokin, 2011). In Kenya, the soda lakes (Bogoria, Magadi and Elmenteita) found in the East African Rift Valley represent the major type of naturally occurring highly alkaline environment.

Soda lakes are alkaline with pH values often ranging between 9 and 12. They are characterized by high concentrations of carbonate salts, especially sodium carbonate and related salt complexes. Many soda lakes also contain high concentrations of sodium chloride and other dissolved salts, making them saline or hyper saline (Gunde-Cimerman *et al.,* 2000; Litchfield & Gillevet, 2002). These hyper saline and highly alkaline soda lakes are considered some of the most extreme aquatic environments on earth. Hot springs are scattered all over the globe. They are produced by geo-thermally heated groundwater (Kauze *et al.,* 2006) with extreme temperatures of 45°C and above (Bhavesh Kumar *et al.,* 2004). Temperature is one of the most important factor controlling the activity and evolution of microorganisms (Namsaraev *et al.,* 2015)

Microbial communities can be found in the most diverse conditions of temperature, pressure, salinity and pH (Kumar *et al*., 2010), as they are not limited to specific environments. Fungi have a worldwide distribution, and grow in a wide range of habitats,

including extreme environments such as deserts or areas with high salt concentrations (Vaupotic *et al*., 2008) or ionizing radiation (Dadachova *et al.,* 2007), as well as in deep sea sediments. Fungi play vital roles in the ecosystem, they are decomposers, symbionts and pathogens that live closely with bacteria, plants and animals. Despite their functional importance, diversity, distribution and ecology of fungi is much less studied compared to bacteria (Desprez- Loustau *et al.*, 2007). Traditional culturing methods that rely on morphological and other phenotypic characteristics as the main criteria for fungal classification (Bartnicki-Garcia, 1987) are heavily biased towards fast-growing species. Many fungi have specialized growth requirements, so this approach recovers only a small proportion of the community sampled (O'Brien *et al.,* 2005). Similarly, fruiting body collection is limited to the detection of species that frequently reproduce sexually unless long-term studies are conducted **(**Straatsma & Egli**,** 2001). Therefore, these traditional methods alone do not enable reliable identification of fungi at lower taxonomic levels (Feau *et al.,* 2009). Molecular taxonomy has partially solved this problem, allowing better classification of fungal species (Fávaro *et al.,* 2011; Gehlot *et al.,* 2012). Recent studies carried out using illumina sequencing revealed that the phyla *Ascomycota* and *Basidiomycota* were the dominant and diverse groups of fungi within the sediments and water samples collected from Lake Magadi and little Magadi (Kambura *et al*., 2016). In order to comprehensively determine the fungal diversity within the hot springs of Kenyan Soda lakes, 454 amplicon pyrosequencing approach was used (Bates *et al.,* 2011; Dumbrell *et al.,* 2011), that is not selective and biased for specific microbial growth like the previously used culture based methods.

4.2 Materials and methods

Research authorization, Study site, Measurements of physicochemical parameters, Sample collection, Nucleic acid extraction, P C R Amplification, 454 sequencing, sequence analysis and statistical analysis are as described in **chapter 3**. Initial **s**equence quality control was done using a proprietary pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) developed at the service provider's laboratory. *De novo*

Operational Taxonomic Unit (OUT) clustering was done with standard UCLUST method using the default settings as implemented in QIIME pipeline Version 1.8.0 at 3 % genetic distance (Caporaso *et al*., 2010a). Taxonomic classification was done using BLASTn against SILVA SSU Reference 119 database at default e-value threshold of 0.001 in QIIME (Quast *et al*., 2013).

4.3 Results

4.3.1 Sampling

Microbial mats, sediments and water samples were randomly collected at three different locations in the hot springs of the soda lakes in Kenya. The metadata collected before sampling included the geographical position of each site in terms of latitude, longitude and elevation, temperature, pH, electrical conductivity, total dissolved solids and dissolved oxygen. The various samples collected from the four soda lakes and their parameters are summarized in **Table 4.1** below where EC stands for Electrical Conductivity and TDS is Total dissolved salts

Table 4.1: Summary of samples collected from the four soda lakes and their parameters

Soda Lake	Latitude ºS	Longitude ºE	Elevation (m)	Sampling point	Sample label	Sample type	Temperature (°C)	pH	Dissolved oxygen (Mg/l)	Electrical Conductivity (EC) (mS/cm)	Total Dissolved Solids (TDS) (mg/l)
Little Magadi	1° 43'08.9"	36° 16'15.8"	606	1	LM1	Mats	81.90	8.5	0.29	1	1
				3	LM3	Mats	76.30	8.5	0.01		
				2	LMb	Sediment	67.90	8.6	0.98		
				3	LMc	Sediment	76.30	8.5	0.01		
				1	LMd	Water	81.90	8.6	0.29		
Lake Magadi	2° 00′08.1 S	36° 13′53.0″	616	1	MM1	Mats	43.80	8.8	1.17	1	1
				2	MM2	Mats	41.00	8.8	6.04		
				3	MM3	Mats	37.90	8.8	4.80		
				1	MMa	sediment	43.80	8.8	1.17		
				2	MMb	sediment	41.00	8.8	6.04		
				3	MMc	sediment	37.90	8.8	4.80		
				1	MMd	Water	43.80	8.8	1.17		
Lake Bogoria	0°13′46.1″ N	36° 05′34.8″	1,000	1	B1	Mats	84.60	8.2	0.10	1	1
				2	B2	Mats	77.70	8.3	0.23		
				3	B3	Mats	54.00	8.7	0.53		
				1	Ba	sediment	84.60	8.2	0.10		
				2	Bb	sediment	77.70	8.3	0.23		
				1	Bd	Water	84.60	9	0.10		
Lake Elmenteita	0º28'21.6″ S	36°15'27.9″	1,789	1	E1	Mats	45.00	8.7	2.67	0.03	1
				2	E2	Mats	44.70	8.7	2.45		
				3	E3	Mats	33.80	8.8	3.12		
				1	Ea	sediment	45.00	8.7	2.67		
				2	Eb	sediment	44.70	8.7	2.45		
				3	Ec	sediment	33.80	8.8	3.12		

4.3.2 Estimators for Diversity and Species Richness of Fungal Communities

For the twenty four sequenced samples, five from Little Magadi, seven from Lake Magadi and six each from both L. Bogoria and L. Elmenteita (**Table 4.1** sample label column), exclusion of low-quality and short sequence reads yielded 139,023 fungal ITS reads. Out of these, L. Elmenteita recorded the highest reads (51,913) while Little Magadi had the lowest reads (25,958), Lake Magadi recorded 34,718 reads while L. Bogoria had 26,434 reads. The wet sediments, sample Ea at 45 ^{o}C from L. Elmenteita, had the highest reads (14,929). Among the microbial mats, sample MM2 at 41 ^{o}C from L. Magadi recorded the highest reads (10,574) followed by sample B1 at 84.6 ^{o}C from L. Bogoria that had 8204 reads while sample LM1 at 81.9 ^{o}C from Little Magadi had 7678 reads. Species richness (S) estimated the sediments at L. Magadi, sample MMb at 41 ^{o}C, to be the richest site with 633 species. The evenness (J′) value was closer to 0 with the highest value (0.258) recorded in sample MM1 at 43.80 ^{o}C which revealed less distribution in abundance among species. Simpson (1/D) and Shannon's index (H') indicated the sediments in sample MMc at 37.90 $^{\circ}$C to harbor the most diverse taxa with 11.66 and 3.739 values, respectively (**Table 4.2**).

Table 4.2: Diversity indices computed on all OTU-based fungal taxonomic units

	Variable	LM (Site 1)	MM (Site 2)	B (Site 3)	E (Site 4)	F (3,20) Statistic	P-value
No. of sequence reads	Before quality check	5614.8±773.4a	5337.7±1350.4a	4715.0±1262.4a	9331.2±2015.4a	2.038	0.141
	After quality check	5191.6±722.6a	4959.7±1247.3a	4405.7±1188.6a	8652.2±1879.4a	2.001	0.146
Diversity indices	Richness (S)	65.40±19.21a	242.71±213.32b	63.50±13.14a	70.00±26.23a	3.360	0.039*
	Shannon (H')	1.35±0.348a	2.24±0.308a	1.37±0.307a	1.30±0.322a	2.196	0.120
	Simpson (I/D)	3.05±0.657a	4.86±1.510a	3.01±0.697a	2.51±0.523a	1.113	0.367
	Evenness (J')	0.079±0.014a	0.085±0.032a	0.074±0.011a	0.093±0.022a	0.117	0.949

*Notes: The means, followed by the same letter in a row are not statistically different at (P<0.05) using one way ANOVA with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).*

4.3.3 Comparison of Fungal Communities between different sampling sites

Using a 3 % dissimilarity cut-off for clustering, the reads were grouped into Operational Taxonomic Units (OTUs). The Fungal OTUs common to the four sampling sites were then presented using a Venn diagram to compare the relationships between the four communities (**Figure 4.1**). The numbers of fungal OTUs obtained for each region were as follows: The hot spring at L. Elmenteita recorded the highest OTUs (1196) while the one at L. Bogoria had 294, the least OTUs in this study. The Lake Magadi hot spring had 394 OTUs and that of little Magadi had 295 OTUs. The hot springs at L. Bogoria and Little Magadi shared 87 OTUs, L. Elmenteita and L. Magadi (Main Magadi) shared 82 OTUs, L. Bogoria and L. Elmenteita shared 71 OTUs and Little Magadi and L. Magadi shared 95 OTUs. All the four sites (hot springs) shared 31 OTUs (**Figure 4.1**).
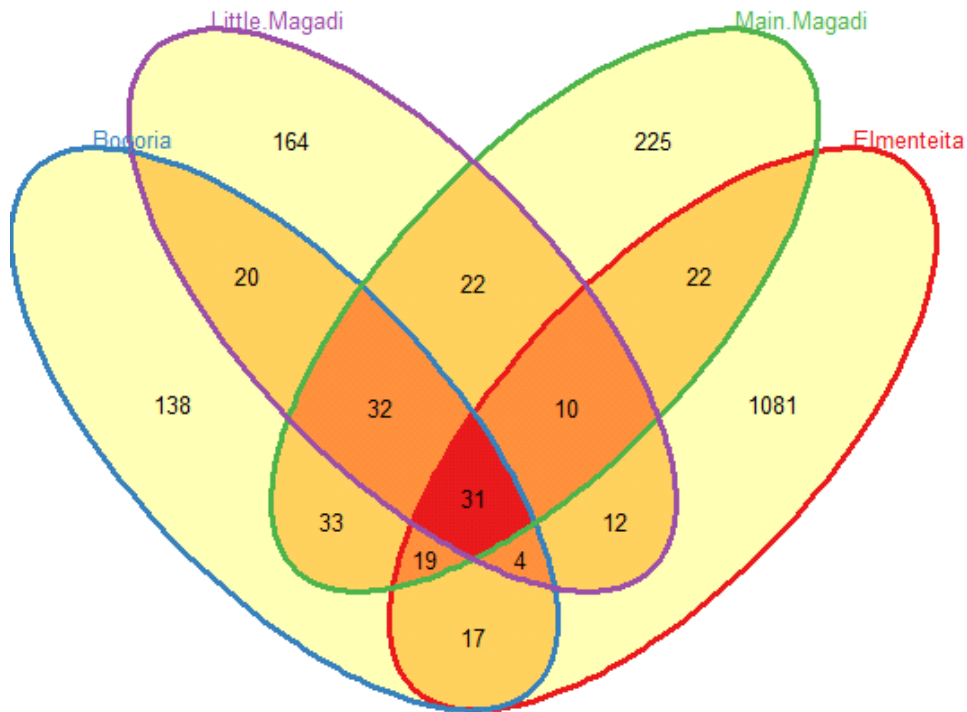
Figure 4.1: Venn diagram representing the number of fungal OTUs that are unique and shared between the samples from 4 different sampling sites. The number of OTUs in each hot spring is indicated in the respective circle. Main Magadi stands for Lake Magadi.

4.3.4 Fungal Community Composition and Structure Analysis

All analyzed sequences were classified into three known fungal phyla namely *Ascomycota*, *Basidiomycota*, *Glomeromycota* and *unclassified fungal* phylum. *Ascomycota* represented the most dominant and diverse phyla while *Glomeromycota* was the least dominant **(Figure 4.2)**.
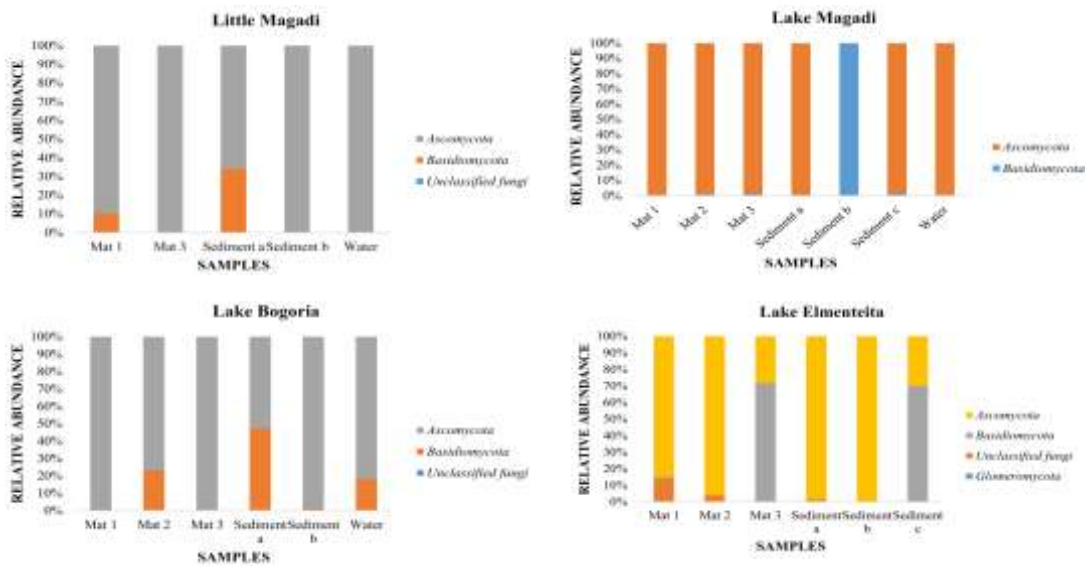
Figure 4.2: Taxonomic Composition Analysis at phylum level within samples collected from the hot springs of four soda lakes.

4.3.4.1 Ascomycota in hot springs of soda Lakes

The phylum *Ascomycota* had a relative abundance of 100 % in samples B3 at 54 ºC, LMc at 67.9 ºC, MM1 at 43.9 ºC and MMd at 43.9 ºC, 99 % in samples LM3 at 67.9 ºC, LMd at 81.9 ºC, MM2 at 41 ºC, MM3 at 37.9 ºC, B1 at 84.6 ºC, Bb at 77.7 ºC and Eb at 44.7 ºC. Sample Ec at 33.8 ºC had the lowest relative abundance of 29.9 %. In addition, all samples had relative abundances above 50 % apart from Ec at 33.8 ºC (29 %), E3 at 33.8 ºC (28 %) and MMb at 41 ºC (0 %). Seven classes were identified namely; *Dothideomycetes, Eurotiomycetes, Saccharomycetes, Sordariomycetes, Pezizomycetes, Leotiomycetes* and *Lichinomycetes* (**Figure 4.3**). Out of the 24 samples analyzed, *Dothideomycetes* and *Eurotiomycetes* were the most diverse and dominant (91.7 % and 70.8 %, respectively) classes as they were present in 22 and 17 samples, respectively. The class *Dothideomycetes* was the most dominant in the water samples with the following

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relative abundances; in LMd (99.7 %) at 81.9 ºC, MMd (81.5 %) at 43.9 ºC and Bd (37.3 %) at 84.6 ºC. In the microbial mats the class *Dothideomycetes* accounted for 54 % in sample LM3 at 76.3 ºC, 98 % in MM3 at 37.9 ºC, 89 % in MM1 at 43.8 ºC, 98 % in B3 at 54 ºC, 70 % in B1 at 84.6 ºC, 91 % in E2 at 44.7 ºC and 76 % in sample E1 at 45 ºC. The percentages of the class *Dothideomycetes* in the wet sediments also varied. The highest value was recorded in samples Ea (85.6 %) at 45 ºC, LMc (69.9 %) at 76.3 ºC, Eb (60 %) at 44.7 ºC, MMc (56 %) at 37.9 ºC and Ba (52 %) at 84.6 ºC**.** The class *Eurotiomycetes* was also present in the water samples MMd (16 %) at 43.8 ºC, and Bd (37 %) at 84.6 ºC **(Figure 4.3)**.

Sequences from *Ascomycota* matched 16 known orders, with *Pleosporales* being the most diverse as it had six families affiliated to it. The most abundant fungal order was *Capnodiales* in little Magadi and Lake Bogoria accounting for 99.8 % in sample LMd at 81.9 ºC and 52.7 % in sample Ba at 84.6 ºC, respectively. The order *Dothideales* accounted for 95 % in sample MM1 at 43.9 ºC in Lake Magadi and the order *Pleosporales* had a percentage of 91.6 % in sample E2 at 44.7 ºC. Ascomycota phyla had twenty seven (27) families with *Davidiellaceae* being the most abundant and diverse family (99.8 %) in sample LMd at 81.9 ºC, *Dothioraceae* (95 %) in sample MM1 at 43.8 ºC, *Sporormiaceae* (91 %) in sample E2 at 44.7 ºC, *Didymellaceae* (68 %) in sample MMd at 43.8 ºC, and *Onygenaceae* (55 %) in sample MM2 at 41ºC.

Out of the 62 genera detected in this study, the dominant genera were *Cladosporium* (99.79 % in LMd, 52.79 % in Ba), *Aureobasidium* (79.88 % in MM1), *Aspergillus* (34.37 % in Bb), *Penicillium* (65.90 % in LMd)*, Westerdykella* (91.62 % in E2 and 79.40 % in Ea), *Epicoccum* (42.76 % in B1), *Debaryomyces* (12.67 % in LM3), *Auxarthron (*16.18 % in MMd) and among other varied percentages in many of the samples. Most genera were recorded from Lake Elmenteita in samples Ea at 45 ºC, Eb at 44.7 ºC and E3 at 33.8 ºC with varying percentages. The common fungal species were *Cladosporium* sp. (83.08 % in LMd)*, Cladosporium cladosporioides* (17.90 % in Ba)*, Pleosporales* sp*.* (86.07 % in MM3), *Aureobasidium pullulans* (79.88 % in MM1) and *Aspergillus oryzae* (35.02 % in

B2). *Cladosporium* sp. was present in 17 samples, *Cladosporium cladosporioides* in 14 samples, *Pleosporales* sp. in 13 samples A*ureobasidium pullulans* in 13 samples and *Aspergillus oryzae* in 9 samples out of the 24 samples sequenced in this study.
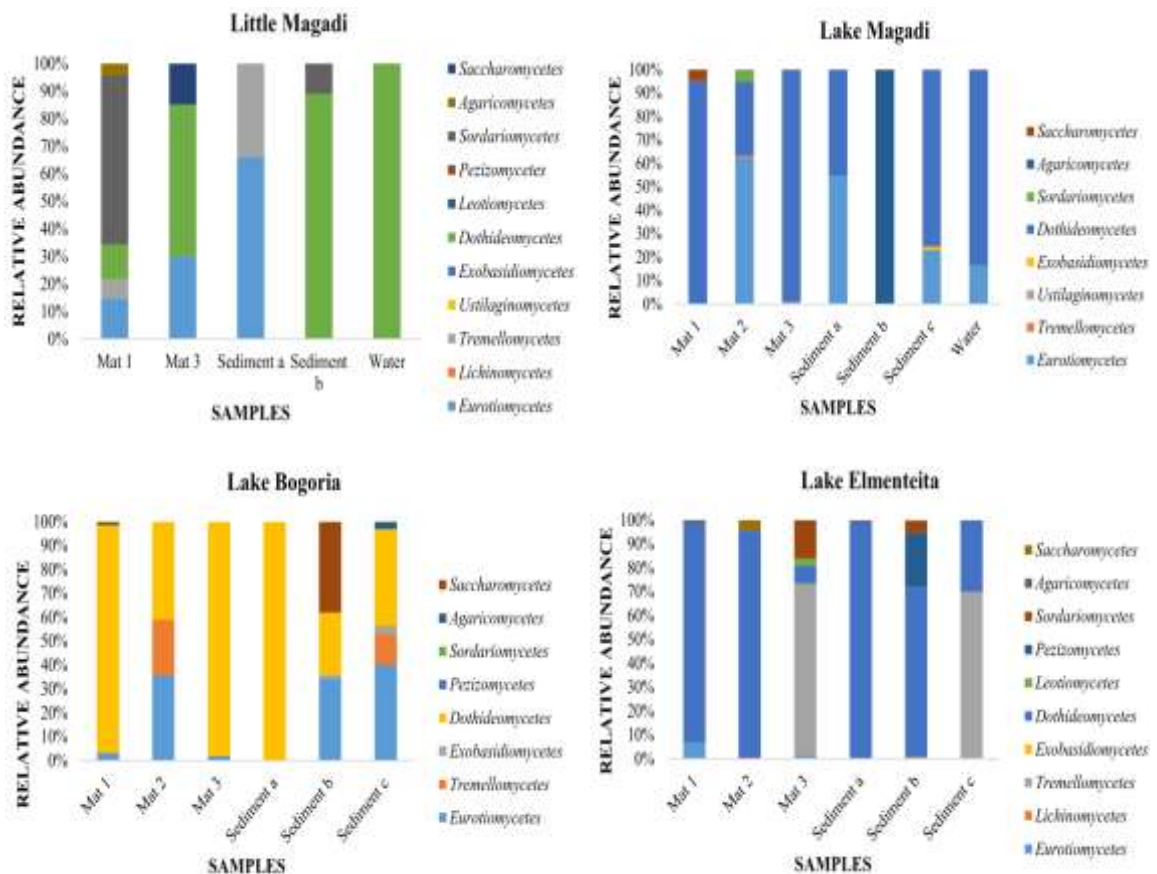
Figure 4.3: Taxonomic Composition Analysis at class level within samples collected from the hot springs of the four soda lakes. The class *Dothideomycetes* from the phylum *Ascomycota* was the most dominant.

