CHARACTERIZATION, IDENTIFICATION AND METABOLITES OF FUNGI FROM THE SODA LAKES IN KENYA

FRANCIS IRERI NDWIGAH

DOCTOR OF PHILOSOPHY

(Microbiology)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2017

Characterization, Identification and Metabolites of Fungi from the Soda Lakes in Kenya

Francis Ireri Ndwigah

A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in microbiology in the Jomo Kenyatta University of Agriculture and Technology

`

2017

DECLARATION

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

Signature..... Date.....

Francis Ireri Ndwigah

This thesis has been submitted for examination with our approval as University supervisors.

Signature..... Date.....

Prof. Hamadi Hidi Boga, PhD
Taita- Taveta University, Kenya

Signature..... Date.....

Prof. Wanjiru Wanyoike, PhD
JKUAT, Kenya

Signature..... Date.....

Dr. Romano M. Kachiuru, PhD
University of Embu, Kenya

DEDICATION

This research work is dedicated to my late parents, Izaak Ndwiga and Abija Ndwiga, my family and more so to my wife, Agnes Ireri and all those who supported this work.

ACKNOWLEDGEMENTS

This research work would not have been accomplished were it not for the JKUAT research fund financial support. My supervisors, Prof. Hamadi I. Boga, Prof. Wanjiru Wanyoike and Dr Mwirichia R. Kachiuru do here by acknowledge their tireless guidance support and encouragement throughout this research study period.

I sincerely wish to thank Prof Sheilla Okoth from the University of Nairobi for allowing me access to the biotechnology laboratory facilities for microscopy.

Special thanks to the technical staff, Muthanga Josphat, Thuo Peter, Kimunge Charles, Mbogo Josphat, Rotich Richard and all those who in one way or another contributed toward the completion of this study.

Mr. Kuja Josiah was of great help in the analysis of some data and i really thank him for his contribution.

Finally, special thanks go to my Wife Agnes Ireri, my children, Nick, Edith and Jeff for their constant support, encouragement and reminder of completing my study.

I glorify the Almighty Father for his amazing grace; it is through him that all has been accomplished.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

CMC	Carboxy Methyl Cellulose
DDE	Dichloro diphenyl dichloro ethylene
DDT	Dichlorodiphenyl Trichloroethane
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamineteraacetic acid (chelating agent)
EI	Enzymatic Index
EL	Elmentaita/ Elmenteita
GC-MS	Gass Chromatograpy-Mass Spectrophotometry
ICIPE	International Center for Insect Physiology and Ecology
ILRI	International Livestock Research Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
Kb	Kilobase
MEA	Malt Extract Agar
MEGA	Molecular Evolutionary Genetic Analysis
Mm	Milimeters
NaCl	Sodium Chloride
NK	Nakuru
PCR	Polymerase Chain Reaction
pH	Hydrogen ion concentration
PKS	Polyketide synthase
RT	Retention Time
SDS	Sodium dodecyl sulphate
µl	Microlitre
UNESCO	United Nations Educational, Scientific and Cultural Organization

ABSTRACT

The soda lakes of Kenya developed as a consequence of geological and topological activities of the earth. These lakes are characterized by great variation in temperature, salt and pH extreme environment where diverse microorganisms inhabit. The physicochemical condtion in the lakes make them an extreme environment. The extreme conditions in the lakes may also provide microbial diversity as they adapt to this environment. However, Information on fungi from the Kenyan soda lakes environment is poorly documented or scanty. Little information is also available on the metabolic potential of the fungal diversity in the soda lakes. The main objective of this study was to isolate, and characterise fungi from the Kenyan Soda lakes and thereafter screen the isolates for enzymes and antimicrobial metabolites. The study sites were Lake, Elmentaita, Nakuru and Sonachi. Sediment samples were randomly collect from each lake, six sediment samples of 20g in polythene bags for each lake were pooled together to make a representative single sample of the lake sediments. The sediments were transported to JKUAT laboratory and stored in a freezer at minus 20 ºC**.** Serial diluted was used in isolation of fungi in triplicate on malt extract agar. The plates were incubated at 28 ºC for 48 hours. Purification of the isolates was done by subculturing on fresh malt extract medium. Cultural and morphological characterization was carried out to identify the isolates. Effect of growth, salt, pH, and temperature was done on malt extract agar agar and incubated at 28 ºC. Molecular characterization was done by amplifying the 18S gene using fungal primers. The amplicons were purified, quantified and checked for quality on an agarose gel and then sent for sequencing. Phylogenetic relationships were determined for the isolates in comparison with sequences retrieved from Nucleotide databse. Substrate utilization assays were carried out using different enzyme substrates to determine the ability of the fungus to degrade the substrate. Enzymatic activity was indicated by clearance zone around the fungal colony. The production of metabolites was carried out by grown isolates in liquid medium in conical flask. The medium was inoculated with a four millimetre agar fungal inoculums disc

culture and incubated at 28 ºC in a shaker at 1000rpm for fourteen days. After the fourteen days of incubation the Crude filtrate was recovered through muslin filtration of the fermented fungal liquid culture. This was subjected to antimicrobial assay. Aportion of the crude filtrate was subjected to solvent extraction, three times.using ethylacetate and hexane (4:1-vol.) respectively. This constituted the solvent extract. This was dryried in a rotary vacuum evaporator. The dry extract was dissolved in 1ml ethylacetate and was used for antimicrobial assay.and GC-MS analysis for fungal metabolites A total of twenty six isolates were recovered, fourteen from Elmentaita, six from Nakuru and six from Sonachi. The recovered isolates were mainly anamorphic fungi in the phylum Ascomycota. *Rhodotorula* was the only genus recovered in the phylum Basidiomycota. Two species were recovered; the conspicuous one was *Rhodotorula mucilaginosa*, a yeast with valuable biotechnological feature and an emergent opportunistic pathogen that might cause disease in immunocompromised individuals. Effect of salt, pH, and temperature on the fungal isolates revealed different range for growth optimum for fungal isolates. Phylogenetic relationships aligned the isolates to the genera, *Acrimonium, scopulariopsis, Verticillium, Rodotorula, Fusarium, Sarocladium, Paeciomyces,* and *Plectosphaerella* among others. Two isolates were affiliated to the genus *Rhodotorula* in the phylum Basidiomycota. All the rest (24 isolates) were affiliated to the phylum Ascomycota. It has been reported that members of this phylum dominate in many extreme environments. Substrate utilization of the fungal isolates revealed different types of enzymes (cellulases, proteases, pectinases, lipases and esterase) as evidenced by the clearing zone. The percentage of isolates with enzymatic activity.were, Protease (58%), Esterase (39%), Lipase (31%) the list activity was amylase (4%). The solvent extract antimicrobial assay revealed that most fungal isolates exhibited antimicrobial activity against the test organisms, *Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aurogenosa, Candida albicans*, *Salmonella typhimurium* and *Streptococcus pneumoiae* of which some of these microorganisms are potential human pathogens used in this study. GC-MS analysis revealed fungal metabolites in the groups (acids, ketones, quinones, alcohols, esters,

aliphatic compounds). Among the revealed comounds like thujaplicin and thymoquinone have antifunal, and floropropione and maltol have antimicrobial effects, Isophorone are anticancer and antioxidants.The revealed high diversity of fungi has a potential of producing enzymes, antimicrobial agents and other metabolites of economic importance in food, agricultural or pharmaceutical industries.

CHAPTER ONE

INTRODUCTION

1.1 Soda lakes of Kenya

Soda lakes are saline and alkaline ecosystems that are believed to have existed throughout the geological record of Earth and are widely distributed across the globe, but are highly abundant in terrestrial biomes such as deserts and steppes and in geologically interesting regions such as the East African Rift Valley. The alkaline saline, soda lakes of Kenyan Rift valley include Lakes Bogoria, Elmentaita, Magadi, Nakuru, Natron and Sonachi (formerly Naivasha Crater Lake), (Figure 1.1).The pH of the Lakes range from 8 to12 (Grant & Mwatha, 1989; Jones et al., 1998). Salinity within the lakes ranges from around 5% total salts but reaches saturation in Lake Magadi and Natron.

The development of soda lakes is a consequence of geological and topological factors (Mwatha, 1991; Sorokin & Kuenen, 2005). A combination of various environmental factors, results in large amount of sodium carbonate as well as a high concentration of Ca^{2+} and Mg^{2+}, which are insoluble as carbonate minerals under alkaline conditions (Sorokin & Kuenen, 2005**).** The ion concentration vary in Lake Bogoria, Nakuru, Elmentaita and Sonachi but reaches saturation in Lake Magadi and Natron with roughly equal proportions of Na_2CO_3 and NaCl as major salts (Green, 1993).

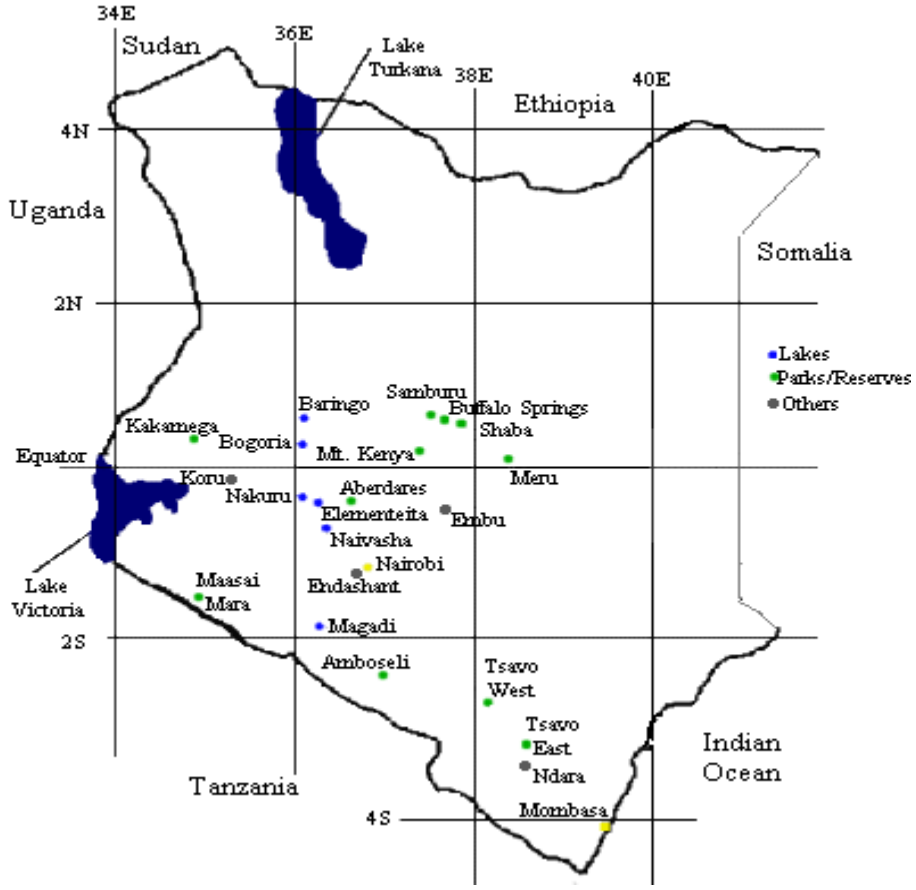


Figure 1.1: A General outline map of Kenya showing the distribution of soda lakes (Lakes: Elmentaita, Nakuru, Bogoria and Magadi); Modified from Grant 2004

The soda lakes in Kenya also exhibit active volcanism with numerous hot springs on the shores of some of the lakes. These hot springs are less alkaline and more dilute but where mixing occurs, complex pH, temperature and salinity gradients occur (Jones et al., 1994). A surveys of soda environments include, the Wadi Natrun lake system in Egypt (Mesbah et al., 2007), Magadi (Alexey et al., 2015; Kambura et al., 2016), soda lakes in the Kenyan-Tanzanian Rift Valley and diversity at extreme pH (Rees et al., 2004), soda lakes in Inner Mongolia in China (Ma et al., 2004), saline, meromictic Lake Kaiike in Japan (Koizumi et al., 2004), saline Qinghai Lake, China

(Dong et al., 2006), and athalassohaline Lake Chaka, China (Jiang et al., 2006), and also soda soils (Alexey et al., 2015).

Different groups of microorganisms have adapted to these extreme environments. They are defined based on the nature of environment where they are found and are collectively referred to as extremophiles (Turk et al., 2007). Extremophiles that are adapted to high temperatures are called thermophiles while acidophiles are adapted to low pH and alkaliphiles are adapted to highly alkaline conditions (Gunde-Cimerman et al., 2009). Some organisms occur in one or more environmental extremes, simultaneously and are called polyextremophiles (Gostinčar et al., 2011; Ali et al., 2015).

Microbial studies on the soda lake have focused mainly on bacteria but least on fungi. Fungi have been isolated from the soda lakes of Ethiopia and other saline alkaline environments such as the Dead Sea. In Kenya some fungi have been isolated from the hypersaline lake Magadi. However, the isolation and characterization of fungi in the Kenyan soda lakes is scanty.

Microorganisms found in the soda lakes endure a combination of extreme environmental conditions in terms of pH, salinity and temperature at the hot springs (Green 1993; Jone et al., 1998; Sorokin & Kuenen, 2005). Such extreme environments could stimulate the microorganisms to produce a range of metabolites of which some may be potential antimicrobial or enzymes that can be applied in industries and in biotechnology.

Historical interest in soda lake microbiology has focused primarily on the isolation and characterization of individual microorganisms with potential industrial applications (Horikoshi, 1999), although anaerobic strains with hypothesized ecological roles have also been described (Zhilina et al., 1996; Zhilina et al., 1997).

The fungi from the extreme environment have adapted to alkaline saline condition and relatively high temperatures. Therefore over the years, they might have changed or modified their metabolic pathways to produce different or improved metabolites, which may have antimicrobial and enzymatic activities (Mouchacca et al., 1995; Kis-

Papo et al., 2003).Thermophilic fungi such as *Rhizumucor miehei, Chaetomium thermophile,* and *Melanocarpus albomyces*, have been isolated from compost, soils and other sources (Tansey & Brock, 1978; Reysenbach et al., 2002). Unlike fungi from other habitats, the fungal communities from the soda lakes of East African Rift Valley have not been explored. In Kenya some fungi have been isolated from the hypersaline lake Magadi. There is little information on the isolation, characterization and metabolites of fungi from the soda lakes of Kenya (Alexy et al, 2015). This study will bridge this gap on fungal diversity; enzyme and metabolites produced by fungi in the soda lakes of Kenya. The main aim of this study was to isolate novel fungi from soda lakes that can be exploited for the production of enzymes and metabolites of economic importance.

1.2 Statement of the problem

Fungi comprise one of the most diverse groups of organisms on Earth. They are ubiquitous in all ecosystems and govern soil carbon cycling, plant nutrition and pathology. There are about 70,000 known species and an estimated 1.5 million species in total; the vast majority comprising of filamentous fungi. They have an enormous diversity and metabolic complexity (Moore et al., 2011; Hawksworth 2001). However, unlike the prokaryotes, the distribution of species, phyla, and functional groups has been poorly documented. The earth's biological diversity is disappearing at an ever increasing rate (Wilson 1988). Evidence exists of an increasing decline in the diversity of the less conspicuous organisms such as the fungi including lichenized fungi, and bryophytes (Lizon 1993). These poorly known but important groups of organisms may actually be more vital to long term ecosystem health than the well-known macrofauna and flora. Researchers throughout the world are also shifting their attention towards the potential of marine microorganism as an alternative source for isolation of novel metabolites (Anke & Erkel 2002; Biabani &Laatsch, 1998). Therefore, there is need to gather more information on the diversity, function and the metabolites produced by these microorganisms especially in less studied biomes such as the soda lakes.

1.3 Justification of the study

Soda lakes provide an alkaline-saline extreme environment where different microorganisms are adapted to these conditions. Extensive studies on bacterial isolation, characterization and identification have been carried out in the Kenyan soda lakes but there is little information on fungi isolated from the Kenyan soda lakes. Fungi have been isolated from saline lakes in the world such as the Dead Sea (Buchalo et al., 2000). 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). Alkaliphilic enzymes are important in detergent industries and account for approximately 30% of total worldwide enzyme production. Fungi are also important in food and pharmaceutical industrial processes such as in fermentation leading to production of organic acids, antibiotics and other secondary metabolite of potential application in biotechnology.

Enzymes from alkaliphiles have long term stability in detergent products, energy cost saving by lowering the washing temperatures, quicker and more reliable product, reduced effluent problems during the process, and stability in the presence of detergent additives such as bleach activators, softeners, bleaches and perfumes (Horikoshi, 1999). Due to the unusual properties of these enzymes they are expected to fill the gap between biological and chemical processes and have been greatly employed in laundry detergents (Horikoshi, 1999; Bordenstein, 2008).

Antimicrobial activity of metabolites produced by fungi in this study would be exploited as antifungal or antimicrobial compounds and this will add to the increasing demand for new antimicrobial compounds or agents to reduce resistance to the existing ones.

The understanding of fungal biodiversity in the Kenyan soda lakes wills also enhance and stimulate bioprospecting for enzymes, antimicrobial compounds and other metabolites with pharmaceutical and biotechnological application.

1.4 Hypotheses

The soda lakes environment is not a habitat for fungi that have potential of producing antimicrobial molecules, exo-enzymes and other metabolites of economic importance.

1.5 Broad objective

To isolate, characterize and identify fungi and their metabolites, in the soda lakes of Kenya.

1.5.1 Specific objectives

i. To isolate, characterize and identify fungi from samples collected from Kenyan soda lakes.
ii. To test the effect of Sodium Chloride, temperature and pH on the isolates
iii. To screen the isolates for the production of enzymes.
iv. To screen the isolates for antimicrobial activity.
v. To extract and identify the produced metabolites

CHAPTER TWO

LITERATURE REVIEW

2.1 The soda lake environment

Soda lakes are characterized by large amounts of sodium carbonate ($Na_2CO_3.10H_2O$ or $Na_2CO_3.Na_2HCO_3.2H_2O$) and very high concentration of calcium and magnesium ions, which are insoluble as carbonate minerals under alkaline conditions (Kempe and Degens, 1985).The salinity varies according to the geographical location and also according to seasonal weather conditions (Kis-Papo et al., 2003).The soda lakes also exhibit active volcanism with numerous hot springs on the shores of some of the lakes and geologically, alkaline trachyte lavas predominate (Jones et al., 1998).

Chemical analyses show Na^+, Cl^-, and $CO_3^{2-}$are the major ions responsible for salinity in the lakes (Kempe & Degens, 1985). In lakes of lower salinity the concentration of CO_3^{2-} usually exceed that of Cl^- but in brines of higher sanity Cl^- exceeds the CO_3^{2-} concentration (Kis-Papo et al., 2003). Lake Magadi has been the subject of many studies because of vast deposits of sodium hydrogen carbonate ($NaHCO_3.Na_2CO_3.2H_2O^-$ the stable crystalline product of Na_2CO_3, $NaHCO_3$ and CO^{2-}equilibrium), which are harvested and kilned to produce anhydrous Na_2CO_3 (soda ash) used in glass manufacture. Chemical analysis of ion concentration has shown varying amounts at different years, Sodium, carbonate, and chloride ions have a relative high concentration in all the soda lakes compared with others at any given time. Other ions concentration varies in different Kenyan soda lakes at any given period. The driving force for the sodium accumulation is depletion of Ca^{2+} trapped by CO_3^{2-} ions leaving sodium ion as the dominant cation (Jones et al., 1998).

2.2 Heavy metal and pesticide pollution of soda lakes

Soda lakes are mainly located in closed drainage basins exposed to high evaporation rates (Kempe & Degens, 1985). Effluent water from industries, sewage and domestic water, pesticides from the surrounding agricultural activities and other human

activities end up in the lakes. Atomic absorption spectrophotometry and gas chromatography have showed that more contaminants are added to the lake during the rainy season than the dry season with heavy metals and pesticide residues occurring in higher concentrations in the sediments than in the water (Koeman et al., 1972; Suarez-Serrano et al., 2010).

Contamination by most of the pollutants has increased over the past 25 years. Dichlorodiphenyldichloro ethane (DDD) was more prevalent in the sediments and particulates in the water, but Dichlorodiphenyl dichloroethylene (DDE) was predominant in fish, suggesting two different mechanisms of Dichlorodiphenyltrichloroethane (DDT) degradation in the biotic and a biotic environment (Suarez-Serrano et al., 2010). Whereas heavy metals tend to be distributed almost uniformly within the lake, pesticide residues are found predominantly in its northern and southern river inlets (Suarez-Serrano et al., 2010). Some contaminants occur in slightly higher concentration upstream, perhaps due to contamination from point sources, followed by dilution, however this may affect biodiversity (Mavura & Wangila, 2003; Suarez-Serrano et al., 2010).

2.3 Biodiversity of the soda lakes

2.3.1 Bacterial and Archaeal Diversity

The East African soda lakes are among the most productive aquatic environments in the world. Aerobic and anaerobic organotrophic bacteria as well as Archaea have been isolated from the soda lakes ((Jones et al., 1998; Melack & Kilham, 1974; Duch Worth et al., 1996; Jones et al., 1998; Mwatha 1991; Kambura et al., 2016). One of the noticeable features of many soda lakes is their colour, due to massive blooms of microorganisms, an indication of very high primary productivity.

Studies report abundant bacterial communities which also act as primary producers, usually dominated by *Cyanobacterium species* (Antony et al., 2013). Despite the high pH and high salinity, these environments have high bacterial and archeal diversity (Mwirichia et al., 2010a; Mwirichia et al., 2010b; Kambura et al., 2013; Kambura et al., 2016; Ntabo et al., 2013). Owing to high salinity, Lake Magadi is

dominated by the bacteria belonging to the genera, *Holomonas, Bacillus, Idiomarina and Alkalibacillus* (Lottie, 2012). Both Gram positive and the Gram-negative organisms have been isolated from the soda lakes (Mwatha, 1991; Nielsen et al., 1995; Groth et al., 1997). Archaea that have been isolated from samples of soils, water and sediments from Kenyan soda lakes include *Halorurumonas vacuolatum, Natronoalba magadii, Natronobacterium gregoryi, Natromonas pharaonis, Natrococcus spp.* and *Natronomonas pharaonis* (Mwatha & Grant 1993; Kamekura et at., 1997; Mwirichia et al., 2011).

2.3.2 Algae Diversity

The lakes of East African rift valley are dominated by dense blooms of cyanobacteria, especially the more dilute lakes. These blooms of cyanobacteria are usually dominated by *Spirulina species* and *S.platensis* are particularly prevalent at higher conductivity, but there are also contributions from *Cyanospira* (*Anabaenopsis*) and unicellular forms, which might be *Synaechococcus* species or *Chroococcus* species (Mwatha, 1991). The genus *Arthrospira* (Spirulina) is the principle food of the fast flocks of Lesser Flamingo (*Phoeniconaias minor*) that inhabit the Kenya soda lakes and contribute highly to the lakes productivity. Other algae dominant in tropical soda lakes in Kenya and Ethiopia include the genera, *Nitzschia, Navicula, Anabaenopsis, Oocystisetc* and *Cyanospira* (Ballot et al., 2005; Dadheech et al., 2013; Krienitz et al., 2003). Haloalkaliphilic cyanobacteria are most dominant at moderate salinity, whilst at higher salt concentrations only extremely salt-tolerant unicellular green algae, such as *Dunaliella viridis* and *Picocystis salinarium*, can thrive (Gerasimenko et al.,1999; Krienitz et al., 2012). Hypersaline soda brines are dominated by the extremely haloalkaliphilic unicellular cyanobacterium '*Euhalothece natronophila'* (Mikhodyuk et al., 2008).

2.3.3 Fungal diversity in saline environments

Fungi are eukaryotic organisms that have a heterotrophic mode of nutrition. They are adapted to different types of environments such as fresh water, high temperatures and alkaline- saline environments (Alexopoulos & Mims, 1979). The literature available

shows little information on the soda lake fungi of the East African Rift Valley. Studies from other extreme environments have shown that fungi can be isolated from thermophilic environment (Magan, 2007). However, some thermophilic fungi such as *Rhizumucor miehei*, *Chaetomium thermophile*, *Melanocarpus albomyces*etc, have been isolated from compost, soils and other sources (Tansey 1978; Reysenbach 2002). Different species of black yeast have been isolated from hyper-saline waters of solar saltans (Gunde-Cimeman et al*.,* 2000). These new fungi were described as new groups of eukaryotic halophiles, and they are represented by *Hortaea werckii*, *Phaeothecatri angularies*, *Trimmasrostroma salinum*, and halotorerant *Aureobasidium pulluns* (De-Hoog et al., 1999). *Cladosporium glycolicum* was found growing on submerged wood in the Great salt lakes. Buchalo et al., (2000) idnentified twenty-six (26) fungal species from thirteen (13) genera of *Zygomycetes* (*Absidiaglauca*), *Ascomycotina* (*Chaetomium aureum*, *C.flavigenum*, *Emericella nidulans*, *Eurotium amstelodami* and mitosporic fungi (*Acrimonies persicinum*, *Stschbotry schartarum*, *Ulocladium chlamydosporum*) from the Dead Sea.

The work on coastal mycology in Puerto Rico by Nieves-Rivera, (2005) explored many aspects of fungi in marine environments. A study on arenicolous filamentous fungi in the Mayaguez Bay shoreline revealed the presence of the genera *Aspergillus, Cladosporium, Dreschlera, Fusarium, Geotricum, Penicillium, Trichoderma, Mucor* and *Rhizopus* (Ruiz-Suarez (2004). The genus *Aspergillus* represented 80 % of total fungal abundance. This data suggested that salinity concentration may regulate the abundance of fungi in the shoreline. The first record of filamentous fungi in Dead Sea by Buchalo et al. (1998) revealed a novel species, *Gymnascella marismortui* (Ascomycota). This fungus was shown to be an obligate halophile that grows optimally at a range of 10-30 % of Dead Sea water. Moreover, they isolated *Ulocladium chlamydosporum* and *Penicilium westlingii* with salt tolerance at the range of 3 to 15 % NaCl and 26 °C. The spores and mycelia from *Aspergillus versicolor, Chaetomium globosum, Eurotium herbariorum, Eurotium amstelodami, Eurotium rubrum and Gymnascella marismortui* isolated from Dead Sea waters were tested for survival in Dead Sea water for prolonged time (Kis-Papo et al., 2001; 2003a), the results suggested that genomic diversity was positively correlated with

stress. The species *Trichosporon mucoides, Rhodotorula larynges, Candida glabrata*-like strain and *Candida atmosphaerica*-like strain have also been isolated from these waters (Kis-Papo et al., 2001).

2.3.4 Halophilic fungal diversity

The biology of the most widespread and most halophilic or halotolerant fungi and yeasts has been reviewed, these include the black yeasts *Hortaea werneckii* which grows in 5 M NaCl, the true halophile *Wallemia ichthyophaga* that requires atleast 1.5 M NaCl and grows up to saturation, and *Aureobasidium pullulans* that grows in 3 M NaCl. All of these are commonly found in hypersaline lakes and in a great variety of other, often unexpected, environments: domestic dishwashers, polar ice, and possibly even on spider webs in desert caves (Gunde-Cimerma et al., 2009). Salt-adapted cells of *Hortaea werneckii* and *Aureobasidium pullulans* are able to keep very low amounts of internal Na^+ even when grown at high NaCl concentrations and can be thus considered Na^+ excluders, suggesting the existence of efficient mechanisms for the regulation of ion fluxes (Kogel et al*.,* 2005).

Halophilic and halotolerant fungi use polyols such as glycerol, erythritol, arabitol, and mannitol as osmotic solutes and retain low salt concentrations in their cytoplasm (Gunde-Cimerman et al., 2009). Molecular studies on osmotic adaptation of *Hortaea werneckii* and *Wallemia ichthyophaga* (Lenassi et al., 2011) have been presented. Identification and structural features of Na^+-sensitive 3′-phosphoadenosine-5′-phosphatase HwHal2, one of the putative determinants of halotolerance in *H. werneckii* and a promising transgene to improve halotolerance in crops, was presented (Vaupotič et al., 2007). An in-depth understanding has been obtained of the high osmolarity glycerol (HOG) pathway, and this understanding may be applied in the future to the development of improved salt-resistant crops. Glycerol-3-phosphate dehydrogenase is involved in glycerol synthesis by both *Wallemia* and *Hortaea* (Lenassi et al**.,** 2011), and heterologous expression of the gene encoding the enzyme can restore halotolerance in *Saccharomyces cerevisiae* deficient in glycerol production (Lenassi et al., 2011).

The strategy of accumulating a mixture of polyols is also common in many fungi (Davis et al., 2000) and is not unique to *H. werneckii* (Lenassi et al., 2011). For example, *Candida sake* accumulates equimolar amounts of glycerol and arabitol (Abadias et al**.,** 2000). Erythritol is produced by osmophilic yeasts such as *Pichia, Candida, Torulopsis, Trigonopsis, Moniliella* and *Aureobasidium sp*. (Kim et al., 1997). All four polyols glycerol, erythritol, arabitol and mannitol, have been detected in some *Aspergillus species* (Beever and Lracy, 1986, Abadias et al., 2000).

