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EXTREMOPHILIC FUNGI AND CHEMICAL ANALYSIS OF HYPERSALINE, ALKALINE LAKES OF WADI-EL-NATRUN, EGYPT

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Abstract- Mesophiles, halophiles and thermophiles fungi were isolated from water, mud and newly reclaimed soil of the biggest eight hypersaline, alkaline lakes of Wadi-El-Natrun, Egypt. Morphological techniques were used to identify the isolated fungi and in many cases molecular technique was used to confirm the identification. Mesophiles showed the highest number of taxa from newly reclaimed soil, followed by mud and water. Aspergillus was almost the most common genus recovered on the three isolation media contributing regularly the highest CFUs. Acremonium was the most dominant genus recovered from water on Czapek's + 10 % NaCl medium. Penicillium was the second most dominant behind Aspergillus in the mud and soil and it was completely absent at 45° C. A distinctive isolate was recovered from a water sample representing a new species of Ramophialophora based on its morphology and rDNA analysis. A 281 strains of mesophiles and halophiles were screened for their ability to grow on Czapek's broth medium supplemented with different NaCl concentrations (5, 10, 15, 20, 25 and 30 %) and all could grow up to 15 % NaCl (weakly halotolerant), while 248 isolates up to 20 % NaCl (moderately halotolerant), 32 up to 25 % NaCl (highly halotolerant) and 12 could grow up to 30 % NaCl (extremely halotolerant). These 12 strains could further survive 30 % salt concentration for up to18 months.

Key words: Alkaline lakes, Extremophiles, Halophiles, Hypersaline, Thermophiles, Wadi-El-Natrun.

1. INTRODUCTION

Hypersaline lakes are characterized by extreme concentrations of NaCl, often high concentrations of other ions, and in some cases extremes in pH. After evaporation, soda soils are formed with high pH values (> 8) which represent an example of extreme habitats. Soda soils usually develop in arid and semi-arid lands worldwide, and may be varied in salt concentrations from low to saturation [28]. The driving force for the soda accumulation is the loss of Ca^{+2} trapped by CO_3^{-2} ion, leaving Na^+ as the dominant cation [36]. Soda soils are formed as a result of carbonate accumulation under poor Ca^{+2} and Mg^{+2} conditions [28, 36].

Hypersaline waters (lakes) can be divided into thalassohaline and athalassohaline, the first have a marine origin as they have a composition similar to that of sea water and the second, whose composition reflects the composition of the surrounding geology, topography and environmental conditions and its composition is varied widely [57]. Dead Sea, Great Salt Lake, some cold hypersaline lakes in Antarctica or alkaline lakes such as Lake Magadi or the lakes of Wadi-El-Natrun are typical examples of athalassohaline waters [27, 57].

Fungi are globally distributed over a wide range of ecological extreme habitats, and to conquer them they must be able to rapidly germinate and become established to produce the necessary extracellular enzymes in the encirclement environment [59]. The study of such these extremes are very important for the egression of new species and evolution of various ecological relations among organisms which compensate certain environmental extremes. New metabolites and metabolic pathways of organisms from such rigorous habitats can be expected [52].

These extreme habitats are occupied by highly specialized organisms which termed extremophiles. Halophiles are considered an important organisms that existed in hypersaline habitats and they can be divided on the basis of their optimal growth as 1) non-halophiles are those that grow best in media containing less than 1.16 % NaCl, 2) slightly halophiles grow best in media with 1.16-2.9 %, 3) moderately halophiles grow best with 2.9-14.5 % and 4) extremely halophiles show optimal growth in media containing 14.5-30.16 % [39]. Another classification proposed was based on the halophilic abilities of the tested fungi which categorized them as 1) halophilic, which could grow on 5-25 % NaCl, 2) highly halotolerant, which could grow on 0-20 %, 3) moderately halotolerant, which could grow on 0-15 % and 4) weakly halotolerant, which could grow on 0-10 % [47].

1.1 Objectives of the Study

- To study the chemical analysis of the water, mud and newly reclaimed soil around the hypersaline, alkaline lakes of Wadi-El-Natrun.
- To study the biodiversity of the mesophilic, halophilic/halotolerant and thermophilic/thermotolerant fungi that inhabit these habitats.
- To assess the mesophilic and halotolerant/halophilic fungal isolates for their ability to survive different levels of NaCl and different storage periods.

2. MATERIALS AND METHODS

2.1 Site Description

Wadi-El-Natrun ("Natrun" is derived from the Latin word "natrium" for the element sodium) is a part of the Western Desert adjacent to the Nile Delta and belongs to Behira Governorate, Egypt. It is a narrow depression located approximately 90 km south of Alexandria and 110 km northwest of Cairo between Lat. 30º17' and 30º38' N and between Long. 30º2' and 30º30' E [65]. It is narrow at both ends (2.6 km in the north and 1.24 km in the south) and wider in the middle (8 km) and lies 23 m below sea level and 38 m below the water level of Rosetta branch of the Nile [7]. It is characterized by a series of twenty small disconnected shallow lakes in its bottom aligned with its general axis in the northwesterly direction for a distance of about 30 km, most of them no deeper than half a meter and they have become hypersaline by evaporative concentration [32]. These lakes are fed by two sources: the springs in the bottom (e.g. in Lake Hamra) and the underground seepages from the Nile River [32]. Groundwater (the source of the salts in the lakes) derived from the Nile delta infiltrates the Wadi in small trickles due to the presence of a hydrostatic connection between the Nile (Rosetta branch) and the Wadi [32, 60].

2.2 Samples Collection

40 water, 40 mud (5 samples/each lake) and 33 newly reclaimed soil samples were collected during February 2012 from the 8 main biggest lakes in the Wadi, namely Fasida, Umm-Rishah, Rosetta, Hamra, Zugm, Beida, Khadra and Gaar.

2.3 Sample Preparation for Chemical Analysis

As soon as samples were collected, they were transferred to the laboratory. Moisture content was determined for all the mud and soil samples investigated before sample preparation for chemical and mycological analysis. The mud and soil samples were spread out on separate polyethylene sheets and left to air dry for 3 days. The dried samples were ground by means of wooden mortar and pestle to reduce particle size to pass through 2-mm sieve, then were thoroughly mixed and put in plastic bags and preserved in a refrigerator at 4°C till chemical and mycological analysis.

2.4 Chemical Analysis

pH, organic matter content [56], total dissolved solids (TDS) [67], sodium and potassium [15], calcium and magnesium [14], carbonates, bicarbonates and chlorides [66] were determined for all samples investigated.

2.5 Media Used for Isolation of Fungi

- 1 % glucose-Czapek's agar medium of the following composition (g/l): glucose, 10; Na_2NO_3, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01; $CuSO_4$, 0.005, agar, 15 and final pH 7.3.
- 1 % glucose–Czapek's agar of the above composition + 10 % NaCl.
- Yeast-Starch agar (YpSs) [24] of the following composition (g/l) of: Soluble starch, 15; powdered yeast extract, 4.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; agar, 15; water (1/4 tap and 3/4 distilled), up to 1000 ml. and final pH 7.0. To each of these media, Rose Bengal at 0.05 g/l and chloramphenicol at 250 mg/l [10, 63] were added to suppress the growth of bacteria and to restrict the fungal colonies which facilitate the isolation of slow-growing fungi.

2.6 Isolation of Fungi from the Water Samples

Pour plate technique was used for isolation of fungi from the water samples. Three ml of each water sample were pipetted into each 9.0 cm Petri dish (5 dishes/sample). Three isolation media and two incubation temperatures were used for isolation of mesophiles, halophiles and thermophiles. The inoculated plates were incubated for 7-15 days at 25ºC for mesophiles and halophiles and at 45ºC for thermophiles fungi. Counts of CFUs of each fungus were calculated per 600 ml water in all samples.

2.7 Isolation of Fungi from the Mud and Soil Samples

Dilution plate method [34] was used for isolation of fungi from mud and soil samples. The inoculated plates were incubated for 7-15 days. Counts of CFUs of each fungus were calculated per 1 gm oven-dry mud or soil in every sample. Five plates were used for each treatment.