4.3.3.2 Basidiomycota in hot springs of soda Lakes

The distribution of *Basidiomycota phylum* was diverse within the samples, accounting for 100 % in sample MMb at 41 ºC, 71 % in sample E3 at 33.8 ºC and 70 % in sample Ec at 33.8 ºC while the rest of the samples had relative abundances of below 50 %. Sequences matching with *Basidiomycota* were affiliated to the following classes; *Agaricomycetes,*

Exobasidiomycetes, *Tremellomycetes* and *Ustilaginomycetes* (**Figure 4.3**). *Tremellomycetes* was the most abundant and diverse, representing 70.6 % relative abundance in E3 at 33.8 ℃ and 69.9 % in sample Ec at the same temperature. *Agaricomycetes* recorded a relative abundance of 100 % in sample MMb at 41 ℃. Sequences from *Basidiomycota* matched 9 known orders, with A*garicales* being the most abundant recording 100 % in sample MMb at 41 ℃. *Malasseziales* and *Tremellales* were the diverse orders as they were present in 7 samples out of the 24 samples analyzed in this study. *Malasseziales* recorded 3.1 % in sample Bd at 84.6 ℃ while *Tremellales* had 70.5 % in sample E3 and 70.05 % in sample Ec both at 33.8 ℃.

At family level, OTUs were distributed in seven (7) fungal families with the most abundant (100 %) belonging to *Lyophyllaceae* in sample MMb at 41 ℃, *Tremellaceae* (33 %) in sample LMb at 67.9 ℃, *Lachnocladiaceae* (2.8 %) in sample Bd at 84.6 ℃ and *Malasseziaceae* (1.3 %) in sample Bb at 77.7 ℃. Out of the 62 genera in this study, few were affiliated to *Basiodiomycota* phyla. This included *Termitomyces* (100 %) in sample MMb at 41 ℃, *Rhodotorula* (47.2 %) in sample Ba at 84.6 ℃, *Tremella* (32.95 %) in sample LMb at 67.9 ℃ and *Malassezia* (1.3 %) in sample Bb at 77.7 ℃. Among the dominant species were; *Termitomyces* sp. (100 %) in sample MMb at 41 ℃, *Tremella aurantialba* (25 %) and *Tremella encephala* (7.95 %) both in sample LMb at 67.9 ℃, *Dioszegia hungarica* (1.7 %) in sample MM2 at 41 ℃ and *Malassezia globosa* (1.25 %) in sample MMc at 37.9 ℃.

4.3.4.3 Glomeromycota and Unclassified fungi in hot springs of soda Lakes

The phylum *Glomeromycota* was present only in samples Ea at 45 ℃ and Eb at 44.7 ℃ with 0.4% and 0.1% relative abundances, respectively. The proportion of *unclassified fungi* was relatively small and was recorded in eight samples only with the highest relative abundance in sample E1 (13% at 45 ℃) and sample E2 (4% at 44.7 ℃) with the rest having relative abundances below 1% (**Figure 2**). Notable was the presence of order *Mortierellales* (0.07%) from the subkingdom *Incertae sedis* in sample Eb at 44.7 ℃.

4.3.5 Relationships between Fungal Communities and Environmental Variables

To test which environmental or geographical parameters correlated with community dissimilarity, a non metric dimensional scaling (NMDS) plot was drawn for the sampling sites or lakes (**Figure 4.4A**) and sampling types (**Figure 4.4B**). These showed that the sample types had less influence on the fungal communities than temperature and/or the sampling sites, supporting environmental variation as the major determinant of fungal community structure. The Bray–Curtis clustering indicated a tendency of the communities to group by sample types but differed in temperature and sampling sites. As the sampling sites were in different climatic regions, the changes in salinity, temperature and latitude appeared to have contributed to these differences.
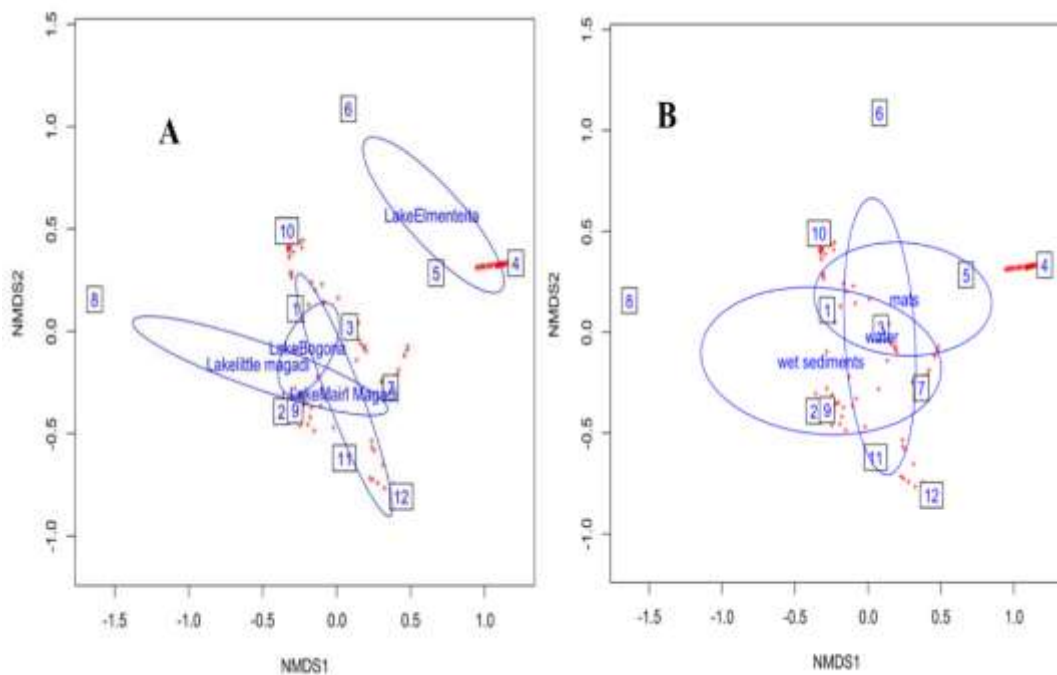
Figure 4.4: Non-metric dimensional scaling (NMDS) based on Bray Curtis dissimilarities between microbial compositions within various samples. A and B represents different lakes and sample types of each site respectively. References 1, 2 and 3;

4, 5 and 6; 7, 8 and 9; and 10, 11 and 12 represents mats, sediments and water samples from lake Bogoria, Elmenteita, Little Magadi and Magadi, respectively.

Hierarchical clustering of various samples and fungal taxa at family level revealed the most dominant families to be *Entylomataceae*, *Halosphaeriaceae*, *Xylariaceae* and *Bionectriaceae* in microbial mats (Sample E3) at lake Elmenteita at 33.8 °C and the families *Sordariaceae*, *Leptosphaeriaceae*, *Microascaceae*, *Peltulaceae*, *Mortierellaceae* and *Ascobalaceae* in wet sediments (Sample Eb) at the same lake at a temperature of 44.7 °C. Lake Elmenteita was found to harbor the most dominant fungal taxa compared to the other three soda lakes. This could be attributed to the lower temperatures that were favorable for the growth of fungi (**Figure 4.5**). The phylogenetic diversity of fungi revealed in this study is relatively low compared to that of studies done on terrestrial or marine habitats like soils, plants and mangroves. Although a total of 2179 OTUs were recorded in this study there was a distinct discrepancy in the number of OTUs per sampling site. Notably lake Elmenteita had the highest number of fungal OTUs at 1196 followed distantly far by lake Magadi with 394 OTUs (**Table 4.2)**.
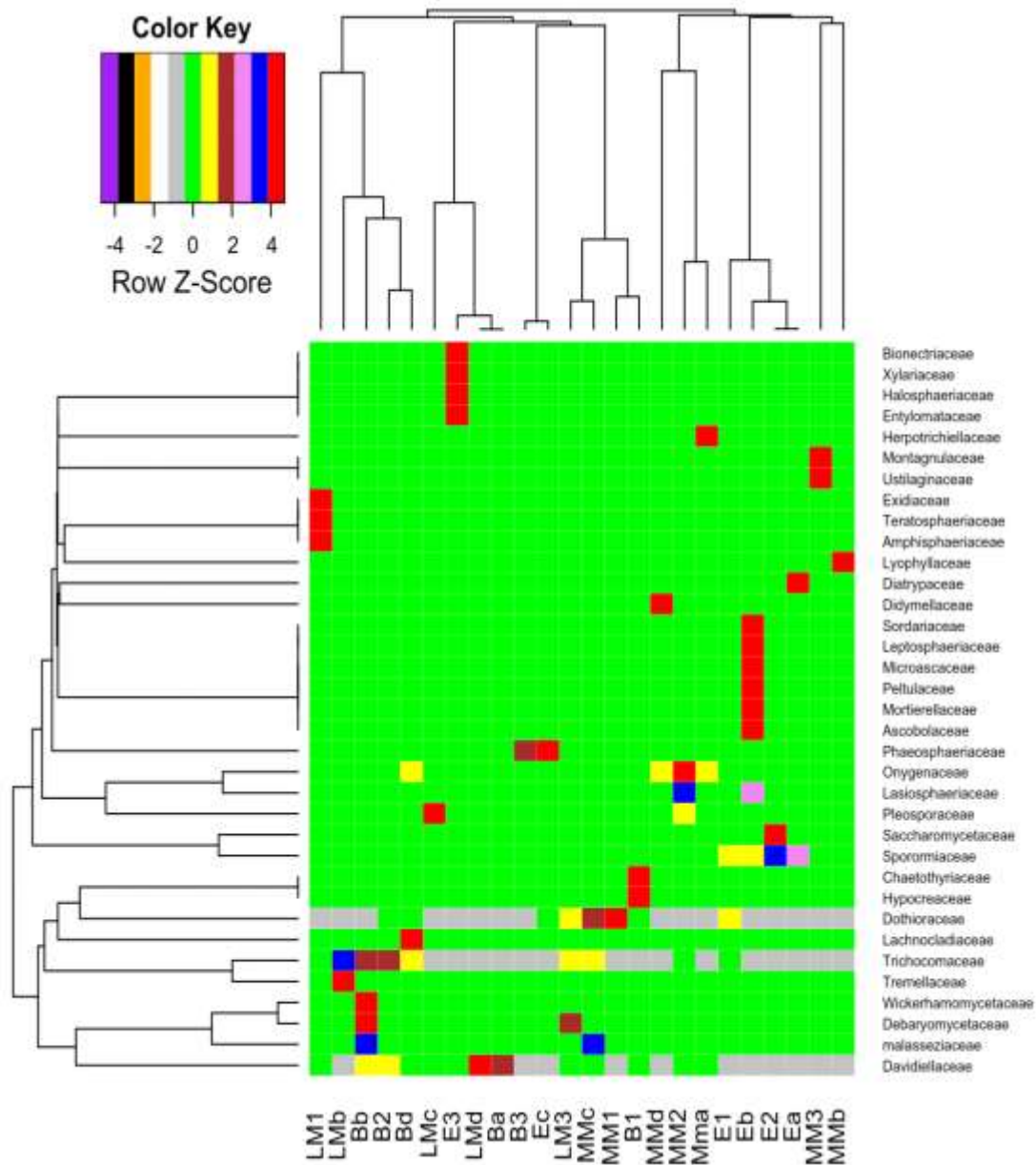
Figure 4.5: Hierarchical clustering of 18S rDNA of the 24 samples collected from the hot springs of the four soda lakes under investigation. Family level was chosen to be used in hierarchical clustering to assess the relationships between samples and taxa.

4.4 Discussion

There has been significant interest in finding life in extreme environments characterized by high temperatures, salinity and pH like hot springs and soda lakes. To the best of our knowledge, this is the first report on the use of 454-pyrosequencing approach to investigate fungal diversity and community structure at different temperature gradients along the flow of hot springs of four Kenyan soda lakes. This ecological study aimed at examining fungal indicators of life in such extreme environments, their biogeography, diversity and distribution within hot springs of selected soda lakes in Kenya.

Sequences analyzed in this study revealed that the majority of recovered fungal sequences belonged to the Domain *Eukaryota* and comprised of the phyla *Ascomycota*, *Basidiomycota, and Glomeromycota* in varying percentages while unclassified fungi represented only a small proportion of the fungal communities (**Figure 4.2**). A study by Schadt *et al.* (2003) found a large proportion of the members of the phylum *Ascomycota* in 125 cloned fungal sequences from Tundra soils. Similar studies on hyper saline environments conducted by Santini *et al.* (2015) on fungal communities found that 73 % of the total OTUs were dominated by members of the phylum *Ascomycota* (52 to 100 %) with minor contributions from the phylum *Basidiomycota*. Contrary to many reports on hyper saline environments, Singh *et al*. (2011) and Bass *et al.* (2007) found basidiomycete yeasts to be the most dominant fungal forms in deep-sea environments. Generally members of the phylum *Ascomycota* occur naturally in all terrestrial ecosystems worldwide (Egidi *et al.,* 2019).

Chytridiomycota, a phylum of fungi distinguished by having zoospores were evidently missing from the sequences obtained in this study. A previous study on frequency and distribution of zoosporic fungi from moss covered and exposed forest soils reported that members of the phylum *Chytridiomycota* could be found in freshwater or wet soils, with most species being infrequent and scarce to rare (Letcher & Powell, 2001; Letcher *et al.,* 2004). Members of *Chytridiomycota* phylum are the simplest and most primitive

Eumycota. Although many ecotypes are adapted to extreme environments, most members are ubiquitous in many ecosystems, especially in cool, moist soils and freshwater habitats that are rich in organic matter. This could explain why the phylum *Chytridiomycota* could not be detected in saline environments with high temperatures and pH as these conditions appear to be harsh for their survival. However, a previous study that applied illumina DNA sequencing analysis of samples collected from the hot springs of Lake Magadi and Little Magadi showed members of *Chytridiomycota* to represent only a small proportion of the hot spring fungal communities (Kambura *et al*., 2016).

In this study, the most commonly identified classes within the phylum *Ascomycota* were *Sordariomycetes* and *Dothideomycetes*. The class *Sordariomycetes* were represented by seven (7) orders and nine families affiliated to it while the class *Dothideomycetes* had 3 orders and nine families. Previous studies on fungal communities in the deep-sea sediments of the Pacific Ocean (Xu & Luo, 2014) and that of diversity and distribution of fungal communities in marine sediments of Kongsfjorden, Svalbard (Tao *et al.,* 2015) found that the two classes *Sordariomycetes* and *Dothideomycetes*, were the most diverse and abundant. The classes *Sordariomycetes* and *Dothideomycetes* are so far the largest and most phylogenetically diverse classes within the phylum *Ascomycota* (Kirk *et al.,* 2008). The members are a heterogeneous group of fungi that subsist in majority of niches where fungi can be found.

The dominant genera across the four lakes studied were *Cladosporium, Aureobasidium, Aspergillus*, *Penicillium*, *Westerdykella*, *Epicoccum, Debaryomyces*, *Auxarthron* and *Malassezia*. The common fungal species were *Cladosporium sp., Cladosporium cladosporioides, Pleosporales* sp., *Aureobasidium pullulans* and *Aspergillus oryzae*. Kambura *et al.* (2016) also found that *Cladosporium cladosporioides* species were unique to sediment samples that were collected at 83.6 °C from the hot springs of Little Magadi. In the same study, Kambura *et al.* (2016) observed that sediment samples collected at 81 °C had *Aspergillus* within the phylum *Ascomycota* as the most abundant species. This is also similar to previous studies in hypersaline waters of salterns that revealed different

species of *Aspergillus, Penicillium* and diverse non-melanised yeasts (Gunde-Cimerman *et al.,* 2005). Studies done by Razieh *et al.* (2015) indicated that most strains isolated from coastal waters of the southern Caspian Sea, belonged to the genus *Cladosporium*. Also, Damare *et al.* (2006) showed that the genera *Penicillium*, *Aspergillus* and *Cladosporium* were the most abundant in aquatic environments. Jaouani *et al.* (2014) isolated fungi belonging to the genera *Cladosporium, Alternaria, Aspergillus, Penicillium, Ulocladium, Engyodontium* and *Cladosporium cladosporioides* that were able to grow in media containing 10 % of salt with an initial pH of 10 from Sebkha El Melah, a Saharan Salt Flat in Southern Tunisia. A study done by Purnima Singh *et al.* (2011) on the phylogenetic diversity of culturable fungi from the deep-sea sediments of the Central Indian Basin grouped the fungal microorganisms into seven (7) clusters belonging to *Aspergillus*, *Sagenomella* sp, *Exophiala* sp, *Capronia* sp, *Cladosporium*, *Acremonium* sp. and *Tritirachium* sp. Another study that used morphological and molecular techniques to identify a series of halotolerant fungi from hypersaline environments of solar salterns revealed 86 isolates of 26 species from salt ponds, which were identified as *Cladosporium cladosporioides*, nine *Aspergillus* sp, five *Penicillium* spp. and the black yeast *Hortaea werneckii* (Cantrell *et al*., 2006). In this study, most of the fungal taxa such as *Aspergillus*, *Cladosporium* and *Penicillium* species are derived from terrestrial habitats like soils. This could be attributed to previous run off waters from adjacent areas that may have brought large numbers of terrestrial fungi in form of spores and fungal hyphae into the hot spring rivulets. Therefore, the fungi detected in this study may have originated from other environments and adapted to saline conditions, high temperatures and alkaline pH by developing effective strategies to tolerate stress in the hot springs.

4.5 Conclusion

The study of fungi has been given little attention compared to other microorganisms like bacteria and archaea. The results obtained using high-throughput analysis indicate that sediments, mats and water from the studied hot springs of soda lakes in Kenya are important niches that harbor unexpectedly high richness of fungal species, most of which

possibly originated from terrestrial environments. However, the ecological roles of these fungi and their adaptive mechanism remain poorly investigated. It is also unclear if these fungi are actively growing in these environments or being dormant propagules (spores) that are washed into the sediments, microbial mats and water during the rainy seasons. Therefore, a combination of different technologies including traditional culture-based method, metagenomics, metatranscriptomics and metaproteomics may reveal the functions of the genes present in these extreme environments. The use of these technologies will have a huge impact in understanding the functions of fungal communities in the ecosystem of Kenyan soda lakes and may also serve as a useful community model for further ecological and evolutionary study of fungi in these extreme environments.

CHAPTER FIVE

CHANGE ON FUNGAL DIVERSITY ACROSS A WIDE RANGE OF TEMPERATURES WITHIN THE HOT SPRINGS

5.1 Introduction

Hot springs are one of the extreme environments on the earth planet (Merino *et al*., 2019). Their micro biomes play a critical role in shaping the ecosystems (Poddar & Das, 2018). Hot springs are often abundant in thermophilic, hyperthermophilic and thermoresistant microorganisms (Urbieta *et al.,* 2014a). Microbial eukaryotes, especially microbes from the phyla of *Ascomycota* and *Basidiomycota* are important components of hot springs micro biomes (Liu *et al.*, 2018). The stability of hot spring environment is determined by the steady state of their microbial diversity in a specific environment, where temperature, pH, and chemical composition are often the most important factors that influence fungal diversity (Poddar & Das, 2018).

Fungi are eukaryotic microorganisms that play fundamental roles in regulating key processes in the ecosystem (Frąc *et al.,* 2018). Fungal diversity and community composition are shaped by factors such as dispersal, temperature, salinity and even composition of media. Such factors limit growth of certain taxa. Most studies indicate that the global distribution of fungi is shaped by multiple environmental factors but temperature is the primary one for the most common fungal species. Temperature directly accelerates metabolic rates and biochemical processes of microorganisms and this could determine community level microbial diversity and distribution.

One of the most influencing factors for fungal growth is temperature (Carlile *et al.,* 2001). The understanding of the effect of temperature on fungal growth is an essential part of fungal physiology. The complex effects of physiological parameters such as pH, salt concentration and temperature are vital in shaping the composition of fungal species within the soda lakes (Kambura *et al*., 2016). As fungi cannot control their internal temperature,

this is governed by the ambient climate. Fungi can live in a relatively large range of temperatures, but their growth rate and metabolism are different at different temperatures even when other conditions like nutrient and water activity are constant. The understanding of the effect of temperature on fungal growth is an essential part of fungal physiology. In this study, we studied the impact of temperature on the growth of fungal species from hot springs of four soda lakes in Kenya.

5.2 Methodology

NGS sequence data was further assessed to evaluate the change on fungal diversity across a wide range of temperatures within hot springs. The main temperatures considered for this analysis included; Little Magadi; 81.70 °C, 67.90 °C and 76.30 °C, Lake Magadi; 43.80 °C, 41.0 °C, 37.90 °C, Lake Bogoria; 84.60 °C, 77.70 °C and 54. 0 °C; and Lake Elmenteita; 45.0 °C, 44.70 °C and 33.80 °C. Fungal taxonomic classification obtained from the blastn assignment were grouped at genus level and unique groups were visualized to express their relationships. Groups unique to specific temperatures and those common across the hot springs were assessed. Respectively, the shared and unique groups revealed through culture dependent methods for hot springs in lakes Magadi, Bogoria and Elmenteita were assessed across a wide range of temperatures.

5.3 Results

Sediment samples collected from the four hot springs had the highest number of OTUs (46 OTUs), followed by mats that had 37 OTUs and water samples with only 11 OTUs. Among the microbial mats, the hot spring at Lake Elmenteita had 11 unique OTUs, Lake Magadi had 4 while both Lake Bogoria and Little Magadi had 3 unique OTUs. The mats at the four hot springs shared 5 OTUs. Sediment samples at Lake Elmenteita had 26 unique OTUs, Lake Magadi had only 4 while both Lake Bogoria and Little Magadi had 3 unique OTUs. The sediment samples shared only 1 OTU. The water samples also shared 1 OTU

among the hot springs though Little Magadi had 2 unique OTUs while Lake Magadi and Lake Bogoria each had 1 Unique OTU (Figure 5.1).