2.3.5 Alkaliphilic fungal diversity

The ability to grow under alkaline conditions has been demonstrated in five species by the genera *Alternaria, Aspergillus, Fusarium, Peacilomyces* and *Penicillium* which exhibited growth profile of facultative alkaliphilic fungi (Dumestre et al., 1997). These fungi have the potential to produce different alkaline detergent enzymes. *Aspergillus flavus, Fusarium oxysporum, Paecilomyces varioti***i** and *Penicillium chrysogenum* are known to be the best producers for the alkaline - amylase, cellulase, lipase and protease, respectively (Mohamed et al., 2012). Growth and production of alkaline detergent enzymes by these four fungi are favorably affected by addition of sodium chloride to the fermentation media. In 2005; an alkaliphilic-holomorphic fungus from hyper-saline soda soils (pH around 10) was isolated, described and placed among members of the genus *Heleococcum* as *Heleococcum alkalinum*. *Heleococcum* (order *Hypocreales*) seemed appropriate based on morphological and ecological features (Bila-nenko et al., 2005).

Fungi growing at extreme pH values are of scientific interest for the general study of fungal evolution as well as for the evaluation of their potential in producing commercially valuable substances. Obviously, the fungi adapting to alkalinity must have metabolic pathways that have become modified with respect to those seen in related neutrophilic fungi. For instance, enzymes that are being secreted into the ambient environment should work optimally in alkalinity in order to provide sufficient amounts of nutrients (Kladwang et al., 2003).

2.4 Fungal enzymes

Fungi produce enzymes, which break substrate into simple utilisable substances. Most of the enzyme production by fungi comes from the fungus, *Aspergillus niger* (Narasimha et al., 2006). The enzyme α-amylase is used to convert starch to maltose and maltotriose in bread making and is used in conjunction with amyloglucosidase to obtain glucose and maltose from starchy materials (Aquino, 2003). Pectinases are used to clarify fruit juice and wine (Deacon, 1984). Moreover, proteases are also very important in food and pharmaceutical industries.

Both aerobic and anaerobic fungi are known to degrade the various leaf constituents by producing enzymes whose action may be synergistic to each other (Wood 1989). Such enzymes include exo-1, 4-β-D-glucanase, endo –1, 4- β -D-glucanase, and β – glucosidase produced by *Trichoderma viride, Trichoderma reecei, Trichoderma kiningii, Fusarium solani, Penicillium pinophilum, Thermoascus aurantiacus, Phanerochaete chrysporium* and *Talaromyces emersoniito* act on crystalline cellulose (Wood, 1989; Linko et al., 1989.

Peroxidase is known to act on lignin degradation; probably Mn-dependent peroxidase and a copper laccase enzyme degrade lignin by oxidation of phenolic compounds (Leisola et al., 1989).

Cellulose requires the enzymes, Endoglucanase (EG or CX) which hydrolyses internal β-1, 4 glucan chain of cellulose at random, primarily within amorphous regions and display low hydrolytic activity toward crystalline cellulose (Walsh, 2002; Grassin & Fauquembergue, 1996). Pectin degrading enzymes includes; pectinases, exopolygalacturonase, pectatelase from fungi such as *Apergillus, Fusarium, Penicillium* and *Trichoderma* species (Linko et al., 1989).

Fungi are well known to produce lipids and increased attention has been paid to filamentous fungi which can accumulate up to 80% of lipid and also produce value added fatty acids (Subramaniam et al., 2010; Andre et al., 2010). Lipids produced from filamentous fungi show great promise for biofuel production, but a major limiting factor is the high production cost attributed to feedstock. The fungi,

Aspergillus terreus, Chaetomium globosum, Thermomyces lanuginosus among others do produce lipids (Subramaniam et al., 2010; Andre et al., 2010).

Fungi produce proteases that catalyze hydrolytic reactions in which protein molecules are degraded to peptides and amino acids. These constitute a very large and complex group of enzymes, which differ in properties such as substrate specificity, active site and catalytic mechanism, pH and temperature optima and stability profile (Baisuo, 2014; Grant & Heaphy, 2010). The entomopathogens and *Verticillium fungicola* degrade a broad spectrum of proteins, such as elastin and mucin (Ramírez-Coutiño et al., 2006). The saprophytes (*N. crassa* and *A. nidulans*) and the opportunistic pathogens (*A. fumigatus* and *A. flavus*) produce the broadest spectrum of protein degrading enzymes. Enzyme production is based on the adaptation of fungi to the requirements of their ecological niches (Raymond et al., 1997; Alagarsamy et al., 2005).

2.5 Secondary metabolites of fungi

Secondary metabolites of fungi are organic compounds produced when growth is restricted. They are derived from primary metabolic pathways mainly, Embden-Myerhof-Panas pathway (EMP), (Hexose monophosphate pathway (HMP), and Entner-Doudoroff pathway (EDP) (Author, 2012). These intermediates of primary metabolism have no specific role in basic life processes of fungi. Secondary metabolic products constitute a wide array of natural products and are more complex than primary metabolic products. They are derived from the primary products, such as amino acids or nucleotides by modifications, such as methylation, hydroxylation, carboxylation, condensation and glycosylation (Bentley & Bennet, 1988). Although the estimated 3000 to 4000 known fungal secondary metabolites have been isolated (Keller et al., 2005), possibly not more than 5000 to 7000 taxonomic species have been studied in this respect. Genera such as *Aspergillus, Penicillium, Fusarium*, and *Acremonium* are among fungi highly capable of producing a high diversity of secondary metabolite (Dreyfuss & Chapela, 1994).

Most fungal secondary metabolites are synthesized from only a few key precursors in pathways that comprise a relatively small number of reactions and which branch off from primary metabolism at a limited number of points. Acetyl-CoA is the most precursors of fungal secondary metabolites, leading to polyketides, terpenes, steroids, and metabolites derived from fatty acids. Other secondary metabolites are derived from intermediates of the shikimic acid pathway, the tricarboxylic acid cycle, and from amino acids (Dreyfuss & Chapela, 1994; Martin & Demain, 1978). The data on the consistence of secondary metabolites in fungi are by contrast, rather scanty (Dreyfuss & Chapela, 1994).

The main industrial use of fungi has been associated with the production of fermentation products, including antibiotics, enzymes and a range of biochemicals. Ethanol, citric acid, gluconic acid, itaconic acid, amino acids, vitamins, nucleotides and polysaccharides provide examples of primary metabolites produced by fungi, while antibiotics such as penicillin, the cephalosporins, fusidic acid and griseofulvin are important secondary metabolites (Namikoshi et al., 2002).

2.6 Polyketides

Polyketides are secondary metabolites from fungi, bacteria, plants and animals (Pfeifer & Khosla, 2001). They are usually biosynthesized through the decarboxylation condensation of malony-l CO A derived extender units in a similar process to fatty acid synthesis (Claisen condensation) (Pfeifer & Khosla, 2001). The polyketide chains produced by a minimal polyketide synthase are often further derived and modified into natural products (Robinson, 1991; Cox, 2007). While structurally diverse, all polyketides are assembled by successive rounds of decarboxylative Claisen condensations between a thioesterified malonate derivative and an acyl thioester. The enzymes that catalyze these condensations are referred to as polyketide synthases (PKSs). Polyketides provide a wide range of clinically effective drugs (Pfeifer & Khosla, 2001). These include antibiotics (erythromycin A, monensin A, rifamycin S), immune suppressants (rapamycin, FK506), antifungal (amphotericin B), antiparasitic (avermectin) and anticancer (doxorubicin) drugs (Weissman & Leadlay, 2005).

Fungal PKSs are responsible for the biosynthesis of mycotoxins and other secondary metabolites. They are multi-domain enzymes, which are named type I PKSs in analogy to multi-domain fatty acid synthases. A typical fungal PKS consists of a set of -ketosynthase (KS), acyl transferase (AT) and acyl carrier protein (ACP) domains. Many PKSs, especially those for reduced polyketides; also contain additional domains, such as -ketoacylreductase, dehydratase, enoylreductase, and methyltransferase (Schmitt, et al., 2005). Fungal PKSs are classified into three groups according to their architecture: (i) PKSs for nonreduced polyketides, (ii) PKSs for partially reduced), type II polyketides (often aromatic molecules produced by the interative action of dissociated enzymes), and type III polyketides (often small aromatic molecules produced by fungal species).

2.7 Regulation of fungal secondary metabolism

Most secondary metabolites are formed via enzymatic pathways rather than by ribosomal mechanism (Pfeifer & Khosla, 2001). The enzymes occur as individual proteins, free or complex, or as parts of modules of large multifunctional poly-peptides carrying out a multitude of enzymatic steps such as, in the cases of polyketide synthases and peptide synthases. Whether chromosomal or plasmid-borne, the secondary metabolism genes are often clustered, but not necessarily as single operons. Clusters of fungal biosynthetic genes have been found encoding enzymes for the production of penicillin, cephalosporin and others (Aharonowitz et al., 1992; Carlsen 1990; Hohn et al., 1995; Keller et al., 1997).

2.7.1 Regulation by carbon source

Glucose, usually an excellent carbon source for growth, often interferes with the formation of secondary metabolites. Instead, polysaccharides (e.g. starch), oligosaccharides (e.g. lactose) and oils (e.g. soybean oil, methyloleate) are often preferable for fermentations yielding secondary metabolites (Demain, 1996). In media containing a mixture of a rapidly used and a slowly used carbon source, the former is utilized first to produce cells but little to no secondary metabolites are

formed. After the rapidly assimilated compound is depleted, the ‘‘second-best’’ carbon source is used for the production phase, known as the idiophase.

2.7.2 Regulation by nitrogen source

Nitrogen regulation affects both primary and secondary metabolism (Demain, 1996). The control of enzyme synthesis is generally exerted by the intracellular nitrogen pool. Many secondary metabolic pathways are negatively affected by nitrogen sources favorable for growth, e.g. ammonium salts. As a result, a slowly assimilated aminoacid is often used as the nitrogen source to encourage high production of secondary metabolites. Information concerning the mechanism(s) underlying the negative effect(s) of ammonium and certain amino acids on industrial processes is scarce.

2.7.3 Regulation by phosphorus source

A rather specific negative effect of inorganic phosphate arises from its ability to inhibit and/or repress phosphatases. Because biosynthetic intermediates of certain pathways are phosphorylated whereas the ultimate product is not, phosphatases are sometimes required in biosynthesis. Although only little is known about the mechanism of general phosphate control of secondary metabolism, there is a strong possibility that phosphate regulation also works by affecting enzyme activities (Liras et al., 1990).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

3.1.1 Lake Nakuru

Lake Nakuru is a small, shallow, alkaline-saline lake located in a closed basin without outlets in the Eastern Rift Valley of equatorial East Africa, GPS-position 37175279E, 9962082N and elevation of 1760M above sea level. The present maximum depth is about three meters, but the lake water level is still quite variable; the whole lake had been almost dried up several times during the past 50 years due to unknown reasons. The lake has a surface area of about 40-km^2 and depth of about 0.092 km^3. It is a soda-lake with a water pH value of 10.5 and an alkalinity of 122 meq l-1. The lake's catchment area amounts to some 1,800 km^2 and is extensively utilized for agriculture and livestock farming (Vareschi, 1978) (Fig. 3.1).

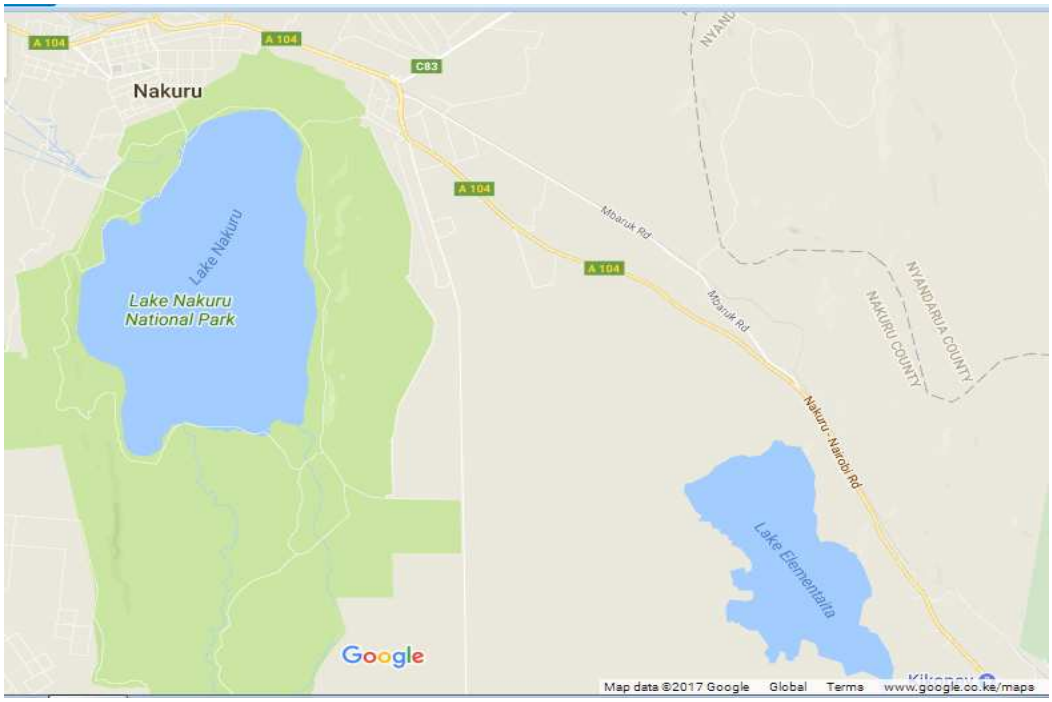


Figure 3.1: Google map of Lake Nakuru and Lake Elmentaita

3.1.2 Lake Elmentaita

The lake is located in a basin whose water budget is maintained by recharge from hot springs located on the southern lakeshore, two inflowing rivers, surface run off and direct rainfall. It has a GPS-position; 37195473E, 9947486N and elevation of 1787M above sea level. The lake has no surface outlet or underground seepage for releasing its water to other aquifers (Mwaura, 1999). The lake is not protected and is surrounded by private and communal lands. It is also part of a wider human catchments basin where human population has been increasing rapidly in recent years (Mwaura, 1999).

Various anthropogenic disturbances have resulted to the degradation of the catchments through deforestation, grazing, cultivation, mining, fires and human settlement (Mwaura, 1999). The absence of sewage treatment, Pollution from the factories like the Diatomite Company that undertakes diatomite mining and the treatment of poles by Cabro East African Ltd Company involves in seasoning of poles contributes to pollution of the lake (Mwaura, 1999) (Fig. 3.1).

3.1.3 Lake Sonachi

The lake is a small meromictic volcanic crater lake in Naivasha, Kenya (Ballot et al., 2005). It has a GPS-Position; 37195036E and 9913347N and elevation of 1885M above sea level. The factors that contribute to the maintenance of meromixis are basin morphometry, the diurnal periodicity of the winds and of thermal stratification, biological decomposition, and seasonal yearly changes in rainfall. Lake Sonachi is sheltered from wind by crater walls 30-115 M above its surface. Higher values of hydrogen sulfide, soluble reactive phosphate, and ammonia in the deeper waters, as well as a lower pH value, suggest that biological processes contributed to the meromixis. The Lake is dominated by cyanobacterium, *Synecoccus bacilaris* (Njuguna, 1988), (Fig. 3.2).

Figure 3.2: Google map of Lake Sonachi (Naivasha Crater Lake).

3.2 Sample collection

Sediment samples were collected by randome sampling method from the three soda lakes in the month of September 2009. Sediment samples were randomly collected at six different points in waters close to the lake shores. A motorised boat was use to facilitate sample collection in the deep waters of the lakes. Six sediment samples of 20g each in polythene bags for each lake were pooled together to make a representative single sample of the lake sediments. The sediments were transported to JKUAT laboratory and stored in a freezer at minus 20 ºC for subsequent analysis. Water temperature, pH and dissolved oxygen were recorded on site. Isolation of fungi from lake sediments

Fungi were isolated from the pooled sediment sample of the soda lake. Water from the lake was used to prepare culture medium; 50gm of malt extract agar (MEA) was dissolved in one litre of the lake water and sterilized in the autoclave. After cooling, 300 mg/L of penicillin was added before pouring medium on plates. For the recovery of some fungi, the baiting techniques using sesame and hemp seeds were used (EI-Hissy & Khallie, 1989). The seeded plates (5 plates for each sample) were incubated at 28 ºC for one to two weeks during which the growing fungal colony were examined and recorded.

3.3 Isolation of fungi from sediments by serial dilution method

Pooled sediment sample were subdivided into 2 g samples. This was suspended in 10 ml sterile saline water (with triton) vortexed and allowed to settle for about 10 min. A sequence of 1:10 dilution in sterile saline water was performed. 0.1 ml aliquots of highest dilutions were drawn separately and plated on sterile agar medium (Malt extract, glucose, 300 mg/L of penicillin and streptomycin) aseptically and spread through glass rod. The plates in triplicates were incubated at 28 ºC until visible colonies sporulated. Pure cultures were sub-cultured into fresh agar plates. Each isolate was preserved on agar slants in universal bottles for downstream studies.

3.4 Morphological and physiological characterization

Morphological features were used to identify the fungal isolates under a light microscope. The number of isolates isolated from lake were, Elmentaita fourteen, Nakuru six and Sonachi six. Morphological featutres used in identification were, hyphae, conidia, conidiophores structures and mycelia colour. Identification was aided by keys described by Barnett and Hunter (1987); Hanlin (1992); Raper and Fennell (1965). Micrographs of fungal isolates were made from a camera mounted on a microscope at magnification of 1000mg.

The fungal isolates were grown on malt extract agar to test the effect of sodium chloride at 0%, 5%, 10% and 15%), pH at 4, 7, 9, and 12, and temperature at 26 ºC, 28 ºC, 30 ºC and 35 ºC Plates were inoculated with six mm agar disc fungal inoculum. Plates were incubated at $28^{\circ C}$ for five days, and growth measurements recorded.

3.5 Substrate uterization by fungal isolates

Qualitative determination of the enzymatic assays of fungal isolates was carried out using Carboxymethyle-cellulose (CMC), Starch, Gelatin, Tween 20 and Tween 80 and pectin as enzyme substrate by agar diffusion methods (Ruijassenaars, 2001).

The fungi isolated were subjected to enzymatic assays to determine the type of enzyme(s) each fungus produces. The fungus was grown on Toyama's mineral solution containing (g/l): $(NH_4)_2SO_4$, 10.0; KH_2PO_4, 3.0; $MgSO_47H_2O$, 0.5; and $CaCl_2$ $2H_2O$, 0.5. To the Toyama's solution was added appropriate quantity of cellulose, pectin, starch, or protein and then 15g agar. The components were mixed in 1 litre distilled water, pH adjusted to 9 using carbonate buffer and then sterilized at 121 ºC for 45 min. The medium was dispensed into sterile petri dishes, allowed to set and then inoculated using different fungal isolates. The plates, in triplicates were incubated at 28 ºC and growth characteristics monitored.

3.5.1 Determination of amylolytic activity

The ability to degrade starch was used as the criterion for determination of ability to produce amylase enzyme. The medium used contained malt extract plus 0.2% soluble starch, pH 9. After 3-5 days of incubation the plates were flooded with an iodine solution for analysis (Hankin & Anagnostakis, 1975).

3.5.2 Cellulolytic activity

Screening of cellulase-producing fungi was done on CMC selective agar containing 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% carboxymethyl cellulose sodium salt, 0.02% peptone and 1.7% agar. Plates were inoculated with the fungus and incubated at 28 ºC for five days. Plates showing growth were flooded with Gram's Iodine solution for 3 to 5 min. (Kasana et al., 2008). Zones of clearance around the colony indicated a positive test.

3.5.3 Determination of lipolytic activity

The medium described by Sierra (1957) was used to detect production of lipolytic enzymes. Sorbitanmonolaurate (Tween 20, Fisher Scientific Co., Fairlawn, N. J.) was used as the lipid substrate. Test medium contained Difco peptone, 10 g; NaCl, 5 g; $CaC1_2$ $2H_20$, 0.1 g; agar, 20 g, pH9 per liter. The Tween 20 was sterilized separately by autoclaving for 15 min. and 1 ml added per 100 ml of sterile and cooled basal medium. Lipolytic activity by a colony was seen as either a visible precipitate due to the formation of crystals of the calcium salt of the lauric acid liberated by the enzyme, or as a clearing of such a precipitate around a colony due to complete degradation of the salt of the fatty acid.

3.5.4 Determination of Esterase Activity

The media used was described by Sierra (1957), containing (gl-1): peptone 10.0, NaCl 5.0, $CaCl_2.2H_2O$; 0.1, agar 18 pH 9. To the sterilized culture media, previously sterilized Tween 80 was added in a final concentration of 1 % (v/v). This was incubated at 28 ºC for 5 days and clear zones were observed around the colonies.

3.5.5 Determination of Protease Activity

To determine the hydrolysis of gelatin (Frazier's gelatin agar) the medium contained malt extract agar and bacteriological gelatin (4.0g/1). This was incubated at 28 ºC for 5days. The plates were flooded with Frazier's revealers (distilled water100ml, Hull 20.0g and mercury dichloride 15.0g), modified from Smibert and Krieg (1994). The presence of a clear halo around the fungal colony indicated a positive result.

3.5.6 Determination of pectinolytic activity

To Malt extract medium was added 5g pectin and incubated at 28 ºC for 5 days and then followed by addition of HCl (2ml l^{-1}) to the plate. The presence of a clear halo around the fungal colony was indicative of the degradation of pectin (Andro et al., 1984).

3.6 Determination of the Enzymatic Index

The isolates were grown in modified agar media for 5 days for each enzyme to be investigated. The cultures were incubated at 28 ºC for 120h. Enzymatic index (EI) was expressed by the relationship between the average diameters of the degradation halo over time, modified from Hankin et al. (1971).

3.7 Antimicrobial Activity

3.8 Cultivation of fungi in liquid medium

Each of the fungal isolate was grown in liquid medium composed of 15g malt extract, 5g Bacteriological peptone, 5g Glucose, 2% NaCl in 1L of distilled sterile water at pH 9. 100ml of the sterile medium was dispensed into sterile 250ml conical flasks. Each flask was inoculated with a four-millimetre agar disc cut from two days' fungal isolate culture and incubated at 28 ºC in a shaker (1000rpm) for fourteen days. The crude filtrate was recovered for each fungal isolate and later used for antimicrobial bioassay.

3.9 Screening the isolates for production of antimicrobial activity

Fungal isolates were grown on malt extract broth at 28 ºC in an incubator shaker at 100rpm for fourteen days and the crude fitrate used to screen the isolates for antimicrobial activity against the test organisms- *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 11778), *Escherichia coli* (NCTC 10418), *Candida albicans* (ATCC 90028) *Streptococcus pneumoiae* (ATCC 49619 and *Staphylococcus aureus* ATCC 25923). The test organisms were spread on malt extract agar plates (Paterson & Bridge, 1994). Three sterile cellulose discs of a diameter of 0.6cm were then dipped in each isolates crude filtrate product, air dried and then placed at three points on the plates. The plates were then incubated at 28 ºC for a period of 24 hours. Isolates that tested positive for antimicrobial activity were indicated by clearing zones around the discs while the negative isolates were indicated by lack of clearing zones around the discs (Cappuccino & Sherman, 2002). Growth inhabitation was measured as, mean diameter of fungal colony and clearing zone minus colony mean diameter in triplicates for each fungal isolate and the test organism. The control tests discs were not loaded with isolate crude filtrate but distilled water in all the tests.

3.10 Extraction of fungal metabolites

Each of the fungal isolate was grown in liquid medium composed of 15g malt extract, 5g Bacteriological peptone, 5g Glucose, 2% NaCl in 1L of distilled sterile water at pH 9. 250ml of the sterile medium was dispensed into sterile 500ml conical flasks. Each flask was inoculated with a four-millimetre agar disc diameter cut from two days' fungal isolate culture using a cork borer and incubated at 28 ºC in a shaker (1000rpm) for twenty-one days, modified from (Lauer et al., (1991) and Sawa et al., (1994). Aqueous phases of all crude fitrate were subjected three times to solvent extraction using ethyl acetate and hexane (4:1-vol.). The extract was passed through a pad of anhydrous sodium sulphate to remove excess water and thereafter evaporated to dryness using a rotary vacuum evaporator (RE 100B, Bibby Sterilin, United Kingdom). Yields of the extracts were determined and recorded. The isolates solvent crude extracts were used for biological activity tests as well as Gas

Chromatography - Mass Spectrophotometry (GC - MS) analysis of the compounds contained in them.

3.11 Bioassay of the solvent extracts against test organisms

Agar diffusion method (Anke et al., 1989) was used to determine the antimicrobial activity of the extracts. The micro-organisms used comprised, *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 11778) *Staphylococcus aureus* (ATCC 25923) *Pseudomonas aurogenosa* (ATCC 27853), *Candida albicans* (ATCC 9008), *Salmonella typhimurium* (ATCC 700931) and *Streptococcus pneumoiae* (ATCC 49619). Filter paper discs of 6mm were prepared and impregnated with10µl of solvent extract prepared by dissolving the dry extract in 1ml ethyl acetate. The impregnated discs were allowed to dry in a fume chamber and then placed on agar seeded with the test organism. The control was not impregnated with the extract. The plates were incubated at 28 ºC for 24h and the diameter of zones of inhibitions measured in millimetres and recorded.

3.12 GC-MS analysis of fungal metabolites from the isolates

Chemical screening of the active compounds present in the solvent extracts was done by use of a GC - MS to detect the compounds, their quantity and abundance. Each sample was reconstituted using 1 ml DCM (Dichloromethane (≥ 99.8%; Aldrich chemical co. Ltd., USA.) and passed through a glass wool to remove solid materials. 40 µl of the collection in triplicate was transferred into auto sampler glass vials having Teflon caps and analyzed using GC - MS whose conditions are given below. Agilent Technologies 7890A system was used (Sasagawa, et al., 2015). Oven conditions set during the analysis were: 1 min for equilibration time; 35 ºC for 5 min, 10 ºC / min to 280 ºC for 10.5 min and 50 ºC / min to 285 ºC for 9.9 min as the oven program while the running time was 50 min. Injection was done in splitless mode and the conditions used were as follows: 250 ºC for the heater, 8.8271 Psi as the pressure, a total flow of 10.2 ml / min, septum purge flow of 3 ml / min, gas saver at 20 ml / min after 2 min and Purge flow to split vent at 6ml / min at 0.8min. The column used was HP-5MS, (5 % methyl silox), (30 m × 250 µm × 0.25 µm). The

compounds identified were generated from a computer program that involved calculation by the data system of a similarity index, match factor or purity between the unknown spectrum and library (reference) spectra. For this analysis, NIST/EPA/NIH MASS SPECTRAL LIBRARY (NIST 05) and NIST MASS SPECTRAL SEARCH PROGRAM Version 2.0d were used (ICIPE).

3.13 Molecular characterization

3.13.1 DNA Extraction and Sequencing

DNA from pure cultures was extracted using the bead beater machine method and two lyses 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). 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). 2.8 g of fungal mycelia were scrapped using a sterile surgical blade put into separate 2 ml Eppedorf tube (vial) containing 100µl solution A and then half filled with 0.5 mm silicon beads. The vials were then inserted securely in the arms assembly of the mini bead-beater machine, which was then run at 400rpm for 3 min. At the end of the process, beads quickly settle to the bottom of the vial by gravity. The homogenate containing crude DNA was removed using a micropipette. To the homogenate was added 30µl of 20mg/l Lysozyme and 15µl of RNase, gently mixed and incubated at 37^{o}C for two hours to lyse the cell wall completely. 600µl of Solution B was then added and gently mixed by inverting the tubes severally, followed by the addition of 10µl of Proteinase K (20mg/l). The mixture was there after incubated in a water bath at 60 ºC for 1h. Extraction followed the phenol/chloroform method (Sambrook et al., 1989). The presence of DNA was checked on 1% agarose gel and visualized under ultraviolet by staining with ethidium bromide. The remaining volume was stored at -20 ºC.

Total genomic DNA from each isolate was used as a template for amplification of the 18S rDNA genes. Nearly full-length 18S rDNA gene sequences were amplified using fungal primer pair Fung5f (5'-GTAAAAGTCCTGGTTCCCC-3') and FF390r (5'-CGATAACGA ACGAGA CCT-3'), as explained elsewhere (Vainio and Hantula 2000; Lueders et al., 2004). Amplification was performed using Peqlab primus 96 PCR machine. Amplification was carried out in a 40µl mixture containing 5µl of PCR buffer (×10), 3µl dNTP's (2.5mM), 1µl (5 pmol) of Fung5f forward primer, 1µl (5pmol) of FF390r reverse primer, 0.3µl taq polymerase, 1.5µl of template DNA and 28.2µl of water. The control contained all the above except the DNA template.

Standard PCR conditions were followed. Initial activation of the enzyme at 96 ºC for 5 min, denaturation at 95 ºC for 45 secs, primer annealing at 48 ºC for 45 seconds, extension at 72 ºC for 1.30 min and a final extension at 72 ºC for 5 min. All were repeated for 36 cycles. PCR products (5µl) were separated on a 1 % agarose gel in 1× TBE buffer and visualized under ultraviolet by staining with ethidium bromide (Sambrook et al., 1989). PCR products for each isolate were purified using the QIAquick PCR purification Kit (Qiagen, Germany) following the manufacturer's protocol. The amplicons were sent to SEGOLIP, ILRI, for Sequencing.