2.8 Halophilic Activity

This test was performed using Czapek's solution supplemented with NaCl concentrations of 0, 5, 10, 15, 20, 25 and 30 % [47]. 281 fungal strains (144 from saline water, 37 from mud and 100 from soil) were tested. 15 ml of Czapek's solution was transferred into 32-ml screw-capped tubes, autoclaved at 121° C for 20 min and inoculated with spore mass of 7-day-old culture of the tested strains and incubated at 25° C for 15-60 days. The visual growth was recorded for positive strains. The growth of the highest halophiles strains that grow at 30 % NaCl was tested on Cz agar after different storage periods of 3, 6, 9, 12, 15 and 18 months.

2.9 Phenotypic Identification of Fungi

The identification of fungal genera and species was based on macroscopic and microscopic features following the keys and descriptions of Aspergillus species [55], Penicillium and its teleomorphs [54], Dematiaceous Hyphomycetes [21, 22], Fusarium species [11, 40], fungi in general [19, 42] and thermophilic and thermotolerant fungi [16, 58].

2.10 Molecular Identification of the Isolated Fungi

The fungus was grown on Czapek's yeast extract agar (CYA) plates and incubated at 25° C for 7 days. A small amount of the fungal growth was scraped and suspended in 200 µl of distilled water and boiled at 100° C for 15 minutes, and sent to SolGent Company, Daejeon, South Korea, for PCR and rDNA sequencing.
Fungal DNA was extracted and isolated using SolGent purification bead in SolGent Company (SolGent, Daejeon, South Korea). Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using the universal primers ITS 1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then amplification was performed using the polymerase chain reaction (PCR) (ABI, 9700). The PCR reaction mixtures were prepared using Solgent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mM dNTPs (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5U) 0.25µl, template1.0 µl, distilled water to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 1 min, with a final extension step of 72 °C for 5 min.
The PCR products were then purified with the SolGent PCR Purification Kit-Ultra prior to sequencing. Then the purified PCR products were reconfirmed (using size marker) by electrophoreses of the PCR products on 1% agarose gel. Then these bands were eluted and sequenced. Each sample was sequenced in the sense and antisense direction.
Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center for Biotechnology Information (NCBI) website. Sequences obtained with those retrieved from GenBank database were subjected to Clustal W analysis using Meg Align (DNA Star) software version 5.05 for the phylogenetic analysis. Sequence data were deposited in GenBank and accession numbers are given for them.

3. RESULTS AND DISCUSSION

3.1 Chemical Analyses

3.1.1 Water samples

In the present study, the water samples showed alkaline nature of pH ranging from 8.5 to 9.54 (Table 3.1). In agreement with the current results nearly the same range of pH (8.51- 9.45) was registered in the water of Wadi-El-Natrun lakes [9, 26, 48, 65], (9.4 - 9.8) in the water of Mono Lake, California [64], (7.9 - 9.1) in the water of Sutton Salt Lake, east Otago, New Zealand [17], and (8.66 - 9.27) in the water of the Chaplin Lakes [12]. In the current study, the water samples contained TDS ranging from 1.92 % in Lake Khadra to 26.3 % in Lake Umm-Rishah (Table 3.1) and these TDS range was disagreed with the higher levels of TSS previously recorded in the water of Wadi-El-Natrun lakes during 2006/2007 [9, 26, 65]. These differences may be due to the difference in the season of sampling. In agreement with these results almost similar TDS levels (9.0 %) in Mono Lake in California and (8.8 %) in Big Soda Lake in Nevada were obtained [53]. Also values of TDS ranging from 2.487 % in Lake Big Quill to 25.18 % in Lake Ingebright were recorded [12].
In the current investigation, the water samples registered sodium cations and chloride anions of (1.13 - 17.64 g/l) and (0.72 - 13.52 g/l) respectively. In contention with these results, higher levels were reported in the water of Wadi-El-Natrun lakes ranged from 17 - 44 g/l of sodium and 10.5 - 23 g/l of chloride [9, 48]. Also higher contents of sodium and chloride of (17.6 g/l and 29.5 g/l respectively) in Mono Lake in California and (28.0 g/l and 27.0 g/l respectively) in the Big Soda Lake in Nevada were recorded [53]. However much higher levels of sodium (78.41 g/l) and chloride (47.54 g/l) were reported in Lake Katwe in Uganda [38] and (30.8 g/l and 49.1 respectively) in Sevier Lake Utah [30].
Carbonates showed its peak (38.1 g/l) in Lake Umm-Rishah while the highest value of bicarbonates (29.2 g/l) was reported in Lake Fasida (Table 3.1). This high level of carbonates and bicarbonates may explain the high

alkalinity of Lake Fasida and Lake Umm-Rishah. These values are much higher than those reported in the water of Wadi-El-Natrun lakes during 2006/2007 which registered lower values of carbonates (0.2 - 1.3 g/l) and bicarbonates (0.11- 2.0 g/l) [9, 48]. Also, a higher sum of carbonates and bicarbonates of 30.1 g/l in Mono Lake and 24.0 g/l in the Big Soda Lake was recorded [53].
Other cations: K^+ (0.0186-0.45 g/l), Ca^{+2} (0.03 - 0.64 g/l) and Mg^{+2} (0.01- 0.09 g/l) were found at very low levels compared to those reported in the Great Salt Lake, Dead Sea, Sevier Lake Utah and ocean water [30]. In agreement with the current results, similar concentrations of calcium (0.15 g/l), magnesium (0.3 g/l) and potassium (0.6 g/l) in the water of Wadi-El-Natrun lakes were previously found [50]. Also lower levels of potassium (0.1- 0.3 g/l), calcium (0.01- 0.5 g/l) and magnesium (0.02 - 0.3 g/l) in the water of Wadi-El-Natrun lakes were reported during 2006/2007 [9, 48].

3.1.2 Mud samples

In the present study, pH of the mud samples showed its peak (10.24) in Lake Fasida while the least (8.78) was recorded in Lake Rosetta (Table 3.2). In this respect, similar results of pH of the mud samples of Wadi-El-Natrun lakes ranging from 9.0 to 9.4 [9, 26], the mud samples of Mono Lake ranging from 8.6 - 9.45 [64] and Playa Lake sediments ranging from 8.5 - 9.5 [61] were previously recorded.
In the current study organic matter content ranged from 0.45 % in Lake Fasida to 5.94 % in Lake Zugm, and this was higher than those recorded from the mud samples of Wadi-El-Natrun lakes during 2006-2007 [9, 26]. TDS values ranged from 0.66 % in Lake Khadra to 20.36 % in Lake Rosetta mud, however higher TSS values ranged from 15.8 - 38.3 % in the mud samples of Wadi-El-Natrun lakes during 2006-2007 were registered [9, 26]. Sodium cation (g/kg) in the mud registered a range of 76.5 in Lake Khadra to 281.11 in Lake Beida which is much higher compared to those previously recorded during 2006/2007 in the mud samples of Wadi-El-Natrun lakes [9, 26] and those reported in the mud samples of Mono Lake, California [64].
The current study showed that Lake Hamra registered the peak of potassium cations (7.1 g/kg) while the trough (0.97) was recorded in Lake Khadra. However, relatively lower levels of 0.2 g/kg in Lake Fasida and 2.3 g/kg in Lake Zugm were previously reported in the mud of Wadi-El-Natrun lakes during 2006/2007 [9, 26]. Nearly similar results were obtained from the sediments of Lake Abert, Oregon, USA which contained 1.7 - 7.7 g/kg potassium [35] while the mud samples of Mono Lake registered much lower potassium content ranging from 0-0.265 g/kg [64].
Low calcium amounts of 0.18 - 2.3 g/kg and magnesium of 0.05 - 0.17 g/kg were registered in the mud samples (Table 3.2) and this is concordant with those previously obtained from the mud of Wadi-El-Natrun lakes during 2006/2007 where low calcium (0.01 - 0.1 g/kg) and magnesium (0.02 - 0.2) content was recorded [9, 26]. Higher values of magnesium content ranging from 16.1-41.5 g/kg have been reported from sediments of Lake Abert [35]. Much higher calcium content of 18.95-38 g/kg and magnesium of 1.5 - 1.75 g/kg have been reported in the mud samples of Mono Lake, California [64].
In the present study, carbonates, bicarbonates and chlorides yielded their peaks (22.98, 16.06 and 90.9 g/kg respectively) in the mud of Lake Rosetta which registered the highest TDS while their trough (0.12, 1.67 and 2.5 g/kg respectively) were recorded in Khadra which recorded the least TDS level. These current results are in disagreement with the previously reported range of carbonates (0.02 - 0.45 g/kg), bicarbonates (0.18 - 0.76) and chlorides (5.7 - 16.4) in the mud of Wadi-El-Natrun lakes during 2006/2007 [9, 26].