The mats at sampling point 2 at Lake Magadi at 41 ℃ and Little Magadi at sampling point 1 at 81.9 ℃ had the genus *Auxarthron Alternaria* as the most abundant taxa at 54 % *and* 53 % respectively. The genus *Aspergillus, cladosporium, Penicillium, Aureobasidium*, *Phoma*, *Fusarium*, *Penicillium* and *Alternaria* were among the unique fungal taxa at the two points. The genus *Pestalotiopsis* was the most abundant taxa at sampling point 1 at the hot springs at Little Magadi at 81.9 ℃ and genus *Aureobasidium* at sampling point 2 at Lake Magadi at 41 ℃. Other genus that were unique at the two points included *cladosporium, Penicillium, Auxarthron* and *Debaryomyces* (**Figure 5.2**)
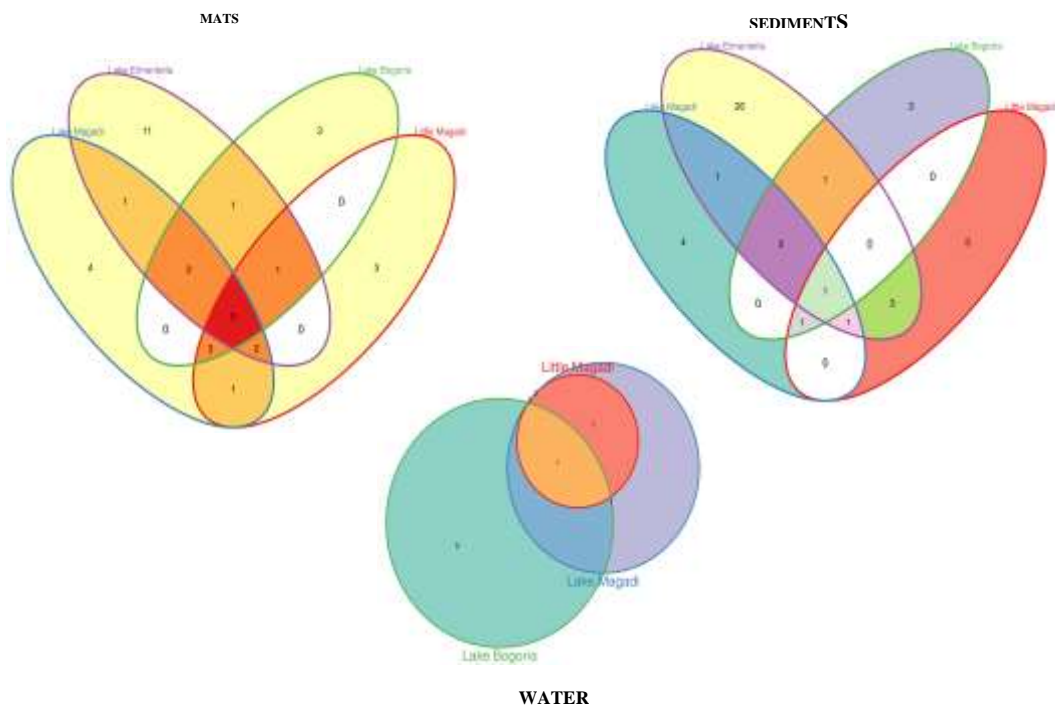
Figure 5.1: Shared and unique fungal taxa at genus level within sample types across hot springs.
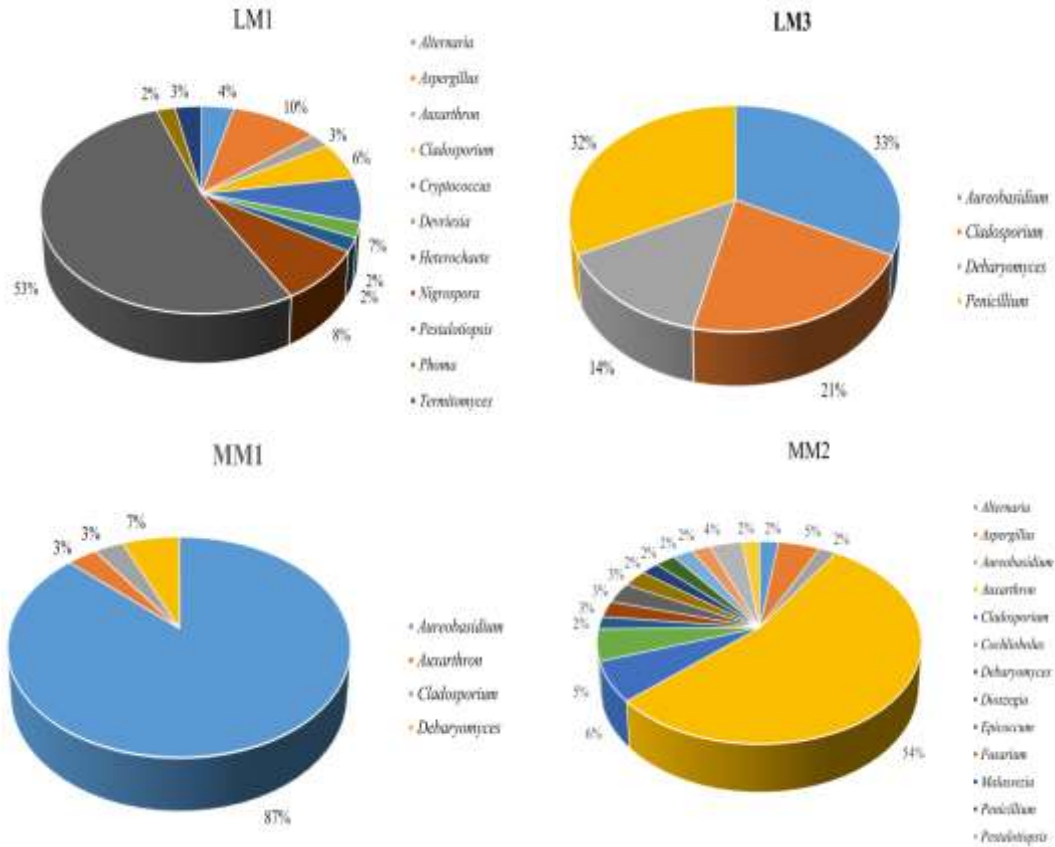
Figure 5.2: Pie diagrams showing most abundant and unique fungal taxa at genus level across mat samples within the hot springs. LM1 represents mats at 81.9 ℃, LM3 represents mats at 76.3 ℃, MM1 represents mats at 41.8 ℃, and MM2 represents mats at 41 ℃.

The hot spring at Lake Elmenteita had diverse fungal microorganisms as the temperatures were favourable for growth. The mats at sampling point 1 at 45 ℃ and sampling point 2 at 44.7 ℃ had the genus *Westerdykella* at 61% and 94% as the most abundant taxa. Sampling point 3 at a temperature of 33.8 ℃ had the genus *Cladosporium* at a lower

percentage of 18% as the most abundant taxa. Sampling point 3 had several taxa that were unique and this included *Aspergillus, Alternaria, Fusarium, Malassezia, Periconia, Phoma, Cryptococcus, Penicillium, Aureobasidium, Acremonium, Oidium, Epicoccum, Ascochyta, Cochliobolus, Podosphaera* among others (**Figure 5.3**).
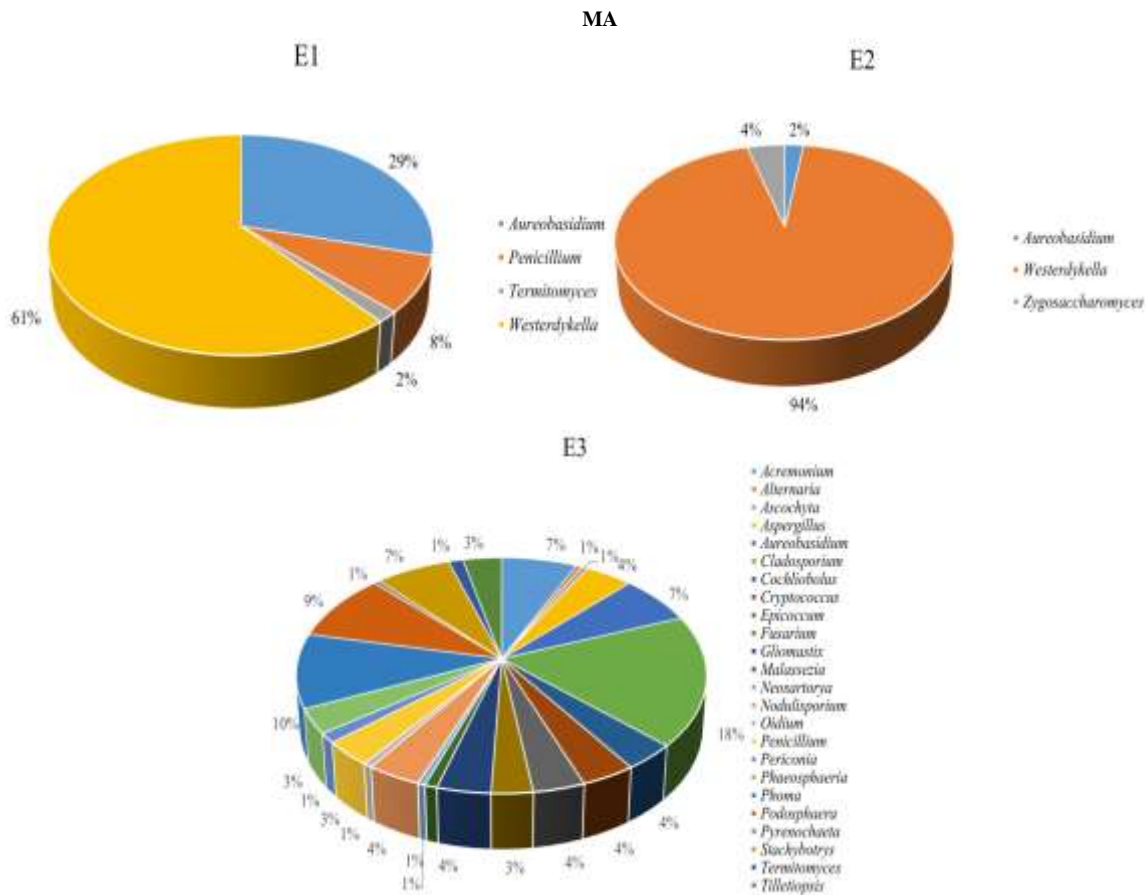
Figure 5.3: Pie diagrams showing most abundant and unique fungal taxa at genus level across mat samples within the hot springs. E1 represents mats at 45 ℃ E2 represents mats at 44.7 ℃, and E3 represents mats at 33.8 ℃.

High temperatures were recorded at the hot spring at Lake Bogoria compared to Lake Magadi and Lake Elmenteita. The mats at sampling point 2 at 77.7 ℃ and sampling point 3 at 54 ℃ had the genus *Aspergillus* and the genus *Phaeosphaeria* as the most abundant at 37% and 54% respectively. At sampling point 1, the genus *Epicoccum* was the most

abundant at 84.6 ℃ and at a percentage of 53 % *Cryptococcus, Penicillium Debaryomyces*, *Cladosporium, Didymella, Aureobasidium*, *Phoma, Malassezia*, *Trichoderma* were among the unique fungal taxa within the mats at Lake Bogoria (**Figure 5.4**)

MAT
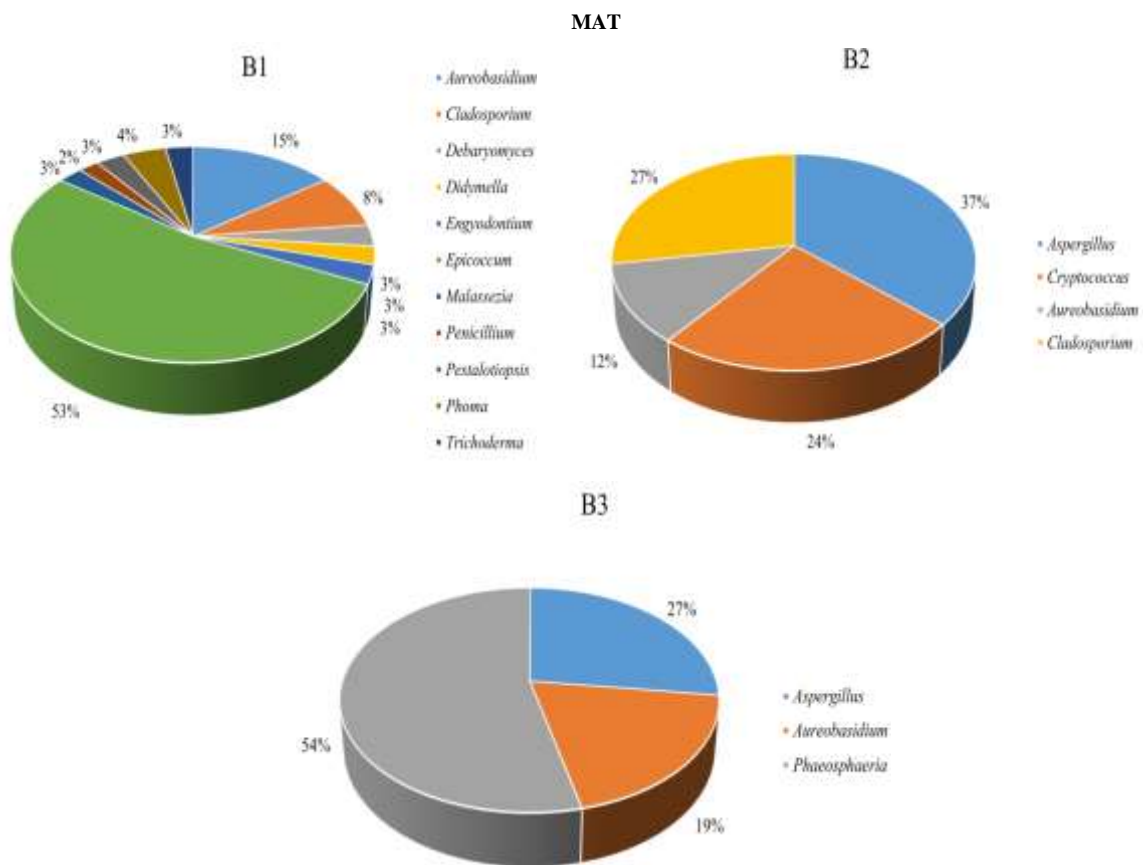
Figure 5.4: Pie diagrams showing most abundant and unique fungal taxa at genus level across mat samples within the hot springs. B1 represents mats at 84.6 ℃, B2 represents mats at 77.7 ℃, and B3 represents mats at 54 ℃.

The sediment samples collected at the hot spring at Lake Magadi (sampling point 2, 41 ℃) had the genus *Termitomyces* as the most abundant at 100 %. Those

at sampling point 1, 43.8 ℃ had the genus *Auxarthron* as the most abundant at 47%. At Lake Bogoria, the genera *Cladosporium,* 54 % and *Aspergillus*, 34 % were the most abundant at 84.6 ℃ and 77.7 ℃ respectively (**Figure 5.5**)
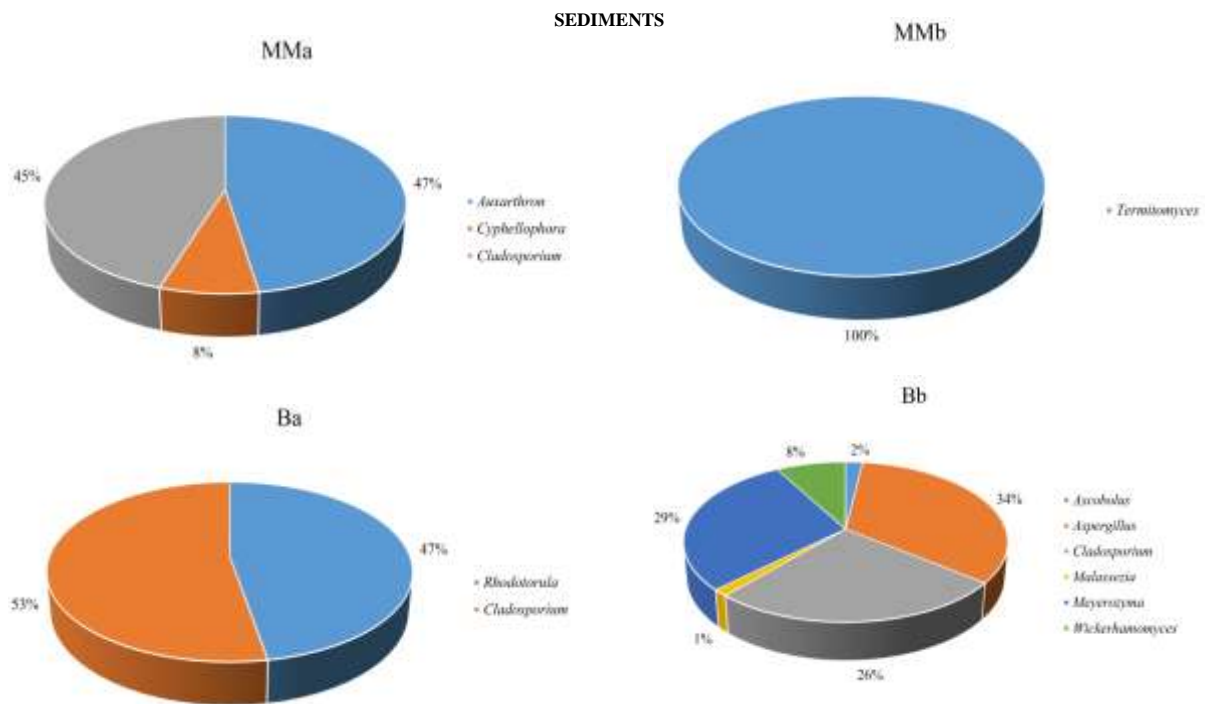
Figure 5.5: Pie diagrams showing most abundant and unique fungal taxa at genus level across sediment samples within the hot springs. MMa represents sediments at 43.8 ℃, MMb represents sediments at 41 ℃, Ba represents sediments at 84.6 ℃, and Bb represents sediments at 77.7 ℃.

The sediment samples collected at Lake Elmenteita at temperatures 45 ℃, 44.7 ℃ and 33.8 ° C had diverse groups of fungal taxa. Sampling point 1 at 45 ℃ and sampling point 3 at 33.8 ℃ had the genus *Westerdykella* and genus *Hannaella* at 48 % and 68 % respectively as the most abundant taxa while sampling point 2 at 44.7 ° C had genus *Westerdykella* as the most abundant fungal taxa at 21 % (**Figure 5.6**).
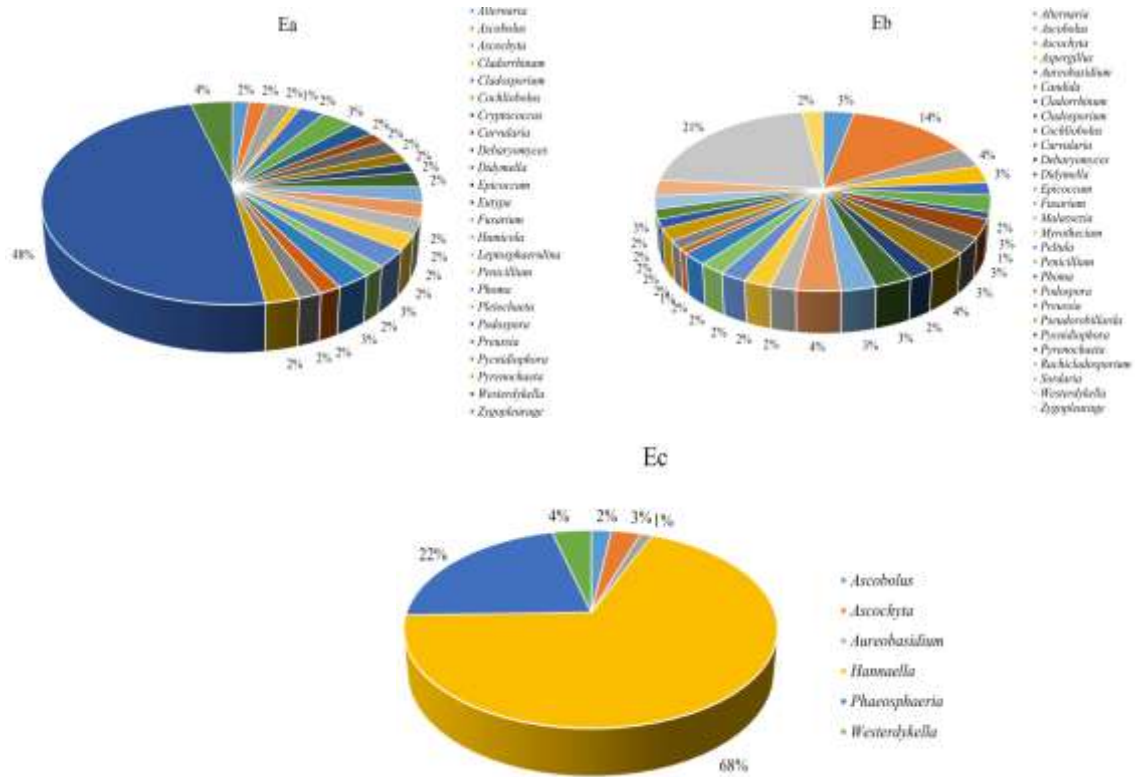
Figure 5.6: Pie diagrams showing most abundant and unique fungal taxa at genus level across sediment samples within the hot springs at Lake Elmenteita. Ea represents sediments at 45 ℃, Eb represents sediments at 44.7 ℃ and Ec represents sediments at 33.8.