3.14 Cellulase and Polyketide synthase gene analysis from fungal isolates

DNA extraction was done as in section 3.6.1. Genomic DNA was used as template DNA for Taq polymerase. Degenerated primers KAF (5-KS-GAR KSI CAY GGI ACI GGI AC-3); KAR2 (5-AT-CCA YTG IGC ICC YTG ICC IGT RAA-3) and fungcbhIF (5-ACC AA TGC ACI G-3); fungcbhIR (5-GC TCC CAT-3) were used for amplification of the polyketide synthase and cellulase genes respectively. PCR products were purified using the QIAquick Gel Extraction Kit (QIAgen) shipped for sequencing using FEDEX, international commercial shipping agency to Macrogen Company, Republic of Korea.

CHAPTER FOUR

RESULTS

4.1 Physicochemical characteristics

The physicochemical parameter; atmospheric and water temperature, pH and dissolved oxygen of the lakes were recorded at the site (Table 4.1).

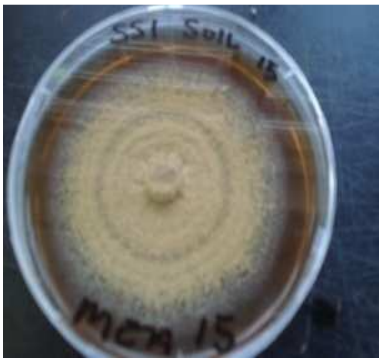
Table 4.1: The water and atmospheric temperature, pH and dissolved oxygen of water.

Parameter	Elmentaita	Nakuru	Sonachi
Weter Temperature. ºC	32-32.8	25-26	20-23
AtmosphericTemperature. ºC	26	23	23
Water pH	9.1-9.5	9.9-10.4	9.1-10.4-
Dissolved Oxygen in mg/L	8.2-8.3	8.3-8.8	8.5-8.8

4.2 Morphological characteristics of fungal Isolates

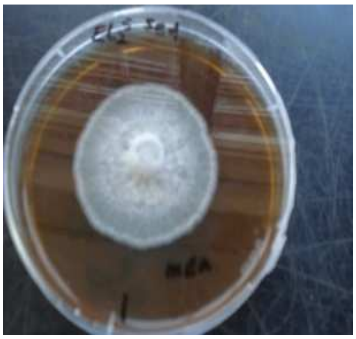
A total of 26 fungal isolates were isolated, 14, from Elmentaita, 6 from Nakuru and 6 from Sonachi. There was no targeted number of isolates from each lake during the isolation period otherwise the numbers can be enormous. The mycelia colour of isolates on MEA ranged from brown-white, green-light green, grey-white, cream-white, cottony and yellow-white (Plate 4.1). Table 4.2 illustrates the sequence number, medium and morphological description of fungus. All the 26 isolates were anamorphs. Sexual structures were absent.

The characteristic features of hyphae, conidia and conidiophores structures placed the fungal isolates in the phylum Ascomycota and Basidiomycota (Plate 4.2).



EL1

SN19



SN31

EL6

Plate 4.1: Mycelial growth of some selected fungal isolates on malt extract agar in petridishes. (EL1, SN19, SN31 and EL6)

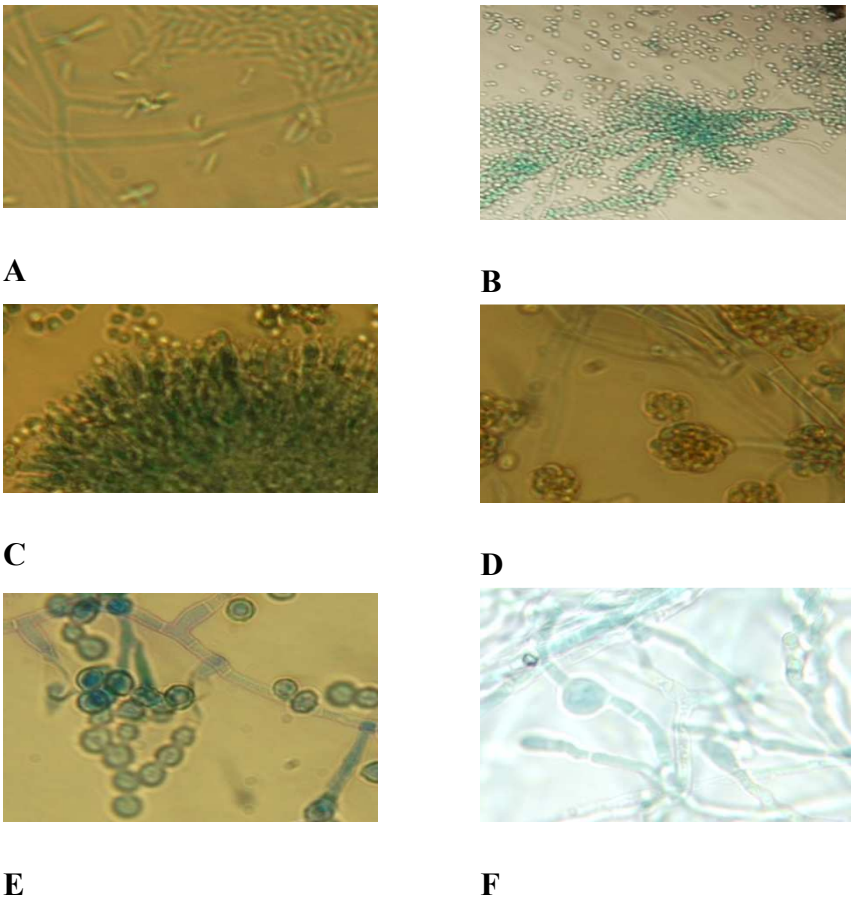


Plate 4.2: Anamorphic features of some fungal isolates showing conidia and conidiophores structures from the three lakes, 1000x magnification. (A-EL25-*Fuasarium sp*, B-SN5- *Rhodotorula sp,* C-EL15-*Aspergillus sp*, D-EL4-*Verticillium sp*. E-NK20-*Scopulariopsis sp* and F-SN19- *Fusarium sp*).

4.2.1 Presumptive identification of fungal isolates

The identification of fungal isolates was based on mycelium, conidiophores and conidial cell characteristics using different fungal identification keys and illustrations to genera level. (Plate 4.1 and 4.2). Table 4.2 shows the cultural and morphplogical characteristics of the fungus isolate.

Table 4.2: Growth and morphological characteristics of fungal isolates from Lakes Elmentaita, Nakuru and Sonachi after 5 days inoculation in MEA (EL – Elmenteita; NK – Nakuru; SN – Sonachi)

Isolate	Sequence No.	Colour on MEA	Mean growth (mm).	Morphological description	Fungus
EL I	1FF390R	White cream, rough	16	Conidiophere, phialides, hyphal coils,septate and hyaline hyphae	*Plectosphaerella sp*
EL 4	4FF390R	Brown,white margins, ridged	13	Conidiophores branched, phialides verticillate, conidia in small moist clusters.	*Verticillium sp.*
EL6	6FF390R	White, pink mycelia	48	Budding spherical cells, single pseudo hyphae	*Rhodotorula sp.*
EL8	8FF390R	Whitish-yellow, cottony	43	Hyphae hyaline, phialoconidum, micro and macro-conidia	*Fusarium sp.*
EL9	9FF390R	Sand-yellow, cottony mycelia	17	Phialoconidia, micro and macro-conidia, septate hyphae	*Fusarium sp*
EL12	12FF390R	Brownish mycelia	19	Conidiophores branched, annellation, conidia annellospores, septate hyphea	*Scopulariopsis sp.*
EL13	13FF390R	White mycelia, smooth margin	81	Hyphae hyaline, phialoconidum, micro and macro-conidia	*Fusarium sp.*

Isolate	Sequence No.	Colour on MEA	Mean growth (mm).	Morphological description	Fungus
EL14	14FF390R	White grey, yellow	30	Hyphae hyaline septate, conidiogenous cell, phialide present.	*Sacrodium sp.*
EL15	15FF390R	Grey white	160	Conidiophore upright, simple. Clavate swelling, phialides, basipetal phialospores	*Aspergillus sp*
EL16	16FF390R	yellow white, cottony mycelia	75	Phialoconidia, micro and macro-conidia, septate hyphae	*Fusarium sp.*
EL25	25FF390R	Whitish mycelia	78	Phialoconidia, micro and macro-conidia, septate hyphae	*Fusarium sp*
EL26	26FF390R	White grey	38	Conidiophore up right, simple. Clavate swelling, phialides, basipetal phialospores	*Aspergillus sp.*
EL27	27FF390R	Yellow brown, ridged	16	Branching conidiophores, hyaline septate hyphae, phialospores in chains,ovoid to fusoid	*Paecilomyces sp.*
EL11	11FF390R	Orange mycelia	16	Conodiophere, phialides,hyphal coils,septate and hyaline hyphae	*Plectosphaerella sp.*
NK2	2ff390R	Brown cream mycelia	20	Conidiophores branched, annellation, conidia annellospores, septate hyphea	*Scopulariopsis sp*

Isolate	Sequence No.	Colour on MEA	Mean growth (mm).	Morphological description	Fungus
K10	10FF390R	Whitish mycelia	35	Conodiophere, phialides, hyphal coils,septate and hyaline hyphae	*Plectosphaerella sp*
NK20	20FF390R	Brown mycelia	21	Conidiophores branched, annellation, conidia annellospores, septate hyphea	*Scopulariopsis sp*
NK7	7FF390R	light grey mycelia	12	Conidiophores erect, single branched, single celled spores, septate hyphae	*Acremonium sp.*
NK18	18FF390R	Grey cottony mycelia	9	Conidiophores erect, single branched, single celled spores, septate hyphae	*Acremonium sp.*
NK23	22FF390R	grey, white margins	15	Phialoconidia, micro and macro-conidia, septate hyphae	*Fusarium sp.**
SN21	21FF390R	white cream	35	Phialoconidia, micro and macro-conidia, septate hyphae	*Fusarium sp.*
SN24	24FF390R	Grey White cream	1118	Branching conidiophores, hyaline septate hyphae, phialospores in chains,ovoid to fusoid	*Paecilomycetes*
SN3	3FF390R	White cream	18	Conidiophere, phialides, hyphal coils, septate and hyaline hyphae	*Plectosphaerella sp.*
SN5	5FF390R	Whitish-yellow spores	34	Budding spherical cells, single pseudo hyphae	*Rhoditorula sp*
SN19	19FF390R	Grey whitish mycelia	20	Phialoconidia, micro and macro-conidia, septate hyphae	*Fusarium sp*
SN31	31FF390R	Cream yellow mycelia	35	Conidiophores branched, annellation, conidia annellospores, septate hyphea	*Scopulariopsis sp.*

4.3 Effect of salt, pH, and temperature

Each of the fungal isolates was grown on malt extract agar (MEA) and incubated at 28 ºC at different, percentage of sodium chloride, pH and temperature. Mean growth of each isolate was done after five days.

4.3.1 Effect of Sodium chloride

Table 4.3: Effect of Sodium Chloride on fungal growth (mm) ***(EL - Elmenteita; NK – Nakuru; SN – Sonachi)***

Isolate	0 % NaCl	5%NaCl	10% NaCl	15%NaCl
EL I	20	21	2	0
EL 4	27	21	2	0
EL6	18	13	0	0
EL8	28	40	14	0
EL9	75	76	21	1
EL12	61	65	43	3
EL13	71	49	32	1
EL14	71	71	26	0
EL15	26	32	4	0
EL16	71	62	26	0
EL25	71	32	3	0
EL26	21	15	0	0
EL27	35	18	1	0
EL11	29	29	5	0
NK2	71	46	1	0
NK10	33	4	0	0
NK20	64	76	51	0
NK7	28	24	0	0
NK18	11	31	3	0
NK23	15	32	3	0
SN3	13	29	8	0
SN21	70	40	12	0
SN24	18	4	2	1
SN5	8	34	2	0
SN19	71	69	24	2
SN31	71	44	2	0

4.3.2 Effect of pH on fungal isolates growth

Table 4.4: Effect of pH on fungal growth (mm): *(EL – Elmenteita; NK – Nakuru; SN – Sonachi)*

Isolate	pH4	pH7	pH9	pH12
EL I	12	33	35	43
EL 4	27	33	30	36
EL6	14	18	21	28
EL8	39	71	61	63
EL9	60	72	76	74
EL12	25	52	60	61
EL13	64	78	85	66
EL14	61	80	85	75
EL15	19	28	23	40
EL16	60	80	85	77
EL25	40	80	85	76
EL26	23	27	36	11
EL27	45	72	63	37
EL11	16	26	29	31
NK2	25	48	56	65
NK10	15	22	26	42
NK20	22	54	60	63
NK7	26	32	36	29
NK18	14	24	25	45
NK23	13	22	29	38
SN3	28	25	28	34
SN21	35	67	73	60
SN24	25	78	35	30
SN5	12	23	22	36
SN19	57	80	85	74
SN31	24	45	62	65

4.3.3 Effect of temperature on fungal isolates growth

Table 4.5: Effect of temperature on fungal growth (mm): (EL – Elmenteita; NK – Nakuru; SN – Sonachi)

Isolate	26 ºC	28 ºC	30 ºC	35 ºC
EL I	35	20	2	0
EL 4	21	25	27	17
EL6	20	22	25	16
EL8	62	59	69	27
EL9	70	71	61	13
EL12	47	57	43	22
EL13	68	71	71	13
EL14	71	71	71	45
EL15	39	26	21	3
EL16	66	71	71	20
EL25	71	71	52	4
EL26	29	31	26	9
EL27	22	24	27	12
EL11	34	12	1	0
NK2	46	56	43	22
NK10	32	9	1	0
NK20	46	64	45	23
NK7	23	28	25	11
NK18	36	11	11	12
NK23	45	24	2	0
SN3	34	13	9	6
SN21	55	70	64	41
SN24	18	18	27	29
SN5	36	8	3	0
SN19	71	71	71	31
SN31	47	62	44	22

4.4 Screening of fungal isolates for enzymatic activity

4.4.1 Lipolytic activity

Enzymatic activities of isolates from the three lakes were variable based on the substrates as shown in Table 4.6. Only one isolate (EL25) from Lake Elmentaita was able to degrade lipids with enzymatic index of 0.017 (Table 4.6). Two isolates (NK20 and NK23) from Lake Nakuru were able to degrade lipids with enzymatic index of 0.017 and.0.054), respectively Out of the 6 isolates from Lake Sonachi, five isolates had high effect on degrading lipids with maximum and minimum enzymatic index of 0.042 (SN5) and 0.013 (SN3) respectively (Table 4.6).

Table 4.6: Lipolytic activity with tween 20 as substrate (EL – Elmenteita; NK – Nakuru; SN – Sonachi).

Isolate	Colony diameter	Clear zone Diameter	Enzymatic index(EI)
EL I	33.5	0	0
EL 4	30.5	0	0
EL6	28.4	0	0
EL9	55.5	0	0
EL12	38	0	0
EL13	49	0	0
EL14	59	0	0
EL15	38	0	0
EL16	34	0	0
EL25	29.5	31.5	0.017
EL26	50	0	0
EL27	32.5	0	0
EL11	34.5	0	0
NK2	28	0	0
NK10	28.5	0	0
NK20	29.0	31.5	0.021
NK7	39.0	0	0
NK18	31.5	0	0
NK23	28.5	35.0	0.054
SN3	18.5	20	0.013
SN21	35.5	39	0.029
SN24	38.0	0	0
SN5	40.0	45	0.042
SN19	36.0	28.5	0.021
SN31	28.5	32	0.029

4.4.2 Esterase activity

Three isolates (EL6, EL13 and EL15) from Lake Elmentaita were able to degrade ester substrate, tween 80. However, they had variable enzymatic index (Table 4.7). Isolates from Lake Nakuru, however, had exceptional growth patterns with only one isolate (NK18) degrading ester compounds with the highest enzymatic index of 0.183. Two isolates (SN3 and SN5) from Lake Sonachi indicated enzymatic activity on ester compounds with index of 0.029 and 0.05 respectively. The isolate EL6, EL13, NK18 and SN5 were observed to grow on the ester substrate exceptional indicating high enzymatic activities.

Table 4.7: Esterase activity with tween 80 as substrate (EL – Elmenteita; NK – Nakuru; SN – Sonachi)

Isolate	Colony Diameter(mm)	Clear zone Diameter (mm)	Enzymatic Index (EI).
EL I	36.5	0	0
EL 4	48	0	0
EL6	14.6	20.5	0.05
EL8	40.5	0	0
EL9	38.0	0	0
EL12	45.0	0	0
EL13	49	53.5	0.038
EL14	59	0	0
EL15	13	14.5	0.013
EL16	42	0	0
EL25	36.5	0	0
EL26	40.0	0	0
EL27	35.6	0	0
EL11	38.0	0	0
NK2	36.5	0	0
NK10	28.0	0	0
NK20	42.2	0	0
NK7	30.5	0	0
NK18	32.0	54	0.183
NK22	28.5	0	0
NK23	36	0	0
NK2	34.5	0	0
SN3	24	27.5	0.029
SN21	45	0	0
SN24	38.5	0	0
SN5	44.5	50.5	0.05
SN19	48.0	0	0
SN31	46.5	0	0

4.4.3 Starch hydrolysis by isolates from Lake Sonachi

Test for amylase activity was carried out with 6 isolates from Lake Sonachi (Table 4.8). These were the only isolates that were able to grow on starch substrate constituted with amylose components. Only one isolate (SN3) was able to degrade amylose with enzymatic index of 0.108.

Table 4.8: Starch hydrolysis by isolates from Lake Sonachi

Isolate	Colony Diameter(mm)	Clear zone Diameter (mm)	Enzymatic Index(EI)
SN3	21.5	34.5	0.108
SN21	22	0	0
SN24	23	0	0
SN5	30	0	0
SN19	28	0	0
SN31	30.5	0	0

4.4.4 Proteolytic activity

Enzymatic activity of isolates on gelatin was observed to vary across isolates and the three lakes (Table 4.9). Lake Elmentaita had the highest number of isolates to degrade gelatin components. 6 isolates (EL1, EL4, EL6, EL14, EL15 and EL11) had remarkable enzymatic activity of 0.133, 0.05, 0.033, 0.188, 0.042, 0.05 and 0.079 respectively. Three isolates from Lake Nakuru, NK20, NK18 and NK22, showed activity on gelatin with an enzymatic index of 0.125, 0.183 and 0.017 respectively. All isolates from Lake Sonachi exhibited enzymatic activity ranging from 0.088 to 0.196 on gelatin. These isolates are the unique colonies with a steady and highest range of enzymatic activity.

Table 4.9: Proteolytic with gelatin as substrate. (EL – Elmenteita; NK – Nakuru; SN – Sonachi)

Isolate	Colony Diameter(mm)	Clear zone Diameter (mm)	Enzymatic Index(EI)
EL I	46.5	62.5	0.133
EL 4	30	36	0.05
EL6	16	30	0.033
EL8	35	0	0
EL9	35.5	0	0
EL12	40	0	0
EL13	36.5	0	0
EL14	26.5	49	0.188
EL15	12.5	18.5	0.05
EL25	34.0	0	0
EL26	38.5	0	0
EL27	40.1	0	0
EL11	12	21.5	0.079
NK7	28	0	0
NK20	48.5	63.5	0.125
NK18	32	54	0.183
NK22	11.5	13.5	0.017
NK23	34	0	0
NK2	28	0	0
SN3	23	46.5	0.196
SN21	46.5	64.5	0.15
SN5	42.5	53	0.088
SN19	57.5	71.5	0.117
SN31	48.5	48.5	0.125

4.4.5 Pectinolytic activity

Enzymatic activity on Pectin (Table 4.10) was observed to have relatively lower enzymatic index for most of the isolates. Three isolates (EL1, EL14 and EL15) from Lake Elmentaita were able to degrade pectin compounds. The isolates (NK10, NK20, NK22 and NK23) from Lake Nakuru also had enzymatic activity on pectin. Low enzymatic index was also observed on isolates from Lake Sonachi that had 6 isolates and three were able to degrade pectin substrate (SN3, SN21 and SN31. The isolates had enzymatic index of 0.013, 0.021 and 0.021 respectively.

Table 4.10: Pectinolytic activity with pectin as substrate. (EL – Elmenteita; NK – Nakuru; SN – Sonachi)

Isolate	Colony Diameter(mm)	Clear zone Diameter (mm)	Enzymatic Index(EI)
EL 1	55	57	0.017
EL 4	38	0	0
EL6	42	0	0
EL8	40.5	0	o
EL9	34.5	0	0
EL12	30.5	0	0
EL13	34.0	0	0
EL14	28.3	31.5	0.025
EL15	58	62.5	0.038
EL16	36	0	0
EL25	40	0	0
EL26	38	0	0
EL27	36.5	0	0
EL11	45.0	0	0
NK2	54	0	0
NK10	57.5	61.5	0.033
NK20	59.5	64	0.038
NK7	18	0	0
NK18	22	0	0
NK22	14	15.5	0.013
NK23	27	28.5	0.013
SN3	28	29.5	0.013
SN21	53.5	56	0.021
SN24	26	0	0
SN5	58	0	0
SN19	54	0	0
SN31	69	71.5	0.021

4.4.6 Cellulolytic activity

Degradation of complex carbon compounds, Carboxymethyl cellulose (Table 4.11) was observed to be low in a few isolates. Two isolates (EL1 and EL6) from Lake Elmentaita had an enzymatic index of 0.092 and 0.125 respectively. Three isolates (NK7, NK18 and NK23) from Lake Nakuru had enzymatic index of 0.075, 0.013 and 0.063 respectively. Four isolates from Lake Sonachi had enzymatic index ranging from 0.01 to 0.083.

The selected plates below reveal the clearing zones due to enzymatic activity on the substrate for some fungal isolates.

EL1 NK8 EL14

Plate 4.3: Enzymatic activity showing clearing zones on medium around the fungal colony

Table 4.11: Cellulolytic activity with carboxymethyl cellulose as substrate

Isolate	Colony Diameter(mm)	Clear zone Diameter (mm)	Enzymatic Index(EI)
EL I	18	29	0.092
EL 4	34.5	0	0
EL6	30	44	0.125
EL8	28	0	O
EL9	32	0	0
EL1	28	0	0
EL13	36	0	0
EL14	43	0	0
NK2	21	0	0
NK10	28	0	0
NK20	32	0	0
NK7	29	38	0.075
NK18	20	31.5	0.013
NK22	14	0	0
NK23	10	18	0.063
SN3	15.5	34.5	0.175
SN24	30	41.5	0.1
SN5	20	31.5	0.083
SN19	28	0	0
SN31	33	0	0

4.5 Molecular characterization of fungal isolates

4.5.1 PCR amplification of fungal DNA

PCR amplification of 26 isolates fungal DNA using Fungi primers yielded products of approximately 1400bp as shown in plate 4.4. The bands below the isolate numbers (1 to 31) indicate the presence of amplicon 18s rDNA.

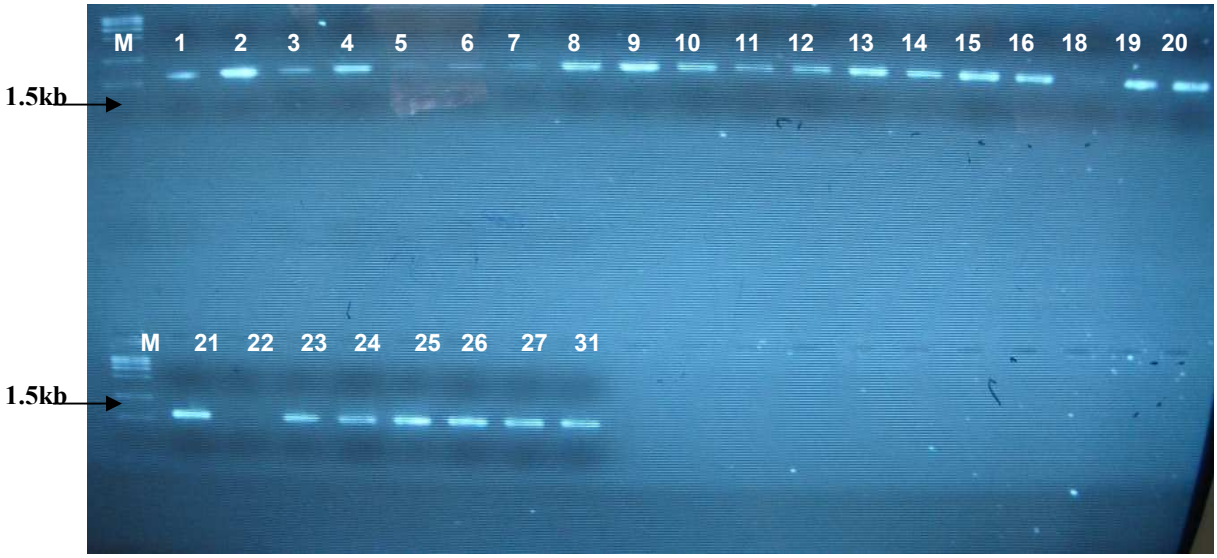


Plate 4.4: PCR amplification of fungal isolates 18s rDNA. The bands below the isolate number prove a positive amplification

4.5.2 Sequencing and Phylogenetic analysis

Sequence data obtained had a consistent percentage similarity almost 99% to their representatives from the NCBI public genebanks (Appendix 1.2). Isolates capture on the phylogenetic tree formed congruent clusters with the haplotypes as shown in figure 4.1. Captured haplotypes include *Scopulariopsis spp., Petriellasetifera spp., Halosapheialotica spp., Rodotorula spp., Fusarium spp., Artomyces spp., Verticillium spp., Acrimonis spp.,* and *Aspergillus spp*

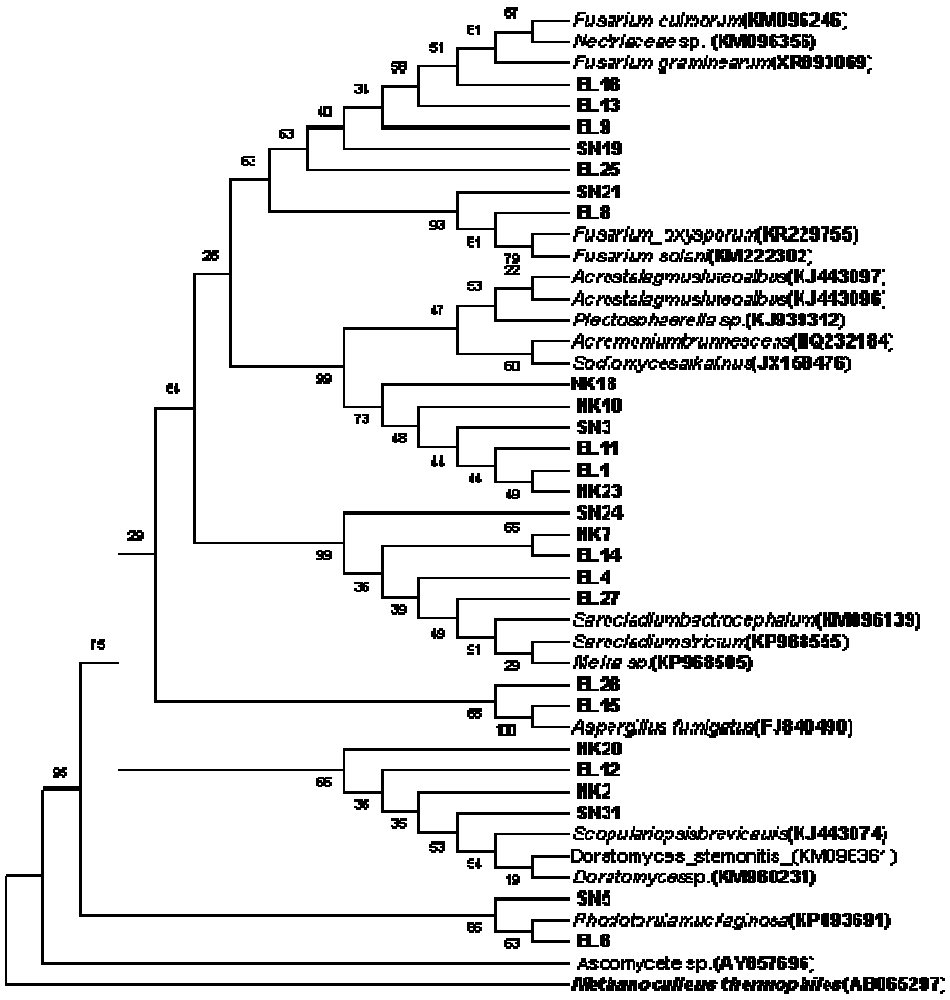


Figure 4.1: Phylogenetic tree of the soda Lakes isolates (EL-Elmentaita, NK-Nakuru and SN-Sonachi)

Method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 2.54253789 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felseesetein et al., 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The differences in the composition bias among sequences were considered in evolutionary comparisons (Tamura & Kumar, 2002). The analysis involved 46 nucleotide sequences. The codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for

each sequence pair. There were a total of 569 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

4.6 Screening for antimicrobial activity

The fungal crude filtrates were observed to have antimicrobial effect on the selected bacterial species; the tests were in triplicates (Plate 4.5. and Table 4.12.).

