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Diversity of fungi in sediments and water sampled from the hot springs of Lake Magadi and Little Magadi in Kenya

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Lake Magadi and Little Magadi are saline, alkaline lakes lying in the southern part of Kenyan Rift Valley. Their solutes are supplied by a series of alkaline hot springs with temperatures as high as 86°C. Previous culture-dependent and independent studies have revealed diverse prokaryotic groups adapted to these conditions. However, very few studies have examined the diversity of fungi in these soda lakes. In this study, amplicons of Internal Transcribed Spacer (ITS) region on Total Community DNA using Illumina sequencing were used to explore the fungal community composition within the hot springs. Operational taxonomic units (OTUs) were analyzed using QIIME 1.8.0, taxonomy assigned via BLASTn against SILVA 119 Database and hierarchical clustering was done using R programming software. A total of 334, 394 sequence reads were obtained from which, 151 OTUs were realized at 3% genetic distance. Taxonomic analysis revealed that 80.33% of the OTUs belonged to the Phylum *Ascomycota,* 11.48% *Basidiomycota* while the remaining consisted of *Chytridiomycota, Glomeromycota* and early diverging fungal lineages. The most abundant *Ascomycota* groups consisted of *Aspergillus* (18.75%), *Stagonospora* and *Ramularia* (6.25% each) in wet sediment at 83.6°C, while *Penicillium* and *Trichocomaceae (*14.29% each) were dominant in wet sediment at 45.1°C. The results revealed representatives of thermophilic and alkaliphilic fungi within the hot springs of Lake Magadi and Little Magadi. This suggests their ability to adapt to high alkalinity, temperature and salinity.

Key words: Fungi, hot springs, temperature, DNA, diversity.

INTRODUCTION

Fungi have colonized diverse habitats such as tropical regions (Hawksworth, 1991), extreme environments such as deserts, areas with high salt concentrations (Vaupotic et al., 2008), ionizing radiation (Dadachova et al., 2007), deep sea sediments (Raghukumar and Raghukumar, 1998) and ocean hydrothermal areas (Le Calvez et al.,

2009). Most fungi grow in terrestrial environments, though several species live partly or solely in aquatic habitats, such as the chytrid fungus *Batrachochytrium dendrobatidis*, a parasite that has been responsible for a worldwide decline in amphibian populations (Brem and Lips, 2008). In most ecosystems, fungi are the major decomposers, playing an essential role in nutrient cycling as saprotrophs and symbionts that degrade organic matter into inorganic molecules (Barea et al., 2005; Lindahl et al., 2007; Gadd, 2007). While there are well-known examples of bacteria that can grow in a variety of natural environments including hot springs and geysers where temperatures can reach 100°C, eukaryotes are much more sensitive because, above 65°C, their membranes become irreparably damaged (Magan and Aldred, 2007). However, mesophilic thermo-tolerant fungi exist. For example, some *Deuteromycetes* isolated from thermal springs have maximum growth temperature of 61.5°C (Magan, 2006).

The presence of fungi in extreme alkaline saline environments has been recognized by culture-dependent methods, with the majority showing similarity to terrestrial species (Mueller and Schmit, 2006; Salano, 2011; Ndwigah et al., 2015). Culture-independent methods have revealed highly novel fungal phylotypes such as *Chytridiomycota* and unknown ancient fungal groups (Yuriko and Takahiko, 2012).

pH tolerance in fungi has been attributed to efficient control of proton movement into and out of the cells, and is able to meet necessary energy requirements (Magan, 2006). The exact diversity and function of fungi in extreme environments is still poorly understood. The aim of this study was to explore the fungal diversity within the hot springs of Lake Magadi and Little Magadi in Kenya using metagenomic analysis.

MATERIALS AND METHODS

Study site

Lake Magadi is a hyper saline lake that lies in a naturally formed closed lake basin within the Southern part of the Kenyan Rift Valley. It is approximately 2 °S and 36 °E of the Equator at an elevation of about 600 m above sea level (Behr and Röhricht, 2000). The solutes are supplied mainly by a series of alkaline springs with temperatures as high as 86°C, located around the perimeter of the lake. Samples analyzed in this study were collected from 3 hot springs: one hot spring within the main Lake Magadi (02° 00′ 3.7″S 36° 14′ 32″ E at an elevation of 603 m, a temperature of 45.1°C and pH 9.8), and two hot springs within Little Magadi "*Nasikie eng'ida"*: Hot spring 1 - 01° 43′ 28″ S 36° 16′ 21″ E, at an elevation of 611 m, a temperature of 83.6°C and pH 9.4); and Hot spring 2 - 01° 43′ 56″ S 36° 17′ 11″ E, at an elevation of 616 m, temperature of 81°C and pH of 9.2 (Table 1).