3.1.3 Soil samples

In the present study, the soil samples collected from around the lakes exhibited slightly alkaline pH values ranging from 7.83 to 8.12 which harmonize with the results previously obtained from soil samples around lakes of Wadi-El-Natrun [9, 20, 26, 49] and soil around the Mono Lake, California [64]. The soils around lakes of Wadi-El-Natrun are newly reclaimed and depend on humus and different types of fertilizers which may explain its high range of organic matter (1.5 - 2.49 %) compared with that previously recorded from different types of soil in Egypt [47] and Libya [23].
In the current investigation, moisture content of the soil samples ranged from 6.9 % in Lake Khadra to 12.6 % in Lake Fasida. Other parameters (TDS, Na^+, K^+, CO_3^{-2}, HCO_3^-, Ca^{+2}, Mg^{+2}, Cl^-) were reported in lower levels in the soil than those registered in the water and mud (Table 3.3) and this may explain the highest number of propagules in the soil than in the water and mud, since stresses from pH, TDS, Na^+ and other cations and anions are much lesser. A slightly higher range of TSS (0.5 - 6.2 %) during 2006/2007 was reported [9, 26]. However, soils of Mono Lake area revealed much higher content of chlorides (53.25 - 61.75 g/kg), calcium (50.85 - 53.05) and magnesium (2.45 - 2.95) while potassium was at zero level and sodium ranged from 0.0 to 1.85 [64].

3.2 Biodiversity of Fungi

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Three media and two incubation temperatures were employed. 44 genera, 129 species and 4 varieties were recorded from the water, mud and newly-reclaimed soil samples on the three media at 25º and 45º C. As expected, the CFUs were consistently higher on Cz than on Cz + 10 % NaCl at 25º C and YpSs at 45° C. In the hypersaline waters of Wadi-El-Natrun lakes the mean density of fungi was 76.66 cells/l on Cz + 10 % NaCl at 25° C, 78.33 cell/l on YpSs at 45° C and 831.66 cells/l on Cz at 25° C. Fungal counts of about 10 cells/l were typical for ocean water [62]. On Cz at 25º C, the mud was the richest source in fungal genera (28) while the water was the richest in fungal species (61 + 1 variety). On Cz + 10 % NaCl, mud gave the highest number of genera (8) while soil contributed the highest number of species (27 + 1 variety). Soil was also the richest in fungal genera (14) and species (19 + 3 varieties) on YpSs at 45º C.
Identification of fungi was based mainly on the macroscopic and microscopic characteristics, but in many suspected cases molecular methods (ITS sequence analysis of the rDNA) were used to confirm their identification (Table 3.4).

3.2.1 Mesophiles

A total of 105 species and 1 variety appertaining to 35 genera were isolated from the three habitats. Aspergillus contributed a total of 23 species and one variety from the water, mud and soil. The biodiversity of Aspergillus species was more pronounced in water (18 species + 1 variety) than in mud and soil (13 species + 1 variety each). A. fumigatus was the most dominant species from water followed by A. sulphureus which contributed 7.21 % and 6.61 % of total fungi respectively (Table 3.5). A. terreus was the most dominant species from soil (26.15 %) and mud (12.8 % of total fungi). A. flavipes came next to A. terreus in its dominance in mud (11.2 % of total fungi) and A. flavus came next in soil (6.67 % of total fungi). Penicillium was the runner up of Aspergillus, represented by 20 species recovered from the three habitats. Similarly, Penicillium from the water yielded more species (12) than the mud (10) and soil (5) (Table 3.5).
The current results are in agreement with those previously recorded from water samples collected from the 8 lakes of Wadi-El-Natrun during 2006/2007 where 15 species related to 7 genera were reported and it was found that Aspergillus was the most dominant genus followed by Acremonium and Penicillium [48]. Also 81 species and 2 varieties from the alkaline soil around Wadi-El-Natrun lakes were recorded and it was found that Aspergillus, Fusarium, Myrothecium, Stachybotrys, Penicillium and Emericella were the most common genera [49]. Also, these results are in accordance with those recorded from alkaline soil (pH 8.1-8.6) around Basrah, Iraq where Aspergillus and Penicillium were the most common fungal genera isolated [33].
Some Aspergillus species have been recorded from the water of solar salterns of Cabo Rojo in Puerto Rico, namely A. caespitosus, A. candidus, A. flavipes, A. flavus, A. melleus, A. nidulans, A. ochraceus, A. penicillioides and A. unguis [13]. Penicillium citrinum, P. chrysogenum, P. oxalicum and P. variabile were also isolated from water of solar saltern of Cabo Rojo in Puerto Rico, while A. japonicas, Chaetomium globosum, Cladosporium cladosporioides and P. variabile were recorded from sediments [13]. It was found that A. flavus, A. janus, A. japonicas, A. niger, A. sydowii and A. terreus were the most common species from alkaline soil samples collected from Casa Caiada and Bairro Novo Beaches, Brazil [25]. Aspergillus and Penicillium were also isolated in high frequencies from salt marsh soils in Egypt where A. flavus, A. fumigatus, A. niger and A. ochraceus were the most common species [6].
Some fungal species were recorded for the first time from hypersaline alkaline water in Egypt; these were Acremonium alternatum, A. zonatum, Alternaria chlamydosporigena, Aspergillus insulicola, A. roseoglobulosus, Chordomyces antarcticum, Penicillium dendriticum, P. melinii, P. sublateritium, Phaeoacremonium sp., Plectosphaerella oligotrophica and Stilbella fimetaria. A distinctive isolate was also recovered from the water of Lake Fasida probably representing a new species of Ramophialophora based on morphology and rDNA analysis (Tables 3.4, 3.5).

3.2.2 Halophiles

In the present study, 38 species and one variety related to 11 genera were isolated on Cz + 10 % NaCl at 25° C from the three habitats contributing a total of 3670 CFUs per 1 g or 1 ml in all samples. The soil was the richest in the number of species (27 species and 1 variety) followed by mud (17 species) while the water registered the least number (3 spp.). Aspergillus was the most frequent genus obtained from the soil (93.9 % of the samples) followed by mud (32.5 %) contributing 81.0 % and 85.46 % of total fungi respectively and was missing in water (Table 3.6). A. flavipes was the most dominant species recovered from mud samples contributing 36.8 % of total fungi and A. flavus was the most dominant from the soil comprising 37.2 % of total fungi and this is in agreement with the previously obtained results from soil of Wadi-El-Natrun lakes [49]. 14 species of Penicillium were isolated from desert soils of Saudi Arabia on 5 % NaCl agar medium [2]. It was found that P. chrysogenum and P. citrinum were among the common species in soils from the Red Sea shore and Wadi Bir-El-Ain on 5-10 % NaCl agar [8, 46]. Acremonium was the most dominant fungus from water samples exhibiting 93.48 % of total fungi (Table 3.6).

Previous studies revealed that at low water content the active mycobiota were dominated by species of Aspergillus and Penicillium, which were the most common taxa [18] as observed in studies of Mono Lake, California [64], Arctic [29] and Cabo Rojo in Puerto Rico [13]. Only 2 species of Scopulariopsis (S. halophilica and S. brumptii) and Acremonium hyalinulum were previously recorded from water samples during one season of study of the water of Wadi-El-Natrun lakes [48]. However, 41 species and one variety related to 16 genera were isolated from the soil samples collected from around lakes of Wadi-El-Natrun during 2006/2007 [49] and it was concluded that the majority of fungi of Wadi-El-Natrun are osmophiles and halophiles and they are able to accommodate themselves to exist under conditions of water stress but a lower number can tolerate water stress with the toxic effect of excessive concentrations of Na^+ and Cl^- ions.