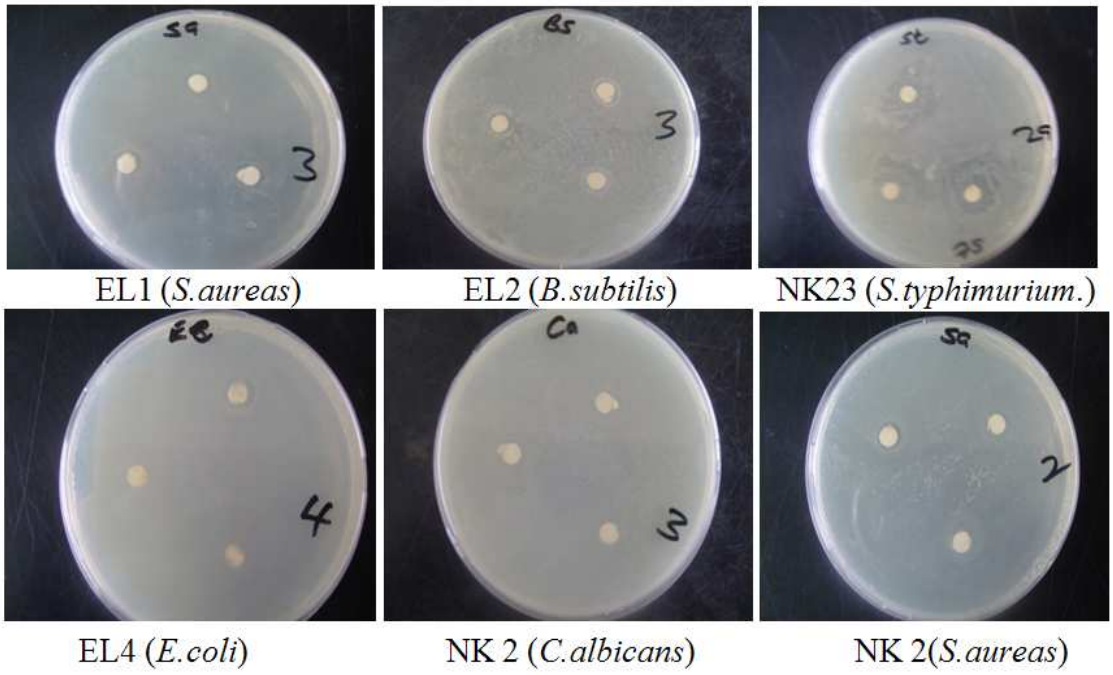


Plate 4.5: Antimicrobial activity of some plates showing inhibition clearing zones around the 6mm paper disc absorbed with metabolite filtrate on medium seeded with test organism

The antimicrobial activity of the crude filtrates from Lake Nakuru isolates ranged from 6.83± 0.44 to 10.83±0.44 which was low as compared to Lake Elmentaita isolates (6.33±0.33 to 12.00± 0.29). Antimicrobial Activity of crude filtrate from Lake Sonachi isolates ranged between 7.00±0.00 to11.50±0.29. It was observed that the level of antimicrobial activity was high from the Lake Elmentaita isolates followed by the Lake Sonachi and isolates from Lake Nakuru had the lowest crude filtrate activity against the selected bacteria species (Table 4.12). The control test recorded no inhibition and was 6.00±0.00 which was actually the diameter of inoculation disc.

Some isolates crude filtrate did not show any antimicrobial activity on specific bacterial species. Isolate EL1 did not produce crude filtrate that was effective against *Escherichia. coli*. The crude filtrates from isolates EL4, 6 and 8 did not have quantifiable inhibition activity against *Streptococcus pneumoniae,* and *Candida albicans* showed total resistance to crude filtrates from isolates NK10, NK18, NK20 and NK23. *Streptococcus pneumoniae* also showed antimicrobial activity to crude filtrates from isolates SN3, SN5, SN19, and SN24.Table 4.2 shows

Table 4.12: Antimicrobial activity of fungal isolates crude filtrate from the lakes on selected test microorganisms

	Microorganisms					
Isolate	***B. sbtilis***	***C.albicans***	***E.coli***	***P.aeruginosa***	***S.aureas***	***S.pneumoniae***
EL 1	6.33±0.33^{d}	7.17±0.17^{bc}	6.00±0.00^{e}	7.05±0.00^{d}	7.50±0.29^{bc}	9.50±0.57^{ab}
EL 4	6.00±0.00^{d}	7.17±0.17^{bc}	9.50±0.29^{abc}	7.50±0.00^{d}	7.50±0.29^{bc}	9.50±0.57^{ba}
EL6	7.00±0.00^{bc}	8.00±0.58^{abc}	7.50±0.29^{cde}	8.50±0.50^{bcd}	9.17±0.17^{ba}	6.00±0.00^{c}
EL8	6.00±0.00^{d}	7.33±0.17^{bc}	8.00±0.00^{bcde}	7.50±0.00^{d}	6.00±0.00^{c}	9.50±0.50^{ab}
EL9	8.00±0.58^{bcd}	8.33±0.33^{abc}	7.5.0±0.29^{cde}	7.00±0.00^{d}	8.33±0.44^{b}	8.50±0.76^{b}
EL11	9.00±0.00^{abc}	8.10±0.56^{abc}	I0.33±0.60^{ab}	10.33±0.33^{ab}	10.50±0.00^{a}	10.83±0.60^{a}
EL12	8.00±0.58^{bcd}	7.00±0.58^{de}	9.17±0.00^{abcd}	7.00±0.00^{d}	10.50±0.00^{a}	8.50±0.29^{bc}
EL13	8.00±0.00^{bcd}	10.00±0.58^{a}	8.00.00±0^{bcde}	7.83±0.44^{ab}	9.00±0.50^{ab}	7.83±0.44^{bc}
EL14	8.50±0.29^{bc}	6.00±0.00^{c}	9.50±0.00^{abc}	9.50±0.50^{bc}	8.00±0.00^{bc}	6.00±0.00^{c}
EL15	8.67±0.33^{bc}	8.60±0.33^{ab}	9.00±0.00^{abcd}	8.00±0.58^{cd}	8.67±0.33^{ab}	9.50±0.29^{ab}
EL16	7.00±0.00^{dc}	8.00±0.76^{abc}	8.00±0.00^{bcde}	8.00±0.58^{cd}	8.00±0.58^{bc}	9.50±0.29^{ab}
EL25	11.00±0.58^{a}	8.50±0.76^{ab}	8.67±0.00^{abcd}	12.00±0.29^{a}	9.00±0.58^{ab}	6.00±0.00^{c}
EL26	6.00±0.00^{d}	8.50±0.76^{ab}	9.17±0.00^{abcd}	8.50±0.29^{bcd}	6.00±0.00^{c}	8.00±0.00^{bc}
EL27	9.33±0.67^{ab}	8.00±0.50^{abc}	10.50±0.00^{a}	10.33±0.33^{ab}	9.00±0.50^{ab}	10.83±0.60^{a}
NK2	7.33±0.33^{b}	6.83±0.44^{a}	7.30±0.333^{b}	7.00±0.00^{bc}	7.00±0.00^{b}	6.00±0.00^{bc}
NK10	6.00±0.00^{c}	6.00±0.00^{a}	7.50±0.29^{b}	8.00±0.58^{ab}	7.00±0.00^{b}	6.00±0.00^{c}
NK18	7.00±0.00b^{c}	6.00±0.00^{a}	7.00±0.00^{b}	8.50±0.29^{ab}	8.50±0.29^{a}	8.00±0.00^{b}
NK20	8.50±0.29^{a}	6.00±0.00^{a}	10.00±0.58^{a}	9.33±0.67^{a}	8.50±0.29^{a}	6.00±0.00^{c}
NK23	7.50±0.29^{ab}	6.00±0.00^{a}	10.83±0.44^{a}	8.50±0.29^{ab}	7.50±0.58^{ab}	10.00±0.58^{a}
SN3	9.00±0.58^{a}	9.50±0.50^{a}	8.33±0.33^{b}	7.50±0.29^{ab}	10.00±0.58^{a}	6.00±0.00^{b}
SN5	6.00±0.00^{b}	9.50 ±0.29^{a}	7.50±0.29^{b}	6.00±0.00^{b}	7.00±0.58^{b}	6.00±0.00^{b}
SN19	7.30±00.44^{b}	6.00±0.00^{b}	8.67±0.88^{b}	6.00±0.00^{b}	7.33±0.44^{b}	6.00±0.00^{b}
SN21	10.17±0.44^{a}	9.50±0.29^{a}	11.00±0.58^{a}	10.00±0.87^{a}	11.50±0.29^{a}	6.00±0.00^{b}
SN24	7.00±0.00^{b}	7.67±0.88^{ab}	8.00±0.00^{b}	8.50±0.29^{ab}	7.00.00±0.44^{b}	6.00±0.00^{b}
SN31	9.00±0.00^{a}	10.50±0.87^{a}	8.33±0.00^{b}	10.00±0.00^{a}	10.00±0.87^{a}	8.50±0.76^{a}

EL. Elmentaita: NK. Nakeru: SN: Sonachi. Inhibition (Mean ± SE,) followed with the same superscript letter within a column are not significantly difference ($p< 0.05$, n=3 Tukeys tests)

4.6.1 Antimicrobial activity using crude filtrate

The fugal isolate with the highest inhibition against *Bacillus subtilis* was EL25, while the lowest was NK 2.There was no antimicrobial activity from isolates, EL4, EL8 and EL26 against the same test microorganism. The fungal isolate crude filtrates that had the highest inhibition for *Candida albicans* was SN21, least being NK19, isolate EL1 did not show any inhibition. All the fungal isolates crude filtrate were active against *Escherichia.coli* except EL1. The fungal isolate, EL25, had the highest inhibition and the lowest being EL9 and EL12, while isolates SN5, and SN19 were not active against *Pseudomonas aeruginosa*. The highest fungal isolate with the highest inhibition for *Staphylococcus.aureus* was SN21, lowest being SN24, NK2 and NK10, while, isolates EL8 and EL26 had no inhibition effects on the microorganism. The fungal isolate EL11 and EL 27 had the highest mean of inhibition, for *Staphylococcus.Pneumoniae*, the lowest being EL13. This test microorganism was not sensitive to eleven fungal isolates crude filtrates. The inhibition of test microorganism were significantly different ($p<0.05$). Table 4.12 shows statistical analysis of mean inhibition of the isolates crude filtrate on the test organisms. The (Mean ± SE,) followed with the same superscript letter within a column are not significantly different ($p< 0.05$, n=3 Tukeys tests).

4.6.2 Antimicrobial activity using solvent extract

Antimicrobial bioassay of the solvent extracts was carried out by measuring the inhibition zones in millimetres in triplicates. The test microorganism, *Salmonella typhimurium* was added to the six test organism. The highest inhibition was from isolate EL12 and 15, while the lowest was EL1, EL8, EL11, EL14, EL25, EL26 and NK7 and NK10. All the isolated were effective against *Bacillus.subtilis*. The fungal isolate solvent extracts that had the highest inhibition on *Candida albicans* was NK18, the minim inhibition was from the isolates EL1, EL4, EL6, EL12, EL25, EL26 and NK23 while isolates EL18,EL11,EL14,NK23 and SN19 did not show any inhibition effects. The fungal isolate, SN3, had the highest inhibition, lowest EL1, EL11, and NK2. The isolates EL8, EL13, EL14, EL26, NK7, NK10 and SN31 were not active against *Escherichia.coli* The fugal isolates with the highest inhibition was

EL27, lowest EL14, 26, NK23 and SN31., The isolate NK10 had no effect on *Pseudomonas aeruginosa*. The fungal isolate with the highest inhibition for *Staphylococcus.aureus* was EL13, lowest being EL11 and EL2, NK10 and SN31, The isolates EL11 and NK2 had no inhibition effects against the microorganism. The fungal isolate EL18 had the highest mean of inhibition, lowest EL1, EL4, EL6, NK2, NK7, NK10 and SN3.on *Staphylococcus.Pneumonia*, isolate EL8 and EL9 were not active against this test microorganism. The highest inhibition on *Salmonella typhimurium* was from the fungal isolate EL14, lowest EL12, EL13, NK18 and NK23, isolate EL8,.EL24, EL27, NK10, SN24 and SN31 had no inhibition effect on this microorganism. Inhibition of the test organism from the solvent fungal isolates extract were significantly different ($p<0.05$). Table 4.13 shows statistical analysis of mean inhibition of the isolates solvent extract filtrate on the test organisms. The (Mean ± SE,) followed with the same superscript letter within a column are not significantly different ($p< 0.05$, n=3 Tukeys tests).

Table 4.13: Antimicrobial activity of fungal isolates solvent extracts from soda lakes

	Microorganisms						
Isolate	***B. sbtilis***	***C.albicans***	***E.coli***	***P.aeruginos***	***S.aureus***	***S.pneumoniae***	***S typhi***
EL 1	7.00 ± 0.00^{d}	7.00 ± 0.00^{d}	7.00 ± 0.00^{c}	8.50 ± 0.29^{bc}	9.00 ± 0.00^{bc}	7.00 ± 0.00^{dc}	8.50 ± 0.29^{b}
EL 4	13.03 ± 0.67^{b}	7.00 ± 0.00^{d}	9.30 ± 0.89^{ab}	8.50 ± 0.29^{bc}	9.00 ± 0.00^{bc}	7.00 ± 0.00^{dc}	8.50 ± 0.29^{b}
EL6	7.00 ± 0.00^{d}	7.00 ± 0.00^{d}	7.50 ± 0.29^{abc}	8.50 ± 0.29^{bc}	9.00 ± 0.00^{bc}	7.00 ± 0.00^{dc}	8.50 ± 0.29^{b}
EL8	7.00 ± 0.00^{d}	6.00 ± 0.00^{e}	6.00 ± 0.00^{c}	7.50 ± 0.29^{c}	8.00 ± 0.58^{cd}	6.00 ± 0.00^{d}	6.00 ± 0.00^{b}
EL9	6.00 ± 0.00^{d}	10.00 ± 0.00^{a}	7.50 ± 0.29^{abc}	9.50 ± 0.29^{b}	9.50 ± 0.29^{b}	6.00 ± 0.00^{d}	8.50 ± 0.29^{b}
EL11	7.00 ± 0.00^{d}	6.00 ± 0.00^{e}	7.00 ± 0.00^{bc}	7.67 ± 0.33^{bc}	6.00 ± 0.00^{e}	7.00 ± 0.00^{dc}	8.00 ± 0.58^{b}
EL12	26.00 ± 2.90^{a}	7.00 ± 0.00^{d}	8.70 ± 1.33^{ab}	7.67 ± 0.33^{c}	7.00 ± 0.00^{de}	8.00 ± 0.00^{bc}	7.00 ± 0.00^{b}
EL13	9.30 ± 0.67^{bcd}	7.50 ± 0.50^{cd}	6.00 ± 0.00^{c}	8.67 ± 0.33^{bc}	12.67 ± 0.33^{a}	8.00 ± 0.00^{bc}	7.00 ± 0.00^{b}
EL14	7.00 ± 0.00^{d}	6.00 ± 0.00^{e}	6.00 ± 0.00^{c}	7.00 ± 0.00^{C}	7.67 ± 0.33^{cd}	9.00 ± 0.58^{ab}	15.33 ± 2.40^{b}
EL15	26.00 ± 2.30^{a}	8.50 ± 0.29^{b}	9.00 ± 0.58^{ab}	7.67 ± 0.33^{c}	8.67 ± 0.33^{bc}	9.67 ± 0.33^{a}	15.30 ± 2.40^{a}
EL24	7.70 ± 0.00^{cd}	$8.00\pm0.00b^{c}$	8.00 ± 0.00^{abc}	8.50 ± 0.50^{bc}	6.00 ± 0.00^{e}	9.00 ± 0.00^{ab}	6.00 ± 0.00^{b}
EL25	7.00 ± 0.00^{d}	7.00 ± 0.00^{d}	9.50 ± 0.29^{ab}	8.67 ± 0.33^{bc}	8.67 ± 0.33^{bc}	8.67 ± 0.67^{ab}	7.50 ± 0.50^{b}
EL26	7.00 ± 0.00^{d}	6.00 ± 0.00^{e}	6.00 ± 0.00^{C}	7.00 ± 0.00^{C}	9.50 ± 0.50^{b}	7.67 ± 0.33^{bc}	7.5 ± 0.50^{b}
EL27	13.00 ± 0.60^{bc}	$7.50\pm.29^{cd}$	9.67 ± 0.67^{a}	12.67 ± 0.67^{a}	10.00 ± 0.00^{b}	9.85 ± 0.17^{a}	6.00 ± 0.00^{b}
NK2	8.00 ± 0.00^{b}	8.50 ± 0.50^{ab}	9.33 ± 0.33^{b}	9.33 ± 0.33^{a}	6.00 ± 0.00^{b}	7.00 ± 0.00^{b}	8.00 ± 0.00^{ab}

Isolate	Microorganisms						
	B. sbtilis	***C.albicans***	***E.coli***	***P.aeruginos***	***S.aureus***	***S.pneumoniae***	***S typhi***
NK7	7.00 ± 0.00^{b}	6.00 ± 0.00^{b}	6.00 ± 0.00^{C}	8.00 ± 0.00^{b}	8.00 ± 0.00^{a}	7.00 ± 0.00^{b}	9.00 ± 1.15^{a}
NK10	7.00 ± 0.00^{ab}	8.50 ± 0.29^{ab}	6.00 ± 0.0^{C}	6.00 ± 0.00^{d}	7.00 ± 0.00^{b}	7.00 ± 0.00^{b}	6.00 ± 0.00^{ab}
NK18	8.00 ± 0.00^{b}	11.00 ± 1.73^{a}	7.00 ± 0.00^{b}	$7.00\pm0.00_{C}$	7.67 ± 0.33^{a}	12.50 ± 1.44^{a}	7.00 ± 0.00^{ab}
NK20	8.33 ± 0.33^{b}	8.50 ± 0.77^{ab}	7.00 ± 0.00^{b}	7.00 ± 0.00^{C}	8.00 ± 0.00^{a}	7.50 ± 0.29^{b}	7.67 ± 0.00^{b}
NK23	18.00 ± 1.15^{a}	7.00 ± 0.00^{b}	9.50 ± 0.29^{a}	7.00 ± 0.00^{C}	7.67 ± 0.33^{a}	12.00 ± 1.15^{a}	7.00 ± 0.00^{ab}
SN3	12.00 ± 0.51^{b}	8.00 ± 0.00^{a}	11.00 ± 0.51^{a}	9.50 ± 0.29^{ab}	8.00 ± 0.00^{abc}	7.00 ± 0.00^{b}	10.50 ± 0.29^{ab}
SN5	16.50 ± 0.29^{a}	8.00 ± 0.58^{a}	9.50 ± 0.20^{b}	9.00 ± 0.00^{b}	8.00 ± 0.00^{abc}	7.67 ± 0.33^{b}	14.00 ± 2.31^{a}
SN19	10.67 ± 0.33^{bc}	6.00 ± 0.00^{b}	9.00 ± 0.00^{bc}	8.50 ± 0.29^{b}	9.00 ± 0.508^{a}	7.50 ± 0.29^{b}	8.50 ± 0.50^{b}
SN21	10.00 ± 0.0^{d}	8.50 ± 0.29^{a}	8.00 ± 0.00^{c}	10.50 ± 0.29^{a}	7.50 ± 0.29^{bc}	7.50 ± 0.00^{b}	8.00 ± 0.00^{b}
SN24	7.50 ± 0.29^{e}	8.00 ± 0.00^{a}	8.00 ± 0.00^{c}	8.33 ± 0.44^{b}	8.50 ± 0.29^{ab}	7.50 ± 0.29^{a}	6.00 ± 0.00^{b}
SN31	8.50 ± 0.29^{de}	7.50 ± 0.50^{ab}	6.00 ± 0.00^{d}	7.00 ± 0.00^{C}	7.00 ± 0.00^{C}	9.50 ± 0.29^{a}	6.00 ± 0.00^{b}

EL: Elmentaita, NK: Nakuru, SN: Sonachi. Inhibition (Mean ± SE,) followed with the same superscript letter within a column are not significantly difference ($p< 0.05$, n=3 Tukeys tests).

4.7 Identification of fungal isolates metabolites

The chromatogram for each isolate shows time in minutes (min) and abundance, the area occupied by the ions in the 40µl sample of fungal extract injected in the Gas Chromatography-Mass Sptrophotometer (GC-MS) in triplicates

4.7.1 Lake Elmentaita Isolates

A total of twelve different compounds were identified for isolate EL.1. Benzonitrile, 2-amino occurred in the highest proportion (0.8%) followed by N-Acetyltyramine (0.7%) and Mesitylene (0.5%) with the least two being Benzene, 1-ethyl-2-methyl- and Geranyl linalool (E,Z)- each accounting for (0.1%) respectively (Table 4.14 and Fig 4.2). The fungal extract for isolate 1 comprised of various classes of chemical compounds like: terpenes, example Geranyl linalool (E, Z); esters, Carbamic acid, methyl-, 3-methylphenyl ester and ketones, 2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone among others.

Table 4.14: Profile for isolate EL.1 showings the compounds and their abundance

Peak no.	RT(Min)	Compound name	Area %
1	7.9024	2-Furanmethanol	0.4875
2	9.1869	Benzene, 1-ethyl-2-methyl-	0.1479
3	10.3212	2-Furancarboxaldehyde, 5-methyl-	0.2262
4	10.814	Mesitylene	0.5345
5	11.5755	Cyclopentanedione<3-methyl-1,2->	0.379
6	21.8782	Cyclopropane, 1-methyl-2-octyl-	0.2615
7	22.6845	N-Acetyltyramine	0.7451
8	24.0059	Benzonitrile, 2-amino	0.8384
9	25.55737	Octadecanol<n->	0.3387
10	26.233	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	0.2012
11	28.0374	Carbamic acid, methyl-, 3-methylphenyl ester	0.1744
12	30.747	Geranyl linalool<E,Z->	0.1345

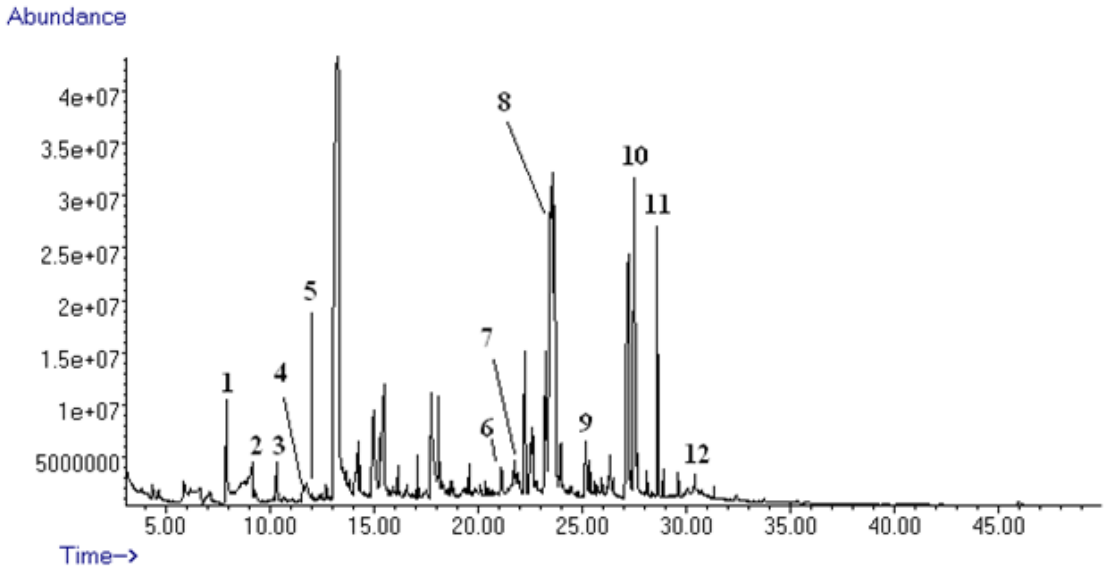


Figure 4.2: Total ion chromatogram profile for isolate El.1

Twelve different compounds were identified in fungal isolate El.6. Butanediol<2, 3-> (21.4%) occurred in the highest proportion followed by Cresol acetate <para-> (10.3%) and 5-Nitroso-2,4,6-triaminopyrimidine (9.7%) with the least in abundance being (2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans- (0.2%) (Tab.4.15, Fig. 4.3). Alcoholic class of compounds lead with the highest candidates totaling to five (Butanediol<2,3->, 2-Furanmethanol, Maltol, Propanol<3-methylthio-> and Phenol, 3-methyl-). The extract also contained ketones, such as Isophorone among other classes.

Table 4.15: Profile for isolate EL.6 showings the compounds and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	3.803	Propanol<3-methylthio->	0.789
2	6.558	Butanediol<2,3->	21.411
3	7.230	1H-Pyrazole, 3,5-dimethyl-	0.379
4	7.902	2-Furanmethanol	3.402
5	13.053	Maltol	1.987
6	13.243	Isophorone	1.863
7	21.430	N-BOC-trans-3-methyl-l-proline	0.474
8	23.535	5-Nitroso-2,4,6-triaminopyrimidine	9.704
9	23.893	2,4-Dimethoxyamphetamine	0.601
10	27.880	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	0.231
11	28.037	Cresol acetate <para->	10.282
12	28.373	Phenol, 3-methyl-	0.658

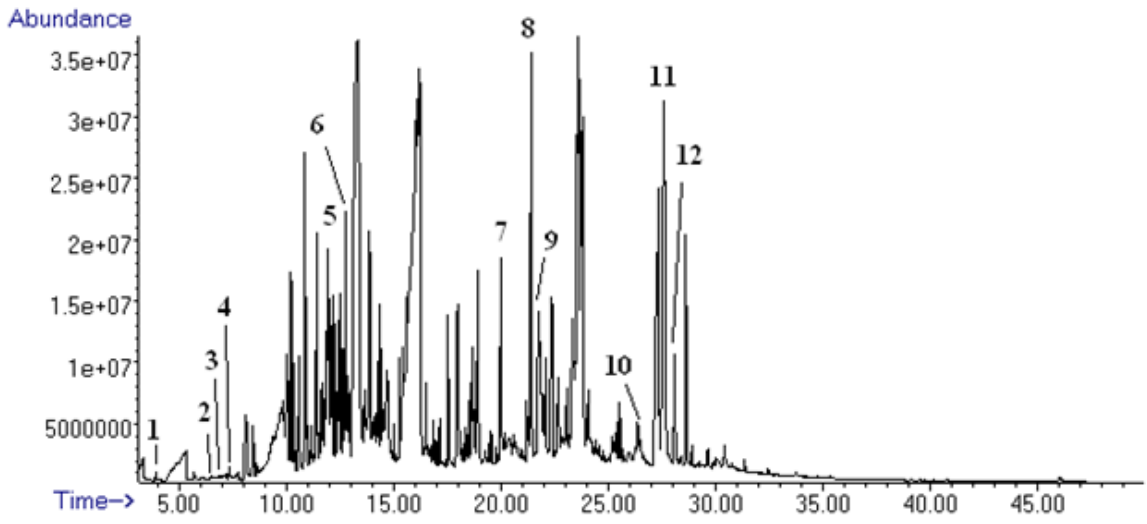


Figure 4.3: Total ion chromatogram profile for isolate El.6

Eleven compounds were identified in fungal isolate El. 8 (Tab.4.16 and Fig. 4.4) Concentration of compounds in this isolate ranged 0.2% to 12.5%. Benzene acetic

acid (12.5%) occurred in the highest proportion followed by Isovaleric acid (6.1%) and Menthatriene<1,3,8-para-> (1.3%). The second least in proportion was 1H-Indole, 5-methyl-(0.2%) and the least was 2,4,7-Trioxabicyclo[4.4.0]dec-9-ene, 8-decyloxy-3-phenyl- (0.2%). Carboxylic group of compounds had two members and are the one in which abudance were first and second. The extract also had a terpene, Menthatriene<1,3,8-para-> (1.3%) among other classes.

