Terrestrial fungi tolerating the hypersaline water of Wadi El-Natrun Lakes, Egypt

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Abstract: Chemical analysis revealed that water samples collected from Wadi El-Natrun Lakes were highly alkaline, of pH ranging from 8.4 -9.5 and of high levels of total soluble salts, chlorides, sodium and potassium. Water collected from El-Zugm Lake showed the highest levels of organic matter, sodium, calcium, magnesium and chlorides among the 8 lakes investigated. On the other hand, some parameters showed their peak in other lakes e.g. pH (9.4) and total soluble salts (87%) in Fasida. A total number of genera (16) and species (33) were recorded from water samples collected from all lakes during the seasons of study, with the widest spectrum of species being isolated on the control medium (14) and the lowest on 10% NaCl medium (3). Aspergillus, Acremonium followed by Penicillium were the most dominant genera possessing the highest proportions of propagules on all isolation media except on 10 % NaCl. On the other hand, only species of the genera Scopulariopsis and Acremonium were isolated on 10% NaCl medium. Aspergillus showed its count peak in Al-Beida Lake during winter 2007 on both acidic and alkaline media while in spring 2007 on control medium (from Khadra Lake) and on 40% sucrose (from El-Zugm Lake). From Aspergillus, A. terreus followed by A. flavus and A. niger were the most common on all the isolation media, but A. ochraceus was dominant on acidic media only. Other most common species, namely Penicillium chrysogenum and P. puberulum were encountered on all media but not on 10 % NaCl medium. Some species were isolated on one medium but not on the others: Scopulariopsis halophilica (on 10% NaCl), Emericella quadrilineata (on 40% sucrose), Staphylotrichum coccosporum (on medium adjusted at pH 4) and Acremonium hyalinulum (on alkaline media).

Key words: Hypersaline waters, lakes, Wadi El-Natrun, extremophilic fungi, terrestrial.

Introduction

Extreme environments, such as acidic or hot springs, saline and/or alkaline lakes, deserts and the ocean beds are found in nature, which are too harsh for normal life to exist (Satyanarayana, et al. 2005). Hypersaline environments can be found in all continents and in most countries. They consist of two primary types: thalassohaline and athalassohaline systems. Thalassohaline systems arose from seawater evaporation and hence contain sodium chloride as the predominant salt. The Great Salt Lake, Utah, is an example of such, but other examples are salt mine drainage waters, playas, natural coastal splash zones and tide pools, brine springs from underground salt deposits, and solar salterns (Litchfield and Gillevet 2002). Athalassohaline systems arose from non-seawater sources and contain different ion ratios. These athalassohaline systems are dominated by potassium, magnesium, or sodium and are frequently the sources of potash, magnesium metal, soda, and even borax if the waters were high in boron. Some examples of these are the alkaline soda lakes of Egypt (e.g., Wadi El-Natrun), the Dead Sea, the soda lakes of Antarctica, and Big Soda Lake and Mono Lake in California (Gunde-Cimerman et al. 2000,

Ulukanli and Digrak 2002, Litchfield and Gillevet 2002, Oren 2002 and Cantrell *et al.* 2006).

Alkaline environments e.g. East African Rift Valley, Wadi El-Natrun in Egypt, Indian Sambhar Lake (Satyanarayana et al. 2005) are considered type of athalassohaline systems. Soda lakes and soda deserts represent the major type of naturally occurring highly alkaline environments. The pH of these environments fluctuates due to their limited buffering capacity and therefore, alkalitolerant microbes are more abundant in these habitats than alkaliphiles. These lakes are often closed basins with no obvious outflow, forming semipermanent standing bodies of water. Surface evaporation rates exceed the rate of inflow of water allowing the dissolved minerals to concentrate with CO3 $^{-2}$ and Cl⁻¹ as major anions creating a pH 8.5 to > 12 (Grant et al. 1990, Jones et al. 1994, 1998, Jones and Grant 1999 and Grant 2006).