Water samples were collected at the mouth of the hot springs. The samples at the little Magadi at 81.9 ℃ had the genus *Cladosporium* as the only dominant and abundant fungal taxa at 100 %. The water samples at Lake Magadi were collected at a temperature of 43.8 ℃ and the genus *Phoma* was the most abundant at 77 %. Other genera at Lake Magadi included *Epicoccum*, *Auxarthron* and *Cladosporium.* At Lake Bogoria the most abundant genus was *Aspergillus* at 84.6 ℃ and at a percentage of 28%. The genera *Aureobasidium*, *Auxarthron, Cladosporium*, *Neosartorya* and *Westerdykella* were the

unique ones within the water samples at Lake Bogoria at varying percentages (**Figure 5.7**).
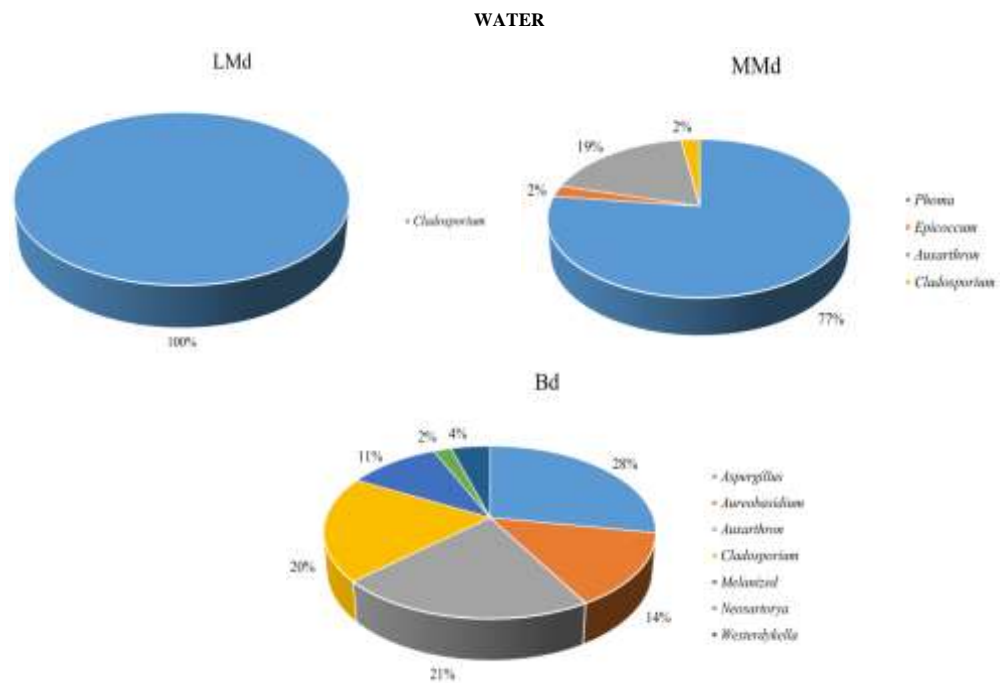
WATER

Figure 5.7: Pie diagrams showing most abundant and unique fungal taxa at genus level across water samples within the hot springs. LMd represents water samples at Little Magadi at 81.9 ℃, MMd represents water samples at Lake Magadi at 43.8 ℃ and Bd represents water samples at Lake Bogoria at 84.6 ℃.

At species level, the unique taxa across the temperature range were picked in both culture dependent and and culture independent approaches and summarized in **Table 5.1.**

Table 5.1: Change on fungal diversity across a wide range of temperatures

Hot spring	Temperature range	Culture dependent approach	Culture independent approach
Lake Bogoria	54 ℃ - 84.6 ℃	*Aspergillus versicolor, Aspergillus fumigatus, Penicillium pinophilum, Alternaria sp., Trametes sp.*	*Aspergillus oryzae* *Cladosporium cladosporioides*
Lake Elmenteita	33.8 ℃ - 45 ℃	*Aspergillus fumigatus, Aspergillus flavus* *Aspergillus sp. Penicillium sizovae, Penicillium chrysogenum Penicillium pinophilum, Alternaria tenuissima Alternaria sp.*	*Cladosporium sp.*
Lake Magadi	37.9 ℃ - 43.9 ℃	*Aspergillus flavus, Aspergillus oryzae, Aspergillus sp. Aspergillus tamarii, Neurospora sp.* *Penicillium sp., Beauveria sp., Tetrapisispora fleetii, Parmastomyces transmutans*	*Aureobasidium pullulans* *Pleosporales sp.* *Termitomyces sp.* *Dioszegia hungarica* *Malassezia globosa*
Little Magadi	67.3 ℃ - 81.6 ℃	NA	*Morchella esculenta, Verticillium dahliae*

5.4 Discussion

This present study reports the presence of diversity of fungi thriving in microbial mats, sediments and water samples collected from hot springs of four alkaline soda lakes in the Kenyan Rift valley. The lakes are of different sizes, alkalinity and salinity and of varying temperatures. They are also characterized by gorges, escarpments, craters on the rift floor that have gushing geysers and hot springs. Hot springs in this study showed moderate to high temperatures. The samples were treated to a wide range of temperatures using both culture-dependent and culture-independent approach. Fungi from extreme environments such as the soda lakes have adapted to elevated temperatures and alkaline saline conditions, which may lead to evolution and modifications of various fungal pathways (Brakhage & Schroeckh, 2011).

Among the microbial mats at the genus level, a diverse group of fungi were revealed and they included the following with their relative abundance, *Westerdykella* 94 %, *Cladosporium* 18 %, *Phaeosphaeria 54* %, *Aspergillus* 37 %, *Epicoccum* 37 %, *Pestalotiopsis* 53 %, *Aureobasidium* 87 % *Auxarthron 54* %. The mat communities from different lakes were similar in fungal communities with the genus *Westerdykella* as the most abundant.

At genus level, potentially thermophilic genera (Fraç *et al.*, 2018) were recovered from sediment samples. They were as follows *Termitomyces 100* %, *Westerdykella* 48 %, *Rhodotorula* 47 %, *Hannaella* 68 %, *Cladosporium* 53 %, *Auxarthron 47%, Aureobasidium* 54 %, and *Penicillium* 66 %. The genus *Aspergillus* was found to be the dominant genus in the sediments from the hot springs of Lake Magadi (Kambura, 2016).

Three groups of fungi at genus level were recorded among the water samples. These were *Cladosporium* at 100 %, *Phoma at* 77 % and *Aspergillus* at 28 %. Fungal genera known to inhabit waters in saline environments are *Fusarium*, *Aspergillus*, *Acremonium* and *Penicillium* (Wilson & Brimble, 2009). Previous studies on the hypersaline waters of

salterns revealed that the genera *Penicillium* and *Aspergillus* species were present in diverse levels (Gunde-Cimerman *et al*., 2005). Several strains of genus *Cladosporium* have also been isolated from Caspian Sea waters (Sadati *et al*., 2015).

5.5 Conclusion

This study revealed that mats, sediments and water samples collected from the hot springs had differences in fungal communities. Comparison of the fungal taxa identified by the two approaches resulted in an overlap fungal genera. Diverse fungal genera were recovered in this study which have been isolated from other different environments.

CHAPTER SIX

ISOLATION AND CHARACTERIZATION OF FUNGI FROM HOT SPRINGS ON THE SHORES OF THREE SODA LAKES IN KENYA

6.1 Introduction

Lake Bogoria, Elmenteita and Magadi are soda lakes found in the Kenyan Rift valley. Their development is a consequence of geological and topological factors (Mwatha, 1991). The pH of the lakes range from 8 to12 (Grant & Mwatha, 1989, Jones *et al*., 1994), while the salinity of these lakes ranges from around 5% total salts (W/V) in lake Elmenteita, but saturated in Lake Magadi with roughly equal proportions of Na_2CO_3 and NaCl as major salt components (Mwatha, 1991). Soda lakes are extreme environments that are characterized by steep fault escarpments, deep gorges, canyons, and craters on the rift floor, some of which have gushing geysers and hot springs. They are unique sites for extremophilic microorganisms (Kvist *et al*., 2007) that readily grow and multiply (Rothschild & Mancinelli, 2001). They exhibit high productivity rates (410g cm^2 per day) more than their freshwater counterparts with salinity that is up to saturation concentrations (Grant, 2006). The soda lakes also exhibit active volcanism with numerous hot springs on the shores of some of the lakes (Wilson & Brimble, 2009) like Lake Bogoria and Lake Elmenteita. Microorganisms from such regions have to endure extreme environmental conditions in terms of pH, salinity and temperature in the hot springs.

Hot springs are a type of extreme environments that are unique with respect to physical, chemical and geographical characteristics (Spear *et al.,* 2005). They are widely distributed all over the world and represent a wide range of microbial niches of highly diverse microorganisms (Song *et al.,* 2013). Most of the hot springs within the Kenyan lakes harbor extremophiles. Most of the described extremophiles are characterized only by one distinctive extreme condition such as temperature or pH. However, others are multi-extremophiles, for example alkalithermophiles (Kevbrin *et al.,* 1998) that grow under the

combined extremes of high salinity, alkaline pH (greater than or equal to 8.5), and temperatures greater than 50 °C (Bowers & Wiegel, 2011).

Lake Bogoria is a host to flamingoes and a variety of geochemically distinct hot springs (McCall, 2010). It's a deep, hyper alkaline and saline habitat that lies at an altitude of 1,000 metres above sea level. The shoreline of the lake is fringed by geysers and hot springs with water temperatures ranging from 39 °C to 98.5 °C. The hot springs and geysers found on the shores supplement water recharge for the lake. Lake Bogoria has been adversely affected by climate change that has resulted in long periods of drought that have led to fluctuation in the water level. The shoreline of the lake is fringed by geysers and almost 200 hot springs that discharge water into the lake and this confirms the volcanic origin of this harsh landscape (Renaut & Owen, 2005).

In Kenya, the haloalkaline soda lakes like Lake Elmenteita are characterized by exceptionally rich productivity rates presumably because of the high ambient temperatures, high light intensities, availability of phosphates and unlimited access to CO_2 in these carbonate rich waters (Melack, 1974; Grant, 1990). Lake Magadi on the other hand is 80% covered by soda and is well known for its wading birds, including flamingoes. The lake has natural springs to its south that bubble at 35 °C. The northern part of the lake has more hot springs too; these springs are however different as they boil at around 90 °C (Behr, 2002).

Recent studies have demonstrated that a wide range of fungal diversity inhabit the hyper alkaline and saline hot springs of soda lakes in Kenya (Kambura *et al.,* 2016; Salano *et al.,* 2017). Previously, combinations of both culture-dependent and culture-independent methods were used to document the microbial diversity of soda lakes (Baumgarte, 2003; Kambura *et al.,* 2016) and some other lakes (Rees *et al.,* 2004). Culture-independent methods have revolutionized the general view of microbial ecology and thus revealed diverse communities in the ecosystem (Bell *et al.,* 2014; Kambura *et al.,* 2016; Salano *et al.,* 2017).

Although the use of next-generation sequencing (NGS) is playing a major role in the exploration and detection of new species, in this study, four different growth media were used to assess the effectiveness of culture-dependent method at recovering fungal microorganisms from sediments, mats and water collected from hot spring found along the shores of three soda lakes in the Kenyan Rift valley. Earlier studies conducted using culture-dependent methods on the soda lakes of the East African Rift Valley have shown that there are dense and diverse populations of halophilic, alkaliphilic and alkalitolerant microorganisms (Zavarzin *et al.,* 1999; Grant *et al.,* 1999).

6.2 Materials and methods

Research authorization, study sites, measurements of physicochemical parameters, sample collection, isolation of fungi, morphological characterization of the isolates, physiological characterization, enzymatic characterization and nucleic acid extraction, amplification, 454 sequencing, sequence analysis and statistical analysis are as described in **chapter 3**.

6.3 Study site

The study was conducted on hotsprings found on the shores of Lake Bogoria, lake Elmenteita and Lake Magadi. At Lake Bogoria, it was done at a hot spring at Chemurkeu area **(Figure 6.1)** along the western shores of the lake. **Lake Bogoria** (formerly Lake Hannington) is situated within a depression of the Gregory Rift Valley. The lake forms part of the Lake Bogoria National Reserve which is a proposed world heritage site. The reserve is not only a popular tourist destination but also provides resources such as grazing and access to medicinal plants for the local community (Jones, 2000). It's also one of the lakes upon which the lesser flamingoes (*Phoeniconaias minor* Gray) depend for their life strategy (Krienitz & Kotut, 2010). The geographical position and elevation of Lake Bogoria above sea level are as described in **chapter 3**. It's one of the harshest volcanic places in the region and a hot spot of cyanobacterial life (Owino *et al.,* 2001). Numerous boiling (hot) springs, geysers and fumaroles are present at three onshore sites: Loburu,

Chemurkeu and a southern group (Ng'wasis, Koibobei and Losaramat). The hot springs at the shore discharge fresh to moderately saline water into the lake (Hinda, 2001).

Lake Elmenteita derived from the Masaai word *muteita*, meaning "dust place", is located approximately 30 km south of Lake Nakuru. The lake is in a basin whose water budget is maintained by recharge from hot springs located on the southern lakeshore, small in-flowing rivers (the Mbaruk, Chamuka and Kariandus), surface run off, direct rainfall and evapo-transpiration. The lake has no surface outlet or underground seepage for releasing its water to other aquifers (Mwaura, 1999). Hot springs **(Figure 6a)** at the southern end of the lake discharge slightly alkaline and saline water (Mwaura & Moore, 1991).

Lake Magadi on the other hand is a saline alkaline lake in Kenyan Rift Valley. The lake is located 2000 feet below sea level forming one of the lowest points in the valley (Gierlowski-Kordesch *et al.,* 2004). It is approximately 2° S and 36° E of the Equator and lies in the lowest part of the trough in a naturally formed closed lake basin. The lake covers an area of 90 km^2 and it is one of the smaller Rift Valley lakes **(Figure 6b)**. Lake Magadi is famous for its cherts from sodium silicate precursors. It has a pH of 10 and alkalinity of 380 mmol L-1. It also has a surface area of 100 km^2 and a depth that ranges from 1-5 m. Mineral composition consists mostly of trona mixed with halite and either kogarkoite or villaumite, resulting in fluoride concentrations up to 8.7 mg F$^-$. The high fluoride concentration in Lake Magadi is strongly related to the weathering of volcanic rocks enriched in fluoride and alkalis which are found in the same area as Lake Magadi (Behr, 2002).

Figure 6.1: The hot spring at Chemurkeu area, the springs divert the water directly into Lake Bogoria. The sampling points are labelled as numbers 1, 2 and 3. Point 1 represents the source of the spring while points 2 and 3 represent the path taken by the spring water as it flows into the lake.

Figure 6.2: The hot springs at (a) Lake Elmenteita and (b) Lake Magadi. These hot springs flow and discharge slightly alkaline and saline water into the main lakes

6.4 Sample collection

Samples used in isolation and characterization in this study comprised microbial mats, wet sediments and water **(Table 6.1)** that were collected at three distinct points along the flow of the hot springs. At Lake Elmenteita the sampling points were at an elevation of 1776 m above sea level and position 0º 27'S, 36° 15'E, Lake Magadi at 1° 52′S 36° 16′E, at an elevation of about 616 m above sea level, while at Lake Bogoria (00° 13′ 46.1″N 36° 05′ 34.8″ E) at an altitude of 1,000 metres above sea level, at Chemurkeu area, at a place called "Mawe moto". Water samples were collected in triplicates using one litre sterile bottles from the mouth (sampling point 1) of the hot springs at 84.6 ℃ and pH 9.0 at lake Bogoria, lake Magadi at 43.8 ℃ and pH 8.8 and 45 ℃ and pH 8.7 at Lake Elmenteita. Wet sediments (500 g) and microbial mats (500 g) were collected randomly from the floor of the rivulet in triplicates using sterile containers at three distinct points (rivulet point 1, 2 and 3) that differed in temperature and alkalinity levels. The samples were labeled and transported on dry ice in cool boxes to the laboratory at the Jomo Kenyatta University of Agriculture and Technology. Once in the laboratory, the samples were divided into two sets. One set was preserved at -80 ℃ as a backup while the other was used for work on the isolation, characterization and screening of the fungal isolates.

6.5 Preparation of culture media

The four culture media used in this study were: Potato glucose agar (PGA), Malt extract agar (MEA), Potato dextrose agar (PDA) and Sabourand dextrose agar (SDA). Their preparation is as described in Chapter 3 section **3.5.**

6.6 Isolation of fungi from the sediments, water and mats

As described in chapter 3 section **3.6**

Fungi were isolated from the different samples obtained from the hot springs of the three soda lakes. Isolation of fungi from sediments and microbial mats was performed using the serial dilution technique in combination with four selective media: Potato glucose agar (PGA), Malt extract agar (MEA), Sabourand dextrose agar (SDA) and Potato dextrose agar (PDA) separately prepared using lake water. Antibiotic, namely streptomycin (100 mg/litre) was used to inhibit bacterial growth. The inoculated plates were incubated at 30°C for one to two weeks during which the growing fungal colonies were examined and identified. Individual colonies which grew on the plates were re-inoculated on PGA, MEA, PDA, and SDA media several times until pure colonies were obtained. The pure colonies were selected based on morphological characteristics.

6.7 Morphological characterization of the isolates

As described in chapter 3 section **3.7.**

Colony colour, shape, size, elevation and form of the pure cultures were observed and noted. In cell morphology, the most commonly used prior art method and apparatus comprised of a round petri dish which was filled with water to a depth partially submerged by spaced pair of toothpicks. A standard microscope slide was placed on top of the toothpicks and a block of suitable culture nutrient medium (PGA) was applied to the upper surface of the slide. The culture medium was then inoculated with the fungi and a standard cover slip placed on top of the inoculated medium. The petri dish was then covered with a standard petri dish lid, and after 4-7days incubation period, the petri dish lid was removed and the cover slip, cultured fungi, agar, and microscope slide lifted as a unit from the petri dish and placed upon the stage of a microscope for examination purposes. A permanent record of the cultured fungi was made by staining with cotton blue in lactophenol dye (Cappuccino & Sherman, 2002).

6.8 Physiological and enzymatic characterization

As described in chapter 3 section **3.8 and 3.9** respectively

6.9 Statistical analysis

An analysis was conducted to determine the effect of Temperature, pH, salinity and media on the growth of fungal isolates. The analysis was divided into 4 sections to show each variable. In each section the following was done: First the descriptive statistics for each level that included mean, standard deviation, minimum score and maximum score were presented in form of a table. A boxplot for each level of temperature, salinity, pH and media was then plotted to show the distribution of the data under the different levels. An analysis of variance was conducted to test for significance difference among the different levels on growth. The null hypothesis tested was that there was no significant difference among the different levels on the growth of fungal isolates. A significance value (p-value) less than 0.05 indicated that the difference is significant while a p-value greater than 0.05 indicated that there was no significant difference. Finally, a post hoc test was conducted to check which level was significantly different from the others in case there existed a significant difference. In this case Tukey honest significant difference (HSD) post hoc test was used to separate means.

6.10 DNA extraction from fungal cells

This was done to identify the fungal isolates. Each of the isolate was grown on potato glucose agar for 7 days. Total genomic DNA of the isolates was extracted from these cells in duplicate using two lysis buffers as solution A (50mM Tris pH 8.5, 50mM EDTA pH 8.0 and 25 % sucrose solution) and solution B (10mM Tris pH 8.5, 5mM EDTA pH 8.0 and 1 % SDS). The cells were scrapped aseptically using a sterile surgical blade taking care not to pick the media. These were crushed separately in 200 µl solution A using sterile mortar and pestle, and resuspended in 100 µl of solution A. This was followed by addition of 30 µl of 20mg/l Lysozyme and 15 µl of RNase, gently mixed and incubated at 37 ºC for

two hours to lyse the cell wall. Solution B (600µl) was then added and gently mixed by inverting the tubes severally, followed by the addition of 10µl of Proteinase K (20mg/l) and the mixture incubated at 60 ºC for 1 hour. Extraction followed the phenol/chloroform method (Sambrook *et al*., 1989). The presence and quality of DNA was checked on 1 % agarose and visualized under ultraviolet by staining with ethidium bromide to determine the relative intensity of bands. The remaining volume was stored at -20º C. The genomic DNA was used as templates for subsequent PCR amplification.

6.11 PCR amplification and sequencing of ITS gene region

The purified DNA from each isolate was used as a template for amplification of the ITS gene region using a fungal primer pair: ITS1 (5´TCCGTAGGTGAACCTTGCGG3´) and ITS4 (5´TCCTCCGCTTATTGATATGC3´) (White *et al.,* 1990). Amplification was performed using DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) PCR machine. Amplification was carried out in a 40 μl mixture containing 5 μl of PCR buffer (×10), 3 μl dNTP's (2.5mM), 1 μl (5 pmol) of ITS1 forward primer, 1 μl (5pmol) of ITS4 reverse primer, 0.3 μl taq polymerase, 1.5 μl of template DNA and 28.2 μl of water. The control contained all the above except the DNA template. Reaction mixtures were subjected to the following temperature cycling repeated for 35 cycles: Initial activation of the enzyme at 95 °C for 5 minutes, denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 30 seconds, chain extension at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes.

The quality of PCR products was assessed on 2 % agarose gel and visualized under ultraviolet by staining with ethidium bromide (Sambrook *et al*., 1989) to determine the success of amplification and the relative intensity of bands. The PCR products were purified using the multiscreen filter plate (Millipore Corp and sequenced by a Big Dye (R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to manufacturer's instructions. Sequencing of the PCR products was performed using a commercial service (Macrogen, Korea).

6.12 Phylogenetic analysis

The ITS partial gene sequences obtained were manually edited. The sequence data were BLAST (www.ncbi.nlm.nih.gov/BLAST/) analyzed against the GenBank 18S rDNA database. Alignments were checked and corrected manually based on conserved regions. The 18S rDNA gene sequences were compared to sequences in the public database using Basic Local Alignment Search Tool (BLAST) in the National Center for biotechnology Information (NCBI) website (http://www.ncbi.nih.gov) in order to determine similarity to sequences in the Gene bank database (Shayne *et al.,* 2003). The 18S rDNA gene sequences with high similarities to those determined in the study were retrieved based on BLASTn results and added to the alignment. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model (Jukes & Cantor, 1969). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.,* 2015).

6.13 Results

6.13.1 Physical characteristics at sampling site

The metadata collected before sampling included the geographical position of each sampling point in terms of latitude, longitude and elevation, temperature, pH, electrical conductivity, total dissolved solids and dissolved oxygen. The samples collected from the soda lake and their parameters are summarized in Chapter 4 **Table 4.1**.