3.2.3 Thermophiles

Twenty-five species and 3 varieties belonging to 14 genera were isolated from water, mud and soil samples on YpSs at 45°. Soil was the richest source yielding 14 genera, 19 species and 3 varieties. Aspergillus was the most dominant genus in the mud and soil represented by 3 species each contributing 30.2 % and 17.76 % of total fungi respectively (Table 3.7). In this respect, almost similar results were obtained from the soil, mud and salt-crusts of Wadi-El-Natrun lakes [9] where it was found that Aspergillus (6 species + 1 variety) and Emericella (5 species) were the most common genera at 45º C. Thermomyces represented by 2 species; T. ibadanensis which was isolated from the mud and soil comprising 4.14 % and 4.5 % of total fungi respectively and T. lanuginosus which was recovered from the water, mud and soil comprising 14.9 %, 11.83 and 7.65 % of total fungi respectively.

Previous studies of thermophilic and thermotolerant fungi in Egypt revealed that A. fumigatus, Chaetomium thermophile, Emericella nidulans, Malbranchea cinnamomea and Talaromyces thermophilus, were recorded from cultivated and desert soils in Egypt [31] and from desert soils in Saudi Arabia [3] and Myriococcum albomyces and Rhizomucor pusillus from Jordanian soils [44]. Several studies reported these previous fungi as thermophilic and thermotolerant from different habitats and sources [1, 4, 5, 41, 43, 45, 51].

The water was the poorest source in fungi yielding 4 genera and 5 species and this is in agreement with the previously reported results during 2006/2007 [9]. Thermoascus aurantiacus var. levisporus and Thermomyces ibadanensis were isolated from the mud and soil for the first time in Egypt (Table 3.7). A. turcosus, Chaetomium thermophile, Corynascus sepedonium, Emericella acristata, E. lata, E. quadrilineata, E. rugulosa, E. variecolor var. astellata, and Paecilomyces variotii were isolated from the soil only while Acremonium alabamensis, A. terreus, Paecilomyces aeruginosus, P. inflatus and P. zollerniae were recovered from the mud only.

3.3 An Overview on the Biodiversity of Fungi

It is worth mentioning that, some fungal species were recovered from the three habitats while others were isolated from one habitat only as follows: from water, Alternaria chlamydosporigena, A. roseoglobulosus, A. terricola, A. unguis and many others, from mud, Acremonium alabamensis, Gymnascella hyalinospora, Isaria felina, Paracremonium inflatum and many others, and from soil, Acremonium curvulum, Aspergillus candidus, Botryotrichum piluliferum, Chaetomium globosum, C. nigricolor, Clonostachys rosea, C. solani and many others.

3.4 Halophilic Ability

A total of 281 isolates related to 20 genera, 72 species and 1 variety were tested for their halophilic ability on media supplemented with 0-30 % NaCl (Table 3.8) and these are classified as: weakly halotolerant that could grow in 0-15 % NaCl (all isolates), moderately halotolerant in 20 % (246 isolates represented by 16 genera, 60 species and 1 variety), highly halotolerant in 25 % (32 isolates represented by 7 genera and 18 species) and extremely halotolerant in 30 % (12 isolates represented by 2 genera and 7 species). The twelve extremely halotolerant isolates could survive 30 % NaCl for up to 18 months, and these include Sarocladium kiliense (1 isolate AUMC 11041 from Beida mud) and the remaining 11 isolates were identified as: A. flocculosus (1 isolate AUMC 11041 from Zugm mud), A. insulicola (3 isolates; AUMC 11036 from Fasida water, AUMC 11044 from Beida soil and AUMC 11046 from Gaar soil), Aspergillus ochraceus (1 isolate; AUMC 11035 from Fasida water), A. roseoglobulosus (2 isolates; AUMC 11037 from Umm-Rishah water and AUMC 11039 from Hamra water), A. sclerotiorum (3 isolates; AUMC 11038 from Khadra water, AUMC 11042 from Rosetta soil and AUMC 11045 from Beida soil), and Aspergillus species section Wentii near to A. terricola var. americana (1 isolate; AUMC 11043 from Rosetta soil) (Table 3.8). The current results are almost in agreement with those previously obtained results [47] where the tested 100 species and 3 varieties were categorized into 4 groups: halophilic (grew on 5-25 % NaCl), highly halotolerant (0-20 %), moderately halotolerant (0-15 %) and weakly halotolerant (0-10 %).

In agreement with the present results, the isolated fungal species from Cabo Rojo solar saltern were found to grow in 0 - 25 % NaCl; however, none of them can be classified as halophilic because they all can grow in

NaCl-free media [13]. Based on their growth rate in the presence of 25 % NaCl, they classified as highly halotolerant species (with a growth rate >3 cm over 10 d in 25 % NaCl), moderately halotolerant species (with a growth rate between 2 - 3 cm over 10 d in 25 % NaCl) and weakly halotolerant species (with a growth rate < 2 cm over 10 d in 25 % NaCl and can grow in up to 15 % NaCl [13].

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Table 3.1 Chemical analysis of 40 water samples collected from lakes of Wadi-El-Natrun in February 2012 (5 samples from each lake)

Lake	pH	% TDS	Na^+	K^+	CO_3^{-2}	HCO_3^-	Ca^{+2}	Mg^{+2}	Cl^-	CFUs	G	S
F	9.54±0.06	6.7±0.36	8.5±1.27	0.066±0.01	38.0±6.0	29.2±6.67	0.04±0.02	0.03±0.02	2.55±0.16	143	11	25
U	9.1±0.24	26.3±2.26	17.64±6.4	0.23±0.06	38.1±12.15	16.9±8.9	0.03±0.01	0.02±0.01	13.5±0.33	80	6	8
R	8.5±0.11	9.8±3.25	9.3±2.26	0.057±0.03	2.2±1.016	2.4±1.8	0.1±0.02	0.02±0.01	12.3±1.34	184	15	25
H	9.3±0.06	11.6±2.5	10.6±1.7	0.22±0.04	29.8±6.66	16.8±5.63	0.04±0.01	0.02±0.01	5.9±1.11	15	4	8
Z	9.27±0.02	14.4±0.8	12.5±4.45	0.45±0.6	34.2±2.066	25.8±6.26	0.1±0.01	0.03±0.01	11.3±0.34	21	3	5+1
B	8.9±0.1	4.0±0.4	3.4±0.43	0.05±0.01	1.1±0.36	2.8±0.96	0.35±0.06	0.03±0.01	1.7±0.1	63	6	11
K	9.3±0.1	1.9±0.08	1.35±0.14	0.02±0.006	4.5±2.0	5.96±1.84	0.04±0.02	0.01±0.01	0.7±0.1	56	3	8
G	8.7±0.2	1.95±0.56	1.13±0.43	0.023±0.01	0.4±0.1	1.38±0.33	0.6±0.1	0.1±0.02	0.8±0.1	30	4	9

Table 3.2 Chemical analysis of 40 mud samples collected from lakes of Wadi-El-Natrun in February 2012 (5 samples from each lake)