Wadi El-Natrun Depression is situated at the western side of the Nile Delta of Egypt and includes some water bodies characterized by high salinity. It is a narrow depression located approximately 90 km south of Alexandria and 110 km North West of Cairo. It is about 50 km long, narrow at both ends and wider in the middle (Zahran and Willis 1992). It lies 23 m below sea

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level and 38 m below the water-level of Rossetta branch of the Nile (Abdel-Malek and Rizk 1963). It is characterized by a series of twenty small disconnected lakes in the bottom of the Wadi. Ten of these lakes are relatively larger in size and have permanent water in all or some of their parts. Inland saline lakes and salt crusts occupy the area surrounded by contour zero (Abu Zeid 1984). The principal lakes of Wadi El-Natrun are Fasida, Umm-Risha, Al-Razoniya (Rosetta), Abu-Gubara, Hamra, El-Zugm (Zaagig), Al-Beida, Khadra and Al-Gaar (Taher 1999 and Zahran and Willis 1992). The Wadi El-Natrun depression gets its water from two sources: the springs in the bottom (e.g. in Lake Hamra), and seepage into the lakes. Pavlov (1962) attributed the source of the water to the radial inflow of underground water towards the lakes. Shata and El Fayoumi (1967) noted that the main source of water to the depression comes from underground water flowing from the Rosetta branch and its branches. It is believed also that the water originates from the Nile, infiltrating through sands and gravels constituting the main strata separating the wadi from the river (Atia et al. 1970). Most lakes reach maximum levels between December and March, with the lowest levels in summer. Shallow saline pools shrink in volume by >60% following evaporation in summer. It is assumed that the underground water from the Nile Delta infiltrating into Wadi El-Natrun has roughly the same relative salt composition when it reaches the lakes. The differences found between the relative composition of different lakes are mainly the result of differing microbial activities in the sediments and brines (Taher 1999). The water of Wadi El-Natrun saline lakes is enriched in dissolved minerals that have accumulated in the brines following solar evaporation. Detailed chemical analysis of the lakes of Wadi El-Natrun depression in Egypt revealed high pH level (> 11.5) and high concentration of carbonate, chloride, phosphate, sodium, potassium and silicon oxide (Grant 2006).

Fungi can also grow in hypersaline waters. Some "terrestrial" fungi had been isolated from sea water or sea water flora and fauna. Aspergillus and Cladosporium were the most frequent isolates found in samples of sand, wood and mangrove in Puerto Rico (Acevedo-Ríos 1987). Others, namely Cephalosporium, Diplodia, Fusarium, Helminthosporium, Penicillium, Trichoderma, Scopulariopsis and Pleospora were found particularly in sand (Acevedo-Ríos 1987). Xerophilic and halophilic fungi are able to grow in media with low water activities (a_w) and they can be expected to survive in this type of environment. In 1977, Cronin and Post reported the isolation of halophilic fungi belonging to the genus Cladosporium were growing in a submerged piece of pine wood in the Great Salt Lake in Utah. Later, Butinar et al. (2005b)

reported the occurrence of the yeasts Debaryomyces hansenii and Metschnikowia bicuspidate in this lake. Aspergillus versicolor, Chaetomium globosum, Eurotium herbariorum, E. amstelodami and E. rubrum were also isolated from Dead Sea waters (Kis-Papo et al. 2001, 2003a). On the other hand, Gymnascella marismortui (an obligate halophile), Ulocladium chlamydosporum and Penicilium westlingii (halotolarant) were recorded in Dead Sea waters by Buchalo et al. (1998a). Several culturable fungi such as Cladosporium spp., Alternaria spp., Aspergillus sydowii, Nigrospora spp., and Penicilliun solitum from deep-sea water samples (below 500 m depth) have been reported from different geographical locations (Roth et al. 1964 and Raghukumar et al. 1992).

To our knowledge, no report was published up till now about mycobiota inhabiting the hypersaline habitat environment of Wadi El-Natrun lakes. So, this work has been designed to highlight on the extremophilic groups of fungi (including osmophilic/ osmotolerant, halophilic/ halotolerant, acidiphilic/ aciditolerant, and alkaliphilic and alkalitolerant fungi) that may be found in the hypersaline waters of Wadi El-Natrun lakes.