Measurements of physicochemical parameters

Geographical position of each site in terms of latitude, longitude and elevation was taken using Global Positioning System (GARMIN eTrex 20). The pH for each sampling point was measured with a portable pH-meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5-10), Temperature, Electrical Conductivity (EC), Total Dissolved Solids (TDS) and dissolved oxygen (DO) were measured on site using Electrical Chemical Analyzer (Jenway - 3405) during sampling. *In situ* temperature was recorded once for each study site and assigned to all the sample types for that site.

Sample collection

All samples were collected randomly in triplicates from each hot spring. Water samples were collected using sterile 500 ml plastic containers that had been cleaned with 20% sodium hypochlorite and UV-sterilized for one hour. Wet sediments were collected by scooping with sterilized hand shovel into sterile 50 ml falcon tubes. All samples were transported in dry ice to the laboratory at Jomo Kenyatta University of Agriculture and Technology. Water for DNA extraction (500 ml) was trapped on 0.22 µM filter papers (Whatman) and stored at -80°C. Pellets for DNA extraction were obtained from water samples by re-suspending the filter papers in phosphate buffer solution (pH 7.5), and centrifuging 5 ml of the suspension at 13000 rpm for 10 min.

DNA extraction

Total community DNA was extracted in triplicates; pellets from water samples and 0.2 g of sediment samples as described by (Sambrook et al., 1989). The DNA extracted from triplicate samples was pooled during the precipitation stage, washed, air dried and stored at -20°C.

Amplicon library preparation and sequencing

PCR amplification of ITS region was done using ITS1 (TCCGTAGGTGAACCTGCGG) and TS4 (TCCTCCGCTTATTGATATGC) primers with barcode according to (White et al., 1990). Amplification proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial heating at 94°C for 3 min, followed by 28 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 40 s and extension at 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. Polymerase chain reaction (PCR) products were visualized on 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA). The pooled and purified PCR product was used to prepare DNA library by following Illumina sequencing protocol (Yu and Zhang, 2012). Sequencing was performed at Molecular Research DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq platform following the manufacturer's guidelines.

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Table 1. Physico-chemical parameters of sampling stations in Lake Magadi and Little Magadi measured before sampling.

Parameter	Latitude °S	Longitude °E	Elevation (m)	Temperature (°C)	pH	EC (mS/cm)	TDS (mg/L)	Dissolved oxygen (mg/L)
Hot spring 1	02° 00′ 3.7″	36° 14′ 32″	603	45.1	9.8	0.03	1	12.4
Hot spring 2	01° 43′ 28″	36° 16′ 21″	611	83.6	9.4	1	1	0.04
Hot spring 3	01° 43′ 56″	36° 17′ 11″	616	81	9.2	1	1	0.71

Sequence analysis, taxonomic classification and data Submission

Sequences obtained from the Illumina sequencing platform were depleted of barcodes and primers using a proprietary pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) developed at the service provider's laboratory. Low quality sequences were identified by denoising and filtered out of the dataset (Reeder and Knight, 2010). Sequences which were < 200 base pairs after phred20-based quality trimming, sequences with ambiguous base calls, and those with homopolymer runs exceeding 6bp were removed. Sequences were analyzed by a script optimized for high-throughput data to identify potential chimeras in the sequence files, and all definite chimeras were depleted as described previously (Gontcharova et al., 2010). De novo OTU clustering was done with standard UCLUST method using the default settings as implemented in QIIME pipeline Version 1.8.0 at 97% similarity level (Caporaso et al., 2010a). Taxonomy was assigned to each OTU using BLASTn against SILVA SSU Reference 119 database at default e-value threshold of 0.001 in QIIME (Quast et al., 2013).

Statistical analysis

Diversity indices (Shannon, Simpson and Evenness) for each sample were calculated using vegan package version 1.16-32 in R software version 3.1.3 (R Development Core Team, 2012). Community and Environmental distances were compared using Analysis of similarity (ANOSIM) test, based upon Bray-Curtis distance measurements with 999 permutations. Significance was determined at 95% confidence interval (p=0.05). Calculation of Bray-Curtis dissimilarities between datasets and hierarchical clustering were carried out using the R programming language (R Development Core Team, 2012) and the Vegan package (Oksanen et al., 2012). To support OTU-based analysis, taxonomic groups were derived from the number of reads assigned to each taxon at all ranks from domain to genus using the taxa_summary.txt output from QIIME pipeline Version 1.8.0. Obtained sequences were submitted to NCBI Sequence Read Archive with SRP# Study accessions: SRP061806.

RESULTS

Wet sediment and water samples were randomly collected at three different locations in hot springs of Lake Magadi and Little Magadi. The hot springs temperatures ranged from 45.1 to 83.6°C while pH ranged from 9.2 to 9.8. The TDS was beyond measurement using the Electrical Chemical Analyzer; hence the readings appeared as one on the sampling equipment. The metadata collected before sampling is summarized in Table 1. Temperature measurement showed a gradient from hot spring in the main Lake Magadi, with the springs at Little Magadi measuring between 81 and 83.6°C. Cation analysis of the water samples showed that the levels of calcium range between 0.33-0.62 ppm, iron (<0.01 - 0.014 ppm) and magnesium (<0.02 - 0.026 ppm). Sodium levels were very high (11,300, 17,300 and 17,700 ppm) and potassium levels were 225, 458 and 287 ppm. Anion analysis showed that phosphorus range between 2.72 to 6.31 ppm. Chloride levels were high in all samples ranging from 4000 to 4640 ppm (Table 2).