Lake	pH	% OM	% MC	% TDS	Na^+	K^+	CO_3^{-2}	HCO_3^-	Ca^{+2}	Mg^{+2}	Cl^-	CFUs	G	S
F	10.24±0.1	0.45±0.1	21.8±4.9	8.96±1.9	173.7±25.2	1.31±0.3	4.0±1.55	7.37±1.94	0.4±0.1	0.2±0.1	4.2±0.6	4062	12	20
U	10.0±0.2	4.74±0.9	22.97±2.57	6.75±1.97	121.35±59.1	6.6±4.6	0.4±0.46	3.63±4.13	0.54±0.1	0.12±0.04	27.34±8.9	5006	7	14
R	8.8±0.26	3.3±1.75	58.5±21.2	20.36±1.7	156.7±11.6	1.85±0.9	22.98±6.6	16.1±3.45	2.3±0.5	0.15±0.1	90.9±11.3	29510	10	17
H	9.74±0.3	5.7±1.7	27.3±3.99	12.6±3.12	185.7±15.2	7.1±1.9	3.9±6.4	7.7±6.25	2.2±0.7	0.15±0.1	59.3±3.98	4552	9	14
Z	9.3±0.7	5.94±2.9	48.0±14.1	12.1±2.9	182.2±35.85	1.85±0.9	5.9± 6.1	9.26±6.13	0.2±0.1	0.05±0.03	47.75±17.0	29098	12	19+1
B	9.8±0.2	0.9±0.6	24.8±4.6	18.2±6.25	281.1±139.5	3.74±1.2	12.6±8.35	10.2±3.45	0.97±0.4	0.13±0.1	59.9±16.0	7346	14	24+2
K	9.6 ± 0.2	0.5±0.5	17.56±4.3	0.66±0.4	76.5±87.9	0.97±0.3	0.12±0.1	1.67±0.75	0.28±0.1	0.1±0.04	2.5±1.4	9306	7	16+1
G	9.0±0.5	5.14±3.5	29.5±11.9	5.88±4.0	114.24±37.95	3.9±1.57	0.32±0.4	1.8±0.84	0.55±0.13	0.1±0.05	19.3±15.5	13480	9	15

Table 3.3 Chemical analysis of 33 soil samples collected from around lakes of Wadi-El-Natrun in February 2012

Lake(n)	pH	% OM	% MC	% TDS	Na^+	K^+	CO_3^{-2}	HCO_3^-	Ca^{+2}	Mg^{+2}	Cl^-	CFUs	G	S

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F(3)	7.8±0.15	1.5±0.8	12.6±4.1	0.12±0.03	13.2±14.14	1.4±0.9	0.02±0.02	0.6±0.06	0.6±0.25	0.06±0.01	0.8±0.26	63860	16	32
U(4)	7.9±0.22	1.97±0.5	8.32±3.26	0.96±0.06	24.6±6.9	2.35±0.6	0.02±0.05	0.64±0.15	1.97±2.01	0.04±0.03	2.02±0.53	51328	19	30+2
R(4)	7.97±0.05	2.06±0.5	9.71±4.93	0.33±0.23	7.9±5.1	1.4±0.9	0.01±0.02	0.55±0.13	0.5±0.26	0.04±0.02	1.22±0.74	67016	14	21+1
H(4)	8.0±0.05	2.35±0.8	9.33±4.84	0.27±0.19	6.9±1.6	0.6±0.2	0.02±0.02	0.65±0.1	0.65±0.57	0.07±0.02	0.7±0.44	45796	14	24+1
Z(3)	8.1±0.01	2.5±0.6	6.97±3.22	0.12±0.04	8.9±1.8	0.7±0.2	0.03±0.03	0.56±0.12	0.43±0.15	0.06±0.01	0.99±0.75	64230	14	23+1
B(5)	8.12±0.25	2.3±0.5	10.6±3.8	0.33±0.14	8.7±3.52	1.0±0.3	0.04±0.03	0.63±0.15	0.54±0.2	0.04±0.03	1.0±0.6	65854	15	27+3
K(3)	7.99±0.25	1.9±0.3	6.9±2.4	0.3±0.02	7.5±2.23	0.84±0.7	0.06±0.03	0.53±0.09	0.23±0.08	0.05±0.02	0.85±0.34	34800	9	21+1
G(7)	8.1±0.2	2.4±0.5	8.84±2.9	0.6±0.4	14.9±7.6	1.5±0.8	0.02±0.02	0.7±0.15	1.7±1.56	0.05±0.02	1.1±0.8	127390	13	32+1

OM, MC and TDS are determined as %, the anions and cations are determined as g/l water and g/kg dry wt. mud or soil; figures in Tables are mean of triplicate of number of water, mud or soil samples collected from each lake. *G = number of genera, S = number of species and varieties, n = number of samples; F=Fasida, U=Umm-Rishah, R=Rosetta, H=Hamra, Z=Zugm, B=Beida, K=Khadra and G=Gaar.

Table 3.4 Assiut University Mycological Centre accession number (AUMC) of fungi isolated from mud, water and soil of different lakes of Wadi-El-Natrun with their accession GenBank numbers given together with the closest match in the GenBank data base and sequence similarity in percent to the match as inferred from blastn searches of ITS sequences.

Lake	Isolation medium	AUMC	GenBank accession No	bp	Closest match	Similarity (%)	Identification
Mud							
Khadra	Cz + 10 % NaCl	11005	KX376276	582	KC466536 KC987176=CBS 49071^T	570/580 (98.27) 494/500 (98.80)	Acremonium zonatum Emericellopsis pallida
Zugm	Cz + 10 % NaCl	11006	KX376277	581	KC466536 KC987176=CBS 49071^T	576/582 (98.96) 494/500 (98.80)	Acremonium zonatum Emericellopsis pallida
Beida	Cz + 10 % NaCl	11007	KX384651	582	KC466536 KC987176=CBS 49071^T	573/582 (98.40) 494/500 (98.80)	Acremonium zonatum Emericellopsis pallida
Beida	Cz + 10 % NaCl	11008	KX376278	580	KC466536 KC987175=CBS 49171^T	577/580 (99.48) 497/499(99.59)	Acremonium zonatum Emericellopsis maritime
Fasida	1 % glucose-Cz	11027	KX384652	615	HM991271=NRRL 2881^T	590/591(99.83)	Gymnascella hyalinospora
Umm-Rishah	1 % glucose-Cz	11028	KX384653	627	HM991271=NRRL 2881^T	590/591 (99.83)	Gymnascella hyalinospora
Rosetta	1 % glucose-Cz	11029	KX384654	571	HG965031=MUCL 9939^T	551/551(100)	Sarocladium subulatum
Hamra	1 % glucose-Cz	11030	KX384655	557	KM231829=CBS 48577^T	540/557 (96.94)	Paracremonium inflatum
Hamra	1 % glucose-	11031	KX384656	582	KC466536	578/581 (99.48)	Acremonium zonatum