6.13.2 Fungal isolates

A total of 61 fungal isolates, 27 from wet sediments, 24 from microbial mats and 10 from water were recovered from the three sampling points along the hot spring at Lake Bogoria, Elmenteita and Magadi as summarized in **Table 6.1**. The fungal isolates were of different colours, margin, elevation and form (**Figure 6.3**)

Table 6.1: Summary of isolates recovered from the three soda lakes and their sampling point and type.

Sampling point	Sampling type	Isolates
1	Wet sediments	B1, B3, B4, B5, MM5, MM19, EL4, EL8, EL11, EL12
2	Wet sediments	B17, MM10, MM11, MM20, EL2, EL15, EL20
3	Wet sediments	B21, B23, B24,B25, MM7, MM16, MM21, EL3, EL17, EL21
1	Water	B31,MM1,MM3, MM8, MM9,EL5, EL6, EL13, EL19, EL22
1	Mats	B41, MM4, MM12, MM13, EL7, EL14
2	Mats	B52, B54, MM6, MM17, MM18, MM21,EL1, EL10
3	Mats	B61, B62, B63, B64, MM2, MM14, MM15, EL9, EL16, EL18

6.13.3 Colony morphology of the isolates

Morphological characterization was based on classical macroscopic techniques of color, form, shape, margin and elevation of the pure colonies (**Table 6.2, 6.3 & 6.4**). Most colonies were able to grow on PGA media within 4-7 days of incubation at 30 °C. The colony characteristics recorded for the various isolates are as shown in **Table 6.2-6.4**. The isolates showed reproductive spores, mycelia and hyphae (**Figure 6.3**). The hyphae were either septate or aseptate and in some, the spores or conidia were in a chain at the end of aerial hyphae or in a sac like structure and in some the spores were formed externally on a base or just scattered in the media.

Table 6.2: Morphological (colony) characteristics of 17 isolates from Lake Bogoria on PGA media

Isolate	Colour Top	Colour Bottom	Margin	Elevation	Form
B1	White	Yellow	Entire	Raised	Circular
B3	Green	Cream	Entire	Flat	Circular
B4	Yellow and white	Red	Undulate	Raised	Irregular
B5	Green with white margin	Cream	Entire	Flat	Circular
B17	Cream	Cream	Entire	Raised	Circular
B21	Cream with purple rings	Brown	Entire	Flat	Circular
B23	Grey	Cream	Entire	Flat	Circular
B24	Grey	Black	Undulate	convex	Irregular
B25	Green	Purple	Entire	convex	Circular
B31	Pink	Pink	Entire	Raised	Circular
B41	Green with black spots	Cream	Undulate	Raised	Irregular
B52	Green and cream	Brown	Entire	Flat	Circular
B54	Green and cream	Green and Cream	Curled	Umbonate	Irregular
B61	Green	Cream	Undulate	Raised	Irregular
B62	Green with yellow margin	Cream	Entire	Flat	Circular
B63	Green and white	Cream	Curled	Raised	Irregular
B64	White	Cream	Entire	Umbonate	Circular

Note: B represents Lake Bogoria

Table 6.3: Morphological (colony) characteristics of the 22 isolates from Lake Magadi (MM) on PGA media

Isolate	Colour Top	Colour Bottom	Margin	Elevation	Origin
MM1	White	Yellow	Entire	Flat	Water
MM2	Grey	Cream	Entire	Flat	Mats
MM3	Cream	Brown	Undulate	Flat	Water
MM4	Brown	Blackish Brown	Entire	Raised	Mats
MM5	Cream	Cream	Entire	Flat	Sediments
MM6	Orange, cream	Orange	Entire	Raised	Mats
MM7	Pinkish cream	Brown	Entire	Flat	Sediments
MM8	Cream and green	Orange	Entire	Raised	Water
MM9	Greenish cream	Brown	Undulate	Crateriform	Water
MM10	Green	Brown	Entire	Raised	Sediments
MM11	Grey	Black	Undulate	Convex	Sediments
MM12	Green and white	Yellow	Entire	Raised	Mats
MM13	Green(slimy)	Black	Undulate	Convex	Mats
MM14	Greenish brown	Black	Entire	Flat	Mats
MM15	Greenish brown	Black	Entire	Raised	Mats
MM16	Green and cream	Brown	Entire	Flat	Sediments
MM17	Green and white	Brown	Entire	Umbonate	Mats
MM18	Brown and cream	Brown	Curled	Umbonate	Mats
MM19	Green and cream	Green and cream	Curled	Umbonate	Sediments
MM20	White and cream	White	Entire	Umbonate	Sediments
MM21	White and green	White	Curled	Raised	Mats
MM22	White and black	Black	Entire	Flat	Sediments

Note: MM represents Lake Magadi

Table 6.4: Morphological (colony) characteristics of the 22 isolates from Lake Elmenteita (EL) on PGA media

Isolate	Colour Top	Colour Bottom	Margin	Elevation	origin
EL1	Grey	Cream	Entire	Raised	Mats
EL2	Pinkish cream	Brown	Entire	Flat	Sediments
EL3	Purple, green, cream	Purple and Cream	Undulate	Umbonate	Sediments
EL4	Orange	Cream	Filamentous	Flat	Sediments
EL5	Green	Purple	Entire	Convex	water
EL6	Green and white	Cream	Entire	Flat	water
EL7	Brown	Blackish Brown	Entire	Raised	Mats
EL8	Brown and cream	Brown	Curled	Umbonate	Sediments
EL9	Greenish brown	Black	Entire	Raised	Mats
EL10	White and green	Brown	Undulate	Crateriform	Mats
EL11	Green and white	Yellow	Entire	Raised	Sediments
EL12	Green	Cream	Entire	Raised	Sediments
EL13	Green and white	Yellow	Entire	Raised	water
EL14	Green and white	Yellow	Undulate	Crateriform	Mats
EL15	Cream	Brown	Undulate	Flat	Sediments
EL16	White	Cream	Entire	Umbonate	Mats
EL17	Yellow	Cream	Undulate	Raised	Sediments
EL18	Cream coiled	Cream	Curled	Raised	Mats
EL19	Cream	Brown	Entire	Raised	water
EL20	Dark Green	Cream	Entire	Raised	Sediments
EL21	Green with cream ends	Cream	Entire	Flat	Sediments
EL22	Cream coiled	Cream	Curled	Raised	water

Note: EL represents Lake Elmenteita
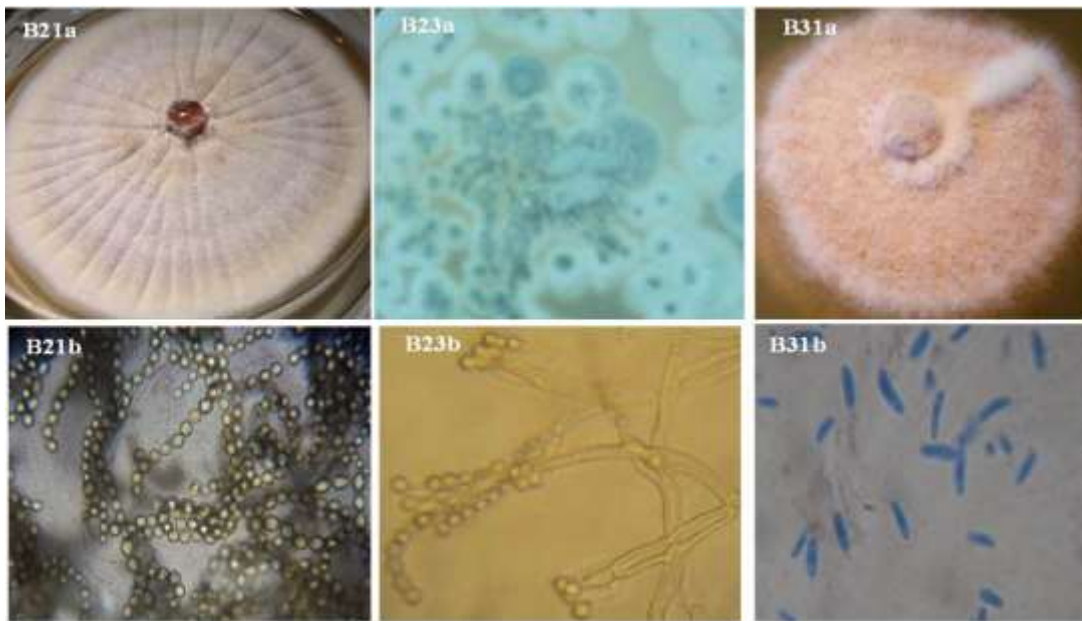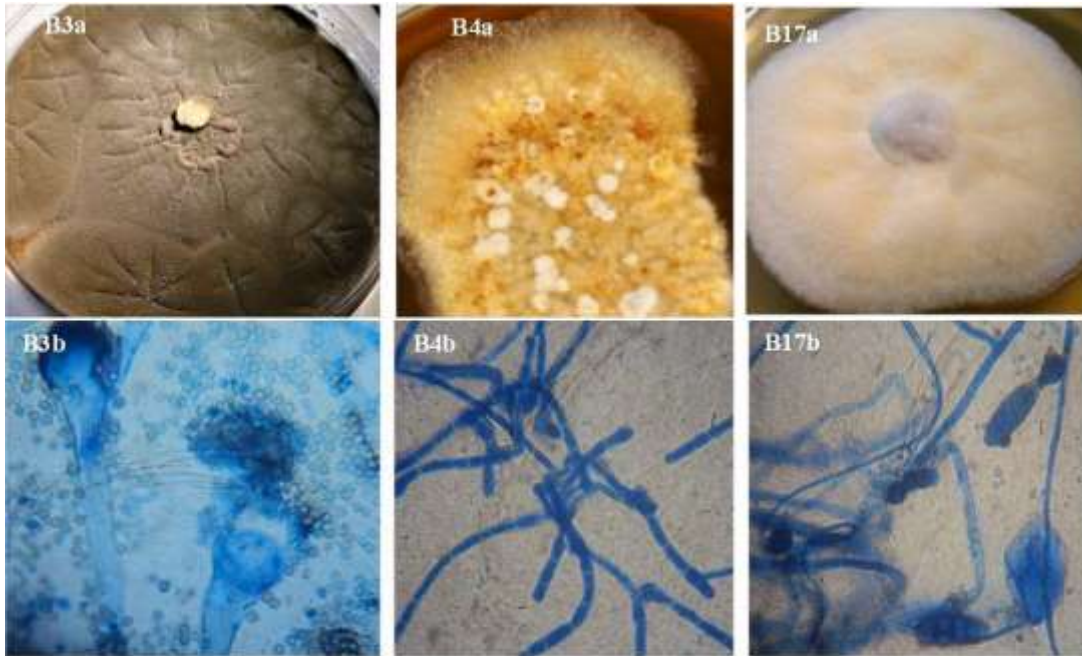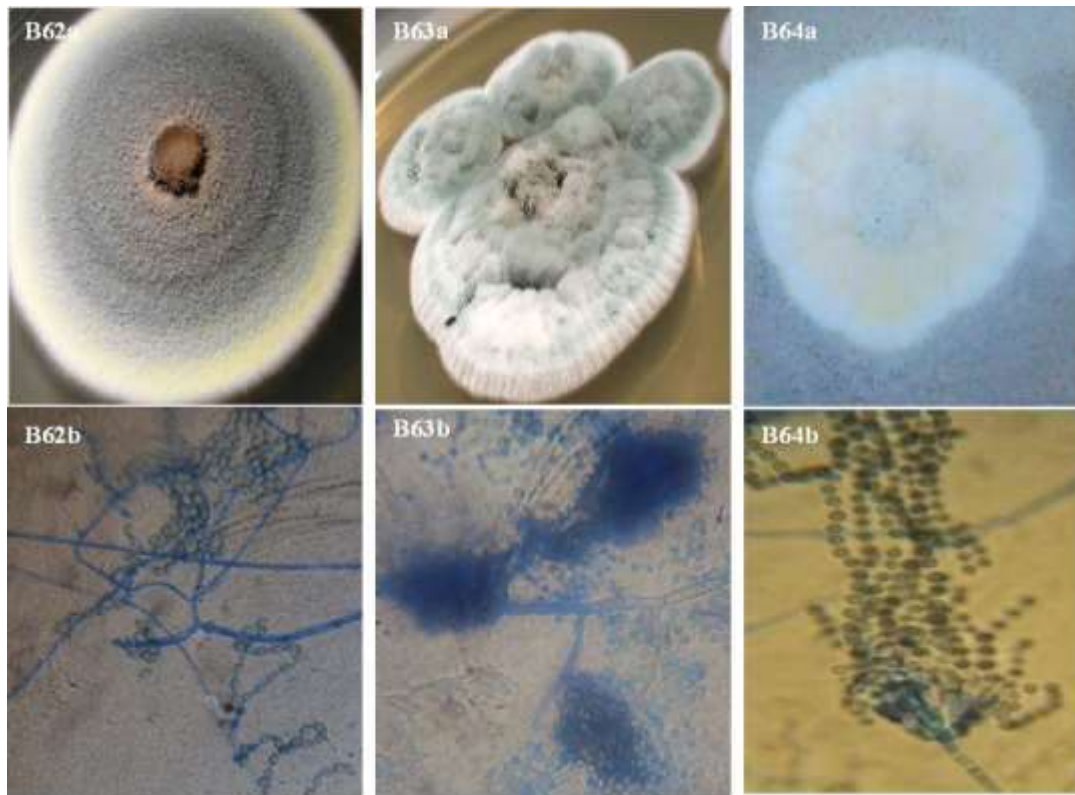
Figure 6.3: Colony and cell characteristics of some of the 17 isolates from the hot spring at Lake Bogoria a) colony characteristics b) cell characteristics

6.13.4 Physiological characterization

6.13.4.1 Hotspring at Lake Bogoria

6.13.4.1.1 Growth at various temperatures

All the isolates grew on PGA media at 25, 30, and 35 °C. Growth at 40 °C was observed only for the isolates B23, B25 and B61. Generally the isolates did not show any growth at 45 and 50 °C. The optimum growth of the isolates was observed between 30 °C and 35 °C. (**Table 6.5**).

Table 6.5: Impact of culture media and temperature on the growth of fungi isolated from Lake Bogoria. The values represent the mean in millimeters ± standard errors for the various isolates.

Fungal Isolates	Media				Temperature			
	MEA	PDA	SDA	PGA	25°C	30°C	35°C	40°C
B1	59±1.5	60.7±1.2[b]	59.33±1.8	79.67±0.9[a]	33±1.2[b]	67.67±0.9[a]	34.67±2.2	0
B3	78.33±1.7	66±8.9	58.67±3.7	80±1.5	72±0.6	68±1.5	67±0.6	0
B4	19.67±1.2[b]	24.3±0.3	30.33±0.9[a]	44.33±2.3	66±1.7	71±1.2	68±1.5	0
B5	80.33±0.3	80.7±1.2[b]	70±2.1	82.67±0.9[a]	52.67±2.0	66±1.5	56.33±1.5	0
B17	47.67±8[c]	30.33±8[c]	19±1.2[b]	50±8.1[c]	59.33±4.3	80±1.2	70.66±1.9	0
B21	39.33±1.5	38.7±3.8	27.67±3.5	55.33±3.2	37±0.6	27.33±2.2	25±1.2[b]	0
B23	64.33±0.9[a]	57±4.7	62.33±2.7	79.33±1.2[b]	71.33±1.2[b]	76.67±0.9	74.33±2.9	23±1.2[b]
B24	44.33±2.3	20.67±1.9	44.33±2.3	60.67±0.9[a]	29±1.2[b]	20.33±0.9[a]	15.33±0.9[a]	0
B25	37±0.6	27.33±2.2	25±1.2[b]	51±3.2	22±1.0	75.33±0.9[a]	63.67±1.5	15.33±0.9[a]
B31	79.33±0.7	66±1.5	56.33±1.5	72±0.6	61.33±2.9	75.67±2.4	72.67±1.2[b]	0
B41	70±5.8	71.33±4.4	66.67±1.2[b]	80.33±1.2[b]	39.67±0.9[a]	18.67±1.5	15±1.5	0
B52	39.67±0.9[a]	18.67±1.5	15±1.5	32.67±2.3	76.67±0.9[a]	26.67±1.2	23±1.2[b]	0
B54	40.67±1.7	26.33±1.8	29.33±2.9	44.33±2.3	17±1.2[b]	24.67±1.5	15.67±0.9[a]	0
B61	80.33±1.5	76.67±0.7	62±2.1	80.67±1.5	51.67±2.3	77±0.6	79.67±0.9[a]	13.33±1.2[b]
B62	47.33±1.5	72±0.6	51±3.2	79±0.6	44.33±2.3	20.67±1.9	44.33±2.3	0
B63	78.33±1.3	71.67±5.9	64±1.5	81.33±0.9[a]	19±1.2[b]	17±1.5	32.67±2.3	0
B64	19±1.2[b]	17±1.5	32.67±2.3	47.67±8	18.67±1.5	33.33±1.2[b]	37±1.2[b]	0

Note: a) Letters designate significant differences at P ≤ 0.05.

b) Means followed by the same letter are not significantly different.

c) B represents Lake Bogoria

The effect of different temperature levels on growth of fungal isolates was investigated. Results showed that there was a significant difference in growth among the four temperature levels at 5 % level of significance, [$F_{(3, 64)}$ = 19.912, p = < 0.0001] (**Table 6.6**). Post hoc comparisons using the Tukey HSD test indicated that the mean score for the 40 °C level (M = 3.0388, SD = 7.00318) was significantly different from the others (**Table 6.7 and Table 6.8**).

Table 6.6: ANOVA Comparisons of Growth from four Temperatures at Lake Bogoria

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	25138.114	3	8379.371	19.912	.000
Within Groups	26932.586	64	420.822		
Total	52070.700	67			

Table 6.7: Descriptive statistics of the different temperatures at Lake Bogoria

Temperature	N	Min	Max	Mean	SD
25°C	17	17.00	76.67	45.3335	20.33290
30°C	17	17.00	80.00	49.7653	25.93499
35°C	17	15.00	79.67	46.7647	23.41349
40°C	17	0.00	23.00	3.0388	7.00318

Table 6.8: Tukey's HSD Comparison for Growth among the different temperature levels at Lake Bogoria

Comparisons	Mean Difference	Std.Error	sig	95 % CI Lower Bound	Upper Bound
40 °C vs. 25 °C	-42.2947*	7.0362	.000	-60.855	-23.734
40 °C vs. 30 °C	-46.7265*	7.0362	.000	-65.287	-28.166
40 °C vs. 35 °C	-43.7259*	7.0362	.000	-62.286	-25.166

* $p < 0.05$

From the boxplot, the median value for growth of all the isolates at 30 °C was at 64mm. The minimum growth value for all the isolates at the the four temperature levels was below 20mm with a maximum value of about 80mm **(Figure 6.4)**
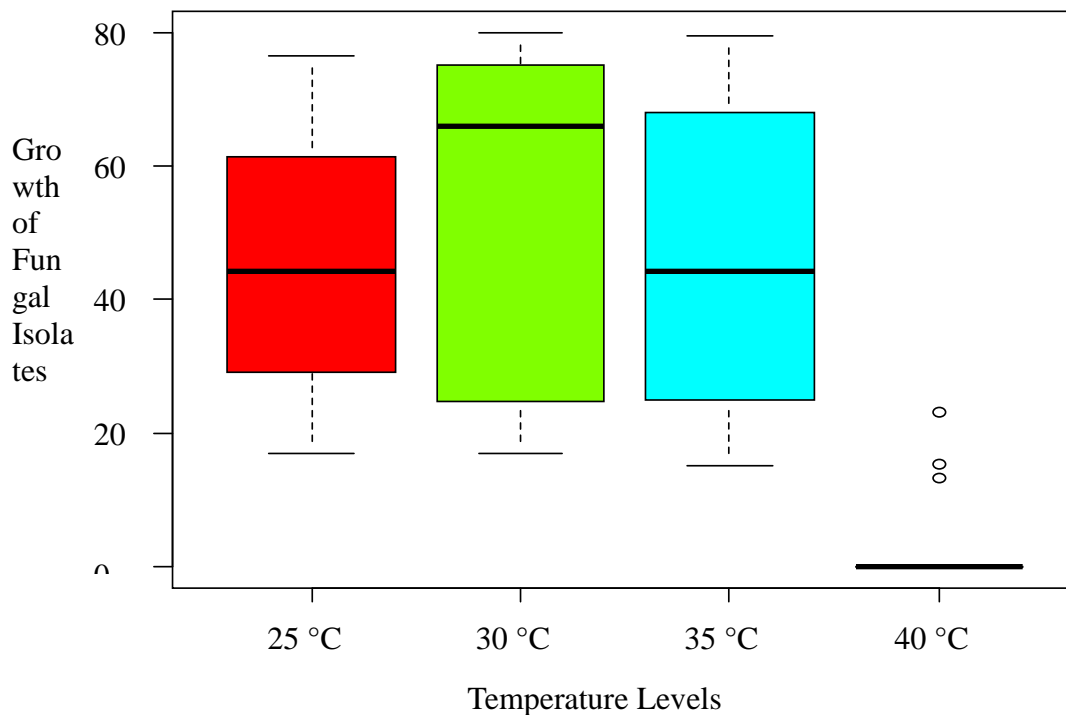
Figure 6.4: Boxplots of fungal isolates growth across the different temperature levels at the hot spring at Lake Bogoria. The growth of the isolates were measured in millimetres (mm).

6.13.4.1.2 Growth on different media

Different isolates showed different growth rates on the four types of media used. Isolates B3, B5, B41, B61 and B63 recorded average growth values of 80mm, 82.67mm, 80.33mm, 80.67mm and 81.33mm respectively on potato glucose agar (PGA). Isolates B5, B41, B63 had average values of 70mm, 66.67mm and 64mm on Sabourand dextrose agar

respectively while isolates B5, B61 and B63 recorded average values of 80.7mm, 76.67mm and 71.67mm on potato dextrose agar media. On Malt extract agar isolates B5, B31 and B61 had average values of 80.33mm, 79.33 and 80.33 respectively. Generally isolates growing on potato glucose agar showed significant growth on the four types of media (**Table 6.5**).