Sequence data

The raw data from the sediments and water samples (three sediment and one water sample) consisted of 548,639 sequences, of which 334, 394 sequences were retained after removing sequences with different tags at each end for quality filtering and denoising. After removing singletons, chimeric sequences and OTUs of non-fungal organisms (<200 base pairs after phred20-based quality trimming, sequences with ambiguous base calls, and those with homopolymer runs exceeding 6 bp), a total of 151 fungal OTUs recovered at 3% genetic distance, were included in the final analysis.

Composition and diversity of fungal communities

Based on BLASTn searches in SILVA SSU Reference 119 database, 151 OTUs were identified, most of which had their best matches against accessions in SILVA database. These 151 OTUs spanned 5 phyla namely; *Ascomycota*, *Basidiomycota, Fungi unspecified phylum*, *Chytridiomycota* and *Glomeromycota*. Sediment samples collected from 81°C had the highest number of OTUs (135 OTUs) while 88 OTUs were shared among all sample types (Figure 1).

The shared OTUs were distributed among the phyla; *Ascomycota* (up to 42.9% relative abundance in sediment sample at 45.1°C), *Fungi unspecified phylum* (up to 6.2% relative abundance in sediment sample at 83.6°C), *Basidiomycota* (up to 3.3% relative abundance in sediment sample at 81°C), *Chytridiomycota* and *Glomeromycota* (up to 1.5% relative abundance each in water sample at 81°C. OTUs belonging to the Phylum *Ascomycota* were the most abundant and were represented

Table 2. Chemical analyses of samples from the Hot Springs Lake Magadi and Little Magadi.

Parameter	Sampling stations		
Chemical properties	**Hot springs 45.1°C**	**Hot springs 81°C**	**Hot springs 83.6°C**
pH	9.61	9.2	9.41
EC (mS cm^{-1})	30.3	30.5	29.9
Ammonium (ppm)	0.94	2.66	2.57
Calcium (ppm)	0.62	0.53	0.33
Magnesium (ppm)	<0.02	0.026	<0.02
Potassium (ppm)	287	458	225
Phosphorus (ppm)	6.31	4.17	2.72
Nitrate N (ppm)	0.53	0.67	0.67
Nitrates (ppm)	2.35	2.98	2.97
Sulphur (ppm)	129	107	58.9
Sulphates (ppm)	387	322	176
Iron (ppm)	<0.01	0.012	0.014
Manganese (ppm)	0.016	0.012	<0.01
Zinc (ppm)	<0.01	<0.01	<0.01
Boron (ppm)	9.3	15.5	8.06
Copper (ppm)	0.043	<0.01	<0.01
Molybdenum (ppm)	0.14	0.12	0.071
Sodium (ppm)	17700	17300	11300
Chlorides (ppm)	4000	4640	4220
Bicarbonates (ppm)	14200	17500	17100

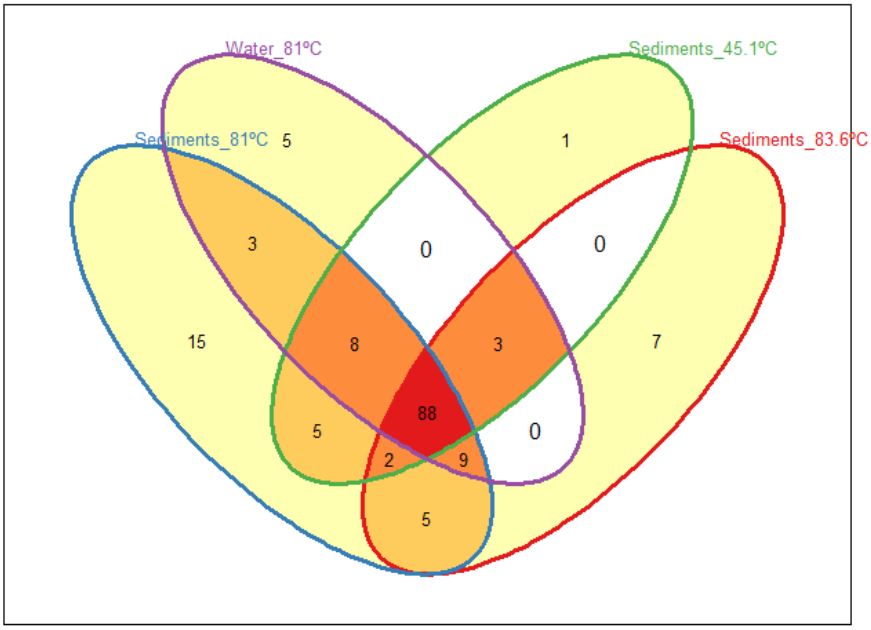

Figure 1. Venn diagram showing the distribution of unique and shared OTUs within various sample types in the three sampling sites. The number of OTUs in each hot spring is indicated in the respective circle.