Table 4.16: Profile for isolate EL 8 showings the compounds and their abudance

Peak no.	Rt (min)	Metabolite	% area
1	9.7929	Isovaleric acid	6.099
2	10.3428	Furfural<5-methyl->	0.301
3	10.8356	Trimethyl benzene<1,2,4->	0.359
4	11.6867	1,2-Cyclopentanedione, 3-methyl-	0.335
5	11.778	Benzene, 1-methyl-4-propyl-	0.633
6	13.6352	Menthatriene<1,3,8-para->	1.288
7	15.0686	4,5-Imidazoledimethanol	1.252
8	15.7853	Benzeneacetic acid	12.464
9	17.1068	1H-Indole, 5-methyl-	0.228
10	21.608	Tridemorph	0.301
11	30.0971	2,4,7-Trioxabicyclo[4.4.0]dec-9-ene, 8-decyloxy-3-phenyl-	0.169

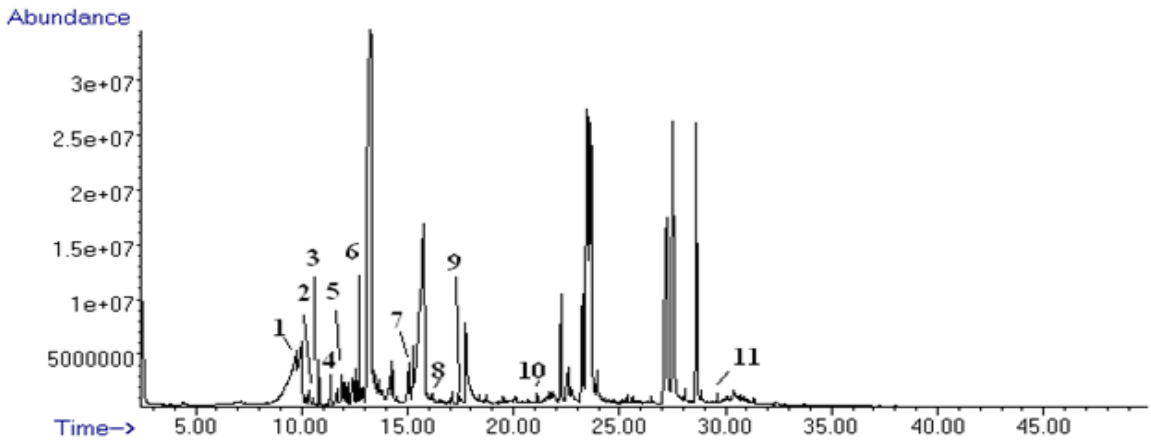


Figure 4.4: Total ion chromatogram profile for isolate El.8.

A total of twelve different compounds were identified in fungal isolate EL.9 (Tab. 4.17 and Fig. 4.5). Concentration of compounds in this isolate ranged from 0.2% to 34.2%. Phenyl ethyl alcohol (34.2%) was the most abundant and Tridemorph (0.2%) was the least. Carboxylic acid had two members, ((Benzeneacetic acid (1.2%) and Benzoic acid (1.2%)); alcohols three ((2-Furanmethanol (1.0%), Butanediol<2,3-> (0.3%) and Phenyl ethyl alcohol (34%)); ketones one (β-Piperidinopropiophenone 1.3%). The extract also had two drugs Tridemorph (0.2%) and Metolachlor (0.4%) among other groups of compounds.

Table 4.17: Profile for isolate EL 9 showings the compounds and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	5.8193	Butanediol<2,3->	0.318
2	7.8798	2-Furanmethanol	1.032
3	10.3211	Furfural<5-methyl->	0.623
4	11.1879	1,2-Cyclopentanedione, 3-methyl-	0.340
5	13.1879	Phenyl ethyl alcohol	34.210
6	14.0838	Benzoic acid	1.169
7	14.8901	1,2-Ethanediol, 1-(2-furanyl)-	2.440
8	15.3605	Benzeneacetic acid	3.546
9	18.0929	β-Piperidinopropiophenone	1.325
10	21.3853	Tridemorph	0.247
11	23.8266	Metolachlor	0.369
12	26.3127	6H-Dibenzo[c,E]1,2-thiazine, 8-methyl-, 5,5-dioxide	0.383

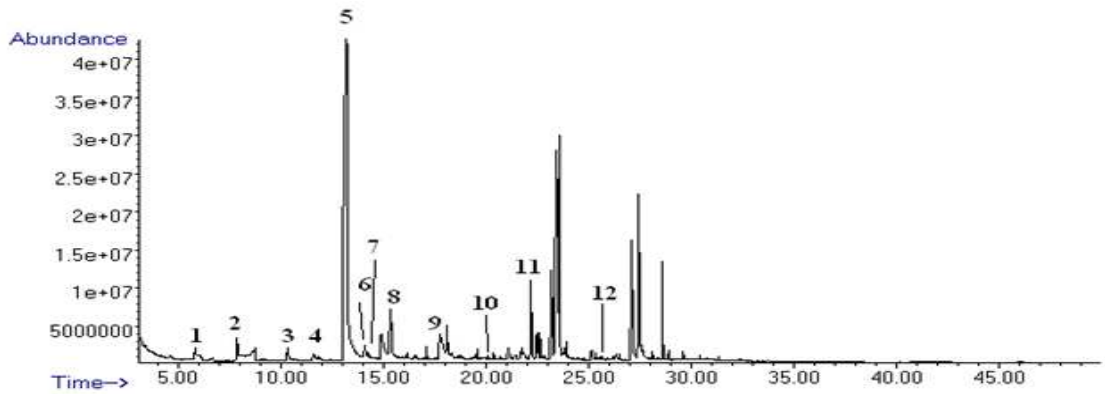


Figure 4.5: Total ion chromatogram profile for isolate El.9

Fourteen different compounds were identified in fungal isolate El.11 (Tab. 4.18 and Fig.4.6). Concentration of compounds for this isolate was in the range, 0.1% to 0.8%. Tetracyclo [3.3.1.1(1,8).0(2,4)] decane (0.8%) was the most abundant compound and Geranyl linalool<E,Z-> (0.1%) was the least. The compounds occurred with almost similar percentages

Table 4.18: Profile for isolate EL 11 showings the compounds and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	7.902	2-Furanmethanol	0.487
2	10.186	Benzene, 1-ethyl-2-methyl-	0.147
3	10.321	2-Furancarboxaldehyde, 5-methyl-	0.226
4	10.814	Mesitylene	0.534
5	11.373	Trimethyl benzene<1,2,4->	0.558
6	11.575	Cyclopentanedione<3-methyl-1,2->	0.637
7	12.551	Cymene<ortho->	0.681
8	12.852	p- Cymene	0.654
9	13.613	Tetracyclo[3.3.1.1(1,8).0(2,4)]decane	0.799
10	14.912	2-Furancarboxaldehyde, 5-methyl-	0.423
11	21.116	(1Z)-1H-Benzo[e]indole-1,2(3H)-dione 1-oxime	0.215
12	22.684	N-Acetyltyramine	0.745
13	26.223	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	0.201
14	30.747	Geranyl linalool<E,Z->	0.134

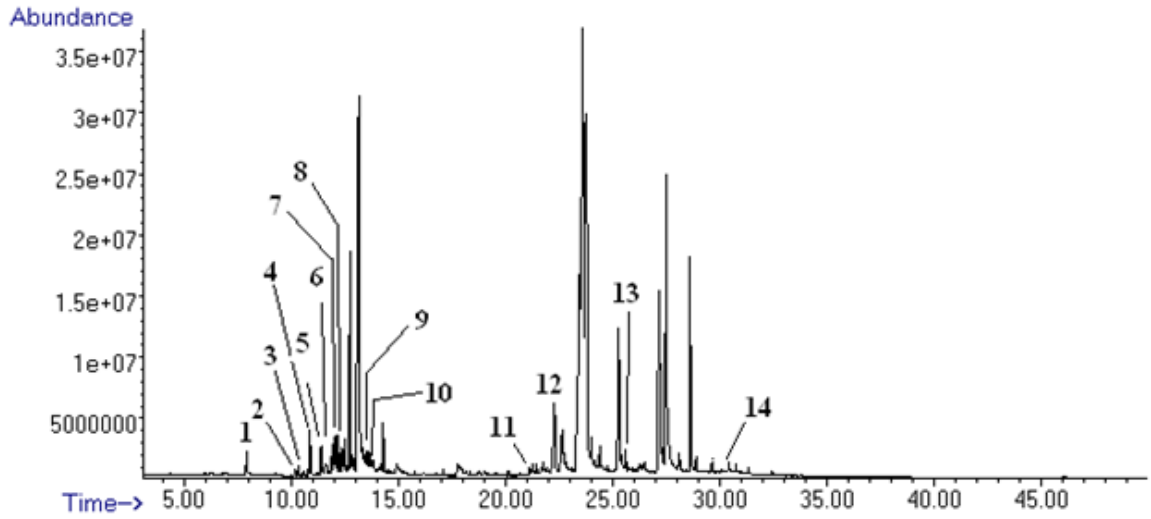


Figure 4.6: Total ion chromatogram profile for isolate El.11.

Sixteen compounds were identified in fungal isolate El.12 (Tab. 4.19 and Fig. 4.7) Concentration of compounds for isolate El 12 ranged from 0.2% to 4.7 %. Butanediol<2,3-> (4.7%) was the most abundant compound followed by Cresol acetate<para-> (4.5%) and Cymene<para-> (4.2%) while 3,4-Dimethoxycinnamic acid (0.2%) was the least.

Table 4.19: Profile for isolate EL 12 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	3.893	3-Hydroxy-2-butanone	1.318
2	3.960	Propyl acetate	01.621
3	4.497	Isopentyl formate	1.894
4	7.095	Butanediol<2,3->	4.686
5	8.954	Acetylcysteine	0.629
6	9.245	Isovaleric acid	0.660
7	10.186	Trimethyl benzene<1,2,4->	1.931
8	10.321	Mesitylene	1.417
9	11.373	Trimethyl benzene<1,2,4->	2.027
10	12.403	Cymene<para->	4.207
11	13.635	Arbozol<endo->	0.744
12	18.692	1,2-Benzisothiazol-5-amine, 3-methoxy-	3.431
13	20.422	Quinoline	0.309
14	21.855	1-Azabicyclo[2.2.2]oct-3-ylamine	0.423
15	28.059	Cresol acetate<para->	4.516
16	30.590	3,4-Dimethoxycinnamic acid	0.244

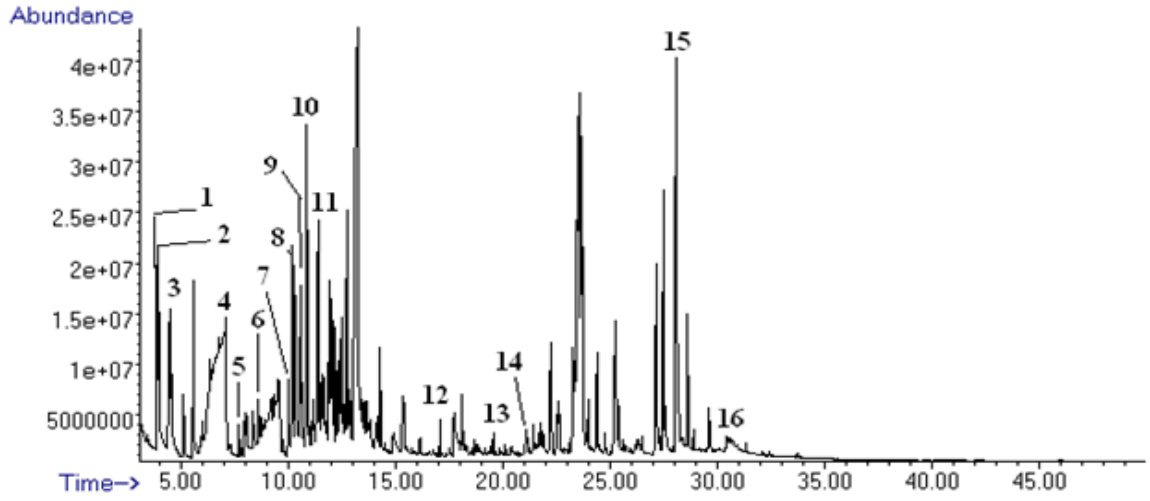


Figure 4.7: Total ion chromatogram profile for isolate El.12

Fourteen different compounds were identified in fungal isolate El.13 (Table 4.20 and Fig .4.8). Concentration of compounds for this isolate ranged from 0.3% to 8.2%. Acetamide, N-(2-phenylethyl)- (8.2%) was the most abundant compound followed by Butanediol<2,3-> (2.5%) while 2-Furancarboxaldehyde, 5-methyl- (0.3%) was the least in proportion.

Table 4.20: Profile for isolate EL 13 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	5.9312	Butanediol<2,3->	2.506
2	10.3211	2-Furancarboxaldehyde, 5-methyl-	0.312
3	11.5529	1,2-Cyclopentanedione, 3-methyl-	0.850
4	12.233	Arbozol<exo->	0.445
5	13.7479	Cymene<ortho->	2.380
6	14.4198	Cineole<dehydro-1,8->	1.401
7	16.6819	Acetophenone<4'-methoxy->	0.406
8	17.1298	Skatole	0.513
9	18.7872	Acetamide, N-(2-phenylethyl)-	8.298
10	20.131	Italicene	0.829
11	21.0741	Pyrimidine, 2-(dimethylamino)-5-nitro-	0.436
12	25.8871	Acetamide, N-[2-(1H-indol-3-yl)ethyl]-	0.559
13	27.335	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	0.589
14	30.8145	2,5-Piperazinedione, 3,6-bis(phenylmethyl)-	0.697

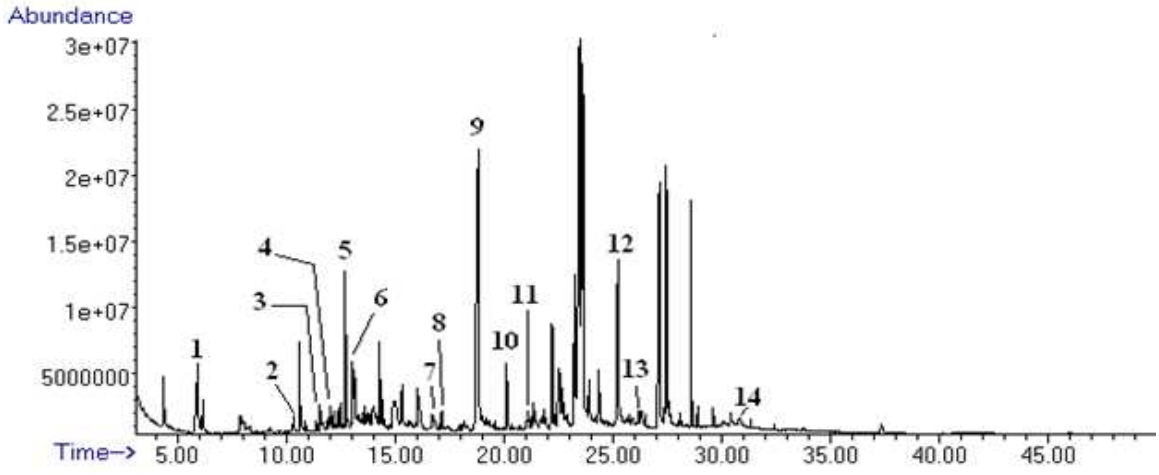


Figure 4.8: Total ion chromatogram profile for isolate El.13

Seventeen compounds were identified in fungal isolate EL.15 (Tab. 4.21 and Fig. 4.9). Concentration of compounds for this isolate ranged frm 0.1 % to 3.9 %. Isopentyl alcohol (3.9%) was the most abundant compound followed by Cresol<meta-> (3.3%) and Propanoic acid, 2-methyl-(2.5%). The least two in abundance were 2,3-Butanediol (0.2%) and N-(1-Cyclopenten-1-yl)-morpholine (0.1%).

Table 4.21: Profile for isolate EL 15 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	3.905	2-Butanone, 3-hydroxy-	0.940
2	4.699	Isopentyl alcohol	3.909
3	5.192	1,8-Nonadien-3-ol	0.197
4	5.617	Propanoic acid	0.540
5	7.611	2,3-Butanediol	0.178
6	8.820	Propanoic acid, 2-methyl-	2.508
7	9.335	Isovaleric acid	1.439
8	11.390	Trimethyl benzene<1,2,4->	1.015
9	12.426	Cyclopentene<3,5-dimethylene-1,4,4-trimethyl->	0.731
10	12.538	Cymene<ortho->	0.510
11	13.613	Pentanoic acid, 2-hydroxy-4-methyl-,	0.728
12	14.979	2-Coumaranone	0.647
13	18.272	4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-	1.875
14	19.772	Benzaldehyde, 2-hydroxy-5-methoxy-	0.614
15	19.862	N-(1-Cyclopenten-1-yl)-morpholine	0.147
16	22.751	Flopropione	2.463
17	28.082	Cresol<meta->	3.348

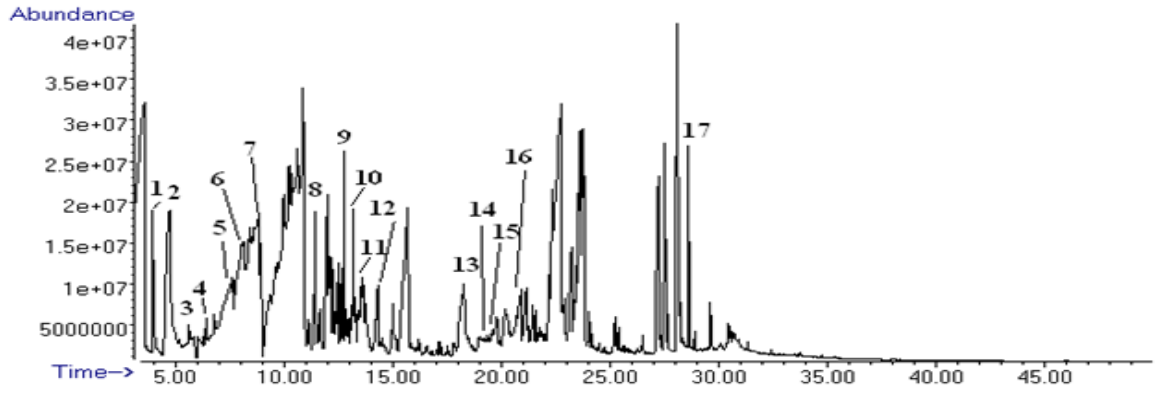


Figure 4.9: Total ion chromatogram profile for isolate El.15

Seventeen compounds were identified in fungal isolate El.26 (Tab. 4.22 and Fig. 4.10). Concentration of compounds for this isolate ranged between 0.2 % and 6.6 %. 5,6-Dihydro-4-methylthieno (2,3-d) pyrimidine (6.6%) was the most abundant compound followed by 1,2-Cyclopentanedione, 3,3,5,5tetramethyl- (5.6%) while the least in proportion was Skatole (0.2%).

Table 4.22: Profile for isolate EL 26 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	5.9985	2,3-Butanediol	3.319
2	7.251	Hydrazine, 1,1-dimethyl-	0.301
3	8.0142	2-Furanmethanol	1.762
4	8.8872	Butanoic acid, 3-methyl-	1.176
5	10.3211	Furfural<5-methyl->	0.866
6	11.665	1,2-Cyclopentanedione, 3-methyl-	0.529
7	13.1656	Maltol	4.560
8	15.4949	Benzeneacetic acid	2.022
9	17.1075	Skatole	0.209
10	19.8847	Furan, 2-(2-furanylmethyl)-5-methyl-	0.324
11	21.7601	1H-Indole-3-ethanol	1.552
12	22.9083	3,6-Diisopropylpiperazin-2,5-dione	0.985
13	23.7818	1,2-Cyclopentanedione, 3,3,5,5-tetramethyl-	5.608
14	24.0506	5,6-Dihydro-4-methylthieno(2,3-d)pyrimidine	6.627
15	26.2455	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	0.760
16	28.0597	Cresol<meta->	2.506
17	32.4271	Benzenamine, 4-octyl-N-(4-octylphenyl)-	0.395

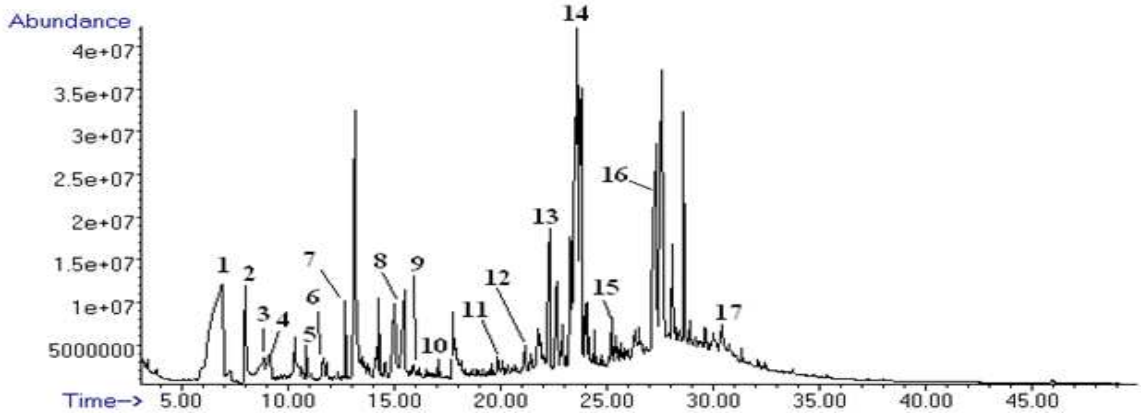


Figure 4.10: Total ion chromatogram profile for isolate EL.26

Twelve compounds were identified in fungal isolate El.27 (Tab. 4.23 and Fig. 4.11). Concentration of compounds for this isolate ranged between 0.3 % and 4.1 %. Triacontane (4.1%) was the most abundant compound and p- Cymene (0.3%) was the least.

Table 4.23: Profile for isolate EL 27 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	11.575	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	0.341
2	12.045	Tumerol<ar->	0.359
3	12.851	p- Cymene	0.322
4	13.859	2-Methylindan-2-ol	0.819
5	14.912	Benzene, 1,3-dimethyl-5-(1-methylethyl)-	1.272
6	16.009	Triacontane	4.172
7	17.756	Benzeneethanol, 4-hydroxy-	1.783
8	21.8323	Cyclooctane, 1,5-dimethyl-	0.323
9	23.669	Phenol, 2-amino-5-nitro-	1.869
10	26.469	o-Cresol	0.446
11	29.462	Phenol, 3-pentadecyl-	1.666
12	30.590	Indole, 5-methyl-2-(4-pyridyl)-	0.873

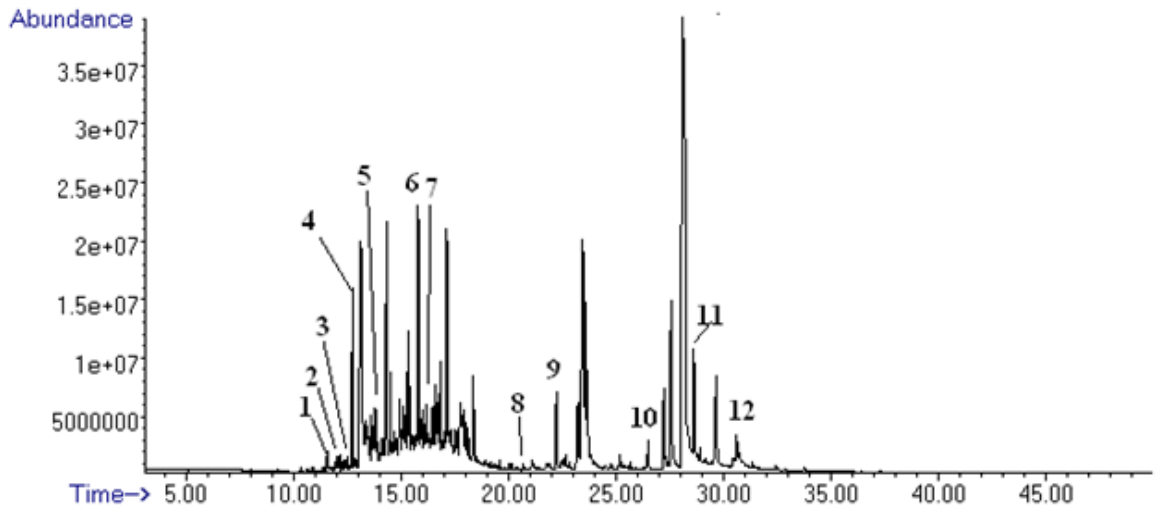


Figure 4.11: Total ion chromatogram profile for isolate El.27.

4.7.2 Lake Nakuru Isolates

Fourteen compounds were identified in fungal isolate Nk.2 (Tab. 4.24 and Fig. 4.12). Propanoic acid, 2-methyl- (2.7%) was the most abundant compound in this extract seconded by Isovaleric acid (1.4%) and closely followed by 2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (1.2 %). The least in proportion was Butanoic acid (0.2%) and l-Alanine, N-allyloxycarbonyl-, isobutyl ester (0.2%). Carboxylic acids formed the largest group of compounds in this extract with their number totaling to three namely (Butanoic acid, Isovaleric acid, Propanoic acid, 2-methyl). This was followed by ketones which had two candidates (2-Coumaranone and 2-Cyclopenten-1-one, 2-hydroxy-3-methyl-). Others included alcohols like Propanol<3-methylthio-> among other classes of compounds.

Table 4.24: Profile for isolate NK.2 showing metabolites and their abundance

Peak no.	RT(MIN)	Compound name	Area %
1	3.803	Propanol<3-methylthio->	0.3616
2	4.364	1-Butanol, 3-methyl-	0.6891
3	7.611	Butanoic acid	0.1636
4	7.725	Propanoic acid, 2-methyl-	2.661
5	8.798	Isovaleric acid	1.4647
6	11.776	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	1.2642
7	12.627	l-Alanine, N-allyloxycarbonyl-, isobutyl ester	0.177
8	14.979	2-Coumaranone	0.6325
9	16.211	N-[Azirid-1-ylmethyl]piperidine	0.5482
10	17.107	Skatole	0.6332
11	21.138	8-Amino-1,3,6-triazahomoadamantane	0.3014
12	21.788	Cyclohexanedione<3-methyl-1,2->	0.5136
13	24.341	trans-2-methyl-4-n-pentylthiane, S,S-dioxide	0.357
14	28.597	Pyridine, 2-methyl-4,6-diphenyl-	0.9062

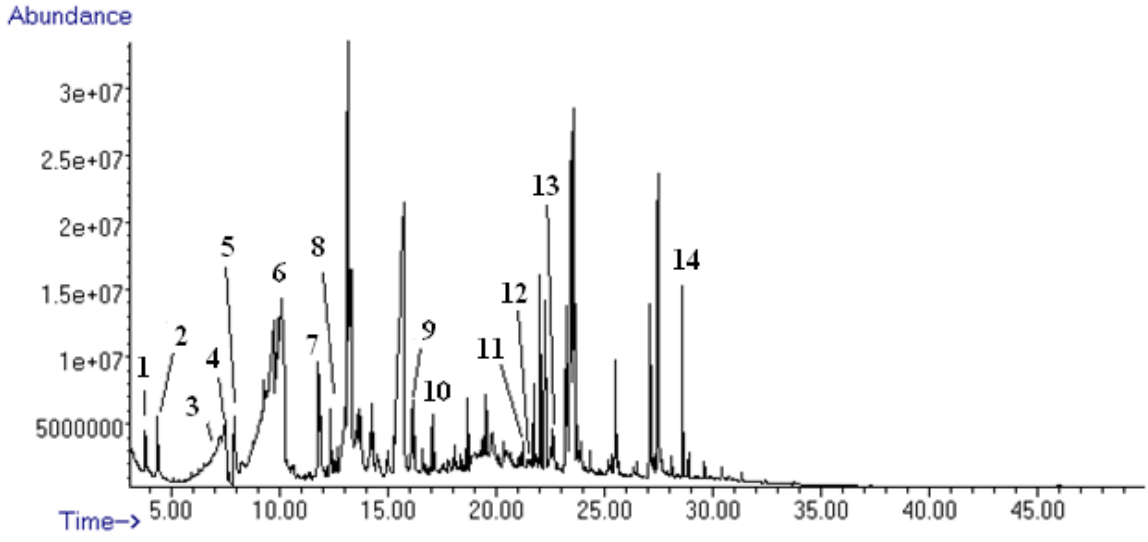


Figure 4.12: Total ion chromatogram profile for isolate Nk.2

Five compounds were identified in fungal isolate NK.20 (Tab. 4.25 and Fig. 4.13). Concentration of compounds for this isolate ranged between 0.2 % and 3.2 %.3-Phenylbicyclo(3.2.2) nona-3,6-dien-2-one (3.2%) was the most abundant compound followed by 2-Thiopheneethanol (2.0%) while Allenyl o-nitrophenyl sulfide (0.2%) was the least in proportion.

Table 4.25: Profile for isolate NK.20 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
4	20.5117	Allenyl o-nitrophenyl sulfide	0.174
1	12.3592	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0.422
3	15.4052	Benzeneacetic acid	1.262
2	14.9796	2-Thiopheneethanol	1.958
5	24.1849	3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one	3.238

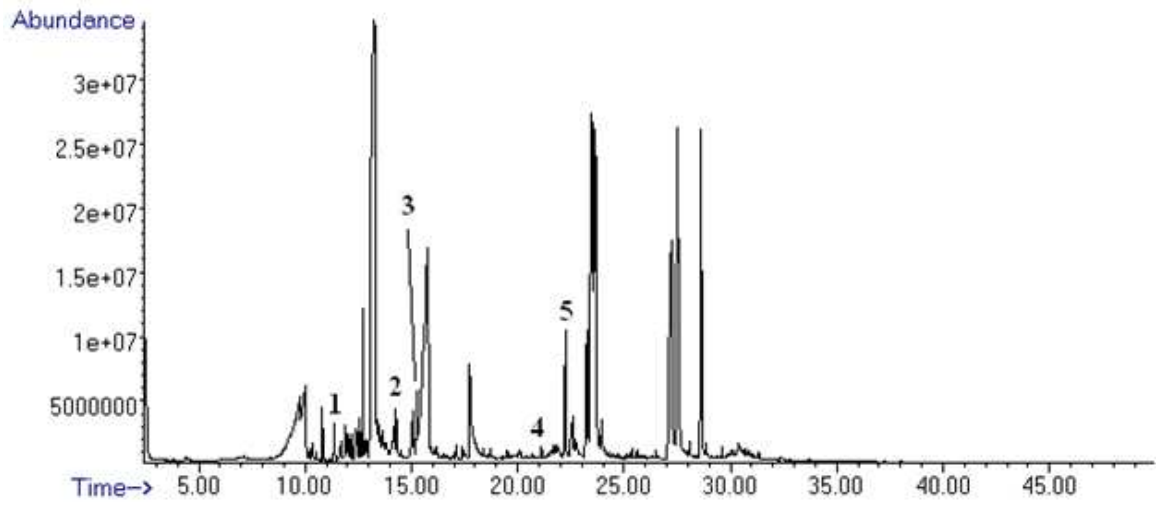


Figure 4.13: Total ion chromatogram profile for isolate Nk.20.