A one-way ANOVA was conducted to compare the effect of the different media type on growth of fungal isolates. Results showed that there was a significant difference in growth among the fungal isolates in the four types of media at 5 % level of significance, [$F_{(3, 64)}$ = 2.997, p = 0.037]. Post hoc comparisons using the Tukey HSD test showed that PGA (M = 64.7647, SD = 17.10699) was significantly different from SDA (M = 45.5094, SD = 18.51799) (**Table 6.9**)

Table 6.9: Descriptive statistics of the different media at Lake Bogoria

Media	N	Min	Max	Mean	SD
MEA	17	19.00	80.33	54.3912	21.01490
PDA	17	17.00	80.70	48.5529	23.56153
SDA	17	15.00	70.00	45.5094	18.51799
PGA	17	32.67	82.67	64.7647	17.10699

6.13.4.1.3 Growth at pH 5.0, 7.0, 8.5 and 10.0

Although the isolates were from an alkaline environment, all showed growth at all the pH values tested including acidic pH 5 and neutral pH 7. There was significant growth for all isolates at pH 10 with five isolates (B3, B5, B17, B21 and B61) recording a mean value of over 78mm (**Table 6.9**).

Table 6.10: Influence of pH and salinity on growth of fungi isolated from the hot spring at Lake Bogoria. The values represent the mean in millimeters ± standard errors for the various isolates.

Fungal Isolates	Salinity				pH			
	5%	10%	20%	30%	pH 5.0	pH 7	pH 8.5	pH 10
B1	25.33±0.9^a	19±1.2^b	12.67±0.9^a	10±0.6	65±1.5	62±1.2	68±1.2	67.67±0.9^a
B3	39.67±0.9^a	32.67±2.3	18.67±1.5	15±1.5	64±2.3	80.33±0.3	81.33±1.2	81.67±1.2^b
B4	47.67±8.0^c	50±8.1^c	30.3±8.0^c	19±1.2	20±1.5	21±1.2^b	72.67±0.9^a	73.67±1.8
B5	60.67±0.9^a	44.33±2.3	44.33±2.3	20.67±1.9	33±1.7	30.67±0.7	31±1.2	81±1.5
B17	75.33±0.9^a	63.67±1.4	22±1	15.33±0.9^a	33±2.7	82±0.6	32.33±0.9^a	81±1
B21	51±3.2	37±0.6	27.33±2.2	25±1.2^b	18±0.6	24±1	18.33±0.7	78±1.5
B23	74±1.7	66.67±1.2^b	24±1.7	20.33±1.9	44±2.7	47.67±0.9^a	62.33±±0.9	62.33±1.2^b
B24	24±1.7	22±1	17.67±1.2^b	17.67±0.9^a	25.67±1.5	28±0.6	67.33±0.9	28.33±1.2^b
B25	28±1	24.33±0.3	21.33±1.5	16.33±1.2^b	22.67±1.2^b	22.67±1.5	25.33±1.2	23±1.2^b
B31	39.67±0.9^a	32.67±2.3	18.67±1.5	15±1.5	28.33±1.2^b	66.67±1.2^b	32.67±1.8	51.33±0.9^a
B41	44.33±2.3	40.67±1.8	29.33±3.0	26.33±1.8	65.33±1.2^b	76.33±0.3	80±0.6	68.33±1.2^b
B52	74.67±1.2^b	54.33±1.5	26.33±1.5	15.33±0.9^a	25±2.5	20±1.2	17.33±0.9^a	22±1
B54	53.67±1.5	43.33±2.0	27.33±0.7	16.67±1.5	28±1.2^b	18±0.6	18.67±0.9^a	61.67±1.2^b
B61	78.33±1.2^b	81.33±0.9^a	71.67±5.9	64±1.6	75.33±1.2^b	80.67±1.2^b	81±1.2	80.67±0.7
B62	19±1.2^b	17±1.5	32.67±2.3	47.67±8.0	67.67±0.9^a	80.33±0.7	73±1.5	81±1.7
B63	48.67±1.9	38±0.6	27.67±1.9	24±1.5	32.33±1.2^b	73±1.2^b	61±1.5	30.33±0.9^a
B64	81.33±0.9^a	51±0.6	27.67±1.2^b	18.67±1.2^b	27.33±0.3	16±1.2^b	17.67±0.9	18.67±0.3

Note: a) Letters designate significant differences at P ≤ 0.05.

b) Means followed by the same letter are not significantly different.

c) B represents Lake Bogoria

The results of the one way ANOVA conducted at various pH levels indicated that there was no significant effect of the different pH levels on the growth of the fungal isolates at 5% level of significance, [F $(3, 64)$ = 1.674, p = 0.181]. (The p-value, 0.181, was greater than 0.05 hence insignificant) (**Table 6.11**)

Table 6.11: Descriptive statistics of the different pH levels at the hot spring at Lake Bogoria

pH	N	Min	Max	Mean	SD
pH 5.0	17	18.00	75.33	39.6859	19.49839
pH 7.0	17	16.00	82.00	48.7847	26.96123
pH 8.5	17	17.33	81.33	49.4112	25.56394
pH 10	17	18.67	81.67	58.2747	24.10417

6.13.4.1.4 Growth of fungal isolates on different sodium chloride concentrations

All the 17 isolates were able to grow in varying concentrations of sodium chloride. Growth was improved with the decrease of salt concentration from 30 % up to 5 %. A one-way ANOVA was conducted to compare the effect of the different sodium chloride concentration on growth of fungal isolates. There was a significant difference in growth among the four salinity levels at 5% level of significance, [$F_{(3, 64)} = 10.344$, $p = < 0.0001$]. Post hoc comparison using the Tukey HSD test indicated that the mean score for the 5% level (M = 50.9024, SD = 20.47441) was significantly different from that of 20% level (M = 28.2141, SD = 13.27129) and 30% level (M = 22.7647, SD = 13.41568) (**Table 6.12**). In addition the 10% level (M = 42.2353, SD = 17.69786) was found to be significantly different from the 30% level (M = 22.7647, SD = 13.41568) (**Table 6.12**)

Table 6.12: Descriptive statistics of the different sodium chloride concentrations at Lake Bogoria

Salinity	N	Min	Max	Mean	SD
5%	17	19.00	81.33	50.9024	20.47441
10%	17	17.00	81.33	42.2353	17.69786
20%	17	12.67	71.67	28.2141	13.27129
30%	17	10.00	64.00	22.7647	13.41568

6.13.4.2 Hot spring at Lake Magadi

6.13.4.2.1 Growth of fungal isolates at various temperature levels

All the isolates grew at temperatures 25 °C, 30 °C, and 35 °C. Growth at 40 °C was observed only for the isolates MM2, MM6 and MM13. The optimum growth of the isolates was observed between 30 °C – 35 °C. A one-way ANOVA was conducted to compare the effect of different temperature levels on growth of fungal isolates. Results show that there was a significant difference in growth among the four temperature levels at 5 % level of significance, [$F_{(3, 84)}$ = 18.324, p = < 0.0001]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for the 40 °C level (M = 3.00, SD = 8.042) was significantly different from the others **(Table 6.13).**

Table 6.13: Descriptive statistics of the different Temperature levels at the hot spring at Lake Magadi

Temperature	N	Minimum	Maximum	Mean	Std. Deviation
25 °C	22	17.00	80.00	39.76	22.434
30 °C	22	18.33	79.00	40.86	23.5435
35 °C	22	18.00	80.00	42.89	25.203
40 °C	22	0.00	30.33	3.00	8.042

6.13.4.2.2 Growth of fungal isolates at different sodium chloride concentrations

All the 22 isolates were able to grow in varying concentrations of sodium chloride. Growth improved with decrease in salt concentration from 30 % up to 5 % sodium chloride. The highest growth was recorded at 5 % sodium chloride concentration. Results from a one-way ANOVA conducted to compare the effect of the different salinity levels on growth of fungal isolates showed that there was significant difference in growth among the four sodium chloride concentration at 5 % level of significance, [$F_{(3, 84)}$ = 14.245, p = < 0.0001]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for

the 5 % level (M = 45.24, SD = 21.26) was significantly different from that of 20 % level (M = 23.58, SD = 4.75) and 30 % level (M = 21.35, SD = 4.61). In addition the 10 % level (M = 36.15, SD = 16.70) was found to be significantly different from that of 20 % level (M = 23.58, SD = 4.75) and 30 % level (M = 21.35, SD = 4.61) **(Table 6.14)**

Table 6.14: Descriptive statistics of the different sodium chloride concentrations

Salinity	N	Minimum	Maximum	Mean	Std. Deviation
5%	22	15.00	75.33	45.24	21.26
10%	22	15.00	75.33	36.15	16.70
20%	22	15.00	33.33	23.58	4.75
30%	22	15.67	33.33	21.35	4.61
Total	88	15.00	75.33	31.58	16.80

6.13.4.2.3 Growth of fungal isolates on different media.

Different isolates showed different growth in the four types of media used. Isolates MM6, MM8 and MM17 recorded higher mean score values in malt extract agar. Isolate MM6 and isolates MM4MM8 and MM15 had the highest mean scores in Sabourand dextrose agar and potato dextrose agar respectively. Potato glucose agar had the highest number of isolates with high mean scores compared to the other three types of media. These included isolates MM1, MM4, MM5, MM6, MM7, MM15, MM16, MM17, MM18 and MM22. A one-way ANOVA was conducted to compare the effect of the different media on growth of fungal isolates. Results show that there was a significant difference in growth among the four types of media at 5% level of significance, [$F_{(3, 84)}$ = 4.258, p = 0.008]. Post hoc comparisons using the Tukey HSD test showed that PGA (M = 68.21, SD = 15.77) was significantly different from SDA (M = 47.71, SD = 19.02) and PDA (M = 51.03, SD = 23.82) **(Table 6.15)**.

Table 6.15: Descriptive statistics of the four types of media at Lake Magadi

Media	N	Minimum	Maximum	Mean	Std. Deviation
MEA	22	19.00	82.67	55.06	22.18
PDA	22	17.00	82.67	51.03	23.82
SDA	22	15.00	82.67	47.71	19.02
PGA	22	32.67	82.67	68.21	15.77

6.13.4.2.4 Growth of fungal isolates at pH 5.0, 7.0, 8.5 and 10

Although the isolates were from an alkaline environment, all showed growth at all the tested pH values including acidic pH 5 and neutral pH. Results from a one way ANOVA showed that there was a significant difference in growth among the four pH levels (**Table 6.16**) at 5 % level of significance, [$F_{(3, 84)} = 6.908$, $p = <0.0001$]. Post hoc comparisons using the Tukey HSD test showed that pH 5.0 (M = 33.44, SD = 20.81) was significantly different from pH 8.5 (M = 53.06, SD = 22.02) and pH 10.0 (M = 54.79, SD = 23.63). In addition, the mean of pH 7.0 (M = 32.97, SD = 18.81) were found to be significantly different from that of pH 8.5 (M = 53.06, SD = 22.02) and pH 10.0 (M = 54.79, SD = 23.63).

Table 6.16: Descriptive statistics of the different pH levels

pH	N	Minimum	Maximum	Mean	Std. Deviation
pH 5.0	22	12.67	81.67	33.44	20.81
pH 7.0	22	12.67	79.67	32.97	18.81
pH 8.5	22	18.67	82.00	53.06	22.02
pH 10.0	22	18.67	82.00	54.79	23.63

6.13.4.3 The hot spring at Lake Elmenteita

6.13.4.3.1 Growth of fungal isolates at various temperature levels

Results from the one way ANOVA conducted to compare the effect of temperature on growth of the isolates show that there was significant difference in growth among the four temperature levels at 5% level of significance, [$F_{(3, 84)}$ = 22.550, $p = < 0.0001$]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for the 40 °C level (M = 2.9541, SD = 6.64544) was significantly different from the 25 °C, 30 °C and 35 °C.

Table 6.17: Descriptive statistics of the fungal isolates at different temperatures

Temperature	N	Min	Max	Mean	SD
25 °C	22	17.00	76.67	41.8791	20.23745
30 °C	22	15.00	80.00	47.0764	26.21493
35 °C	22	15.00	79.67	42.9855	22.81754
40 °C	22	0.00	23.00	2.9541	6.64544

6.13.4.3.2 Growth of fungal isolates on different media.

A one-way ANOVA was conducted to compare the effect of growth of the fungal isolates on PGA, SDA, PDA and MEA media. Results show that there was a significant difference in growth among the four types of media at 5 % level of significance, [$F_{(3, 84)}$ = 5.876, $p = 0.001$]. Post hoc comparisons using the Tukey HSD test showed that PGA (M = 66.3032, SD = 15.30128) was significantly different from the rest **(Table 6.18)**

Table 6.18: Descriptive statistics of the different types of media

Media	N	Min	Max	Mean	SD
MEA	22	18.67	80.33	48.2114	22.03947
PDA	22	17.00	80.67	47.1214	22.18761
SDA	22	15.00	70.00	44.6209	16.35913
PGA	22	32.67	82.67	66.3032	15.30128

6.13.4.3.3 Growth at pH 5.0, 7.0, 8.5 and 10

A one-way ANOVA was conducted to compare the effect of pH on growth of the fungal isolates. Various pH levels namely: pH 5.0, pH 7.0, pH 8.5 and pH 10 were used. The results indicated that there was no significant effect of the different pH values **(Table 6.19)** on the growth of the fungal isolates at 5% level of significance, [F (3, 84) = 1.408, p = 0.246].

Table 6.19: Descriptive statistics of the four different pH values at Lake Elmenteita

pH	N	Min	Max	Mean	SD
pH 5.0	22	17.00	65.00	42.2568	19.88098
pH 7.0	22	18.33	75.33	48.6214	22.50282
pH 8.5	22	22.33	77.00	51.2882	21.24601
pH 10	22	23.00	79.67	55.2114	22.35526

6.13.4.3.4 Growth at different sodium chloride concentrations

A one-way ANOVA was conducted to compare the effect of the different salinity levels on growth of fungal isolates. Results show that there was a significant difference in growth among the four salinity levels at 5% level of significance, [F (3, 84) = 13.981, p = < 0.0001]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for the 5% level and 10% level were significantly different from 20% and 30% levels.

However, 5% level and 10% level were not significantly different just like 20% and 30% levels **(Table 6.20).**

Table 6.20: Descriptive statistics of the different sodium chloride concentrations

Salinity	N	Min	Max	Mean	SD
5%	22	19.00	81.33	50.2582	19.47469
10%	22	17.00	81.33	42.2573	18.62166
20%	22	12.67	71.67	28.3936	12.23307
30%	22	10.00	64.00	22.3482	12.04764

Table 6.21: Impact of culture media and salinity on the growth of fungi isolated from a hot spring at Lake Elmenteita. The values represent the mean in millimeters ± standard errors of the diameter for the various isolates.

Isolates	Salinity				Media			
	5%	10%	20%	30%	MEA	PDA	SDA	PGA
EL1	25.33±0.88	19±1.15	12.67±0.88	10±0.58	57±1.45	60.67±1.20	59.33±1.76	79.67±0.88
EL2	39.67±0.88	32.67±2.33	18.67	15±1.53	64.33±0.88	57±4.7	62.33±2.73	79.33±1.20
EL3	47.67±7.96	50±8.08	30.33±7.96	19±1.15	78.33±1.67	66±8.08.	58.67±3.71	80±1.52
EL4	60.67±0.88	44.33±2.33	44.33±2.33	20.67±1.86	19.67±1.2	24.33±0.33	30.33±0.88	44.33±2.33
EL5	75.33±0.88	63.67±1.45	22±1	15.33±0.88	80.33±0.33	80.67±1.20	70±2.08	82.67±0.88
EL6	51±3.21	37±0.58	27.33±2.18	25±1.15	44.33±2.33	20.67±1.86	44.33±2.33	60.67±0.88
EL7	74±1.73	66.67±1.20	24±1.73	20.33±1.86	37±0.58	27.33±2.19	25±1.15	51±3.21
EL8	24±1.73	22±1	17.66±1.20	17.67±0.88	47.67±7.97	30.33±7.97	19±1.15	50±8.08
EL9	28±1	24.33±0.33	21.33±1.45	16.33±1.20	39.67±0.88	18.67±1.45	15±1.53	32.67±2.33
EL10	39.67±0.88	32.67±2.33	18.67±1.45	15±1.53	39.33±1.45	38.67±3.76	27.67±3.53	55.33±3.17
EL11	44.33±2.33	40.67±1.76	29.33±2.90	26.33±1.76	40.67±1.76	26.33±1.76	29.33±2.91	44.33±2.33
EL12	74.67±1.20	54.33±1.45	26.33±1.45	15.33±0.88	19±1.15	17±1.53	32.67±2.33	47.67±7.96
EL13	53.67±1.45	43.33±2.02	27.33±0.66	16.67±1.45	79.33±0.67	66±1.53	56.33±1.45	72±0.57
EL14	78.33±1.20	81.33±0.88	71.67±5.89	64.±1.53	70±5.77	71.33±4.41	66.67±1.20	80.33±1.20
EL15	19±1.15	17±1.52	32.66±2.33	47.67±7.96	80.33±1.45	76.67±0.67	62±2.08	80.67±1.45
EL16	48.67±1.86	38±0.58	27.66±1.85	24±1.53	47.33±1.45	72±0.58	51±3.21	79±0.57
EL17	81.33±0.88	51±0.58	27.66±1.20	18.67±1.2	78.33±1.2	71.67±5.90	64±1.53	81.33±0.88
EL18	28±1	22±1	18.66±1.45	13.33±1.2	20.67±1.86	26.33±1.76	44.33±2.33	66±1.52
EL19	39.67±0.88	24.33±0.33	29.33±2.91	17.±1.6	27.33±2.19	29.33±2.91	37±0.58	71.33±4.40
EL20	44.33±2.33	32.67±2.33	26.33±1.45	20.67±1.20	30.33±7.97	32.67±2.33	47.67±7.97	76.67±0.67
EL21	74.67±1.20	78.33±1.2	27.33±0.66	24.33±0.33	18.67±1.45	56.33±1.45	39.67±0.88	72±0.57
EL22	53.67±1.45	54.33±1.45	43.33±2.02	29.33±2.03	38.67±3.76	66.67±1.20	39.33±1.45	71.67±5.89

a) Letters designate significant differences at P ≤ 0.05. b) Means followed by the same letter are not significantly different. c) EL represents Lake Elmenteita

Table 6.22: Influence of pH and temperature on the growth of fungi isolated from a hot spring at Lake Elmenteita. The values represent the mean in millimeters ± standard errors of the diameter for the various isolates.

Isolates	Ph				Temperature			
	pH 5.0	pH 7.0	pH 8.5	pH 10.0	25 °C	30 °C	35 °C	40 °C
EL1	58.33±1.2	65.67±2.40	77±0.58	78.33±1.45	33±1.2	67.67±0.88	34.67±2.19	0±0
EL2	64.33±1.67	66±2.31	70.33±2.40	69.33±1.2	72±0.57	68±1.53	67±0.58	0±0
EL3	57.67±2.02	59±2.31	67.67±1.2	79.67±0.88	66±1.73	71±1.55	68±1.53	0±0
EL4	57.33±1.67	58.66±1.45	66.67±0.67	69.33±1.2	52.67±2.02	66±1.53	56.33±1.45	0±0
EL5	64±1.45	59.66±2.72	70.33±1.45	74.33±3.84	59.33±4.2	80±1.55	70.67±1.86	0±0
EL6	26.67±1.20	25.67±2.18	23±1.15	28.33±1.76	37±0.5	27.33±2.19	25±1.15	0±0
EL7	64±2.08	67±7.15	72.33±3.53	79±0.58	71.33±1.2	76.67±0.88	74.33±2.84	23±1.15
EL8	17.33±1.20	20.66±1.20	27.66±2.35	29±0.58	29±1.15	20.33±0.88	15.33±0.88	0±0
EL9	65±2.08	70.66±2.02	73.66±2.18	69.33±0.89	22±1.0	75.33±0.88	63.67±1.45	15.33±0.88
EL10	64.33±2.84	68.66±1.45	71.33±0.88	73.33±3.38	61.33±2.9	75.67±2.40	72.67±1.2	0±0
EL11	17.33±1.76	19.33±0.88	22.67±0.33	29±0.58	39.67±0.88	18.67±1.45	15±1.52	0±0
EL12	22±1.0	23±1.15	24.33±1.67	23±0.58	76.67±0.88	26.67±1.20	23±1.15	0±0
EL13	25.67±2.18	24.66±0.88	27.33±1.67	29±1.53	17±1.15	24.67±1.45	15.67±0.88	0±0
EL14	64±3.51	70±1.15	56.66±1.71	64±1.73	51.67±2.33	77±0.58	79.67±0.88	13.33±1.2
EL15	17±1.53	18.33±0.88	24±0.58	23.33±0.88	44.33±2.33	20.67±1.85	44.33±2.33	0±0
EL16	17±1.53	20.33±0.66	22.33±0.88	24.33±1.76	19±1.15	17±1.53	32.67±2.33	0±0
EL17	24±2.08	32±1.53	27.67±2.60	29.33±1.45	18.67±1.45	33.33±1.2	37±1.15	0±0
EL18	29.33±1.20	71.33±0.88	57.33±1.85	68.67±1.2	17±1.15	15.33±0.88	17±1.15	0±0
EL19	30.33±1.20	21±1.15	56.67±1.76	61.67±2.19	51.67±2.33	63.67±1.45	51.67±2.33	13.33±1.66
EL20	56.33±1.86	75.33±0.88	59±1.15	68.67±1.45	44.33±2.33	72.67±1.2	44.33±2.33	0±0
EL21	30.33±1.33	64.33±1.76	58.66±0.88	69.67±2.03	19±1.15	15±1.52	19±1.15	0±0
EL22	56.67±1.2	65.33±1.45	71.66±3.29	74±3.06	18.67±1.45	23±1.15	18.67±1.45	0±0

a) Letters designate significant differences at P ≤ 0.05.b) Means followed by the same letter are not significantly different. c) EL represents Lake Elmenteita