by the most genera as shown in Figure 2. In sediments at 45.1°C the OTUs were affiliated to the genus *Aspergillus, Penicillium* and *Trichocomaceae*. *Aspergillus oryzae* was the most abundant species with a relative abundance of

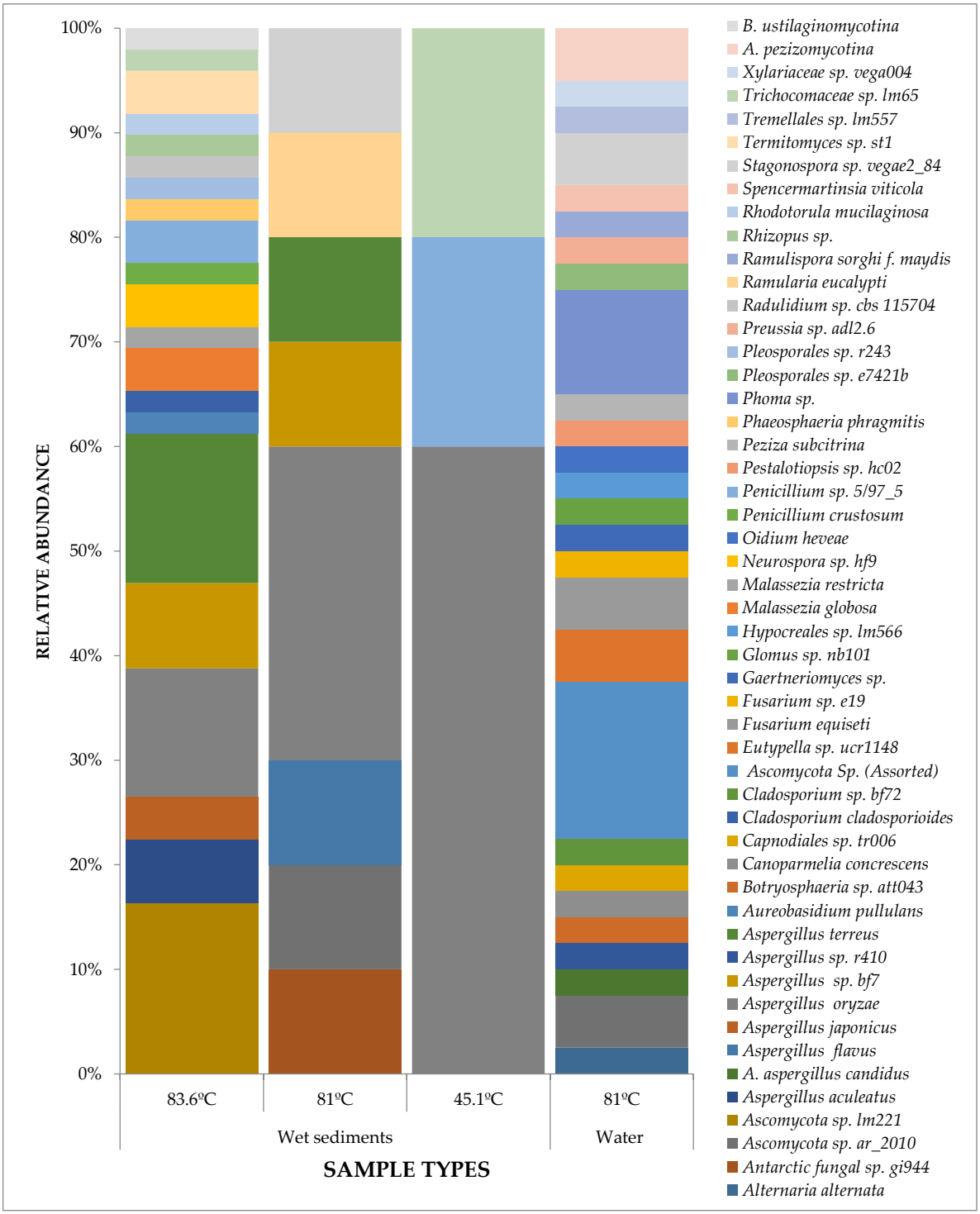

Figure 2. Relative abundance of the most predominant fungal species in various samples collected from the hot springs.