Seven compounds were identified in fungal isolate Nk.23 (Tab. 4.26 and Fig. 4.14). The Concentration of compounds for this isolate ranged between 0.4 % and 70.2 %. Phenol, 3-methyl- (70.2%) was the most abundant compound followed by 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (3.7%) and 2-p-Tolyl-2,3-

dihydro-1H-benzo[1,3,2] diazaborole (3.2% while p-Formophenetidide (0.4%) was the least in proportion.

Table 4.26: Profile for isolate NK.23 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	22.0124	p-Formophenetidide	0.408
2	22.1692	l-Valine, n-propargyloxycarbonyl-, heptadecyl ester	1.310
3	26.4694	Phenol, 3-pentadecyl-	0.458
4	28.082	Phenol, 3-methyl-	70.28
5	28.5972	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	3.740
6	29.6275	Phenol, 2-methyl-	1.850
7	30.5905	2-p-Tolyl-2,3-dihydro-1H-benzo[1,3,2]diazaborole	3.229

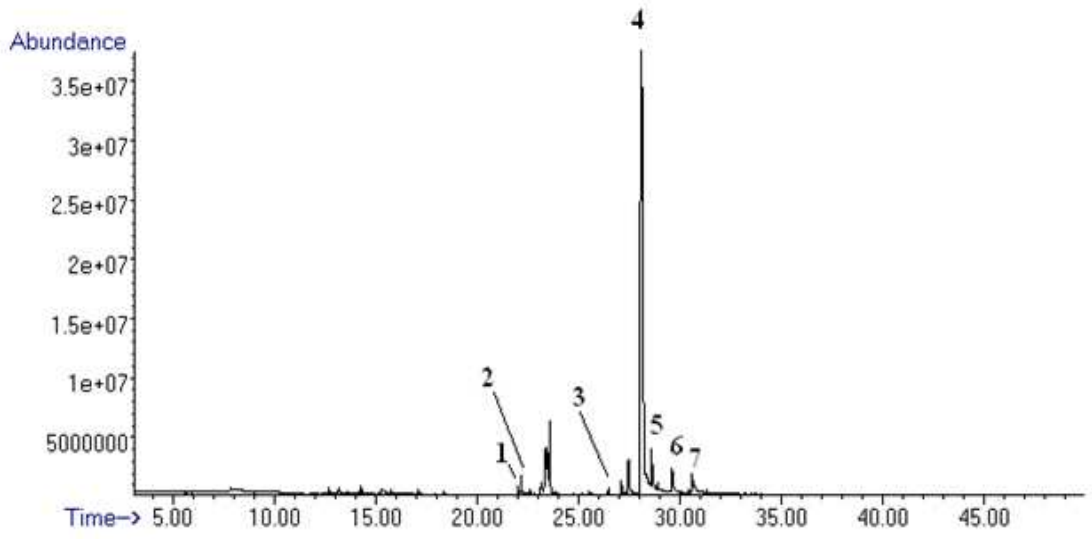


Figure 4.14: Total ion chromatogram profile for isolate Nk.23.

4.7.3 Lake Sonachi Isolates

Six compounds were identified in fungal isolate SN.3 (Tab. 4.27 and Fig. 4.15). Butanediol<2, 3->(61%) was the most abundant compound followed by Benzeneacetic acid (2.6%) with the least being Phenol, 3,5-dimethoxy- (1.3%). Alcoholic class of compounds was the majority with their number totalling to three (Phenol, 3,5-dimethoxy- (1.3%), Propanol<3-methylthio-> (1.5%), Butanediol<2,3-> (61%). The extract also comprised of other groups of compounds like esters, l-Leucine, N-cyclopropylcarbonyl-, hexadecyl ester (1.6%), and carboxylic acid, Benzeneaceticacid (2.6%).

Table 4.27: Profile for isolate SN.3 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	3.804	Propanol<3-methylthio->	1.4699
2	6.670	Butanediol<2,3->	61.4751
3	15.405	Benzeneacetic acid	2.6317
4	22.147	l-Valine, n-propargyloxycarbonyl-, heptadecyl ester	1.8138
5	22.416	Phenol, 3,5-dimethoxy-	1.2722
6	23.132	l-Leucine, N-cyclopropylcarbonyl-, hexadecyl ester	1.6747

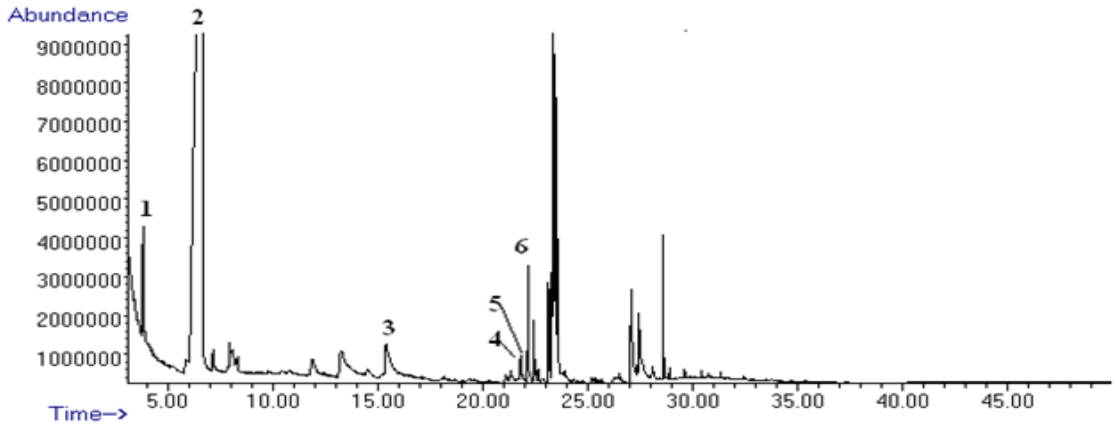


Figure 4.15: Total ion chromatogram profile for isolate SN.3

Nine compounds were identified in fungal isolate SN.5 (Tab. 4.28 and Fig. 4.16). Butanediol<2, 3-> (87.1%) was the most abundant compound followed by 5-Nitroso-2,4,6-triaminopyrimidine (9.7%) and 2-Furanmethanol (3.4%) with the least being (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans- (0.2%). About 50% of compounds were present in very minute quantities in terms of proportion each having less than 1% of total.

Table 4.28: Profile for isolate SN.5 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	3.803	Propanol<3-methylthio->	0.789
2	5.842	Butanediol<2,3->	87.138
3	7.230	1H-Pyrazole, 3,5-dimethyl-	0.3797
4	7.902	2-Furanmethanol	3.4023
5	13.143	Isophorone	1.8639
6	23.535	5-Nitroso-2,4,6-triaminopyrimidine	9.7046
7	23.893	2,4-Dimethoxyamphetamine	0.6012
8	27.883	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	0.2315
9	28.373	Cresol acetate<para-	0.6585

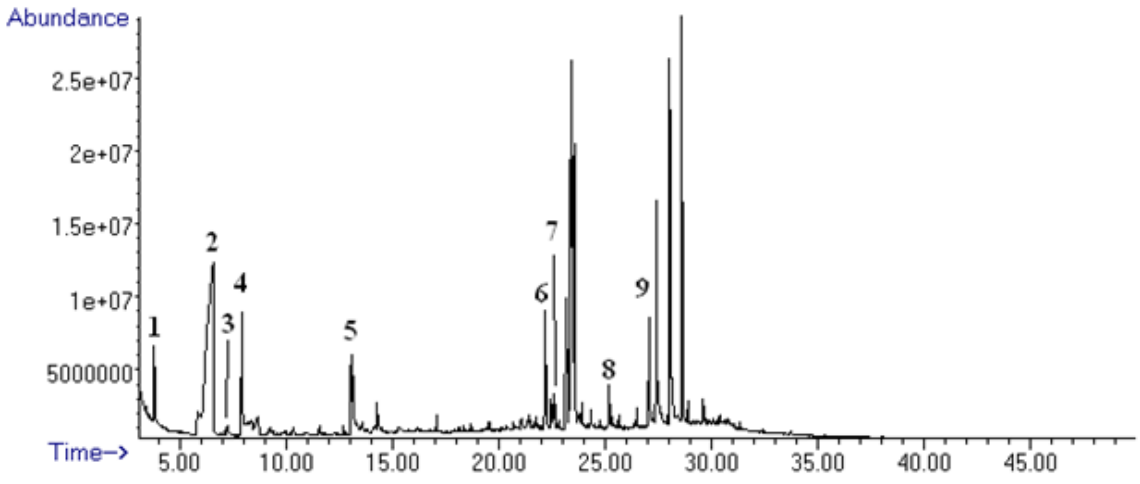


Figure 4.16: Total ion chromatogram profile for isolate SN.5.

Seventeen metabolites were identified in fungal isolate SN.19 (Tab. 4.29 and Fig. 4.17). Concentration of compounds for this isolate ranged between 0.2 % and 10.5 %. Butanediol<2, 3-> (10.5%) was the most abundant compound followed by 2-Furanmethanol (3.0%) and N-Acetyltyramine (1.9%) while Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)- (0.2%) was the least in abundance.

Table 4.29: Profile for isolate SN.19 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	6.5807	Butanediol<2,3->	10.516
2	8.0381	2-Furanmethanol	3.022
3	10.6794	Furfural<5-methyl->	0.405
4	13.1202	Maltol	0.311
5	14.3077	2-Thiophenecarboxylic acid hydrazide	0.894
6	15.5396	3-Pyridinecarboxylic acid, 1,2,5,6-tetrahydro-1-nitroso-	0.449
7	16.1667	Thymohydroquinone	0.789
8	17.1074	Skatole	0.510
9	18.273	N-Isopropylcyclohexylamine	0.385
10	18.6752	Thujaplicin<beta->	0.755
11	19.2127	2-Acetylpyrido[3,4-d]imidazole	0.219
12	20.131	Italicene	1.440
13	21.318	Diaminopyridine	0.871
14	22.7291	N-Acetyltyramine	1.975
15	24.3417	Geranyl-citronellol	0.242
16	26.335	1,3,5-Triazin-2-amine, 4-(2-furyl)-6-(1-piperidyl)-	0.471
17	30.7473	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	0.182

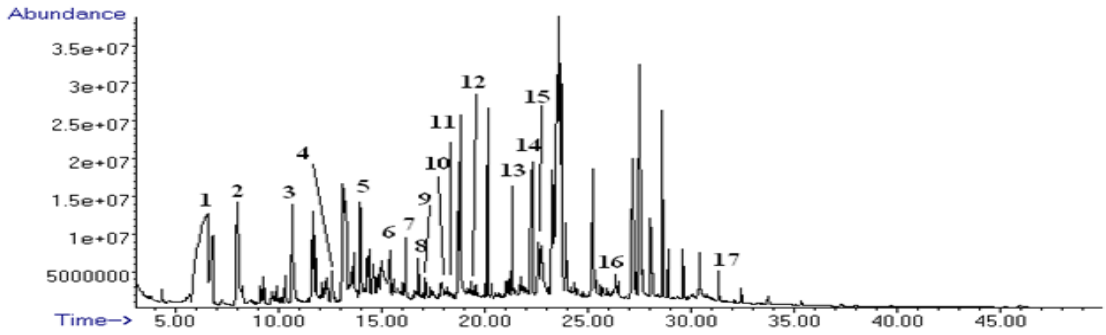


Figure 4.17: Total ion chromatogram profile for isolate SN.19.

Thirteen compounds were identified in fungal isolate SN.21 (Tab. 4.30 and Fig. 4.18). Concentration of compounds for this isolate ranged between 0.2 % and 10.8 %. Benzeneacetic acid (10.8%) was the most abundant compound followed by Thujaplicin<beta-> (1.5%) while Furfural<5-methyl-> (0.2%) was the least in proportion.

Table 4.30: Profile for isolate SN.21 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	6.648	Butanediol<2,3->	1.603
2	8.439	Isopentyl acetate	0.6I5
3	10.186	Mesitylene	0.251
4	10.343	Furfural<5-methyl->	0.203
5	10.679	2-Cyclopenten-1-one, 3-methoxy-4-methyl-	1.038
6	10.837	Mesitylene	0.607
7	12.404	Cymene<para->	0.684
8	13.613	Maltol	2.639
9	15.069	2-Coumaranone	0.792
10	16.160	Benzeneacetic acid	10.80
11	18.787	Thujaplicin<beta->	1.491
12	19.369	N-Acetyltyramine	0.633
13	22.034	4,5,6,7-Tetrahydro-benzo[c]thiophene-1-carboxylic acid allylamide	0.682

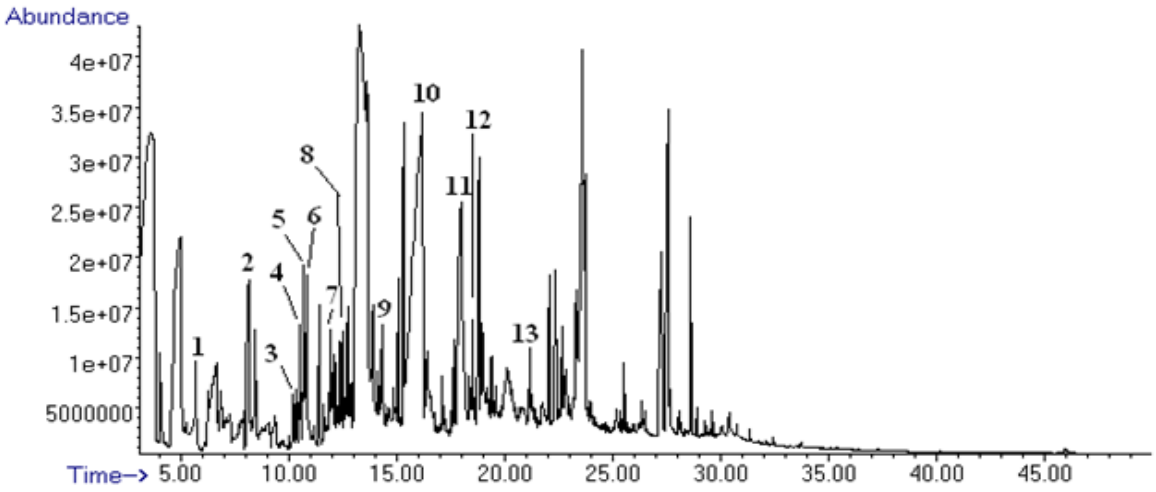


Figure 4.18: Total ion chromatogram profile for isolate SN.21.

Seven compounds were identified in fungal isolate SN.24 (Tab. 4.31 and Fig 4.19). Concentration of compounds for this isolate ranged between 0.4 % and 70.2 %. Phenol, 3-methyl- (70.2%) was the most abundant compound followed by 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (3.7%) and 2-p-Tolyl-2,3-dihydro-1H-benzo[1,3,2] diazaborole (3.2% while p-Formophenetidide (0.4%) was the least in proportion.

Table 4.31: Profile for isolate SN.24 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	22.0124	p-Formophenetidide	0.408
2	22.1692	l-Valine, n-propargyloxycarbonyl-, heptadecyl ester	1.310
3	26.4694	Phenol, 3-pentadecyl-	0.458
4	28.082	Phenol, 3-methyl-	70.28
5	28.5972	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	3.740
6	29.6275	Phenol, 2-methyl-	1.850
7	30.5905	2-p-Tolyl-2,3-dihydro-1H-benzo[1,3,2]diazaborole	3.229

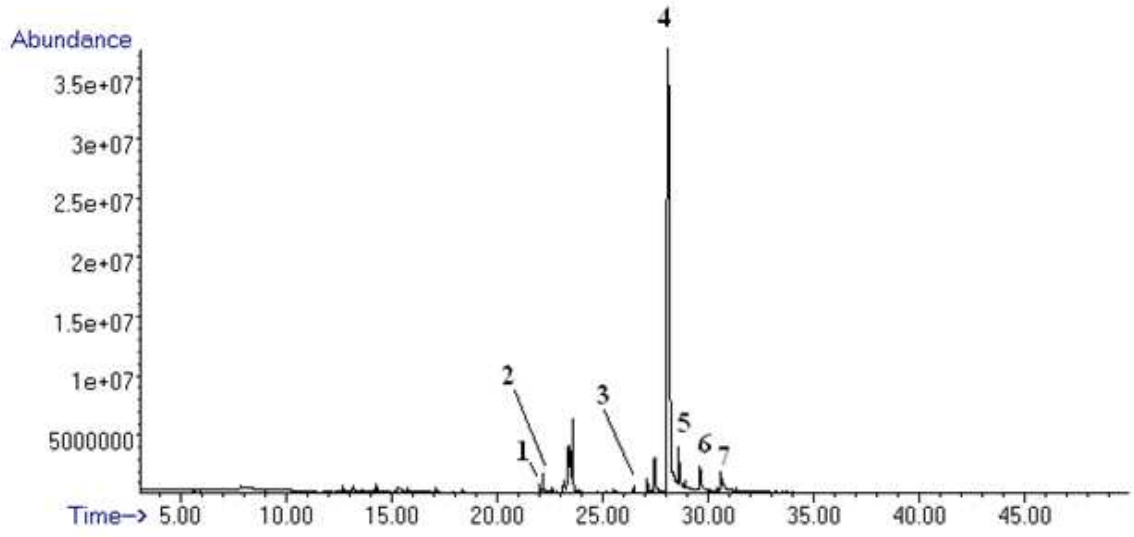


Figure 4.19 Total ion chromatogram profile for isolate SN.24

Twenty compounds were identified in fungal isolate SN.31 (Tab. 4.32 and Fig. 4.20). Concentration of compounds for this isolate ranged between 0.2 % and 3.7%. Maltol (3.7%) was the most abundant compound and Quinolin-2-ol, 4-amino- (0.2%.

Table 4.32: Profile for isolate SN.31 showing metabolites and their abundance

Peak no.	Rt (min)	Metabolite	% area
1	4.341	1-Butanol, 3-methyl-	0.401
2	4.8114	Propanol<3-methylthio->	0.408
3	6.0656	Butanediol<2,3->	3.112
4	7.947	2-Furanmethanol	1.948
5	8.0589	Guanidine	0.431
6	9.246	Butanoic acid, 4-hydroxy-	0.245
7	10.311	Furfural<5-methyl->	0.273
8	11.5977	1,2-Cyclopentanedione, 3-methyl-	0.903
9	13.1655	Maltol	3.770
10	13.3671	5-Acetyl-4-methylthiazole	0.320
11	13.5015	Benzene, 1-methyl-4-(1-methylpropyl)-	0.462
12	13.6134	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.741
13	14.1062	2-(Dimethylaminomethyl)-3-hydroxypyridine	0.449
14	14.5317	N-Aminopyrrolidine	0.464
15	17.1074	Skatole	0.690
16	20.4894	Quinolin-2-ol, 4-amino-	0.204
17	21.0049	Guaicol	0.385
18	21.3181	Diaminopyridine	1.209
19	23.1994	2,5-Dimethylhydroquinone	0.214
20	25.4391	Ethyl Oleate	0.701

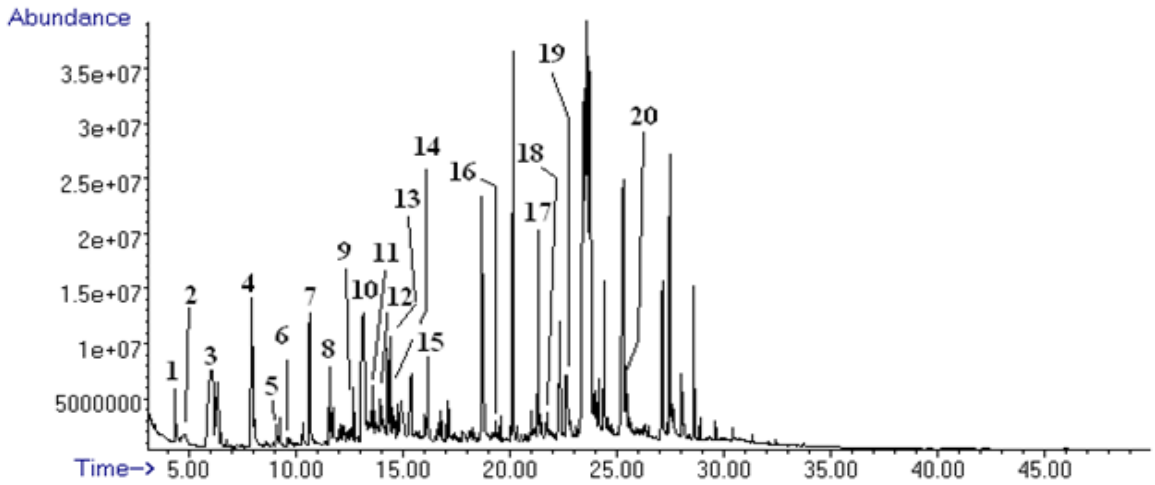


Figure 4.20: Total ion chromatogram profile for isolate SN.31

CHAPTER FIVE

DISCUSSION

5.1 Diversity of fungi in Soda Lakes

The characterization of soda lake fungi was based on morphological, biochemical and molecular analysis. Morphologically twenty six different species of fungi were isolated and identified from the soda lakes. All the isolates were anamorphic fungi in the genera; *Fusarium, Aspergillus, Acremonium, Scopulariopsis and Rhodotorula,* however no sexual stages were identified. The structure and septate hyphae observed indicated that they were anamorphs of the phylum Ascomycota and Basidiomycota. These results agree with the work on the diversity of soda lake fungi (Alexey et al., 2015). Fungal diversity in saline- alkaline lakes remains to be high possibly due high nutrient levels and adaptability in these extreme environments. Fungal species such as *scopulariopsis sp, Acremonies sp* and *Rhodotolula sp* are distributed in different environments. Their presence in the soda lakes is probably an indication of their ability to adapt to extreme environments hence increasing the diversity of fungi. The genera *Apergillus, Scopulariopsis Acremonium* and *Rhodotorula* in this study have fungi species of interest that have been cited to cause diseases in human and opportunistic infection in immune compromised patients (Iwen et al.,2012)

Sediments are believed to be rich in nutrients due to continuous erosion and deposition of rich soil silts (Blomqvist et al*.,* 2004). Production of pigmentation in fungi is influenced by culture medium, stress and other unfavourable conditions. Fungal pigments show important biological activity, such as antioxidants, anticarcinogenic antimicrobial, and immune moderators (Liu & Nizet, 2006; Aberomand et al., 2011). Some of these metabolites may inhibit growth of other microorganisms reducing competition for resources enhancing fungal growth and survival. These factors may also contribute to high fungal diversity in the soda lakes. The chemical concentration within the sediments and varying nutritional aspects of the Kenyan soda lakes creates an environment of high fungal diversity (Woolard & Irvine, 1995). Studies elsewhere have shown that nutrients from the soil silts usually

promote growth of aquatic plants and enhance high microbial diversity (Schallenberg et al., 2003).

5.2 Effects of Sodium Chloride, pH, and Temperature on fungal isolates

Physiologically, isolates seemed to grow relatively well at lower concentrations of sodium chloride. The results indicate that some isolates had lower or high growth rate at low and high salt concentrations of 0% and 15% respectively. The growth of some isolate, however, increased as the salt concentration was increased up to 5%. All the isolates except EL1, EL9, EL12, SN24 and SN19 were unable to grow at 15% Sodium chloride. There fore they could torelate higher salt concentration. Restricted growth on salt concentration is an indication of specified growth conditions and requirements by the saline microorganisms (Brändle et al., 2008; Egamberdieva & Kucharova, 2009).

The fungi identified in the soda lakes have been found in different environments, some are ubiquitous such as the genus *Aspergillus* and *Fusarium*, and other fungi are parasites of plants and animals. The presence of such fungi in the saline environment is an indication of fungi being able to undergo adaptations in hypersaline environments. This phenomenon has been demonstrated in *Hortaea werneckii* and *Wallemia ichthyophaga* which result to the synthesis of glycerol and other osmoregulatory solutes when exposed to high osmolarity (Petelenz-Kurdziel et al., 2011; Kis- papo et al., 2001).

The pH range revealed that isolates from the three lakes were more of alkaliphiles than acidophiles and this has been supported by the work of (Neifar et al., 2015) on alkaliphiles. There was minimal growth of fungi at pH4 for all the isolates However, there was good growth between pH7 to pH12. The pH factor classifies the isolates as alkaliphiles and not acidophiles. This phenomenon has also been supported by other studies (Seckbach & Oren, 2000), that classify most saline fungi as alkaliphiles. The pH signalling pathways are responsible for external pH adaptation in fungi (Calcagno-Pizarelli et al., 2007).

The isolates EL9, EL13 EL14, EL25, SN21 and SN19 had the highest growth rate at temperatures between 26 ºC to 28 ºC but poor growth rates at higher temperatures above 30 ºC. The isolates from Lake Sonachi had a different response based on the growth temperatures. The isolates were observed to have constant growth rate with slow depreciation growth at higher temperatures. The difference in temporal growth pattern might have been caused by the exposure intensity of the soil siltation and the sediments. Sediments are a bit stable and not exposed to alternating heating by the light or continuous physiological saline activities (Zahran, 1997). The biogeochemical mechanisms also contribute to the physiological functions of the alkaliphiles (Kumar et al., 2012). However, the outcome is restricted to the inhabiting organisms like the fungi and other micro-organisms in the saline ecosystem (Borsodi et al., 2005; Kumar et al., 2012).

5.3 Enzymatic activity

Enzymatic activity in halophiles is controlled by the presence of the complex carbon, nitrogen and phosphorus sources (Demain, 1996). Most fungal enzymatic pathways lead to the production of secondary metabolites (Pfeifer & Khosla, 2001). In the current study isolates from the three soda lakes have shown the ability to express enzymes that can degrade a variety of complex compounds. Isolates from Lake Sonachi had an outstanding high percentage of enzymatic activity of the six isolates studied. The highest enzymatic index for various enzymatic activity were, Lipase activity NK23 (EI. 0.054), Protease activity SN3 (EI. 0.196), Pectinase EL15 and NK20 (EI. 038), Esterase activity, NK18 (EI. 0.183), Cellulase activity SN3 (EI. 0.175. The activity of the isolates could have been as result of varying nutrient cycling by the microbiota and the pH levels within the specific lakes. Lipase activity was relatively high for the isolates from Lake Sonachi; this is supported by lipase activity on *Antrodia cinnamomea* and *Rhizopus oryzae* (Shu et al., 2005), Minning et al*.,* 1998). Isolates from Lake Elmentaita and Nakuru did not show any amylase activity with media enriched with starch, possibly starch is limited in the lakes. (Appendix 1.1).

Lakes like Nakuru are known to have a pH value of 10.5 and an alkalinity of 122 meq l-1 (Greichus et al., 1978). Main ions are sodium and bicarbonate-carbonate (Greichus et al., 1978). These conditions might have affected the activity of isolates on the lipid compounds. Lipolytic activity requires acidic reaction which cleaves the complex bonds (McCoy et al., 2002). The biota in the Lake Nakuru is also very simple (Vareschi, 1982).

Like Lake Nakuru, Lake Elmentaita is also characterized by two inflowing rivers with no outlets or surface flows (Mwaura, 1999). It means that there is poor recycling of nutrients and other compounds within such basins. Lack of growth by the isolates from Lake Nakuru and Lake Elmentaita on amylase is unusual. Probably the activity of amylase is regulated among the isolates by the carbon, nitrogen or phosphorus sources (Demain, 1996). Enzymatic pathways can be regulated by these elements to suit the required secondary metabolite, higher values of hydrogen sulfide, soluble reactive phosphate, and ammonia in the deeper waters, as well as a lower pH value, suggest that biological processes contributed to the balanced enzymatic activities for stability and adaptation (Pfeifer & Khosla, 2001).

Lake Sonachi is freshened by rain waters. The freshening by rain waters contributes to the increased stability, conductivity and the volume of water below the chemocline had increased substantially.The lake is known to be dominated by cyanobacterium, *Synecoccus bacilaris* (Njuguna, 1988).which may improve nutrients in the lake. The fungal isolates studied were exceptional with efficient enzymatic activity. The enzymatic activity of the isolates from Lake Sonachi might have been promoted by the freshening and nutrient recycling within the Sonach basin.