6.13.5 Screening the isolates for production of extracellular enzymes

6.13.5.1 The hot spring at Lake Magadi

All the isolates apart from B3, B4, B17, B21, B23, B31 and B41 were positive for the amylase test. Only isolates B21, B23, B31, B41 and B61 gave negative results for esterase test. Five out of the 17 isolates tested were negative for lipolytic activity while 6 isolates gave positive results for protease test **(Figure 6.5)**. Nine isolates were positive for xylanases. All the 17 isolates tested negative for the production of cellulase enzyme as shown in **Table 6.23** below with + and - signs for positive and no production of the enzyme tested respectively.
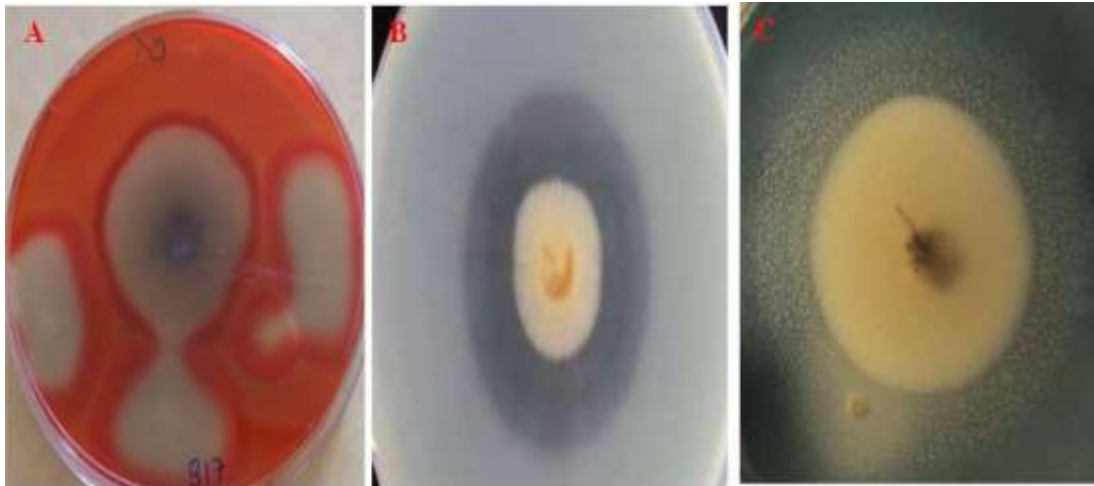
Figure 6.5: (A) shows positive results for xylan utilization in isolate B17, (B) Isolate B4 showing production of protease enzyme and (C) isolate B1 showing precipitates of calcium salts around the colony indicating esterase production

Table 6.23: Biochemical characterization of the 17 isolates from Lake Bogoria

Isolate	Amylases	Esterases	Lipases	Xylanases	Proteases	Cellulases
B1	+	+	-	-	-	-
B3	-	+	+	-	+	-
B4	-	+	+	+	+	-
B5	+	+	+	-	-	-
B17	-	+	+	+	-	-
B21	-	-	-	-	+	-
B23	-	-	+	+	-	-
B24	+	+	+	+	+	-
B25	+	+	-	+	-	-
B31	-	-	+	-	-	-
B41	-	-	+	-	-	-
B52	+	+	-	+	-	-
B54	+	+	+	+	-	-
B61	+	-	+	-	-	-
B62	+	+	+	+	-	-
B63	+	+	+	-	+	-
B64	+	+	-	+	+	-

6.13.5.2 The hot spring at Lake Magadi

Ten (10) isolates out of the 22 isolates tested negative for lipolytic activity while 5 isolates gave negative results for protease test. All the isolates apart from isolates MM2, MM4, MM5 and MM6 were positive for amylase test. Only isolates MM2 and MM6 gave negative results for esterase test. Isolates MM1, MM6 and MM22 were negative for xylanases while the rest of the isolates were positive. All the 22 isolates tested negative for the production of cellulases enzyme **(Table 6.24)**

Table 6.24: Biochemical characterization of the 22 isolates from Lake Magadi

Isolates	Amylases	Esterases	Lipases	proteases	Xylanases	cellulases
MM1	+	+	+	+	-	-
MM2	-	-	-	-	+	-
MM3	+	+	+	+	+	-
MM4	-	+	+	-	+	-
MM5	-	+	-	+	+	-
MM6	-	-	-	-	-	-
MM7	+	+	-	+	+	-
MM8	+	+	+	+	+	-
MM9	+	+	-	+	+	-
MM10	+	+	+	+	+	-
MM11	+	+	+	+	+	-
MM12	+	+	+	+	+	-
MM13	+	+	-	-	+	-
MM14	+	+	-	+	+	-
MM15	+	+	+	+	+	-
MM16	+	+	-	+	+	-
MM17	+	+	-	+	+	-
MM18	+	+	+	-	+	-
MM19	+	+	+	+	+	-
MM20	+	+	+	+	+	-
MM21	+	+	+	+	+	-
MM22	+	+	-	+	-	-

6.13.5.3 The hot spring at Lake Elmenteita

All the 22 isolates from the hot spring at Lake Elmenteita tested negative for the production of cellulase enzyme. EL2, EL10, EL19, EL20, EL, EL and EL21 gave negative results for amylase enzyme. All the isolates apart from isolates EL2 and EL6 were positive for esterase test. Five and ten out of the 22 isolates tested negative for proteolytic and lipolytic activity respectively. Isolates EL1, EL2, EL8, EL9 and EL21 were negative for xylanases (**Table 6.25**).

Table 6.25: Biochemical characterization of the 22 isolates from Lake Elmenteita

Isolates	Amylases	Esterases	Lipases	proteases	Xylanases	Cellulases
EL1	+	+	+	+	-	-
EL2	-	+	+	-	-	-
EL3	+	+	+	+	+	-
EL4	+	+	+	+	+	-
EL5	+	+	+	+	+	-
EL6	+	+	-	+	+	-
EL7	+	+	+	+	+	-
EL8	+	+	+	+	-	-
EL9	+	+	+	+	-	-
EL10	-	-	-	-	+	-
EL11	+	+	+	+	+	-
EL12	+	+	-	-	+	-
EL13	+	+	-	+	+	-
EL14	+	+	+	+	+	-
EL15	+	+	-	+	+	-
EL16	+	+	-	+	+	-
EL17	+	+	+	+	+	-
EL18	+	+	-	+	+	-
EL19	-	+	+	-	+	-
EL20	-	+	-	+	+	-
EL21	-	-	-	-	-	-
EL22	+	+	-	+	+	-

6.13.6 Molecular characterization of fungal isolates

6.13.6.1 Phylogenetic analysis of sequences

The BLASTn search results showed that all the isolates belonged to the fungal domain. The isolates (90 %) were affiliated to the phylum *Ascomycota* and this suggested that diverse groups of fungi in this phylum have the potential to adapt to extremophilic conditions of the hot springs. Isolates B4, B21, B31, B41 and B63, closely clustered with members of the genus *Aspergillus* including *Aspergillus versicolor* and *Aspergillus*

fumigatus both at 99 % similarity while *Aspergillus flavus* was at 97 % similarity. Isolate B17 was affiliated to *Penicillium pinophilum* (with sequence identity of 99 %), while isolate B61 clustered closely with members of the genus *Alternaria*. The phylum *Basidiomycota* was represented by a single filamentous fungi (Isolate B1) of the genus *Trametes* and family *Polyporaceae* (**Figure 6.6**).

From the hot spring at Lake Elmenteita seven (7) isolates namely EL7, EL10, EL11, EL12, EL13 EL14 and EL20 clustered closely with members of the genus *Aspergillus*. Among these were *Aspergillus fumigatus*, *Aspergillus flavus and Aspergillus* sp. Isolates EL6, EL18, EL21 and EL22 were closely related to members of the genus *Penicilllium*. These included *Penicillium sizovae, Penicillium chrysogenum* and *Penicillium pinophilum*. The genus *Alternaria* was represented by five isolates namely EL5, EL8, EL9, EL16 and EL19. The four clustered closely with members of *Alternaria tenuissima* and *Alternaria* sp*.* Isolate EL17 is the only isolate that clustered closely with members of *Debaryomyces hansenii.* All the isolates from the hot spring at Lake Elmenteita were affiliated to the phylum *Ascomycota* (**Figure 6.7**).

Half of the isolates (MM3, MM5, MM6, MM8, MM9, MM11, MM16, MM18, MM19, MM21 and MM22) from the hot spring on the shores of Lake Magadi clustered closely with members of the genus *Aspergillus*. These members were *Aspergillus flavus, Aspergillus oryzae, Aspergillus sp.* and *Aspergillus tamarii.* The genera *Neurospora* and *Penicillium* were represented by three isolates each namely MM4, MM12 and MM15 for *Neurospora* and MM10, MM13 and MM14 for *Penicillium* while the genus *Beauveria* had two isolates (MM2 and MM17) affiliated to it. One isolate MM1 clustered closely with members of *Tetrapisispora fleetii* while MM7 was closer to *Parmastomyces transmutans*. Apart from *Parmastomyces transmutans* that belongs to the Phylum *Basidiomycota*, the rest of the isolates were affiliated the *Ascomycota* Phylum (**Figure 6.8**).
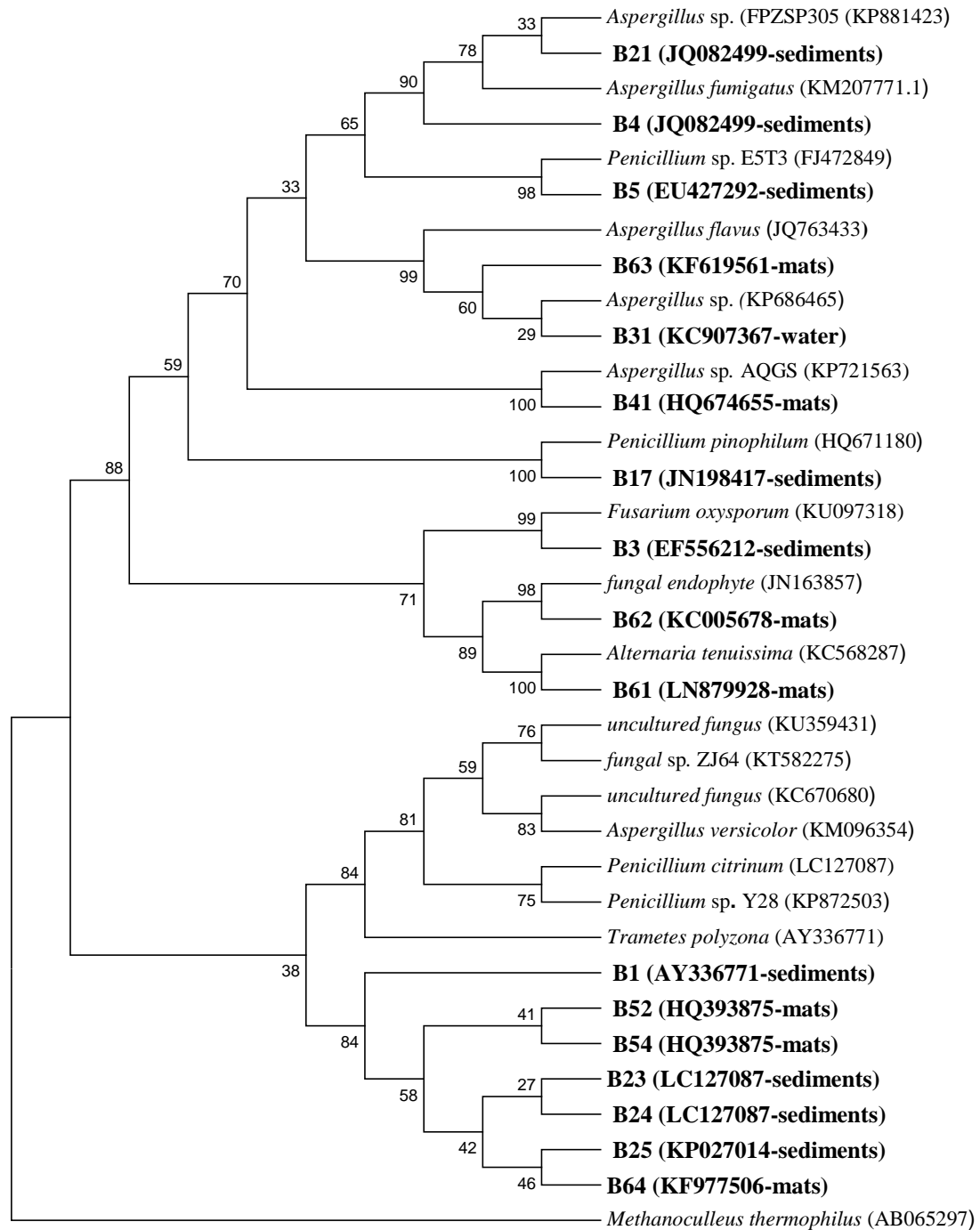
Figure 6.6: Phylogenetic analysis by Maximum Likelihood method of the isolates from Lake Bogoria. Species names are followed by the GenBank accession numbers while the isolates are followed by the accession numbers and the source of isolation.
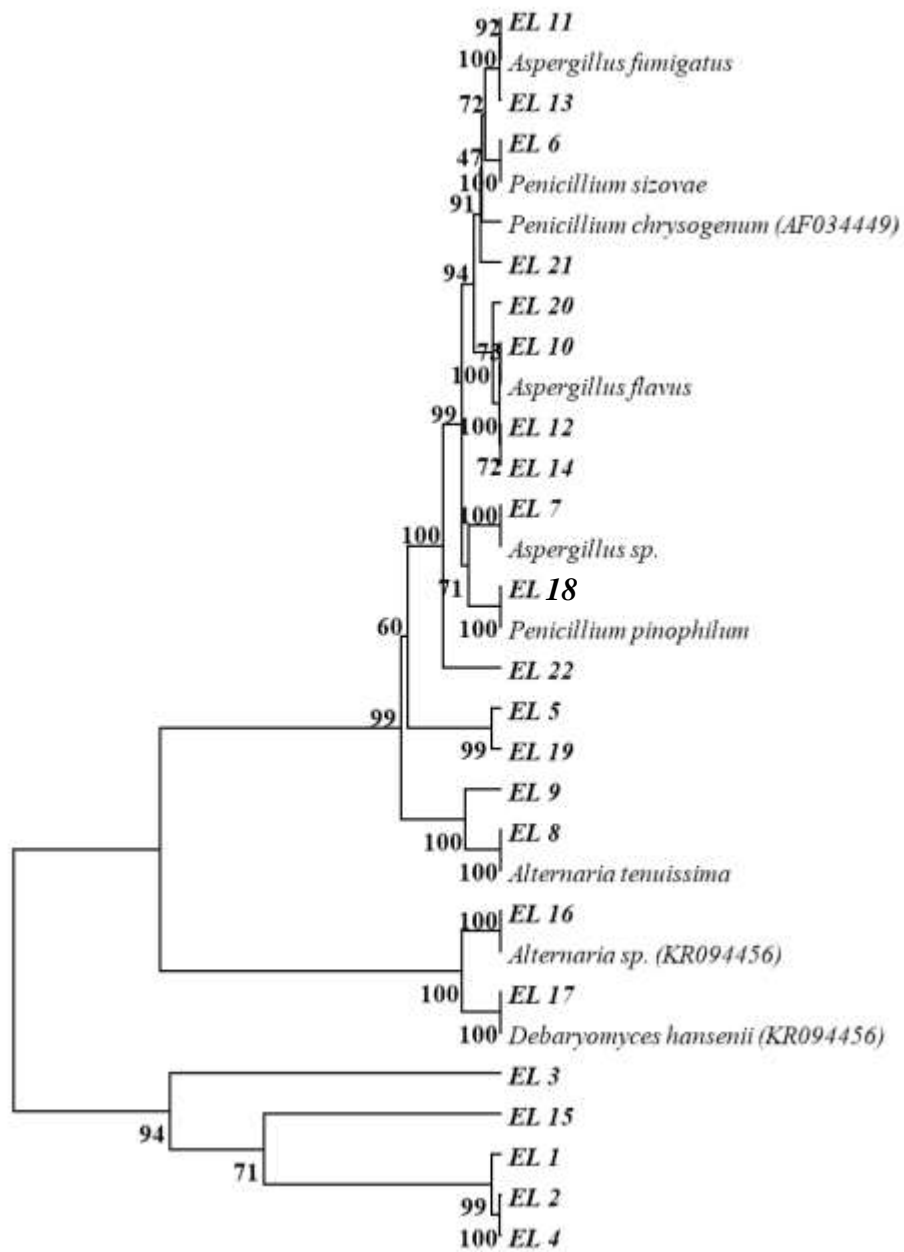
Figure 6.7: Phylogenetic analysis by Maximum Likelihood method of the isolates from Lake Elmenteita. Species names are followed by the GenBank accession numbers.
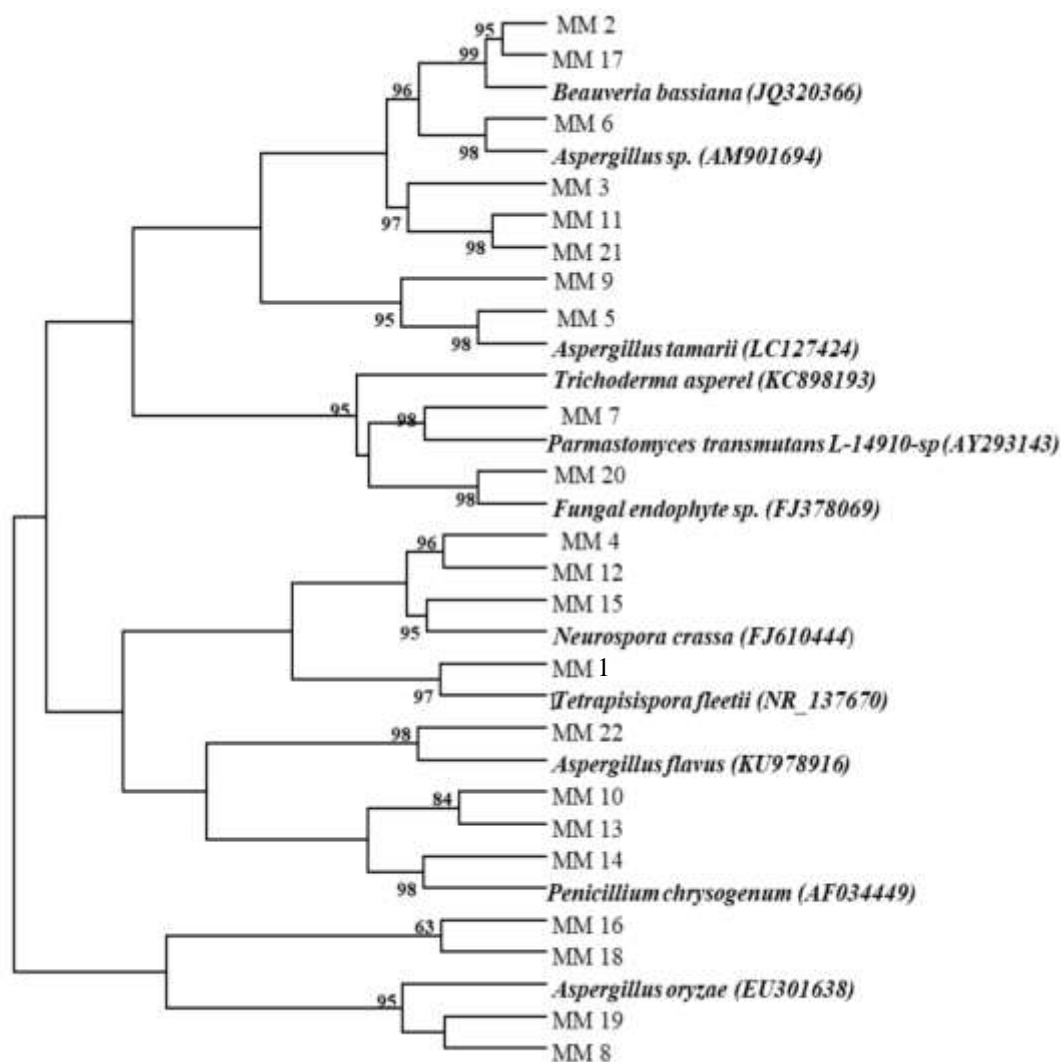
Figure 6.8: Phylogenetic analysis by Maximum Likelihood method of the isolates from Lake Magadi. Species names are followed by the GenBank accession numbers

6.14 Discussion

The aim of this study was to isolate, characterize and identify fungi from hot springs on the shores of three soda lakes (Lake Bogoria, Lake Elmenteita and Lake Magadi) in Kenya using culture-dependent methods and identify the enzymes produced by the isolated fungi. Although culture-dependent methods generally recover only a small portion of the

diversity from environments, they are still a critical component in research (Malaviya & Rathore, 2007).

From this study, morphological observations indicated that different species of fungi were isolated from the hot spring. The isolates exhibited different colours and the spores observed were anamorphic with different shapes and structures belonging to the sub-division of *Ascomycotina* and *Basidiomycotina*. Cellular morphology showed long, branching filamentous (**Figure 6.3**) hyphae (Madigan *et al.,* 2005). According to classical mycology, most species of fungi have been described based on their morphological features such as ascospore morphology, colour, odour, and other organoleptic characteristics (Barseghyan & Wasser, 2010). Toledo et al. (2013) used morphological and molecular characteristics of fungal isolates to identify a fungus of *Hirsutella sp.* from plant hoppers. A recent study conducted in Kashmir (Bandh *et al.,* 2011) reported the identification of five species of *Penicillium* isolated from the water samples obtained from a fresh water lake by use of traditional approach of morphological observation and culture media.

As compared to other microorganisms like bacteria and archaea, fungi are capable of growing and developing over a wide range of pH. Several species of fungi can actively adjust the pH of their environment by secreting acids or bases (Landraud *et al.,* 2013; Vylkova *et al.,* 2017). Swe et al. (2009), affirmed that higher alkalinity in media lowers the colony counts per ml and that although certain alkaline medium (pH 8.0 & 9.0) favour the spore formation of certain fungi species like *Aspergillus parasiticus*, pH values higher than 10 tend to hinder their sporulation.