42.86%. Other species present were *Penicillium* sp. 5/975 and *Trichocomaceae* sp. lm65 with 14.29% relative abundance (Figure 2). However, in the sediment sample at 83.6°C genera represented were *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Malassezia*, *Neurospora*, *Penicillium*, *Phaeosphaeria*, *Pleosporales*, *Radulidium* and *Trichocomaceae*. *Basidiomycota* phylum comprised *Rhodotorula* and *Termitomyces* species (Figure 2). *Malassezia* sp., *Neurospora* sp., *Ascomycota* sp. lm221, *Aspergillus aculeatus, Aureobasidium pullulans* and

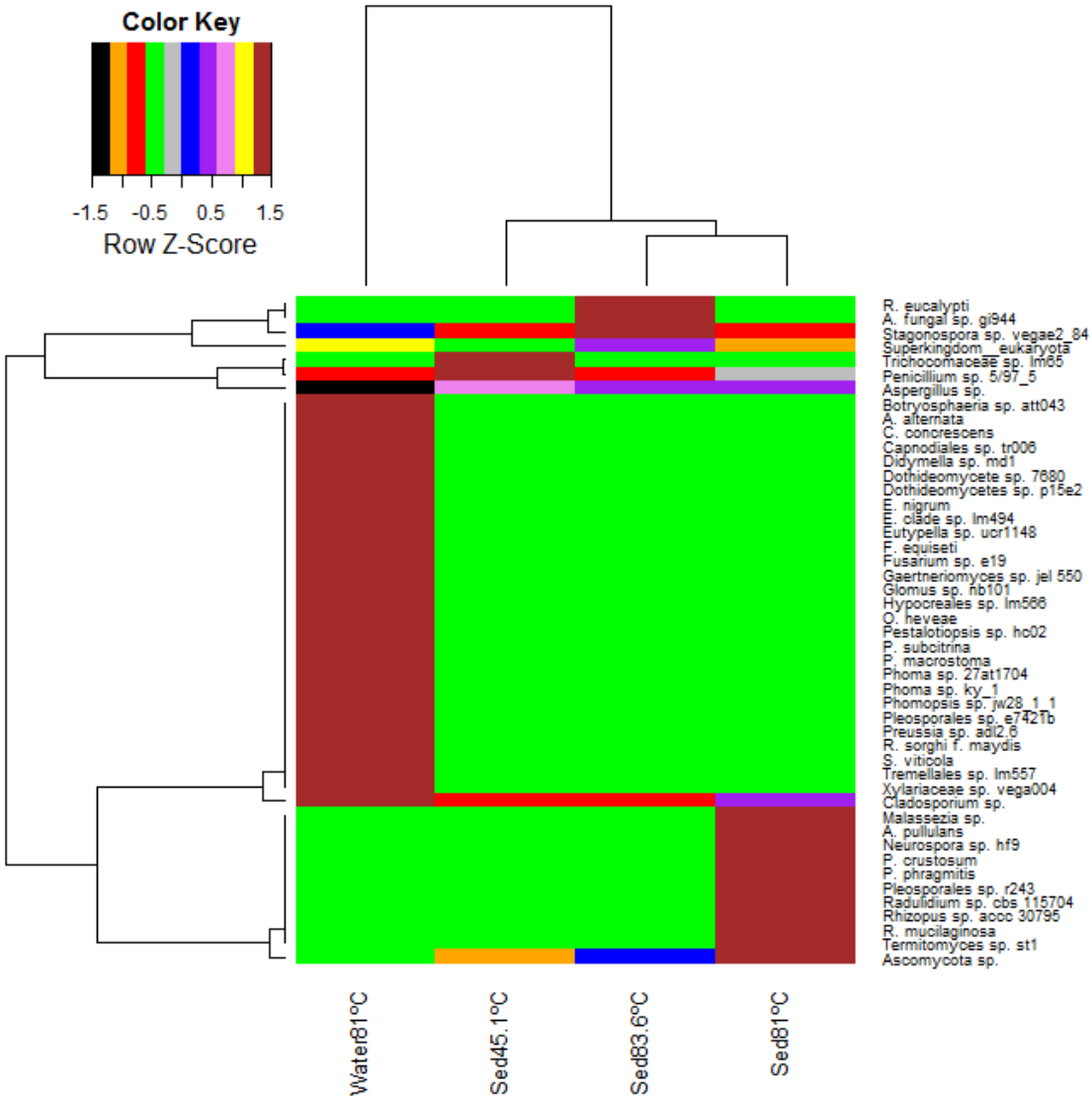

Figure 3. Hierarchical clustering of DNA samples collected from the three hot springs of Lake Magadi and Little Magadi. Species level was chosen to be used in hierarchical clustering to assess the relationships between samples and taxa.

Cladosporium cladosporioides are unique to sediment samples at 83.6°C. In the sediment sample collected at 81°C, *Aspergillus* sp. (*terreus, oryzae, flavus* and *Aspergillus* sp. bf7) within the phylum *Ascomycota* were the most abundant with *Aspergillus oryzae* scoring 18.75%. Other taxa represented in the sample included *Ascomycota* sp. ar_2010, *Ramularia eucalypti,* and *Stagonospora* sp. vegae284 each scoring a relative abundance of 6.25%. *Antarctic fungal* sp. gi944 of *Fungi unspecified phylum* also scored a relative abundance of 6.25% (Figure 2). The water sample collected at 81°C was found to harbor a higher diversity of fungi with low species richness as shown in Figure 2.