5.4 Molecular characterization of the isolates

Phylogenetic tree topology and analysis further illustrated the diversity of fungal species from the saline lakes. Some of the depicted gene bank relatives to the isolates are known to inhabit aquatic and soil environments. Species of the genus *Rhodotorula* are known to be ubiquitous and is a common environmental inhabitant. They can be cultured from soil, water, milk, fruit juice, and air samples (Butinar et

al., 2005). It is able to degrade nitrogenous compounds from the environment (Butinar et al., 2005). Isolate EL6 from Lake Elmentaita and isolate SN5 from Lake Sonachi were identified as haplotypes to *Rhodotorula mucilaginosa* with a 99 and 77 % similarity percentage respectively. All other isolates reviewed haplotypes with percentage similarity of 99% and were all anamorphic fungi in the phylum Ascomycota and Basidiomycota (Appendix 1.2).

Isolate EL15 from Lake Elmentaita was affliated to genus *Aspergillus. Aspergillus flavus* is best known to colonise cereal grains, legumes, and tree nuts. However, in the current study, *Aspergillus flavus* has been isolated from the saline environments. *Aspergillus flavus* is a unique fungus in that it is thermotolerant. By this, it can survive at extreme temperatures that other fungi cannot tolerate (Scheidegger & Payne, 2003). However, this isolate appears to grow under normal temperature ranges (Table 4.5).

Species of the genus *Scopulariopsis* are known to colonize a wide variety of substrates and are common soil saprophytes (Mandeel, 2002). Isolates EL2 and NK20 from Lake Nakuru and isolates SN31 from Lake Sonachi were identified to be closely related to *Scopuloriopsis brevicaulis,* which is an agent of predisposing factors for human mycoses (Zhang et al., 2006), however, its infectivity is not yet established for informative conclusions.

Isolate EL8, EL9 and EL13 from Lake Elmentaita was affliated to subspecies of genus *Fusarium* whereas isolate SN19 from Lake Sonachi was as a close relative to genus *Fusarium*. Species like *F. oxysporum* strains are ubiquitous soil colonies with the ability to exist as saprophytes. Consequently, *F.oxysporum* strains are able to degrade lignin and complex carbohydrates associated with soil debris (Mandeel, 2002; Mandeel, 2006; Smith, 2007). There is a correlation that most of the isolates could be isolated from any of the soda Lakes, because the physical-chemical composition of the lakes is in the same range.

5.5 Antimicrobial activity

The antimicrobial assays conducted revealed the presence of antimicrobial activity from the soda lake fungal isolates. All the isolates solvent extracts from the three Lakes showed antimicrobial activity for at least three test organisms and seven isolates extracts were active against the entire seven test organisms used in this study. Some isolate extracts were more effective on the test micro organisms than others in both solvent extract and crude filtrate tests. The solvent extract gave more effective inhibition to most of the test organisms and this was due to the concentration of antimicrobial compounds as a result of solvent extraction *Escherichia coli* was very sensitive to solvent extract while *Salmonella typhimurium* was the least sensitive

These results are supported by other findings where by the extraction of the fungal broth showed that the diethyl ether extract is the best for antibacterial agent against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, which are important disease agents (Guo et al., 2008; Debbab et al., 2010). A preliminary study of the fungi associated with saltpans in Botswana and their antimicrobial properties do correspond with the results from this study (Lesedi et al., 2009).

Penicillin was the first and the most important known discovery in fungal antimicrobial production with a potent activity against Gram positive bacteria (Demain & Sanchez, 2009). However, the findings in the current study indicate availability of more antimicrobial activity or agents from the soda lakes. The current findings are similar to studies elsewhere (Rodrigues et al., 2005). Rodrigues et al. (2005) found that the fungal extract of *Guignardia species* was active against *Escherichia coli, Staphylococcus aureus*, other fungi and microorganisms. These findings are strongly supported by similar studies by Machalskis et al, (2007), who analyzed the extracts of *Aspergillus ochraceus* and *Penicillium citrinum* that showed wide spectral antibacterial properties against *Pseudomonas aeruginosa* and *Escherichia coli.*

The test microorganisms like *Candida albicans*, *Staphylococcus pneumoniae* and *Salmonella typhimurium* in this study are important human pathogens and therefore the fungal isolates may provide remedy to such pathogens. The only bioactive compounds against these pathogens have been reported to be produced by fungal endophytes from *Spondias mombin* (Rodrigues et al., 2005). Some of the known endophytes are *Guignardia species*, *Phomopsis species* and *Pestalotiopsis guepinii* (Wikee et al., 2011). The study reveals the the isolates do produce antimicrobial agent and therefore a promising source of bioactive compounds for biotechnology applications.

5.6 Diversity of fungal metabolites

Fungal diversity from the Kenya soda lakes is an indication of availabity diverse fungal metabolites. Over a hundred groups of secondary metabolites were analyzed. The several compound groups are sub-groups of phenolic, methanolic, alkaloid, amino acids, proteins and some acetic acidic, alcohols, acids, aldehydes, esters, ketones, hydrocarbons, bases, terpenes, and heterocyclic hydrocarbon compounds among others. The percentage composition of the compounds based on the GC-MS analysis was almost similar across the three lakes. However, percentage compound components were slightly high from Lake Nakuru and Lake Sonachi isolates. The highest percentage compound composition from Lake Elmentaita was Benzene acetic acid (12.464%) by isolate EL8. Isolates SN5 and SN24 had the highest compound production at the ratio of, Butanediol (87.138%) and Phenol, 3-methyl- (70.28%) respectively from Lake Sonachi.whereas the isolate NK23 from Lake Nakuru produced Phenol, 3-methyl- (70.28%).

Isolate EL8 from Lake Elmentaita was characterized as a close relative to genus *Fussarium*. The Benzene acetic acid produced by this fungus has also been reported from terrestrial fungi (Gabriel et al., 2000). In most cases it occurs as a derivative compound of acetyl hodroxybenzamide and has not been documented for any economic or significant industrial application (Mtui and Masalu 2008).

Phenolic and benzoic compounds were produced by the isolates from the three lakes. The same compound and related groups have also been reported to be produced by the sponge fungi from the marine ecosystem (Debbab et al., 2010). Compounds like deoxyterphenylin are elucidated to demonstrate antimicrobial activity (Debbab et al., 2011). Most of the phenolic compounds are rarely found in nature (Debbab et al., 2011), but they are commonly isolated from the Basidiomycetes (Thomson 2012).

Some of the compounds identified from the extracts have been documented to have antimicrobial activity. Thujaplicins, α-thujaplicin, β-thujaplicin and γ-thujaplicin, Flopropione and Isopholone are known for potent anti-fungal and anti-bacterial properties (Chedgy et al., 2009; Ndwigah et al., 2015). Isophorone (3, 5, 5-trimethyl-2-cyclohexen-1-one), a monoterpene, and the structurally related 1, 8-cineole and camphor, have demonstrated a protective effect against cancer, biological activity against a variety of microorganisms, and anti-oxidant properties (Kiran et al., 201). Antifungal effect of thymol, thymoquinone and thymohydroquinone against yeasts, dermatophytes and non-dermatophyte molds isolated from skin and nails fungal infections has been revealed (Taha et al., 2012).

The estimated known fungal metabolites are from 3000 to 4000 (Keller et al*.,* 2005). Most of the known compounds have been isolated from the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Acremonium* (Grewal and Vakhlu 2014). However there is limited information on the analysis of these known compounds. In this study, a good number of the identified compounds have not been reported or analyzed for informative conclusion.

Phenols probably constitute the largest group of fungal secondary metabolites. They are widespread in nature, and found in most classes of aromatic compounds (Cai et al., 2004). Phenolic compounds, occasionally involve the halogens, bromide and iodine that occur frequently in marine environment (Rashid 2012). The current study has also revealed the availability of phenolic compounds from the soda lakes. The phenol, therefore, remains to be ubiquitous in probably all environmental conditions. Though, the compound concentrations vary from the sites, their presence is remarkable, especially in the saline environments. The compounds like bromophenol

are mainly known for their antimicrobial activity (Matanic & Castilla, 2004). Other compounds like 2-(2,4-dibromophenoxy)-4,6-dibromophenol isolated from the marine sponge *Dysidea granulosa* exhibited potent and broad spectrum *in vitro* antibacterial activity, especially against methicillin-resistant and sensitive *Staphylococcus aureus,* vancomycin-resistant and sensitive *Enterococci* and *Bacillus species* (Safdar & Maki, 2002).

Alkaloid compounds represent a group of natural products with a major impact on the economic, medical, and social affairs of humans (Dewick 2002). Alkaloids are difficult to define because they do not represent a homogeneous group of compounds (Bugni & Ireland, 2004). Moreover, they are all nitrogenous compounds with a limited distribution in nature (Dewick 2002; Knölker and Reddy 2002; Kayser et al., 2003). Alkaloid methanolic extracts like 2-methylindan-2-ol are well known for antimicrobial activity (Nostro et al., 2000). Cyclopentedione and heterocyclic compounds that were analyzed from the marine isolates are also important compounds as they are nitrogen containing metabolites (Hamer 2009).

Polyketides were not detected from the GC-MS analysis, but some compounds that are closely linked to polyketides were detected at lower percentage levels. Some of these compounds that are derived from the polyketides include the 3, 6-Diisopropylpiperazin-2, 5-dione (Laport et al., 2009) that were produced by the fungi from soda lakes. Some of the polyketide compounds are strong antimicrobial and antiviral agents (Santos-Gandelman et al., 2014). A few have been isolated by bioassay-guided fractionation from the marine sponge extracts (Laport et al., 2009; Santos-Gandelman, et al., 2014).

Some isolates from the soda lakes were able to produce derivatives of acetylenic fatty acids as 2-Acetylpyrido [3,4-d] imidazole (Fatope et al., 2000). The acetylenic fatty acids have been isolated from the calcareous sponge from the marine waters (Thomæus et al*.,* 2001). The compound has significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (Carballeira 2008).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study results from the soda lakes fungi in the Kenyan Rift valley have demonstrated the presence of fungi and high fungal diversity. A total of twenty six isolates were recovered mainly in the phylum Ascomycota and Basidiomycota. Some of the isolated genera, *Aspergillus, Scopulariopsis,* and *Rhodotorula* have species that may cause human infections. The fungal isolates were alkali-halophilic as they grew well at high pH range and high (5-10%) percentage Sodium Chloride concentration. The soda lakes environment inhabits diverse alkali-halophilic.fungi

The study has revealed that fungi have high potential of producing different types of exo- enzymes such as proteases, lipases pectinases among others that may be exploited for future biotechnological applications in industries. Fungi from the Kenyan soda lakes have the ability to produce a variety of antimicrobial metabolites against bacteria, human pathogenic bacteria and fungi.

The production of diverse bioactive compounds, acids, ketones, phenols, esters among others by the saline- alkaline fungi in the Kenyan soda lakes is important and may provide an additional ecosystem for searching biotechnological tools in proteonomics. The antimicrobial activity of fungal isolates metabolites against the important human pathogens like *Candida albicans, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli* have been demonstrated and this is crucial for disease infection mitigation. The findings in this study should open research on bioprospecting of fungal enzymes and antimicrobial compounds from the Kenyan soda lakes.

The diversity of fungi from soda lakes has a correlation with the availability of fungal metabolites and related derivative compounds. Though some of the compounds were present in lower concentrations, their presence is good enough as an indicator for antimicrobial agents. The Kenyan soda lakes fungi have revealed

high potential of producing high diversity of active/potent and important secondary metabolites that can be a source of antimicrobial agents of economic importance in various industries.

6.2 Recommendations

The Kenyan soda lakes are an ecosystem with high fungal diversity and potential of producing diverse enzymes and secondary metabolites. This study has achieved its objectives by having isolated and characterized some fungi in the soda lakes and also demonstrated that the fungi have the potential of producing enzymes and different antimicrobial molecule. The following recommendations will go a long way to trigger more research on the fungal diversity and function in the soda lake ecosystems:

- Trial of other culture media and optimization of growth conditions for the isolation and characterization is necessary since there is no one medium that is suitable for isolation of all groups of fungi.
- There should be more research on diversity and function of the individual active metabolite molecules that may be of industrial and biotechnological application.
- Molecular tools such as cloning and fosmid libraries for isolation of novel genes from the uncultivable diversity.
- The soda lakes ecosystem are found in an area with high anthropogenic activities such as environmental degradation and pollution, hence the need for conservation and protection to avoid loss in biodiversity, potential enzymes and active molecules that can be of future prospects in industries.
- The fungal diversity may be affected by various factors ranging from industrialization urbanization and human activities. A study to determine the effect of these factors on microbial growth, activity and biodiversity is important.

REFERENCES

Abadias, M., Teixidó, N., Usall, J., Viñas, I. & Magan, N. (2000). Solute stresses affect growth patterns, endogenous water potentials and accumulation of sugars and sugar alcohols in cells of the biocontrol yeast Candida sake. *Journal of Applied Microbiolog, 89*, 1009–1017.

Aberomand, A. (2011). A review article on edible pigment properties and sources as Natural Biological food staffs. Food industry world *Journal of Dairy and food scienc, 6* (1), 71-78.

Aharonowitz, Y., Cohen G. & Martın, J. F. (1992). Penicillin and cephalosporin biosynthetic genes: structure, organization, regulation and evolution. *Annual Review Microbioogy, 46,* 461–495.

Alagarsamy, S., Christian, L. & Ashok, P. (2005). Microbiology and Industrial Biotechnology of Food-Grade. Proteases: A Perspective. *Food Technology and Biotechnology Journal*, 1330-9862.

Alexey, A. G. G., Marina, L. G., Sofiya, A., Bondarenko, S. A., Debets, A. J. M. & Bilanenko, E. N. (2016). On the diversity of fungi from soda soils. An International *Journal of Mycology*, *76* (1) 27-74.

Alexopoalos, C. J. & Mims, C. W. (1979). *Introductory mycology*, (3rd edition). New York: John and sons.

Ali, I., Akbar, A., Anwar, M., Yanwisetpakdee, B., Prasongsuk, S., Lotrakul, P. & Punnapayak, H. (2015). Purification and Characterization of Extracellular, Polyextremophilic α-amylase Obtained from Halophilic Engyodontium album. *Iranian Journal of Biotechnology,12*(4), 35-40.

Andre, A., Diamantopoulou, P., Philippoussis, A., Sarris, D., Komaitis, M. & Papanikolaou, S. (2010). Biotechnological conversions of bio-diesel derived waste glycerol into added-value compounds by higher fungi:

production of biomass, single cell oil and oxalic acid. *India crop production, 31*, 407-416.

Andro, T., Chambost, J. P., Kotoujansky, A., Cattaneo, J., Bertheau, Y., Barras, F., Gijsegem, F., Van., & Coleno, A. (1984). Mutants of *Erwinia chrysanthemidefects* in secretion of pectinase and cellurase. *Journal of Bacteriology, 160,* 1199-1203.

Anke, H., Bergendorff, O. & Sterner, O. (1989). Assays of the biological activities of guanine sesquiterpenes isolated from the fruit bodies of the edible Lactatius species. *Food Chemical Toxicology, 27*, 393-397.

Anke, T. & Erkel, G. (2002). Non- R-Lactan antibiotic: In: Osiewaczitor. *The Mycota Industries Application, 10,* 93-108.

Antony, C. P. & Shouche, Y. S. (2012). Molecular diversity of methanogenes and identification of Metthanoalobus sp. As active methylotrophic Archaea in Lona Crater Lake sediments. FEMS *Microbial Ecology, 81*, 43-57.

Aquino, A.C.M.M., Jorge, J.A., Terenzi, H.F. & Polizeli, M.L.T.M. (2003). Studies on athermostable α-amylase from the thermophilic fungus Scytalidium thermophilum. *Applied microbiology and biotechnology, 61*(4), 323-328.

Author, U. (2012). *Handbook of toxic fungal metabolites*. New York: Elsevier.

Baisuo, Z., Yanchun, Y. & Shulin, C. (2014). How could haloalkaliphilic microorganisms contribute to biotechnology. *Canadian Journal of Microbiology, 60*(11), 717-727.

Ballot, A., Krienitz, L., Kotut, K., Wiegand, C. & Pflugmacher, S. (2005). Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful algae, 4*(1), 139-150.

Barnett, H. L. & Hunter, B. B. (1972). *Illustrated Genera of Imperfect Fungi*. (3rd ed.). USA: Burgess, publishers.

Beever, R. E. & Laracy, E.P. (1986). Osmotic adjustment in the filamentous fungus Aspergillus nidulans. *Journal of Bacteriology, 168*, 1358–1365.

Bentley, R. & Bennet, J.W. (1999). Construction Polyketides: From Collie to Combinatorial Biosynthesis", *Annual Review of Microbiology, 53*, 411-446.

Biabani, A.M.F. & Laatsch, H. (1998). Advances in chemicalstudies on low-molecular weight metabolites of marine fungi. *Advanced Synthesis and Cataly, 340* (7), 589-607.

Bilanenko, E., Sorokin, D., Ivanova, M. & Kozlova, M. (2005). Heleococcum alkali - num, a new alkali-tolerant ascomycete from saline soda soils. *Mycotaxonom, 91*, 497– 507.

Blomqvist, S., Gunnars, A. & Elmgren, R. (2004). Why the limiting nutrient differs between temperate coastal seas and freshwater lakes: A matter of salt. *Limnology and Oceanography*, *49*(6), 2236-2241.

Bordenstein, S. R. (2008). Evolutionary genomics: Transdomain gene transfers. *Current Biology, 17*, 935–936.

Borsodi, A. K., Micsinai, A., Rusznyák, A., Vladar, P., Kovacs, G., Toth, E. M. & Marialigeti, K. (2005). Diversity of alkaliphilic and alkalitolerant bacteria cultivated from decomposing reed rhizomes in a Hungarian soda lake. *Microbial ecology, 50*(1), 9-18.

Brändle, N., Zehnder, M., Weiger, R. & Waltimo, T. (2008). Impact of growth conditions on susceptibility of five microbial species to alkaline stress. *Journal of endodontics, 34*(5), 579-582.

Buchalo, A. S., Nevo, E., Wasser, S. P. & Volz, P. A. (2000). Newly discovered halophilic fungi in the Dead Sea (Israel). *Journey to the Diverse microbial Worlds* (ed. Seckbach, J.), Kluwr, Dordrecht, 2, 241-252.

Bugni, TS. & Ireland, C.M. (2004). Marine-derived fungi: A chemically and biologically diverse group of microorganisms. *Natural Product Reports, 21*(1), 143-163.

Butinar, L., Santos, S., Spencer-Martins, I., Oren, A. & Gunde-Cimerman, N. (2005). Yeast diversity in hypersaline habitats. FEMS. *Microbiology Letters, 244*(2), 229-234.

Cai, Y., Luo, Q., Sun, M. & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life sciences, 74* (17), 2157-2184.

Calcagno-Pizarelli, A.M., Negrete-Urtasun, S., Denison, S.H, Rudnicka, J.D., Bussink, H.J., Múnera-Huertas, T., ... & Peñalva, M.A. (2007). Establishment of the ambient pH signalling complex in *Aspergillus nidulans*: *PalI assists plasma membrane localization of PalH. Eukaryotic Cell, 6*(12), 2365-2375.

Cappuccino, J.G., & Sherman, N. (2002). Microbiology. *A laboratory manual*. San Francisco, U.S.A: Publisher, Pearson Education, Inc.

Carballeira, N.M. (92008). New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents *Progress in Lipid research, 47*(1), 50-61.

Carlsen, S. (1990). Molecular biology in research and produc-ion of industrial enzymes. In: Wolnak B, Scher M (eds). Industrial use of enzymes; technical and economic barriers. Brenard Wolnak and Associates, Chicago, Illinois, 52–69.

Chada, V. & Patel, B. K. (2008). Extremozymes belonging to Glycosyl Hydrolase family from Halothermthrix orenii. 7th International Symposium for subsurface microbiology. *Shizuka*, 1-73.

ChJ. & Lim, Y.W., & Breuil, C. (2009). Effects of leaching on fungal growth and decay of western redcedar. *Canadian Journal of Microbiology*, *55*(5), 578-586.

Cox, R.J. (2007). *The biosynthesis of polyketides, acyl tetramic acids and pyridones by filamentous fungi*. In: Exploitation of Fungi Eds Robson GD, van West, P.and Gadd GM CUP.

Dadheech, P.K., Glockner, G., Casper, P., Kotut, K., Mazzoni, CJ., Mbedi, S. & Krienitz, L. (2013). Cyanobactrial diversity in hot prings pelagic and benthic habitats of tropical soda lake. FEMS, *Microbiology Ecology, 85* (2), 389-401.

Davis, D.J., Burlak, C. & Money, N.P. (2000). Osmotic pressure of fungal compatible osmolytes. *MycologyResearch, 104*, 800–804.

De Hoog, G. S., Zalar, P., Urzi, C., Leo de, F., Yurlova, N. A. & Sterflinger, K. (1999). Relationships of dothideaceous black yeast and meristematic fungi based on 5.8S and ITS2rDNA sequence comparison. *Studies in Mycology, 43*, 31-37.

Deacon, J. W. (1984). Exploitation of fungi: Biotechnology. In *Introduction to modern Mycology.* Oxford: Black well scientific publishers.

Debbab, A., Aly, A.H., Lin, W.H. & Proksch, P. (2010). Bioactive compounds from marine bacteria and fungi. *Microbial biotechnology. 3*(5), 544-563.

Debbab, A., Aly, A. H. & Proksch, P. (2011). Bioactive secondary metabolites from endophytes and associated marine derived fungi. *Fungal Diversity, 49*(1), 1-12.

Demain, A. L. & Sanchez, S. (2009). Microbial drug discovery: 80 years of progress. The *Journal of antibiotics, 62*(1), 5-16.

Demain, A. L. (1996). *Fungal secondary metabolism*: regulation and functions. In: Sutton B (ed) A century of mycology. Cambridge: Cambridge University Press.

Dewick, P. M. (2002). *Medicinal natural products*: a biosynthetic approach. N.J: John Wiley and Sons.

Dong, H., Jiang, H., Zhang, G., Yu, B., Chapman, L.R., Lucas, C.R. & Fields, M.W. (2006). Microbial Diversity in Sediments of Saline Qinghai Lake, China: Linking Geochemical Controls to Microbial Ecology. *Microbial Ecology, 51*(1), 65-82.

dos Santos, V.L., de Souza Monteiro, A., Braga, D.T. & Santoro, M.M. (2009). Phenol degradation by Aureobasidium pullulans FE13 isolated from industrial effluents. *Journal of hazardous materials, 161*(2), 1413-1420.

Dreyfuss, M.M. & Chapela, I.H. (1994). *Fungi as producers of secondary metabolites*. Discovery of natural products with therapeutic potential., Stoneham;Chicago, USA: Butterworth-Heinemann.

Duckworth, A. W., Grant, W. D., Jones, B. E. & Steenberge, R. Van. (1996). Phylogenic diversity of soda Lake alkaliphiles. FEMS. *Microbiology ecology, 19*, 181-191.

Dumestre, A., Chone, T., Portal, J., Gerard, M. & Berthelin, J. (1997). Cyanide Degradation under Alkaline Conditions by a Strain of Fusarium solani Isolated from Contaminated Soils. *Applied and environmental microbiology, 63*(7), 2729-2734.

Egamberdieva, D. & Kucharova, Z. (2009). Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biology and fertility of soils, 45*(6), 563-571.

El-Hissy, F.T. & Khallil, A,M. (1989). Studies on aquatic fungi in Delia region (Egypt). Zentralblatt. *Microbiolog, 144*, 421-432.

El-Hissy, F.T., Khallil, A.M. & Abdel-Raheem, A. (1992). Occurrence and distribution of zoosporic and aquatic Hypomycetes in Upper Egypt. *Bull. Fac. Sci. Assiut Univ, 21*, 45-64.

El-Hissy, F.T., Moubasher, A.H. & El-Nagdy, M.A. (1982). Seasonal fluctuation of the fresh water fungi in River Nile (Egypt). *Zeitschrift fur Allgemeine Microbiology, 22*, 521- 527.

Eliel, E.L., Wilen, S.H. & Mander, L.N. (1994). *Stereochemistry of organic compounds*., New York: Wileyinterscience publication.

Fatope, M.O., Adoum, O. A. & Takeda, Y. (2000). C18 acetylenic fatty acids of Ximenia americana with potential pesticidal activity. *Journal of agricultural and food chemistry, 48*(5), 1872-1874.

Felsenstein, J. (1987).Confidence limits on phylogenies: An approach using the bootstrap. *Evolution, 39,* 783-791.

Gabriel, J., Shah, V., Nesměrák, K., Baldrian, P. & Nerud, F. (2000). Degradation of polycyclic aromatic hydrocarbons by the copper (II)-hydrogen peroxide system. *Folia microbiologica*, *45*(6), 573-575.

Gerasimenko, L.M., Dubinin, A.V., Mityushina, L.L. & Zavarzin, G.A. (1999). A microscopic green alga from soda lakes. *Microbiology* (Moscow, English translation), *68,* 696–700.

Gostinčar, C., Grube, M. & Gunde-Cimerman, N. (2011). Evolution of fungal pathogens in domestic environments. *Fungal biology, 115*(10), 1008-1018.

Grant, D.W. (2004). Half a life in soda lakes. In Hallophilc Microorganisms. *Springer,* 17-31.

Grant, W.D. & Mwatha, W.E. (1989). Bacteria from alkaline saline environment. In recent advances in microbial ecology. *Japan scientific press,* 64-67.

Grant, W.D. & Heaphy, S. (2010). Metagenomics and recovery of enzyme genes from alkaline saline environments. *Environmental Technology, 31,* 1135–1143

Grassin, C. & Fauquembergue, P. (1996).Wine; and Fruit juices. In: Industrial Enzymology (2nd ed.) (Eds): T. Godfrey and S. West, New York: Macmillan Press Ltd.

Green, J. (1993). Zooplankton associations in East African lakes spanning a wide salinity range. In Saline Lakes. *Springer Netherlands*, 249-256.

Greichus, Y.A., Greichus, A., Ammann, B. D. & Hopcraft, J. (1978). Insecticides, polychlorinated biphenyls and metals in African lake ecosystems. III. Lake Nakuru, Kenya. *Bulletin of environmental contamination and toxicology, 19*(1), 454-461.

Grewal, S. & Vakhlu, J. (2014).Assessment of Fungal Diversity and Screening for Antimicrobial Producing Fungi from the Soil Known for its Medicinal Value. *American Journal of Ethnomedicine*, *1*(4), 231-243.

Groth, I., Schumann, P., Reiney, F.A., Martin, K. & Augasten, K. (1997). Bogoriella caseilytica. Novel sp. Novel, new alkaliphilic actinomycetes from soda lakes in Africa. *International Journal of Systematic Bacterioog, 47*, 788-794.

Gunde-Cimerman, N., Ramos, J. & Plemenitaš, A. (2009). Halotolerant and halophilic fungi. *Mycology Research, 113*, 1231-1241.

Gunde-cimerman, N., Zalar, P., de Hoog de, G.S. & Plemenitas, A. (2000). Hypersaline waters insalterns: Natural ecological niches for halophilic black yeasts. (FEMS). *Microbialology Ecology, 32*, 235240.

Guo, B., Wang, Y., Sun, X. & Tang, K. (2008). Bioactive natural products from endophytes: a review. *Applied Biochemistry and Microbiology, 44*(2), 136-142.

Hamer, F. M. (2009). *The Chemistry of Heterocyclic Compounds*. The Cyanine Dyes and Related Compounds (Vol. 18). New York: John Wiley and Sons.

Hankin, L., Zucker, M. & Sands, D.C. (1971). Improved solid medium for the detection and enumeration of pectolytic bacteria. Applied of Microbiology, *22*, 205-209.

Hankins, L. & Anagnostakis, S.L. (1975). The use of solid media for the detection of enzyme production by fungi. *Mycologia, 67*, 597-607.

Hanlin, R.T. (1992). *Illustrated Genera of Ascomycetes*. Minesota, U.S.A: The American Phytopathological Society press.

Hawksworth, D.L (2001). The magnitude of fungal diversity: the 1·5 million species estimate revisited. *British Mycological society*. *105* (12), 1432-1432.

Hohn, T.M, McCormick, S.P. & Desjardins, A.F. (1995). Evidence for a gene cluster involving trichothecene-pathway biosynthetic genes in Fusarium sporotrichioides. *Current Genetics, 24*, 291–295.

Horikoshi, K. (1999). Alkaliphiles, some applications and their products for Biotechnology. *Microbiology and Molecular Biology Review, 64*(4), 735-750.

Iwen, P.C., Schutte, S.D., Florescu, D.F., Noel-Hurst, R.K. & Sigler, L. (2012). Invasive Scopulariopsis brevicaulis infection in an immunocompromised patient and review of prior cases caused by *Scopulariopsis* and *Microascus species*, *Medical Mycology, 50*(6), 561-569.

Jiang, H., Dong, H., Zhang, G., Yu, B., Chapman, L.R. & Fields, M.W. (2006). Microbial Diversity of the Hypersaline Sidi Ameur and Himalatt Salt Lakes of the Algerian Sahara. *Applied and Environmental Microbiology, 72*(6), 3832-3845.

Jones, B.E., Grant, W.D., Duckworth, A.W. & Owenson, G.G. (1998). Microbial diversity in soda lakes. *Extremophiles, 2*(3), 191-200.