Physiochemical characterization of the isolates showed that the highest growth was observed at pH 10. However, the isolates were able to grow at pH 5, which suggests that they are also acido-tolerant (Moreira & Siqueira, 2002)**.** This growth at pH range of 5 to 10 is consistent with the earlier study of Horikoshi, (1998) which showed that a low to high pH range of 5.7 to 9.0, favours growth of alkaliphiles and that a pH range of 9.0 to 10 may

serve as their selective optimum pH. Another study done by Jaouani *et al.* (2014) found that all isolates obtained from ashes collected from Sebkha El Melah, a Saharan salt flat located in southern Tunisia were considered as alkali-halotolerant since they were able to grow in media containing 10% of NaCl with an initial pH 10. Therefore, regarding the stress of pH, the capacity of the majority of isolates to grow at pH 10 implies that some habitats along the hot spring may have a higher pH and secondly, that fungi can tolerate a wide pH range. It also suggests adaptation of the fungi to the alkali environments as is characteristic of alkalophilic fungi (Horikoshi, 1999).

Culture media is an essential growth factor for controlling the growth and sporulation of fungi. Different fungal species can grow on different media, which may be related to the preference of the microorganisms for some nutrients in the different media (Barreto *et al.,* 2011). The composition of a particular medium plays a great role in the growth and sporulation of fungi. Zhao and Shamoun, (2006) suggested that culture media significantly affected the growth, sporulation and conidial discharge in fungi. Results from the one way variance showed that PGA with a minimum diameter of 32.67mm and a maximum of 82.67mm with a mean of 64.7647mm at the hot spring at Lake Bogoria **(Table 6.9)**, 68.21mm at the hot spring at Lake Magadi **(Table 6.15)** and 66.3032 at Lake Elmenteita **(Table 6.18)** was significantly different from the other three types of media (MEA, PDA and SDA). The data obtained from this study revealed that maximum mycelial growth was observed on PGA followed by MEA. On the contrary, Jain (2001) studied the effect of four culture media on filamentous fungal growth and found that SDA medium showed maximum growth and sporulation of all fungi. Also several workers investigating the influence of culture media on growth, colony character and sporulation of fungi stated that PDA is the best media for mycelial growth (Saha *et al.,* 2008). Another study by Sharma and Pandey, (2010) on the influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes revealed that culture media differentially influenced the growth, colony characteristics and sporulation of fungi. They also concluded that a combination of two or more media was more appropriate for routine cultural and morphological characterization of fungi to observe different colony features.

On the contrary, Bandh *et al.* (2012) identified five Aspergillus species by using differential media like CYA and MEA and these demonstrated that it was a simple and reliable technique for identification of *Aspergillus* species.

From this study the optimal temperature for conidial germination of the fungal isolates was approximately 25 °C, with an upper limit at 35 °C, though some grew at temperatures above 35 °C **(Table 6.5 & Table 6.22)**. Studies have also shown that filamentous fungi may experience elevated temperatures throughout their growth period (Kalsbeek *et al.,* 2001).

Most of the fungi isolated in this study were able to produce extracellular enzymes such as amylases, lipases, proteases, esterases and xylanases **(Table 6.23-6.25)**. The stability of these enzymes at alkaline pH is attributed to their habitat (alkaline lake) and growth profile in a wide range of pH. This is a feature that confirms their role in the decomposition of organic matter in these habitats (Kieser *et al*., 2000). Alkaline proteases, chitinases, amylases, lipases and caseinases have also been reported in a wide range of microorganisms isolated from soda lake environments, such as Rift valley soda lakes (Joshi *et al.*, 2008).

In this study, all isolates were sensitive to salt and generally their growth rates were clearly inhibited with increasing concentrations of NaCl (salt concentrations) although the mycelium remained viable. Only a small number of isolates were able to grow at higher NaCl concentrations. It could be possible that Na^+ is poorly taken up by fungi, and could have caused alkaline stress to the fungi inhibiting their growth at high salt concentrations (Qin *et al.,* 2017). This is consistent with earlier data by Maciá-Vicente *et al.* (2012) that fungi isolated from halophytes are more likely halo tolerant but not halophilic.

Phylogenetic analysis of 18S rDNA gene sequences from the hot spring at Lake Bogoria placed most of the fungal isolates in the phylum *Ascomycota* of the genera *Aspergillus* (29.1 %)*, Penicillium* (11.7 %)*, Alternaria* (6 %) and *Fusarium* (6 %)**.** While the phylum

Basidiomycota was represented by a single member of the genus *Trametes* belonging to family *Polyporaceae* (**Figure 6.6**). Jaouani *et al.* (2014) isolated fungi belonging to the genera *Cladosporium, Alternaria, Aspergillus, Penicillium, Ulocladium, Engyodontium* and *Cladosporium cladosporioides* that were able to grow in media containing 10% of salt with an initial pH 10 from Sebkha El Melah, a Saharan Salt Flat in Southern Tunisia. Also, Damare *et al.* (2006) showed that genera *Penicillium*, *Aspergillus* and *Cladosporium* are the most abundant fungal species in aquatic environments. According to studies by Christensen *et al.* (2000) and Asan, (2004) the species of *Aspergillus* and *Penicillium* are among the most abundant and widely distributed microfungi in nature.

From the hot springs at Lake Elmenteita and Lake Magadi the fungal isolates were closely affiliated to the phylum *Ascomycota* with only one isolate MM7, *Parmastomyces transmutans,* clustering closely with members of the Phylum *Basidiomycota* (**Figure 6.7& 6.8**). Mohamed and Martiny (2011), in their study on patterns of fungal diversity and composition along a salinity gradient, recovered more *Ascomycota* (78%), fewer *Basidiomycota* (6%) and a greater number of basal fungal lineages (16%), including *Glomeromycota* and *Chytridiomycota*.

Isolates EL7, EL10, EL11, EL12, EL13, EL14 and EL20 from Lake Elmenteita (**Figure 6.7**) and isolates MM3, MM5, MM6, MM8, MM9, MM11, MM16, MM18, MM19, MM21 and MM22 from the hot spring at Lake Magadi (**Figure 6.8**) clustered closely with the genus *Aspergillus*. Members here included *Aspergillus fumigatus, Aspergillus flavus, Aspergillus oryzae, Aspergillus tamarii* and *Aspergillus* sp. Aspergillus are very cosmopolitan spore-forming genus of fungi. It is a large genus composed of more than 180 accepted anamorphic species, with teleomorphs described in 9 different genera (Pitt and Samson, 2000). According to studies done by Klich, (2002) and Asan, (2004) the species of *Aspergillus* and *Penicillium* are among the most abundant and widely distributed microfungi in nature.

Isolates MM10, MM13 and MM14 and isolates EL6, EL18, EL21 and EL22 from Lake Magadi and Lake Elmenteita respectively were closely related to members of the genus *Penicillium.* This is consistent with previous studies of hyper saline environments, which have showed that genuses of *Penicillium*, *Aspergillus* and *Cladosporium* are the most abundant fungal species in such environments and also spores of *Cladosporium* have often been recovered in aquatic environments (Damare *et al.,* 2006).

Three isolates (MM4, MM12 and MM15) from Lake Magadi were related closely to *Neurospora crassa*. *Neurospora crassa* is a filamentous ascomycete, the red bread mold that reproduces by spores just like all fungi. It is particularly well suited for genetic studies since it can be grown quickly on simple culture medium. It spends most of its life cycle in the haploid condition so any recessive mutations will show up in its phenotype (Galagan *et al.,* 2003). It is important in epigenetics and gene silencing, cell polarity, cell fusion, development, as well as many aspects of cell biology and biochemistry. *Neurospora* is considered a model organism (Davis and Perkins 2002; Aramayo *et al*., 2013) because its characteristics make it ideal for scientific research.

Isolate MM1 clustered closely with *Tetrapisispora fleetii* and MM2 and MM17 with *Beauveria bassiana*. *Tetrapisispora fleetii* is an ascomycetous yeast, a member of the *Saccharomycetaceae,* which was first isolated in a food processing plant in Georgia, USA (Kurtzman *et al.,* 2014). These species play a major role in the food and beverage industry. *Beauveria bassiana,* from the phylum *Ascomycota*, class *Sordariomycetes* is a fungus that grows naturally in soils (Barbarin *et al.,* 2012). *Beauveria bassiana* can be used as a biological insecticide to control a number of pests such as termites, whiteflies (Rehner & Buckley, 2005).

The genus *Alternaria* was represented by five isolates namely EL5, EL8, EL9, EL16 and EL19. From Lake Elmenteita. The four clustered closely with members of *Alternaria tenuissima* and *Alternaria* sp. *Alternaria tenuissima, Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. are moderate and weak alkalitolerant fungi that were previously reported

to grow at neutral or below neutral pH values. These species have previously appeared in existing studies on the alkalitolerant and halotolerant fungi in soda lakes (Kladwang *et al*., 2003; Gunde-Cimerman *et al*., 2009). Since they are also known to inhabit neutral soils worldwide, they have therefore been considered as transition species in the alkaline environments.

6.15 Conclusion

Results showed that culturing recovered a small proportion of the microorganisms from the hot springs. Although isolation and cultivation of microorganisms are essential for studying the physiological and metabolic characteristics of individual microbes, majority of microorganisms from natural environments like hot springs cannot be grown in the laboratory. It is therefore important to combine culture dependent and independent techniques so as to increase the recovery of fungi from these extreme environments. On the other hand, the major advantage of culture dependent over the modern molecular techniques (culture independent) lies in that it provides the researcher with the microbial "material" that can be used in further studies. The findings also revealed that culture media differentially influenced the growth, colony character and sporulation of the isolated fungi. Out of the four test media employed in the present study, PGA was found to be most suitable growth media followed by MEA and PDA respectively.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

The biogeography of microorganisms remains poorly studied and this limits the understanding of how microbial diversity changes in relation to environmental parameters like pH, salinity and temperature. Distant and isolated habitats are expected to have a specific diversity highly confined to that habitat, whereas geographically closer habitats have higher proportion of shared species with very few organisms as reported by Bahram *et al.* (2013) and Hahn *et al.* (2015). However, microorganisms, are considered to be less confined by geographical barriers due to more efficient dispersal capacities (Chong *et al.*, 2015), that enable both the remote and isolated habitats to constantly exchange with both near and distant habitats. In this study, wet sediments, microbial mats and water samples were collected from hot springs of soda lakes that were geographically apart with varying environmental conditions in terms of temperature, salinity and pH. The harsh conditions associated with these lakes prevent survival of most microorganisms not adapted to extreme conditions like mesophiles (Convey *et al.*, 2014); but allow diverse microbial life to thrive and dominate (Vincent & Quesada, 2012). The diversity and biogeography of fungal communities within four soda lakes in the Kenyan Rift Valley were analysed using both culture dependent and independent techniques.

Taxonomic classification of sequences obtained from 454 pyro sequencing platform categorized the fungi into four (4) phyla namely *Ascomycota*, *Basidiomycota*, *Glomeromycota* and *unclassified fungi* phylum. The most predominant fungal genera included *Aureobasidium*, *Auxarthron, Aspergillus*, *Cladosporium, Debaryomyces, Epicoccum*, *Malassezia, Penicillium, Rhodotorula, Termitomyces Tremella and Westerdykella*. The common fungal species were *Aspergillus oryzae, Aureobasidium pullulans, Cladosporium* sp*., Cladosporium cladosporioides, Dioszegia hungarica,*

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Malassezia globosa, Pleosporales sp., *Termitomyces* sp., *Tremella aurantialba* and *Tremella encephala*.

In culture dependent studies on the three soda lakes' hot springs (Bogoria, Elmenteita and Magadi), taxonomic classification of sequences obtained from Sanger sequencing platform grouped the fungi into two (2) phyla namely *Ascomycota* and *Basidiomycota*. Within the hot spring at Lake Bogoria the dominant genera were *Alternaria, Aspergillus, Penicillium, Fusarium* and *Trametes*. These findings revealed presence of moderate and weak alkalitolerant fungi that have been previously reported to grow in aquatic environments and in media containing 10% of salt with an initial pH 10 (Grum-Grzhimaylo *et al.,* 2016).

In Lake Elmenteita the dominant genera included *Aspergillus, Penicillium, Alternaria* and *Debaryomyces* all from the phylum *Ascomycota*. *Alternaria alternata, Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. are moderate and weak alkalitolerant fungi that have been previously reported (Grum-Grzhimaylo *et al.,* 2013) to grow in optimally neutral or below neutral pH values.

Debaryomyces hansenii was the only yeast detected from the hot spring. It is an osmotolerant, halo tolerant and xerotolerant yeast that produce toxins and can survive at a pH range between 4.7 and 6.0 (Safaa *et al.,* 2017). These species has been demonstrated to synthesize useful quantities of D-arabinitol, riboflavin, xylitol, and pyruvic acid under thiamine limitation and can be cultivated in media with up to 25% NaCl (Breuer & Harms, 2006).

Lake Magadi was dominated by the Phylum *Ascomycota,* with 50% of the isolates belonging to the genus *Aspergillus*. Other genera included *Penicillium*, *Beauveria*, *Tetrapisispora* and *Neurospora*. One isolate was closely related to *Tetrapisispora fleetii*, an ascomycetous yeast.

However, another isolate MM7, *Parmastomyces transmutans* (99% similarity level in

Blast) belonged to the phylum *Basidiomycota*. Microbial studies have shown that most *Aspergillus* species are found in a wide variety of environments and substrates on the earth throughout the year (Kwon-Chung & Sugui, 2013).

7.2 Conclusions

- The assessment of total diversity of fungal communities from the hot springs of the four soda lakes in Kenya revealed a total number of 139,023 fungal ITS reads, exclusion of low-quality and short sequence reads.

- Lake Elmenteita recorded 51,913 reads, Lake Magadi 34,718 reads, L. Bogoria had 26,434 reads while little Magadi had the lowest reads (25,958).

- A total of 2,179 fungal OTUs were obtained in this study and were distributed as follows: The hot spring at L. Elmenteita recorded 1196 OTUs, while the one at L. Bogoria had 294 OTUs. The Lake Magadi hot spring had 394 OTUs and that of little Magadi had 295 OTUs.

- The dominant genera from the Phylum *Ascomycota* were *Cladosporium*, *Aspergillus, Penicillium, Westerdykella, Epicoccum, Debaryomyces* and *Auxarthron* while the common fungal species were *Cladosporium* sp.*, Cladosporium cladosporioides, Pleosporales* sp.*, Aureobasidium pullulans* and *Aspergillus oryzae.*

- From the phylum Basiodiomycota, the dominant genera included *Termitomyces*, *Rhodotorula*, *Tremella* and *Malassezia*. Among the dominant species were; *Termitomyces* sp., *Tremella aurantialba*, *Tremella encephala, Dioszegia hungarica* and *Malassezia globosa.*

- This study provides evidence that fungal diversity, that is, taxonomic richness and evenness is greatly influenced by parameters like temperature, salinity, pH and culture media.

- This study has provided new insights into the eukaryotic composition of the hot spring environment. This data could serve as starting points for the development of new culture dependent techniques for the yet uncultivated microorganisms and unrecognized species.

7.3 Recommendations

1. Studies should be done on fungi from soda lakes' hot springs in order to reveal their functional role within the ecosystem.

2. The presence of shared OTUs across a range of temperatures and pH within the soda lakes' hot springs merits further investigation to understand community assembly and succession processes in the ecosystems.

3. Targeted fungal isolation should be carried out to recover, identify and characterize some of the novel groups revealed through pyro sequencing and their role in biotechnology exploited.

4. More research is required to compare the diversity of fungi in different seasons of the year such as the rainy and dry seasons.

5. Further studies should be undertaken to characterize the enzymes screened from isolates in this study to assess their potential in biotechnological application.

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APPENDICES

Appendix I: ANOVA Comparisons of Growth from Four Media at Lake Bogoria

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3669.584	3	1223.195	2.997	.037
Within Groups	26117.391	64	408.084		
Total	29786.975	67			

Appendix II: Tukey HSD Comparison for Growth among the different media at Lake Bogoria

Comparisons	Mean Difference	Std. Error	Sig	95% CI	
				Lower Bound	Upper Bound
PGA vs. SDA	19.25529*	6.92892	.035	.9779	37.5327

* $P < 0.05$

Appendix III: ANOVA Comparisons of Growth from Four pH levels at Lake Bogoria

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2940.698	3	980.233	1.674	.181
Within Groups	37465.936	64	585.405		
Total	40406.634	67			

Appendix IV: ANOVA Comparisons of Growth from four different sodium chloride concentrations at Lake Bogoria

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8444.726	3	2814.909	10.344	.000
Within Groups	17416.376	64	272.131		
Total	25861.102	67			

Appendix V: Turkey HSD Comparison for Growth among the different salinity levels at Lake Bogoria

Comparisons	Mean Difference	Std. Error	Sig	95% CI	
				Lower Bound	Upper Bound
5% vs. 20%	22.68824*	5.65821	.001	7.7628	37.6137
5% vs. 30%	28.13765*	5.65821	.000	13.2122	43.0631
10% vs. 30%	19.47059*	5.65821	.006	4.5451	34.3960

* $p < 0.05$

Appendix VI: Comparisons of growth from four temperature levels at Lake Magadi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24153.157	3	8051.052	18.324	.000
Within Groups	36906.717	84	439.366		
Total	61059.874	87			

Appendix VII: Tukey's HSD Comparison for Growth among the different temperature levels at Lake Magadi

Comparisons	Mean Difference	Std. Error	sig	95% CI	
				Lower Bound	Upper Bound
40 °C vs. 25 °C	-36.76*	6.32	.000	-53.32	-20.19
40 °C vs. 30 °C	-37.86*	6.32	.000	-54.43	-21.29
40 °C vs. 35 °C	-39.89*	6.32	.000	-56.46	-23.33

Appendix VIII: ANOVA comparisons of growth from different sodium chloride concentrations at Lake Magadi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8278.872	3	2759.624	14.245	.000
Within Groups	16273.460	84	193.732		
Total	24552.332	87			

Appendix IX: Tukey's HSD comparison for growth among the different sodium chloride concentrations at Lake Magadi

Comparisons	Mean Difference	Std. Error	Sig	95% CI	
				Lower Bound	Upper Bound
5% vs. 20%	21.67[*]	4.20	.000	10.67	32.67
5% vs. 30%	23.90[*]	4.20	.000	12.89	34.89
10% vs. 20%	12.58[*]	4.20	.018	1.58	23.58
10% vs. 30%	14.80[*]	4.20	.004	3.80	25.80

* $p < 0.05$

Appendix X: ANOVA Comparisons of growth from four types of media at Lake Magadi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5333.246	3	1777.749	4.258	.008
Within Groups	35074.308	84	417.551		
Total	40407.554	87			

Appendix XI: Tukey's HSD Comparison for growth among the four types of media at Lake Magadi

Comparisons	Mean Difference	Std. Error	sig	95% CI	
				Lower Bound	Upper Bound
PDA vs. PGA	-17.182^*	6.161	.033	-33.331	-1.032
SDA vs PGA	-20.50^*	6.161	.007	-36.650	-4.350

* $P < 0.05$

Appendix XII: ANOVA comparisons of growth from four pH levels at Lake Magadi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9479.973	3	3159.991	6.908	.000
Within Groups	38427.217	84	457.467		
Total	47907.191	87			

Appendix XIII: Tukey's HSD comparison for growth among the different pH levels at Lake Magadi

Comparisons	Mean Difference	Std. Error	Sig	95% CI	
				Lower Bound	Upper Bound
pH 5.0 vs pH 8.5	-19.621[*]	6.449	.016	-36.525	-2.717
pH 5.0 vs pH 10.0	-21.348[*]	6.449	.007	-38.252	-4.445
pH 7.0 vs pH 8.5	-20.091[*]	6.449	.013	-36.9945	-3.187
pH 7.0 vs pH 10.0	-21.818[*]	6.449	.006	-38.722	-4.914

* $P < 0.05$

Appendix XIV: ANOVA comparisons of growth from four types of media at Lake Elmenteita

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6521.398	3	2173.799	5.876	.001
Within Groups	31075.348	84	369.945		
Total	37596.746	87			

Appendix XV: Tukey's HSD comparison for growth among the four different types of media at Lake Elmenteita

Comparisons	Mean Difference	Std. Error	Sig	95% CI	
				Lower Bound	Upper Bound
MEA vs PGA	-18.09182[*]	5.79925	.013	-33.2929	-2.8907
PDA vs PGA	-19.18182[*]	5.79925	.007	-34.3829	-3.9807
SDA vs PGA	-21.68227[*]	5.79925	.002	-36.8834	-6.4812

* $p < 0.05$

Appendix XVI: ANOVA comparisons of growth from four different pH values at Lake Elmenteita

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1957.035	3	652.345	1.408	.246
Within Groups	38908.406	84	463.195		
Total	40865.441	87			

Appendix XVII: ANOVA comparisons of growth from four different sodium chloride concentrations at Lake Elmenteita

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10703.885	3	3567.962	13.981	.000
Within Groups	21437.291	84	255.206		
Total	32141.176	87			

Appendix XVIII: Tukey's HSD Comparison for Growth among the different sodium chloride concentrations at Lake Elmenteita

Comparisons	Mean Difference	Std. Error	Sig	95% CI	
				Lower Bound	Upper Bound
5 % vs. 20 %	21.86455*	4.81669	.000	9.2390	34.4901
5 % vs. 30 %	27.91000*	4.81669	.000	15.2844	40.5356
10% vs. 20 %	13.86364*	4.81669	.026	1.2381	26.4892
10 % vs. 30 %	19.90909*	4.81669	.000	7.2835	32.5347

* $P < 0.05$

Appendix XIX: DNA Extraction Reagents

- Solution 1

    - 50 mM Tris pH 8.5

    - 50 mM EDTA pH 8.0

    - 25 % Sucrose solution

- Solution 2

    - 10 mM Tris pH 8.5

    - 5 mM EDTA pH 8.0

    - 1 % SDS

- Lysozyme 2 0 mg/ml

- RNase A   20 mg/ml

- Proteinase K 20 mg/ml

- Phenol

- Chloroform

- Absolute ethanol.

- 3 M NaCl

- Isopropanol