Hierarchical clustering between samples collected from Lake Magadi revealed sediment samples from the two hot springs in Little Magadi "*Nasikie eng'ida"* to be closer than the sample from the hot spring in the main lake. Majority of the groups at species level included *Aspergillus*, *Ascomycota*, *Penicillium*, *Neurospora*, *Termitomyces*, *Malassezia*, *Trichocomaceae*, *Stagonospora*, *Ramularia* and *Hypocreales* (Figure 3). The dendogram shows relationship between the four samples.

Fungal richness and diversity indices

Richness (S) estimated the water sample (81°C) to be the richest site, constituting 35 taxa. Sediment samples from the three sites had Evenness (J') scores close to 1

Table 3. Diversity indices computed on all OTU-based fungal taxonomic units obtained from samples collected from the hot springs of Lake Magadi and Little Magadi.

Sample	No. of sequences after filtering	No. of OTUs	Richness (S)	Shannon (H')	Simpson (1/D)	Evenness (J')
Wet sediment (81°C)	112, 262	61	9	1.90	4.92	0.739
Wet sediment (45.1°C)	59,138	7	21	2.63	9.98	0.663
Wet sediment (83.6°C)	80,702	16	4	1.28	3.27	0.897
Water (81°C)	82,292	67	35	2.66	5.06	0.410
Total	**334, 394**	**151**	**69**			

(0.663 – 0.897), hence showing evenness in their number of taxa members than the water sample (81°C). Simpson (1/D) also indicated the sediment 45.1°C to harbor the most diverse taxa (9.98). The Shannon's index (H' = 1.28–2.63) indicated low variation in the level of diversity among the sediment and water samples (Table 3).

Analysis of similarity and distance based redundancy analysis at genus level showed connectivity of distance matrix with threshold dissimilarity of 1 indicating that data of the four samples are connected ([1] 1 1 1 1), hence there were no significant differences in community structure in the samples at 95% level of confidence (P value=0.05).

DISCUSSION

The significance of fungal communities in the hot springs of hypersaline lakes is unclear, mainly because data on the fungal species in these habitats is limited. Using traditional culture-based methods, researchers reported relatively low levels of diversity for fungal communities in extreme environments (Salano, 2011). In this study we used high-throughput sequencing technology in order to comprehensively analyze fungal communities within the hot springs. The high sensitivity of Illumina sequencing enables the detection of rare species, thus provides more detailed information on fungal diversity in these habitats. Members of *Ascomycota* were more frequently identified in the hot springs than those of *Basidiomycota*, whereas members of *Chytridiomycota* and *Glomeromycota* represented only a small proportion of the hot spring fungal communities. The abundance of *Ascomycota* is similar to the abundance of fungi determined in the previous study on soda soils at the edge of several lake basins, where filamentous fungi that could grow at high ambient pH values were isolated (Alexey et al., 2015). The results in that study revealed 100 strains of fungi with various degrees of alkali tolerance and taxonomic affinity within *Ascomycota* (Alexey et al., 2015). Additionally, 6.2% of the fungi detected in wet sediment 83.6°C were unspecified Phylum. These may be undiscovered and possibly indigenous species in the hot springs. Some of the groups in this study are similar to those recovered from a previous culture dependent study conducted on the Hot spring in main Lake Magadi (Salano, 2011). The isolates recovered in that study belonged to *Aspergillus, Penicillium*, *Neurospora, Polyozellus multiplex, Pycnoporus* sp., *Teratosphaeria, Acremonium, Talaromyces*, *Sagenomella, Paecilomyces* and *Aphanoascus* genera (Salano, 2011). Filamentous fungi like *Aspergillus* and *Penicillium* are attractive organisms for production of useful protein and biological active secondary metabolites. They have high secretion capacity and are effective hosts for the production of foreign proteins (Tsukagoshi et al., 2001).

Penicillium genera were found in wet sediments 45.1 and 81°C with relative abundance of 14.29 and 3.28% respectively. This is similar to previous studies in hypersaline water of salterns that revealed different species of *Aspergillus, Penicillium* and diverse non-melanised yeasts (Gunde-Cimerman et al., 2005). Another study that used morphological and molecular techniques to identify a series of halotolerant fungi from hypersaline environments of solar salterns revealed 86 isolates of 26 species from salt ponds, which were identified as *Cladosporium cladosporioides*, nine *Aspergillus* sp., five *Penicillium* sp. and the black yeast *Hortaea werneckii* (Cantrell et al., 2006). *Rhodotorula mucilaginosa,* a yeast species and *Rhizopus* sp. 30795, a *Zygomycota* were found in wet sediment at 81°C while unclassified *Antarctic fungal* sp. gi944 dominated wet sediment at 83.6°C. Other plant pathogenic fungi recovered included *Fusarium* sp., *Cladosporium cladosporioides, Aspergillus flavus, Aspergillus japonicas* and *Aspergillus oryzae.* Most of these organisms may have found their way to the hot springs through various dispersal mechanisms or may be adapted in these extreme environments.