Jones, B.E., Grant, W.D., Collins, D., & Mwatha, W.E. (1994). Alkaliphiles: diversity and identification. In: *Bacterial diversity and systematic*. Edited by Priest, F.G., Romas-Comzan and Tindall, B.J. New York: Plenum press.

Kambura, A.K., Mwirichia, R.K., Ngaira, J. & Boga, H.I. (2013). Isolation and characterization of bacterial isolates from Lake Magadi. Tropical Microbiology. *Biotechnology, 8*, 17-25.

Kambura, A., Mwirichia, R., Kasili, R., Karanja, E. N., Makonde, H. & Boga, H. (2016). Diversity of fungi in sediments and water sampled from the hot springs of Lake Magadi and Little Magadi in Kenya. *African journal of Microbiology Research, 10*(10), 330-338.

Kambura, A., Mwirichia, R., Kasili, R., Karanja, E. N., Makonde, H. & Boga, H. I. (2016). Bacteria archaea diversity within the hot springs of Lake Magadi and little Magadi in Kenya. *BioMedCenter-Microbiology - MCRO-D-16-00001R2, 16,* 136.

Kamekura, M., Dyall-Smith, M.L., Upsani, V., Ventosa, A. & Kates, M. (1997). Diversity of alkaliphilic halobacteria: proposals for transfer of Natrobacterium vcacuolatum, N. magadii, and Natrobacterium pharaonis to Halorubrum, Natrialba and Natrosomonas gen. Nov., respectively and Nitrosomonas pharaonis com. Nov., respectively. *Integrated Systematic Bacteriology, 47*, 853-857.

Kasana, R.C., Salwan, R. & Dhar, H. (2008). A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. *Current Microbiology, 57*(5), 503-507.

Kayser, O., Kiderlen, A.F. & Croft, S.L. (2003). Natural products as antiparasitic drugs. *Parasitology research, 90*(2), 55-S62.

Keller, N.P., Turner, G. & Bennett, J.W. (2005). Fungal secondary metabolism from biochemistry to genomics. *Nature Reviews Microbiology, 3*(12), 937-947.

Keller, N.P. & Hohn, T.M. (1997). Metabolic pathway gene clusters in filamentous fungi. *Fungal Genenetic Biology, 21*, 17–29.

Kempe, S. & Degens, E.T. (1985). An early soda ocean. *Chemical Geology*. *53*(1), 95-108.

Keresztes, Z., G., Felfoldi, T., Somongyi, B., Dragos, N., Marialigeti, K., Bartha, C. & Voros, L. (2012). First record of picophytoplanktondiversityrtin. Central Europe hypersaline lakes. *Extremophiles, 16*, 759-769.

Kim, S.Y., Lee, K.H., Kim, J.H. & Oh, D.K. (1997). Erythritol production by controlling osmotic pressure in Trigonopsis variabilis. *Biotechnology Letters, 19*, 727–729

Kimani, P.K. (1992). Hydrology and land use of Lake Nakuru catchments. The world wildlife fund and Egerton University report.

Kimunya, M. (2007). *Lake Bogoria National Reserve*. Integrated Management Plan. Baringo and Koibatek-County Council.

Kiran, G., Baburao, B., Rama, A. & Reddy, N. (2010). Antioxidants, analgesic and anti- inflammatory activities of *Leucas cephalotes*. *Brazillian journal of Sciences*, 46(3), 525-529.

Kis-Papo, T., Weig, A.R., Riley, R., Peršoh, D., Salamov, A., Sun, H., Lipzen, A., ... & Nevo, E. (2014). Genomic adaptations of the halophilic Dead Sea filamentous fungus *Eurotium rubrum*. *Nature Communications, 5,* 3745.

Kis-papo, T., Grishkan, I., Oren, A., Wasser, S. P. & Nevo, E. (2001). Spatio-temporal diversity of filamentous fungi in hypersaline Dead Sea. *Mycological research, 105*, 749-756.

Kis-Papo, T., Kirzhner, V., Wasser, S.P. & Nevo, E. (2003). Evolution of genomic diversity and sex at extreme environments: fungal life under hypersaline Dead Sea stress. *Proceedings of the National Academy of Sciences, 100*(25), 14970-14975.

Kis-papo, T., Oren, A., Wasser, S.P. & Nevo, E. (2003). Survival of filamentous fungi in hypersaline Dead Sea water. FEMS, *Microbiology Ecology. 45*, 183-190.

Kladwang, W., Bhumirattana, A. & Hywel-Jones, N. (2003). Alkaline-tolerant fungi from Thailand. *Fungal Diversity. 13*, 69 – 83.

Knölker, H. J. & Reddy, K. R. (2002). Isolation and synthesis of biologically active carbazole alkaloids. *Chemical reviews. 102*(11), 4303-4428.

Koeman, J.H., Pennings, J.H., De Goeij, J.J.M., Tjioe, P.S., Olindo, P.M. & Hopcraft, J. (1972). A Preliminary Survey of the Possile Contamination

of Lake Nakuru in Kenya with Some Metals and Chlorinated Hydrocarbon Pesticides. *Journal of Applied Ecology,* 411-416.

Kogel, T., Romas, J., Plemenitas, A. & Gude-Cimerman, N. (2005).The halophilic fungi, Hortaea werneckii and halotorelant fungus Aureobasidium pullulans maintained in low intracellular cationconcentrationin hypersaline environment. *Environmtal microbiology, 71*, 6600- 6605.

Koizumi, Y., Kojima, H., Oguri, K., Kitazato, H. & Manabu, F.M. (2004). Vertical and temporal shifts in microbial communities in the water column and sediments of saline meromictic Lake Kaiike in Japan, as determined by a 16s rDNA-base analysis and relevant to physicochemical gradient. *Environmental Microbiology, 6*(6), 622-637.

Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J.S., Codd, G.A., & Stephan, P. (2003). Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS. *MicrobiologyEcology, 43*(2), 141-148.

Krienitz, L., Bock, C., Kotut, K. & Luo, W. (2012) Picocystis salinarum (Chlorophyta) in saline lakes and hot springs of East Africa, Phycologia, *51*(1), 22-32.

Kumar, V., Bharti, A., Negi, Y.K., Gusain, O.P. & Bisht, G.S. (2012). Taxonomy and antimicrobial activity of moderately salt-tolerant and alkaliphilic *Streptomyces sp.* MN 9 (V) isolated from solitary wasp mud nest. *Annals of microbiology, 62*(3), 979-985.

Lanzen, A., Simachew, A., Gressesse, A., Chmolowska, D., Jonassen, I. & Ovreas, L. (2013). Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. *Public Library of Science (PLOS), 8*, 72577.

Laport, M.S., Santos, O.C.S. & Muricy, G. (2009). Marine sponges: potential sources of new antimicrobial drugs. *Current pharmaceutical biotechnology, 10*(1), 86-105.

Lauer, U., Anke, T. & Hansske, F. (1991). Antibiotics from Basidiomycetese. Xxxviii.2-methoxymethyl-1, 4-benzoquinone, a thromboxane A2 receptor antagonist from Lentinus adherens. *Journal of antibiotic, 44*(1), 59-65.

Leisola, M.S.A. & Garcia, S. (1989). The mechanism of lignin degradation. In: *Enzymes system for lignocellulose degradation*. (M.P. coughlan, Ed.). England: Elsevier science publishe Ltd.

Lenassi, M., Zajc, J., Gostinčar, C., Gorjan, A., Gunde-Cimerman, N. & Plemenitaš, A. (2011). Adaptation of the glycerol-3-phosphate dehydrogenase Gpd1 to high salinities in the extremely halotolerant Hortaea werneckii and halophilic Wallemia ichthyophaga. *Fungal biology, 115*(10), 959-970.

Lesedi, L., Joanne, E.T. & Tabo, M.J. (2009). A preliminary study of fungi associated with saltpans in Botswana and their antimicrobial properties. *Bioremedial, Biodiversity and Bioavailability, 3*(2), 61-71.

Linko, M., Poutanen, K. & Viikari, L. (1989). New development in the application of enzymes for biomass processing. In: *Enzymes system for lignocellulose degradation*. (M.P. coughlan, Ed.). England: Elsevier Science Publisher Ltd.

Liras, P., Asturias, J.A. & Martín, J.F. (1990). Phosphate control sequences involved in transcriptional regulation of antibiotic biosynthesis. *Trends Biotechnology, 8*, 184-189.

Lizon, P. (1993). Decline of Macro fungi in Europe: An Overview .*Transactions of the Mycological Society of Republican of China, 12*(1), 21-48.

Lottie, E.D. (2012). *An astrobiological study of an alkaline- saline hydrothermal environment, relevant to understanding the habitat if Mars*. Unpublished PhD Thesis, London: University of London.

Ma, Y., Zhang, W., Xue, Y., Zhou, P., Ventosa, A. & Grant, W.D. (2004). Bacterial diversity of the Inner Mongolian Baer Soda Lake as revealed by 16S rRNA gene sequence analyses. *Extremophiles; Microbial Life Under Extreme Conditions, 8*(1), 45-51.

Machalskis, I.C., D'Armas, H., Malaver, N. & Núñez, M. (2007). Actividad antibacteriana de extractos de hongos aislados de raíces del mangle *Rhizophora mangle* (Rhizophoraceae) en Venezuela. *International Journal of Tropical Biology and Conservation*, *55*(3-4).

Magan, N. (2007). Fungi in Extreme Environments. *Environmental and Microbial Relationships, 4*, 85.

Mandeel, Q.A. (2002). Microfungal community associated with rhizosphere soil of Zygophyllum qatarense in arid habitats of Bahrain, Journal of arid Environments, *50*(4), 665-681.

Mandeel, Q.A. (2006). Biodiversity of the genus *Fusarium* in saline soil habitats, Journal of Basic Microbiology, *46*(6), 480-496.

Matanic, V. C. A. & Castilla, V. (2004). Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. *International journal of antimicrobial agents, 23*(4), 382-389.

Mavura, W.J. & Wangila, P.T. (2003). The pollution status of lake Nakuru, Kenya: heavy metals and pesticides. *African jounal of Aquatic Science, 28,* 13-18.

McCoy, M.G., Sun, G.S., Marchadier, D., Maugeais, C., Glick, J.M. & Rader, D.J. (2002). Characterization of the lipolytic activity of endothelial lipase. *Journal of Lipid Research, 43*(6), 921-929.

Meca, G., Soriano, J.M., Gaspari, A., Ritien, I.A., Moretti, A. & Mañes, J. (2010). Antifungal effects of the bioactive compounds enniatins A, A (1), B, B(1). *Springer plus, Toxicology, 56*(3), 480-485.

Mesbah, N.M., Hedrick, D.B., Rohde, A.D., & Wiegel, J. (2007). Natranaerobius thermophilus gen. nov., sp. nov., a halophilic, alkalithermophilic bacterium from soda lakes of the Wadi An Natrun, Egypt, and proposal of Natranaerobiaceae fam. nov. and Natranaerobiales ord. nov. *International Journal of Systematic and Evolutionary Microbiology, 10*, 2507-2512.

Mikhodyuk, O.S., Gerasimenko, L.M., Akimov, V.N., Ivanovsky, R.N., & Zavarzin, G.A. (2008). Ecophysiology and polymorphism of the unicellular extremely natronophilic cyanobacterium *Euhalothece* sp. Z-M001 from lake Magadii. *Microbiology, 64,* 805–813.

Minning, S., Schmidt-Dannert, C. & Schmid, R. D. (1998). Functional expression of Rhizopus oryzae lipase in Pichia pastoris: high-level production and some properties. *Journal of Biotechnology, 66* (2-3), 147–156.

Mohamed, G., Amal, A.K., Hossam, S.H. & Medhat, A.T. (2012). Potentiality of some alkaliphilic fungi isolated from the Egyptian soil as producers of alkaline detergents enzymes. *Egyptian journal of Biomedical Science. 52*, 246-278.

Moore, D., Robson, G. D. & Trinci, A. P. J. (2011). *21st. Century Guide book to fungi*. Cambridge: Cambridge Universiy press.

Mouchacca, J., Allsopp, D., Colwell, R. R. & Hawksworth, D. L. (1995). Thermophilic fungi in desert soils: a neglected extreme environment. In

Microbial diversity and ecosystem function: *Proceedings of the IUBS/IUMS Workshop held at Egham, UK*, 10-13 August 1993. CAB INTERNATIONAL. 265-288.

Mtui, G. & Masalu, R. (2008). Extracellular enzymes from brown-rot fungus Laetiporus sulphureus isolated from mangrove forests of coastal Tanzania. *Science Research Essay. 3,* 154-161.

Mwatha, W.E. & Grant, W.D. (1993). Natronobacterium vacuolata sp. nov., a Haloalkaliphilic Archaeon Isolated from Lake Magadi, Kenya. *International Journal of Systematic and Evolutionary Microbiology . 43*, 401-404.

Mwatha, W. E. (1991). *Microbial Ecology of Kenyan soda lakes*, Unpublished PhD. Thesis, Leicester: university of Leicester.

Mwaura, F. (1999). A spatio-chemical survey of hydrogeothermal springs in Lake Elementaita, Kenya. *International Journal of Salt Lake Research, 8*(2), 127-138.

Mwirichia, R., Muigai, A.W., Cousin, S., Boga, H. I. & Stackebrandt, E. (2010). Archaeal diversity in the haloalkaline Lake Elmenteita in Kenya. *Current Microbiology, 60*, 47-52.

Mwirichia, R., Muigai, A. W., Tindall, B., Boga, H. I. & Stackebrandt, E. (2010). Isolation and characterization of bacteria from the haloalkaline Lake Elmenteita, Kenya. *Extremophiles,14*(4), 339-348).

Mwirichia, R., Muigai, A.W., Cousin, S., Boga, H.I. & Stackebrandt, E. (2011). Bacterial Diversity in the Haloalkaline Lake Elmenteita in Kenya. *Current Microbiology, 62*, 209–221.

Mwura, F. (1999). A Spatial chemical survey of hydrogeothermal springs in lake Elementaita. *International journal of salt lakes research. 55*, 125-138.

Narasimha, G., Sridevi, A., Buddolla, V., Subhosh, C.M. & Rajasekhar, R.B. (2006). *Nutrient effects on production of cellulolytic enzymes by Aspergillus niger;* Mycology. New York: Blackwell scientific press

Ndwigah, F. I., Boga, I. H., Wanyoike, W. & Romano, K. (2015). Characterization, Enzymatic Activity, and Secondary Metabolites of Fungal Isolates from Lake Sonachi in Kenya. *IOSR Journal of Pharmacy and Biological Sciences, 10*(2), 65-76.

Neifar, M., Maktouf, S., Ghorbel, R. E., Jaouani, A. & Cherif, A. (2015). Extremophiles as source of novel bioactive compounds with industrial potential. *Biotechnology of Bioactive Compounds: Sources and Applications*, 245.

Nielsen, P., Fritze, D. & Priest, F.G. (1995). Phenetic diversity of alkaliphilic Bacillus strains: A proposal for nine new species. *Microbiology, 141*, 1745-1761.

Nieves-Rivera, A.M. (2005). In: *Coastal Mycology of Puerto Rico*, (pp. 296). CA: Lulu Press.

Njuguna, S.J., (1988). *Nutrient- Phytoplankton relationship in a tropical meromictic molecular bacteriology*. Washington DC, USA: Williams and Wilkins Company.

Nostro, A., Germano, M.P., D'angelo, V., Marino, A. & Cannatelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in applied microbiology, 30*(5), 379-384.

Ntabo, C. R., Nancy, L. M. B., Romano, K. M. & Hamadi, I. B. (2013). Isolation and characterization of Actinobacteria from Lake Magadi. *Kenya.African Journal of Microbiology Research, 7*(32), 4200-4206.

Paterson, R. R. M. & Bridge, P. D. (1994). Biochemical techniques for filamentous fungi. *CAB International*. 125.

Petelenz-Kurdziel, E., Eriksson, E., Smedh, M., Beck C., Hohmann, S., Goksor, M., Pfeifer, B.A., & Khosla, C. (2001). Biosynthesis of polyketides in heterologous hosts. *Microbiology and Molecular Biology Reviews, 65*(1), 106-1.

Ramírez-Coutiño, L., del Carmen Marín-Cervantes, M., Huerta, S., Revah, S. & Shirai, K. (2006). Enzymatic hydrolysis of chitin in the production of oligosaccharides using Lecanicillium fungicola chitinases. *Process Biochemistry, 41*(5), 1106-1110.

Raper, K.B. & Fennell, D.I. (1965). *The Genus Aspergillus* . Baltimore, USA: Williams and Wilkins Company.

Rashid, M. A. (2012).*Geochemistry of marine humic compounds*. New York: Springer Science and Business Media.

Raymond, J. L., Lokesh, J. & Donald, W. R. (1997). Entomopathogenic fungi to the requirements of their ecological niches. *Microbiology, 146*(6), 1983-1992.

Rees, H. C., Grant, D. W., Jones, B. E. & Heaphy, S. (2004). Diversity of Kenyan soda Lake Alkaliphiles assessed by molecular methods. *Extremophiles Microbial Life Under Extreme Condition, 8*(1), 63-71.

Robinson, J. A. (1991). Polyketide Synthase Complexes: Their Structure and Function in Antibiotic Biosynthesis. *Transactions of the Royal Society of Biological Science, 332*(1263), 107-114.

Rodrigues, K. F., Costa, G. L., Carvalho, M. P. & Epifanio, R. D. A. (2005). Evaluation of extracts produced by some tropical fungi as potential

cholinesterase inhibitors. World *Journal of Microbiology and Biotechnology, 21*(8-9), 1617-1621.

Ruijssenaars, H.J. (2001). Plate screening methods forthe detection of polysaccharase- producing micro organisms. *Applied Microbiology Biotechnology, 55,* 143-149.

Ruiz-Suárez, J. & Jessica, Y. (2004). Arenicolous Filamentous Fungi in Mayagüez Bay Shoreline, Western Puerto Rico. *Digital thesis, University of Puerto Rico* (753 kb).

Rysenbach, A. L., Gotz, D. & Yernool, D. (2002). Microbial life of marine and terrestrial thermal springs. In *Biodiversity of microbial life* (eds Stanley, J.T. and Reysenbatch, A.L.), New York: Wiley-Liss inc.

Safdar, N. & Maki, D. G. (2002). The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant Staphylococcus aureus, enterococcus, gram-negative bacilli, Clostridium difficile, and Candida. *Annalsof Internal Medicine, 136*(11), 834-844.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406-425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular cloning - a laboratory manual.* Cold Spring Harbor, USA: Cold Spring Harbor Laboratory Press.

Santos-Gandelman, F. J., Giambiagi-deMarval, M., Oelemann, M. R. W. & Laport, S. M. (2014). Biotechnological potential of sponge-associated bacteria. *Current pharmaceutical biotechnology. 15*(2), 143-155.

Sasagawa, N., Takushi, K. & Ezai, Y. (2015). *Amethod for producing epoxy compounds*. Japan: Agilent Technologies.

Sawa, R., Takahashi, Y., Itoh, S., Shimanaka, K., Kinoshita, N., Homma, Y., ... & Takeuchi, T. (1994). Aldacalmycin, a new antimicrobial antibiotic from streptomyces. Taxonomy, fermentation isolation, physic-chemical and biological properties. *Journal of antibiotics. 47*, 1266-1272.

Schallenberg, M., Hall, C. J. & Burns, C. W. (2003). Consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes. *Marine ecology. Progress series. 251*, 181-189.

Scheidegger, K. A. & Payne, G. A. (2003). Unlocking the secrets behind secondary metabolism: a review of Aspergillus flavus from pathogenicity to functional genomics. *Toxin Reviews. 22*(2-3), 423-459.

Schmitt, I., Martin, M. P., Kautz, S. & Lumbsch, H. T. (2005). Diversity of non-reducing polyketide synthase genes in the pertusariales (lichenized Ascomycota): a phylogentic perspective. *Phytochemistry. 66*, 1241–1253.

Seckbach, J. & Oren, A. (2000). Extremophilic microorganisms as candidates for extraterrestrial life.In International Symposium on Optical Science and Technology. *International Society for Optics and Photonics*. 89-95.

Selim, K. A., EL-Beith, A. A, Abdel-Rahman, T. M. & EL-Diwang, A. L. (2011). Bioactivity and antimicrobial activityof endophytesassociated with Egyptian medicinal plants. *Mycosphere. 2* (6), 669-678.

Shu, C. H., Xu, C. J. & Lin, G. C. (2005). Purification and partial characterization of a lipase from Antrodia cinnamomea. *Process Biochemistry. 41*(3), 734–738.

Sierra, G. A. (1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substances. *Antonine van Leeuwenhoeck. 28*, 15-22

Smibert, R. M. & Krieg, N. R. (1994). *Phenotypic characterization*. Methods for general and molecular bacteriology. Washington, USA. 607-654.

Smith, S. N. (2007). An overview of ecological and habitat aspects in the genus Fusarium with special emphasis on the soil-borne pathogenic forms. *Plant Pathology Bulletin. 16*, 97-120

Sorokin, D. Y. & Kuenen, J. G. (2005). Haloalkaliphilic sulfur-oxidizing bacteria in soda lakes. Federation of European Microbiogy Society, *Microbiologyreviews. 29*(4), 685-702.

Suarez-Serrano, A., Alcaraz, C., Ibanez, C., Trobajo, R. & Barata, C. (2010). Procambarus clarkii as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology and Environmental Safety. 73*(3), 280-286.

Subramaniam, R., Dufreche, S., Zappi, M. & Bajpai, R. (2010). Microbial lipids from renewable resources: production and characterization. *Indian microbiology and biotechnology. 37,* 1271-1287.

Taha, K. K, Elmahi, R. H., Hassan, E. A., Ahmed, S. E. M. H. & Shyoub, M. H. (2012). Analytical study on three types of gum from Sudan. *Journal of production and Industrial. 1* (1), 11-16.

Tamura, K, Dudley, J, Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution. 24*, 1596-1599.

Tamura, K. & Kumar, S. (2002). Evolutionary distance estimation under heterogeneous substitution pattern among lineages. *Molecular Biology and Evolution. 19*, 1727-1736.

Tamura, K., Nei, M. & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA). 101, 11030-11035.

Tamura, K., Stecher, G., Peterson. D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution. 30*, 2725-2729.

Tansey, M. R. & Brock, T. D. (1978). Microbial life in extreme environments (ed. Kushner, D.J.), Academic press, London. 59-216.

Thomæus, S., Carlsson, A. S. & Stymne, S. (2001). Distribution of fatty acids in polar and neutral lipids during seed development in Arabidopsis thaliana genetically engineered to produce acetylenic, epoxy and hydroxy fatty acids. *Plant science. 161*(5), 997-1003.

Thomson, R. (2012). Naturally occurring quinones IV: Recent Advances. Publisher, Elsevier. 746.

Turk, M., Abramović, Z., Plemenitaš, A. & Gunde-Cimerman, N. (2007). Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. *Federation of European Microbiology Society, Yeast Research. 7*(4), 550-557.

Vainio, E. J. & Hantula, J. (2000). Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycological Research. 104*(8), 927-936.

Vareschi, E. (1978). The ecology of Lake Nakuru (Kenya). Abundance and feeding of the lesser flamingo. *Oecologia.* 11-35.

Vareschi, E. (1982). The ecology of Lake Nakuru (Kenya). *Oecologia. 55*(1), 81-101.

Vaupotič, T., Gunde-Cimerman, N. & Plemenitaš, A. (2007). Novel 3′-phosphoadenosine-5′-phosphatases from extremely halotolerant Hortaea werneckii reveal insight into molecular determinants of salt tolerance of black yeasts. *Fungal Genetics and Biology. 44,* 1109-1122.

Walsh, G. (2002). Industrial enzymes: proteases and carbohydrases. In: *Proteins; Biochemistry and Biotechnology*. John Wiley and Sons.Ltd. 535.

Weissman, K. J. & Leadlay, P. F. (2005). Combinatorial biosynthesis of reduced polyketides. *Nature Reviews Microbiology. 3*, 925-936.

Wikee, S., Udayanga, D., Crous, P. W., Chukeatirote, E., McKenzie, E. H., Bahkali, A. H. & Hyde, K. D. (2011). Phyllosticta an overview of current status of species recognition. *Fungal Diversity. 51*(1), 43-61.

Wilson, E. O. (1989). Biodiversity, in: The current state of Biological Diversity, National Academies press. 521.

Wood, T. M. (1989). Mechanism of cellulose degradation by enzymes from aerobic and anaerobic fungi. In: *Enzyme systems for lignocellulose degradation* (M.P Coughlan, Ed.).

Woolard, C. R. & Irvine, R. L. (1995). Treatment of hypersaline wastewater in the sequencing batch reactor. *Water Research. 29*(4), 1159-1168.

Zahran, H. H. (1997). Diversity, adaptation and activity of the bacterial flora in saline environments. *Biologyand Fertility of Soils. 25*(3), 211-223.

Zantinge, J. L., Huang, H. C. & Cheng, K. J. (2003). Induction, screening and identification of *Coniothyrium minitans* mutants with enhanced β-glucanase activity. *Enzyme and Microbial Technology. 32*(2), 224-230.

Zhang, N., Castlebury, L. A., Miller, A. N., Huhndorf, S. M., Schoch, C. L., Seifert, K. A., & Sung, G. H. (2006). An overview of the systematics of the

Sordariomycetes based on a four gene phylogeny. *Mycologia. 98*(6), 1076-1087.

Zhilina, T. N., Zavarzin, G. A., Rainineyf, A., Pikuta, E. N. & Osipov, N. A. (1997). Desulfonatronovibrio hydrogenovorans *genus*, novel sp., an Alkaliphilic, Sulfate-Reducing Bacterium. Systematic and Evolutionary.47, 144-149.

Zhilina, T. N., Zavarzin, G. A., Rainer, F., Kevbbrin, V. V., Kostrikina, N. A. & Lysenko, R. (1996). Spirochaeta alkalica sp. nov., Spirochaeta africana sp. novel, and Spirochaeta asiatica s. novel, Alkaliphilic Anaerobes from the Continental Soda Lakes in Central Asia and the East African Rift. *Systematic and Evolutionary Microbiology. 10*, 305-312.

APPENDICES

Appendix 1.1: A summary of the percentage of fungal isolates that exhibited enzymatic activity/total number of isolates from the three lakes.

	Lake Elmentaita (%)	**Lake Nakuru (%)**	**Lake Sonachi (%)**
Lipolytic activity	7.7% (1/13)	50% (4/8)	83.3% (5/6)
Esterasic activity	21.4% (3/14)	12.5% (1/8)	33.3% (2/6)
Amylolytic activity	0	0	16.7% (1/6)
Proteolytic activity	38.5% (5/13)	50% (3/6)	100% (6/6)
Pectinolytic activity	21.4% (3/14)	57.1% (4/7)	50% (3/6)
Cellulolytic activity	25% (2/8)	42.9% (3/7)	60% (3/5)

Appendix 1.2: The closest relatives of the soda lakes fungal isolates

Isolate	Closest relative Fungus	Accession N0:NCBI	Phylum/sub-Phylum	Sequence %Similarity
EL1	*Acrimonies sp*	JX273067	Ascomycota	99
EL4	*Sarocladium kiliense*	HQ232197	Ascomycota	99
EL6	*Rhodotorula mucilaginosa*	JQ838010	Basidiomycota; Pucciniomycotina	99
EL8	*Fusarium sp.*	JQ934487	Ascomycota	99
EL9	*Fusarium oxysporum*	KC143070	Ascomycota	100
EL11	*Acrimonies sp*	JX273067	Ascomycota	99
EL12	*Scopulariopsis brevicaulis*	JN157617	Ascomycota; Pezizomycotina	99
EL13	*Fusarium oxysporum*	KC143070	Ascomycota; Pezizomycotina;	100
EL15	*Aspergillus sp*	KC120773	Ascomycota; Pezizomycotina;	99
EL27	*Paecilomyces sp.*	JN546116	Ascomycota; Pezizomycotina;	99
NK2	*Scopulariopsis brevicaulis*	JN157617	Ascomycota' pezizomycotina	99
NK7	*Paecilomyces sp.*	JN546116	Ascomycota;	99
NK10	*Acremonium sp.*	JX273067	Ascomycota; Pezizomycotina;	99
NK20	*Scopulariopsis brevicaulis*	JN157617	Ascomycota	99
SN3	*Acremonium sp.*	JX273067	Ascomycota	99
SN19	*Fusarium sp.*	JX273060	Ascomycota	100
SN21	*Fusarium oxysporum*	JF807402	Ascomycota	100
Sn31	*Scopulariopsissp*	AY773330	Ascomycota	99

Appendix 1.3: (a) Percentage yields of Lake Elmentaita isolates (Final weight of ppt in g after evaporation over initial wt in g of solvent extract filtrate X100).

Isolate	EL1	EL4	E6	EL8	EL9	E11	EL12	EL13	EL14	EL15	EL26	Control
% Yield	39	39	29	38	31	38	30	37	38	31	31	44

Appendix 1.3: (b) Percentage yields of Lakes, Nakuru and Sonachi (Final weight of ppt in g after evaporation over initial wt in g of solvent extract filtrate X 100).

Isolate	NK 2	NK 10	NK 7	NK 18	NK 20	NK 23	SN 2	SN 5	SN 19	SN 21	SN 24	SN 31	Control
% Yield	38	39	39	38	38	31	39	39	38	31	32	31	44