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Lake	Isolation medium	AUMC	GenBank accession No	bp	Closest match	Similarity (%)	Identification
	Cz				NR_077122=CBS 63873^{T}	563/581 (96.90)	Acremonium dichromosporum
Hamra	1 % glucose-Cz	11032	KX384657	581	KC466536 KF032659=DM12	573/580 (98.79) 572/579 (98.79)	Acremonium zonatum Emericellopsis pallida
Zugm	1 % glucose-Cz	11033	KX384658	582	NR_130684=MUCL 9724^{T}	536/538 (99.62)	Sarocladium kiliense
Zugm	1 % glucose-Cz	11034	KX384659	562	LN864540=Kw63-15 NR_130684=MUCL 9724^{T}	556/556 (100) 532/534 (99.62)	Sarocladium kiliense
Fasida	1 % glucose-Cz	10329	KX531010	598	IC-A3 = HQ285562 LC105695=DY10.1.1 NR_135395=NRRL 447^{T}	595/598(99.49) 594/597(99.49) 574/577(99.48)	A. oryzae
Water							
Fasida	Cz + 10 % NaCl	11001	KX384660	581	KC466536 KF032659=DM12 KC987176=CBS 49071^{T}	575/580 (99.10) 574/579 (99.13) 494/500 (98.80)	Acremonium zonatum Emericellopsis pallida
Rosetta	Cz + 10 % NaCl	11002	KX384661	473	KT968535=JCKQF3 KC987176=CBS 49071^{T}	462/472 (97.88) 440/451(97.56)	Acremonium zonatum Emericellopsis pallida
Rosetta	Cz + 10 % NaCl	11003	KX384662	580	KC466536 KC987171=CBS 127350^{T} KC987176=CBS 49071^{T}	570/580 (98.27) 498/499 (99.79) 494/500 (98.80)	Acremonium zonatum Emericellopsis alkalina Emericellopsis pallida
Beida	Cz + 10 % NaCl	11004	KX384663	580	KC466536 KC987171=CBS 127350^{T} KC987176=CBS 49071^{T}	570/580 (98.27) 498/499(99.79) 494/500(98.80)	Acremonium zonatum Emericellopsis alkalina Emericellopsis pallida
Fasida	1 % glucose-Cz	11009	KX384664	563	KJ443241=M27^{T}	491/492 (99.79)	Chordomyces antarcticum
Fasida	1 % glucose-Cz	11010	KX446769	557	KP068972=WM 07.196 JX508810=LC1990^{T}	551/556 (99.10) 547/556 (98.38)	Plectosphaerella cucumerina Plectosphaerella oligotrophica
Fasida	1 % glucose-Cz	11011		556	KJ443241=M27^{T}	487/492 (98.98)	Chordomyces antarcticum
Fasida	1 % glucose-Cz	11012	KX385856	561	KJ443241=M27^{T}	490/492 (99.59)	Chordomyces antarcticum
Fasida	1 % glucose-Cz	11013	KX446768	559	FM955449=FMR 9523^{T}	474/565 (83.89)	Ramophialophora humicola
Rosetta	1 % glucose-Cz	11014	KX446755	557	KU204705=GL11101 616 JX508810=LC1990^{T}	541/546 (99.08) 541/555 (97.47)	Plectosphaerella cucumerina Plectosphaerella oligotrophica
Rosetta	1 % glucose-Cz	11015	KX446756	603	KC466540 KF993392=PAV-M 1.138	591/602 (98.17) 579/588 (98.46)	Alternaria chlamydosporigena Alternaria chlamydospora
Rosetta	1 % glucose-Cz	11016	KX446757	581	KC466536 KC987175=CBS 49171^{T} KC987176=CBS 49071^{T}	572/581 (98.45) 496/499(99.39) 492/500(98.40)	Acremonium zonatum Emericellopsis maritime Emericellopsis pallida

Lake	Isolation medium	AUMC	GenBank accession No	bp	Closest match	Similarity (%)	Identification
Rosetta	1 % glucose-Cz	11017	KX446758	581	KC466536 KC987175=CBS 49171^T KC987176=CBS 49071^T	574/580 (98.96) 495/499 (99.19) 491/500 (98.20)	Acremonium zonatum Emericellopsis maritima Emericellopsis pallida
Rosetta	1 % glucose-Cz	11018	KX446759	582	KC466536 KC987175=CBS 49171^T KC987176=CBS 49071^T	575/581 (98.96) 495/501 (98.80) 492/501(98.20)	Acremonium zonatum Emericellopsis maritima Emericellopsis pallida
Rosetta	1 % glucose-Cz	11019	KX446760	580	KC466536 KC987175=CBS 49171^T KC987176=CBS 49071^T	576/580 (99.31) 497/499 (99.59) 493/500 (98.60)	Acremonium zonatum Emericellopsis maritima Emericellopsis pallida
Rosetta	1 % glucose-Cz	11020	KX446761	602	KC466540 KF993392=PAV-M NR_136039=CBS 49172^T	594/601 (98.83) 580/588 (98.63) 537/544 (98.71)	Alternaria chlamydosporigena Alternaria chlamydospora
Rosetta	1 % glucose-Cz	11021	KX446762	582	KC466536 KF032659=DM12	570/580 (98.27) 569/579 (98.27)	Acremonium zonatum Emericellopsis pallida
Rosetta	1 % glucose-Cz	11022	KX446763	578	KC254090=UOAHCPF 13355 KT878352=07739	565/574 (98.43) 566/576 (98.26)	Sarocladium strictum Acremonium sclerotigenum
Umm-Rishah	1 % glucose-Cz	11023	KX446764	569	FJ430712=MH178 AY952467=D99026	568/569 (99.82) 566/568 (99.64)	Stilbella fimetaria
Gaar	1 % glucose-Cz	11024	KX446765	577	KC254091=UOAHCPF 13842 KF225143=BAFSCH	571/575 (99.30) 571/577 (98.96)	Sarocladium strictum Acremonium alternatum
Khadra	1 % glucose-Cz	11025	KX446766	564	KT192193=00018-1 KT878352=07739	560/561 (99.80) 559/560 (99.82)	Acremonium alternatum Acremonium sclerotigenum
Beida	1 % glucose-Cz	11026	KX446767	580	LN864540=Kw63-15 AY138846=UW 940 NR130684=MUCL 9724^T	574/579 (99.13) 573/578 (99.13) 529/531 (99.62)	Sarocladium kiliense
Soil							
Beida	Cz+10 % NaCl	10331	KX531011	599	AD-B3=HQ285520 CJ-B4=HQ285545 NR_111041=ATCC16883^T	599/599(100) 598/598(100) 593/596(99.49)	A. flavus

Table 3.5 Percent CFUs (calculated to the total fungi per 600 ml water or 40 g dry mud or 33 g dry soil in all samples) and frequency (F) of fungi isolated on Cz agar at 25° C from 40 water, 40 mud and 33 soil samples collected from the 8 lakes of Wadi-El-Natrun.

	Water		Mud		Soil	
Taxa	**%CFUs**	**F**	**%CFUs**	**F**	**%CFUs**	**F**
Acremonium Link	22.84	20M	10.8	6L	0.77	2L
A. alternatum Link	0.6	4L				
A. curvulum W. Gams					0.77	2L
A. zonatum (Sawada) W. Gams	7.41	3L	6.6	6L		
Acremonium spp.	14.83	6L	4.2	4L		
Alternaria	1.6	4L	0.3	1L	0.2	2L
A. alternata (Fries) Keissler	1.0	2L	0.3	1L	0.2	2L
A. chlamydosporigena Woudenberg & Crous	0.6	2L				

Taxa	**Water**		**Mud**		**Soil**	
	%CFUs	**F**	**%CFUs**	**F**	**%CFUs**	**F**
Aspergillus P. Micheli ex Link	36.5	24H	52.2	32H	56.81	33H
A. aegyptiacus Moubasher & Moustafa			0.1	1L		
A. brasiliensis Varga et al.	1.2	3L	0.7	5L	6.1	13M
A. candidus Link					0.04	1L
A. deflectus Fennell & Raper	0.2	2L	0.1	1L	0.04	1L
A. flavipes (Bainier & Sartory) Thom & Church	3.2	3L	11.2	1L	0.23	3L
A. flavus Link			5.6	12L	6.67	21H
A. flavus var. columnaris Raper & Fennell	1.0	1L	0.1	1L	1.1	2L
A. foetidus (Nakaz.) Thom & Raper	0.2	1L			0.04	1L
A. fumigatus Fresenius	7.21	6L	3.7	8L	0.74	4L
A. insulicola Montemayor & Santiago	1.0	1L				
A. lacticoffeatus Frisvad & Samson	0.2	1L			0.11	1L
A.neoafricanus Samson, Peterson, Frisvad & Varga	1.0	2L	12.4	1L	5.0	2L
A. niger van Tieghem			3.8	8L	5.16	14M
A. ochraceus Wilhelm	3.2	3L	1.0	5L	0.9	3L
A. oryzae (Ahlburg) E. Cohn			0.3	1L		
A. parasiticus Speare	0.2	1L			4.1	8L
A. petrakii Vörös- Felkai	0.2	2L			0.31	1L
A. roseoglobulosus Frisvad & Samson	1.2	2L				
A. sclerotiorum G. A. Huber	0.4	1L				
A. sulphureus (Fresen.) Wehmer	6.61	2L	0.3	1L		
A. sydowii (Bainier & Sartory) Thom & Church	1.8	4L	0.1	1L	0.2	3L
A. terreus Thom	6.0	4L	12.8	16M	26.15	26H
A. terricola E. J. Marchal	0.8	2L				
A. unguis (Emile-Weil & Gaudin) Thom & Raper	0.8	1L				
Botryotrichum piluliferum Saccardo & Marchal					1.32	2L
Chaetomium Kunze					0.23	2L
C. globosum Kunze					0.15	1L
C. nigricolor L.M. Ames					0.08	1L
Chordomyces antarcticum Bilanenko, Georgieva, Gum-Grzhimaylo	4.21	2L				
Cladosporium Link	0.4	2L	0.7	3L	0.23	3L
C. cladosporioides (Fresenius) de Vries	0.2	1L	0.2	1L	0.11	2L
C. sphaerospermum Penzig	0.2	1L	0.5	3L	0.15	1L
Cladosporium spp.						
Clonostachys Corda					1.8	3L
C. rosea (Link) Schroers, Samuels, Seifert & W. Gams					1.5	2L
C. solani (Harting) Schroers & W. Gams					0.31	1L
Cochliobolus tuberculatus Sivanesan	1.8	2L	0.6	1L	0.7	2L
Corynascus sepedonium (C.W.Emmons) Arx					0.62	1L
Emericella Berkeley & Broome			0.1	1L	16.1	22H
E. dentata (D.K.Sandhu & R.S.Sandhu) Y. Horie					3.26	3 L
E. lata Subramanian					0.3	2L
E. nidulans (Eidam) Vuillemin			0.1	1L	5.1	22H
E. quadrilineata (Thom & Raper) C.R. Benj					7.2	5L
E. rugulosa (Thom & Raper) C.R. Benj					0.31	1L
Exerohilum rostratum (Drechsler) Leonard & Suggs					0.23	1L
Fusarium Link	2.0	2L	5.9	2L	9.4	12M
F. oxysporum Schlechtendahl & Hansen					2.33	5L
F. sambucinum Füchel			1.9	1L	0.62	3L
F. semitectum Berkeley & Ravenel	1.2	2L				
F. solani (Mart.) Appel & Wollenw. emend. Sny. & Hans.	0.8	1L	4.0	1L	6.25	10L
F. thapsinum Klittich, Leslie, Nelson & Marasas					0.2	1L