According to Frontier (1985), harsh environments experiencing one or more extreme conditions tend to harbor fewer species. In contrast, wet sediments at hot spring 45.1°C were found to have the least OTUs (107 OTUs) as compared to higher temperature samples, distributed within *Aspergillus oryzae* (42.86%), *Penicillium* sp. 5/97_5 (42.28%) and *Trichocomaceae* sp. lm65 (42.28%). Although water samples at 81°C were found to harbor a higher diversity of fungi with lower species richness, wet sediments showed a lower diversity with

high abundance of present groups. This could be due to high abundance of organic matter and lower oxygen levels which favored decomposition processes; hence the groups present have sufficient carbon sources (Neira et al., 2001; Buee et al., 2009). The widespread fungal groups within the wet sediments may therefore be degraders of organic matter (Edgcomb et al., 2011a; Nagahama et al., 2011; Burgaud et al., 2013; Coolen et al., 2013).

This study reveals the presence of moderate and weak alkalitolerant fungi such as *Alternaria alternata, Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. previously reported to grow optimally at neutral or below neutral pH values. These species have previously appeared in existing reports on the alkalitolerant and halotolerant fungi (Kladwang et al., 2003; Gunde-Cimerman et al., 2009). They have therefore been considered as transition species in the alkaline environments, since they are also known to inhabit neutral soils worldwide. *Hypocreales* and *Pleosporaceae* have been reported as strong alkalitolerants and effective alkaliphiles inhabiting soda soils at the edge of lake basins (Alexey et al., 2015). In this study, *Hypocreales* sp. lm 566 was identified in water samples at 81°C while *Pleosporales* sp. was found in wet sediment and water samples at 83.6 and 81°C respectively. Other interesting groups recovered from this study include *Pestalotiopsis* sp., *Neurospora* sp., and *Xylariaceae* sp. These have been reported to have various applications in Biotechnology industries (Russell et al., 2011; Roche et al., 2014; Healy et al., 2004; Posada et al., 2007).

Conclusion

This study presented fungal diversity analysis of samples collected from the hot springs of Lake Magadi and Little Magadi, using Illumina Sequencing Technology. The results revealed representatives of thermophilic and alkaliphilic fungi within the hot springs, suggesting their ability to adapt to a multi-extreme sampling environment due to high pH, temperature, and salinity. Culture dependent studies in future will help us unravel the survival mechanisms used by these polyextremophilic fungi.

Conflicts of Interests

The authors have not declared any conflict of interests. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

ITS, Internal Transcribed Spacer; **OTUs,** Operational Taxonomic Units; **DNA,** Deoxyribonucleic Acid; **QIIME,** Quantitative Insights into Microbial Ecology.

REFERENCES

Alexey AG, Marina LG, Sofiya AB, Alfons JMD, Elena NB (2015). On the diversity of fungi from soda soils. Fung. Divers. 76(1):27-74.

Barea JM, Pozo MJ, Azcón R, Azcón-Aguilar C (2005). Microbial co-operation in the rhizosphere. J. Exp. Bot. 56(417):1761-778.

Behr HJ, Röhricht C (2000). Record of seismotectonic events in siliceous cyanobacterial sediments (Magadi cherts), Lake Magadi, Kenya. Int. J. Earth Sci. 89:268-283.

Brem FM, Lips KR (2008). *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. Dis. Aquat. Org. 81(3):189-202.

Buee M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009). Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol. 184:449-456.

Burgaud G, Woehlke S, Rédou V, Orsi W, Beaudoin D, Barbier G, Biddle J, Edgcomb VP (2013). Deciphering the presence and activity of fungal communities in marine sediments using a model estuarine system. Aquat. Microb. Ecol. 70:45-62.

Cantrell SA, Martí´nez CL, Molina M (2006). Characterization of *fungi* from hypersaline environments of solar salterns using morphological and molecular techniques. Mycol. Res. 110:962-970.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushma FD, Costello E K, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky, J R, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010a). QIIME allows analysis of high-throughput community sequencing data. Nat. Meth. 7:335-336.

Coolen MJL, Orsi WD, Balkema C, Quince C, Harris K, Sylva SP, Filipova-Marinova M, Giosan L (2013). Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene. Proc. Natl. Acad. Sci. USA 110:8609-8614.

Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A (2007). Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One 2(5):e457.

Edgcomb VP, Beaudoin D, Gast R, Biddle JF, Teske A (2011a). Marine subsurface eukaryotes: the fungal majority. Environ. Microbiol. 13:172-183.

Frontier S (1985). Diversity and structure in aquatic ecosystems. Oceanogr. Mar. Biol. 23:253-312.

Gadd GM (2007). Geomycology: Biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bio-weathering and bioremediation. Mycol. Res. 111(Pt 1):3-49.

Gontcharova V, Youn E, Sun Y, Wolcott RD, Dowd SEA (2010). Comparison of bacterial composition in diabetic ulcers and contralateral intact skin. Open Microbiol. J. 4:8-19.

Gunde-Cimerman N, Butinar L, Sonjak S, Turk M, Uršic V, Zalar P, Plemenitaš A (2005). Halotolerant and halophilic fungi from coastal environments in the Arctics. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds) Adaptation to life at high salt concentrations in Archaea, Bacteria and Eukarya. Springer, Netherlands. pp. 397-423.

Gunde-Cimerman N, Ramos J, Plemenitaš A (2009). Halotolerant and halophilic fungi. Mycol. Res. 113:1231-1241.

Hawksworth DL (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol. Res. 95:641-655.

Healy PC, Hocking A, Tran-Dinh N, Pitt JI, Shivas RG, Mitchell JK, Kotiw M, Davis RA (2004). Xanthones from a microfungus of the genus *Xylaria*. Phytochemistry 65(16):2373-2378.

Kladwang W, Bhumirattana A, Hywel-Jones N (2003). Alkaline-tolerant fungi from Thailand. Fung. Divers. 13:69-83.

Le Calvez T, Burgaud G, Mahé S, Barbier G, Vandenkoornhuyse P (2009). Fungal diversity in deep sea hydrothermal ecosystems. Appl. Environ. Microbiol. 75(20):6415-6421.

Lindahl, BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P Stenlid J,

Finlay RD (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol. 173(3):611-620.
Magan N (2006). Fungi in extreme environments: Environmental and microbial relationships. In: Christian PK, Irina SD (eds) The Mycota IV. Springer, Netherlands. pp. 85-104.
Magan N, Aldred D (2007). Environmental fluxes and fungal interactions: maintaining a competitive edge. In: van West P, Avery S, Stratford M (eds) Stress in Yeasts and Filamentous Fungi. Elsevier Ltd, Amsterdam, Netherlands. pp. 19-35.
Mueller GM, Schmit JP (2006). Fungal biodiversity: what do we know? What can we predict? Biodivers. Conserv. 16:1-5.
Nagahama T, Takahashi E, Nagano Y, Abdel-Wahab MA, Miyazaki M (2011). Molecular evidence that deep-branching fungi are major fungal components in deep-sea methane cold-seep sediments. Environ. Microbiol. 13:2359-2370.
Ndwigah FI, Boga IH, Wanyoike W, Kachiuru R (2015). Characterization, Enzymatic Activity, and Secondary Metabolites of Fungal Isolates from Lake Sonachi in Kenya. IOSR J. Pharm. Biol. Sci. 10:65-76.
Neira C, Sellanes J, Levin LA, Arntz WE (2001). Meiofaunal distributions on the Peru margin: Relationship to oxygen and organic matter availability. Deep Sea Res. Part I. 48:2453-2472.
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry MHS, Wagner H (2012) vegan: Community Ecology Package.
Posada F, Aime MC, Peterson SW, Rehner AS, Vega EF (2007). Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycol. Res. 111:748-757.
Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41(DI):D590-D596.
R Development Core Team (2012). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria.
Raghukumar C, Raghukumar S (1998). Barotolerance of fungi isolated from deep-sea sediments of the Indian Ocean. Aquat. Microb. Ecol. 15(2):153-163.
Reeder J, Knight R (2010). Rapidly denoising pyrosequencing amplicon reads exploiting rank-abundance distributions. Nat. Meth. 7:668-669.
Roche CM, Loros JJ, McCluskey K, Glass NL (2014). *Neurospora crassa*: Looking back and looking forward at a model microbe. Am. J. Bot. 101(12):2022-2035.
Russell JR, Huang J, Anand P, Kucera K, Sandova AG, Dantzler KW, Hickman D, Jee J, Kimovec FM, Koppstein D, Marks DH, Mittermiller PA, Núñez SJ, Santiago M, Townes MA, Vishnevetsky M, Williams NE, Vargas MP, Boulanger LA, Bascom-Slack C, Strobel SA (2011). Biodegradation of polyester polyurethane by endophytic fungi. Appl. Environ. Microbiol. 77:6076-6084.
Salano O (2011). Isolation and characterization of fungal communities from L. Magadi. MSc Thesis, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
Sambrook KJ, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd Ed. Cold Spring Harbor Laboratory, New York, USA.
Tsukagoshi N, Kobayashi T, Kato M (2001). Regulation of the amylolytic and hemi-cellulolytic genes in *Aspergilli.* J. Gen. Appl. Microbiol. 47:1-19.
Vaupotic T, Veranic P, Jenoe P, Plemenitas A (2008). Mitochondrial mediation of environmental osmolytes discrimination during osmoadaptation in the extremely halotolerant black yeast *Hortaea werneckii*. Fung. Genet Biol. 45(6):994-1007.
White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. Academic Press, New York, USA. pp. 315-322.
Yu K, Zhang T (2012) Metagenomic and Metatranscriptomic Analysis of Microbial Community Structure and Gene Expression of Activated Sludge. PLoS One 7(5):e38183.
Yuriko N, Takahiko N (2012). Fungal diversity in deep-sea extreme environments. Fung. Ecol. 5:463-471.