Taxa	Water		Mud		Soil	
	%CFUs	F	%CFUs	F	%CFUs	F
Gliocladium Corda					0.62	2L
G. catenulatum Gilman & Abbott					0.11	1L
G. penicillioides Corda					0.5	1L
Gymnascella hyalinospora (Kuehn, Orr & Ghosh) Currah			0.3	1L		
Humicola fusco-atra Traaen	0.2	1L			1.67	4L
Isaria felina (DC.) Fries			0.1	1L		
Microascus manginii (Loubière) Curzi	0.2	1L				
Mucor Fresenius	2.0	4L	0.5	2L	0.04	1L
M. circinellioides van Tieghem	1.0	3L	0.1	1L		
M. hiemalis Wehmer	1.0	2L	0.4	2L	0.04	1L
Myrothecium verrucaria (Albertini & Schweinitz) Ditmer ex Steudel					0.15	1L
Papulaspora irregularis H.H. Hotson					0.11	1L
Paracremonium inflatum Lombard & Crous			0.3	1L		
Penicillium Link	11.42	21H	15.6	19M	8.26	9L
P. aurantiogriseum Dierckx	3.0	4L	0.1	1L	3.9	6L
P. brevicompactum Dierckx	0.2	1L	0.4	1L		
P. chrysogenum Thom	3.61	4L	6.8	9L	1.86	2L
P. citrinum Thom			0.3	1L		
P. corylophilum Dierckx					0.04	1L
P. crustosum Thom	0.6	3L	2.4	2L	0.46	1L
P. dendriticum Pitt	0.2	1L				
P. donkii Stolk	0.2	1L				
P. duclauxii Delacroix			0.1	1L	0.43	2L
P. funiculosum Thom					1.6	3L
P. griseofulvum Dierckx	0.2	1L				
P. italicum Wehmer	0.6	1L				
P. janthinellum Biourge	1.0	3L				
P. melinii Thom	0.6	2L				
P. olsonii Bainier & Sartory	1.0	1L				
P. oxalicum Currie & Thom			2.7	2L		
P. puberulum Bainier			0.3	2L		
P. raistrickii G.Sm.			0.1	1L		
P. sublateritium Biourge	0.2	1L				
P. waksmanii K.M. Zalessky			0.6	1L		
Penicillium spp.			1.8	4L		
Phaeoacremonium sp.	1.2	1L				
Phialophora sp.	0.2	1L	0.2	1L		
Phoma glomerata (Corda) Wollenw. & Hochapfel					0.11	1L
Plectosphaerella oligotrophica Liu, Hu, Liu & Cai	2.0	1L				
Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson			0.1	1L	0.04	1L
Ramophialophora sp. AUMC 11013	0.2	1L				
Sarocladium Gams & Hawksworth	4.41	6L	5.6	3L		
S. kiliense (Grütz) Summerbell	0.2	1L	3.4	2L		
S. strictum (W. Gams) Summerbell	4.21	5L	1.6	1L		
S. subulatum Giraldo, Gené & Guarro			0.6	1L		
Scopulariopsis Bainier	5.0	7L	1.4	3L		
S. acremonium (Delacroix) Vuillemin	0.2	1L				
S. brevicaulis (Saccardo) Bainier	1.6	3L	0.1	1L		
S. brumptii Salv. -Duval	1.0	1L	0.1	1L		
S. carbonaria Morton & G. Sm.	1.8	2L				
S. fusca Zach	0.4	1L	1.2	1L		
Stachybotrys Corda			0.4	4L		

Taxa	Water		Mud		Soil	
	%CFUs	F	%CFUs	F	%CFUs	F
S. dichroa Grove			0.1	1L		
S. ramosa Udaiyan			0.1	1L		
S. verrucispora Matsushima			0.1	1L		
S. virgata Krzemien. & Badura			0.1	1L		
Stilbella fimetaria (Pers.) Lindau	0.2	2L				
Trichoderma Pers.	3.2	4L	4.9	8L	0.15	2L
T. harzianum Rifai	2.0	3L	4.6	7L	0.15	2L
T. koningii Oudem	1.0	1L				
T. longibrachiatum Rifai	0.2	2L	0.3	3L		
Ulocladium Preuss	0.4	2L			0.43	2L
U. atrum Preuss					0.43	2L
U. botrytis Preuss	0.2	1L				
U. tuberculatum E.G. Simmons	0.2	1L				
Total CFUs (615899)	20		28		22	
No. of genera 35 & species 105 + 1 variety	61 + 1 variety		50 + 1 variety		53 + 1 variety	

Table 3.6 Percent CFUs (calculated to the total fungi per 600 ml water or 40 g dry mud or 33 g dry soil in all samples) and frequency (F) of fungi isolated on Cz + 10 % NaCl at 25° C from 40 water, 40 mud and 33 soil samples collected from the 8 lakes of Wadi-El-Natrun.

Taxa	Water		Mud		Soil	
	%CFUs	F	%CFUs	F	%CFUs	F
Acremonium Link	93.48	8L	6.7	7L		
A. zonatum	10.87	2L	3.16	7L		
Acremonium spp.	82.61	7L	3.56	5L		
Aspergillus			85.46	13M	81.0	31H
A. aegyptiacus			0.1	1L	0.12	1L
A. brasiliensis			0.1	1L		
A. flavipes			36.8	3L	0.12	1L
A. flavus			18.9	9L	37.2	9L
A. flavus var. columnaris					2.99	4L
A. flocculosus Frisvad & Samson			7.7	1L		
A. fumigatus			0.1	1L		
A. insulicola					4.5	3L
A. neoafricanus					1.5	1L
A. niger					0.25	1L
A. ochraceus					0.75	5L
A. parasiticus					3.74	7L
A. petrakii					3.24	2L
A. sclerotiorum					1.37	3L
A. sydowii			3.76	4L		
A. tamarii Kita			0.1	1L	2.62	7L
A. terreus					0.12	1L
A. versicolor (Vuillemin) Tirab.			18.0	9L	22.22	13M
Aspergillus sp. near to A. terricola var. americana					0.12	1L
Cladosporium sp.					0.12	1L
Emericella			0.5	1L	2.62	5L
E. nidulans			0.5	1L	0.37	3L
E. quadrilineata					2.25	4L
Eurotium Link					0.75	2L
E. amstelodami (Mangin) Thom & Church					0.25	1L
E. rubrum Thom & Church					0.5	1L
Fusarium			0.4	2L	5.61	3L
F. semitectum					5.37	2L
F. solani			0.4	2L	0.25	1L
Microascus manginii					0.25	2L
Penicillium	2.17	1L	1.4	4L	9.36	14M

Taxa	Water		Mud		Soil	
	%CFUs	F	%CFUs	F	%CFUs	F
P. aurantiogriseum					0.25	2L
P. chrysogenum	2.17	1L	1.3	3L	7.24	12M
P. donkii			0.1	1L		
P. funiculosum					1.87	5L
Sarocladium			4.94	6L		
S. kiliense			4.55	5L		
S. strictum			0.4	2L		
Scopulariopsis	4.35	2L			0.37	2L
S. brevicaulis					0.12	1L
S. brumptii					0.25	1L
S. halophilica Tubaki	4.35	2L				
Ulocladium lanuginosum (Harz.) Simmons			0.4	1L		
Total CFUs (3670)	100 (46)		100 (2022)		100 (1602)	
No. of genera 11 & species 38 + 1 variety	3 & 3		8 & 17		7 & 27 + 1	

Table 3.7 Percent CFUs (calculated to the total fungi per 600 ml water or 40 g dry mud or 33 g dry soil in all samples) and frequency (F) of fungi isolated on YpSs at 45° C from 40 water, 40 mud and 33 soil samples collected from the 8 lakes of Wadi-El-Natrun.

Taxa	Water		Mud		Soil	
	%CFUs	F	%CFUs	F	%CFUs	F
Acremonium			2.36	4L	0.06	1L
A. alabamensis Morgan-Jones			0.6	1L		
A. thermophilum W. Gams & J. Lacey			1.2	3L	0.06	1L
Acremonium sp. (chlamydospores)			0.6	1L		
Aspergillus	78.72	15M	30.2	15M	17.76	25H
A. fumigatus	76.6	13M	28.4	13M	17.3	23H
A. terreus			0.6	1L		
A. turcosus Hong, Frisvad & Samson					0.26	1L
A. viridinutans Ducker & Thrower	2.13	1L	1.2	1L	0.2	1L
Chaetomium thermophile La Touche					2.53	1L
Corynascus sepedonium (C.W.Emmons) Arx					0.77	1L
Emericella Berkeley & Broome			0.6	1L	25.92	9L
E. acristata Fennell & Raper					0.06	1L
E. lata					1.68	1L
E. nidulans			0.6	1L	8.88	2L
E. quadrilineata					14.45	6L
E. rugulosa					0.77	1L
E. variecolor var. astellata (Fennell & Raper) Benjamin					0.06	1L
Humicola grisea var. thermoidea Cooney & Emerson			1.2	3L	2.33	1L
Malbranchea cinnamomea (Lib.) Oorschot & de Hoog	2.13	1L	8.9	7L	23.4	21H
Myriococcum albomyces Cooney & Emerson			0.6	1L	3.7	7L
Paecilomyces Bainier			3.55	4L	0.6	2L
P. aeruginosus			0.6	1L		
P. inflatus (Burnside) J.W. Carmich.			0.6	1L		
P. variotii Bainier					0.6	2L
P. zollerniae Stolk & Samson			0.6	1L		
Paecilomyces spp.			1.77	2L		
Rhizomucor pusillus (Lindt) Schipper			2.36	2L	1.0	7L
Scytalidium thermophilum (Cooney & Emerson) Austwick			11.24	4L	5.38	2L
Talaromyces thermophilus Stolk	4.25	1L	21.9	6L	4.3	8L
Thermoascus aurantiacus var. levisporus Upadhyay, Farmelo, Goetz & Melan			1.18	2L	0.13	1L
Thermomyces Tsikl	14.9	2L	15.97	8L	12.12	20M
T. ibadanensis Apinis & Eggins			4.14	1L	4.5	7L

Taxa	**Water**		**Mud**		**Soil**	
	%CFUs	**F**	**%CFUs**	**F**	**%CFUs**	**F**
T. lanuginosus Tsikl	14.9	2L	11.83	8L	7.65	18M
Total CFUs (3471)	100 (47)		100 (338)		100 (3086)	
No. of genera 14 & species 25 + 3 varieties	4 & 5		12 & 18 + 2		14 & 19 + 3	

Table 3.8 Number of isolates of fungal genera and species tested for their halophilic abilities on Cz medium supplemented with different NaCl concentrations

Taxa	isolates tested	0-15%	20 %	25 %	30 %
Acremonium	18	18	16	3	
A. alternatum	1	1	1		
A. zonatum	17	17	15	3	
Alternaria	3	3	2		
A. alternata	1	1			
A. chlamydosporigena	2	2	2		
Aspergillus	139	139	132	21	11
A. aegyptiacus	1	1	1		
A. brasiliensis	5	5	4		
A. deflectus	1	1			
A. flavipes	12	12	12		
A. flavus	30	30	30	3	
A. flavus var. columnaris	6	6	6		
A. flocculosus	1	1	1	1	1
A. foetidus	1	1	1		
A. fumigatus	8	8	4		
A. insulicola	3	3	3	3	3
A. lacticoffeatus	1	1	1		
A.neoafricanus	2	2	2		
A. niger	2	2	1		
A. ochraceus	9	9	9	1	1
A. parasiticus	7	7	7		
A. petrakii	2	2	2		
A. roseoglobulosus	2	2	2	2	2
A. sclerotiorum	3	3	3	3	3
A. sulphureus	4	4	4		
A. sydowii	12	12	12	4	
A. tamarii	1	1	1		
A. terreus	22	22	22	2	
A. terricola	1	1	1	1	
A. unguis	1	1	1		
A. versicolor	1	1	1		
Aspergillus sp. near to A. terricola var. americana	1	1	1	1	1
Chordomyces antarcticum	2	2	2		
Cladosporium	2	2			
C. cladosporioides	1	1			
C. sphaerospermum	1	1			
Cochliobolus tuberculatus	4	4	4		
Emericella	8	8	8		
E. nidulans	4	4	4		
E. quadrilineata	4	4	4		
Eurotium	5	5	5	1	
E. amstelodami	2	2	2		
E. rubrum	3	3	3	1	
Fusarium	13	13	13	1	
F. semitectum	8	8	8	1	
F. solani	5	5	5		
Humicola fusco-atra	1	1	1		
Microascus manginii	2	2	2		

Taxa	isolates tested	0-15%	20 %	25 %	30 %
Mucor	4	4			
M. circinellioides	3	3			
M. hiemalis	1	1			
Penicillium	50	50	48	3	
P. aurantiogriseum	7	7	7		
P. brevicompactum	1	1	1		
P. chrysogenum	24	24	24	1	
P. crustosum	3	3	3	1	
P. dendriticum	1	1	1		
P. donkii	2	2	1		
P. funiculosum	5	5	5		
P. griseofulvum	1	1	1		
P. italicum	1	1	1		
P. melinii	2	2	2		
P. olsonii	2	2	1		
P. sublateritium	1	1	1	1	
Plectosphaerella oligotrophica	2	2	2		
Ramophialophora near to humicola	1	1	1		
Sarocladium	6	6	4	3	1
S. kiliense	2	2	1	1	1
S. strictum	4	4	3	2	
Scopulariopsis	10	10	7		
S. acremonium	1	1	1		
S. brevicaulis	2	2	1		
S. brumptii	4	4	3		
S. carbonaria	1	1	1		
S. fusca	1	1			
S. halophilica	1	1	1		
Stilbella fimetaria	1	1			
Trichoderma	7	7			
T. harzianum	2	2			
T. koningii	4	4			
T. longibrachiatum	1	1			
Ulocladium	3	3	1		
U. botrytis	1	1			
U. lanuginosum	1	1			
U. tuberculatum	1	1	1		
Total	281	281	248	32	12
No. of genera (20)	20		16	7	2
No. of species (72 + 1 variety)	72+1 variety		60+1	18	7