Materials and Methods

Collection of water samples

Water samples were collected during three seasons naming autumn of year 2006, winter and spring of year 2007, from the big eight lakes (Fasida, Umm-Risha, Rosetta, Hamra, El-Zugm, Al-Beida, Khadra, Al-Gaar) of Wadi El-Natrun depression. The 24 water samples were collected in sterile bottles at a depth of about 10-20 cm near the lake shore from different sites. Samples were brought into the laboratory and kept in a cold place (5°C) till chemical and fungal analysis.

Chemical analysis of water samples

PH value: The pH meter (Orior Research Model GOHL Digital Ionalyzer) electrode was immersed directly in water sample for the determination of water pH (Jackson 1958).

Total soluble salts (TSS): The specific electrical conductance (EC expressed in mmhos /cm) was measured in the samples using the conductance meter (YSI, model 35). The percentage total soluble salts in a sample were estimated using the following equation:

% TSS in the dry sample = $0.064 \times \text{EC} \times \text{extract}$ ratio. The conversion factor to percentage salts (0.064) is fairly applied for solutions extracted from alkaline and saline soils (Richards 1954 and Jackson 1958).

Carbonate and bicarbonate: Total carbonate was determined directly in water according to the method described by Jackson (1958).

Chloride (CI'): Soluble chloride was estimated by applying the silver nitrate titration method using potassium chromate as an indicator (Jackson 1958).

Calcium and magnesium ($Ca^{+2} \& Mg^{+2}$): The versene (disodium dihydrogen ethylene diamine tetraacetic acid) titration method as recommended by Schwarzenbach and Biedermann (1948) was employed for Ca^{+2} and $Ca^{+2} + Mg^{+2}$ determination.

Sodium and potassium (Na⁺ & K⁺): Flame photometer method (Williams and Twine 1960) using Carl Zeiss flame photometer was used for the determination of Na⁺ and K⁺ cations.

Isolation of terrestrial fungi from water samples

Seven agar media types (5 plates each) were used for enumeration and isolation of terrestrial fungi from lakes water. One ml of lake water was transferred into each of Petri-dish and mixed by rotation with agar medium. After solidification of the agar, the plates were incubated at 28°C for 7-15 days. The developing fungal colonies were counted and calculated per ml water and preserved in agar slants for identification.

The seven media types used for isolation of fungi are: 1. Modified Czapek Dox agar in which glucose (10 g/l) replaced sucrose, of the following composition (g/l) sodium nitrate 3.0, potassium dihydrogen phosphate 1.0, magnesium sulphate 0.5, potassium chloride 0.5, ferrous sulphate 0.01, glucose 10.0, agar 15.0), to which rose bengal (1/15000) and chloramphenicol (25 µg/ml) were used as bacteriostatic agents (Smith and Dawson 1944, Al- Doory 1980). This medium was adjusted to pH7.3 and was used as a control medium; 2. Czapek Dox agar supplemented with 40% sucrose for isolation of osmophilic and osmotolerant fungi; 3. Modified Czapek Dox agar medium (in which glucose, 10g/l, replaced sucrose) supplemented with 10% sodium chloride was used for isolation of halophilic and halotolerant fungi; 4 and 5. Modified Czapek Dox agar media in which pH was adjusted at 4 or 5 using diluted HCl were used for isolation of acidiphilic and aciditolerant fungi: 6 and 7. Modified Czapek Dox agar in which pH was adjusted at 10, 13 using NaOH were used for isolation of alkaliphilic and alkalitolerant fungi.

Identification of fungi

The identification of fungal genera and species (purely morphologically) was based on macroscopic and microscopic features following the keys and descriptions of Ellis (1971, 1976), for Dematiaceous Hyphomycetes, Pitt (1979), for Penicillium and its teleomorphic states Eupenicillium and Talaromyces, Raper and Fennell (1965),for Aspergillus species, Moubasher (1993), Domsch et al. (2007) for fungi in general and Booth (1971), Leslie and Summerell (2006) for *Fusarium* specie.

Results and Discussion

Chemical analysis of water samples

Chemical analysis of water samples collected during spring 2007 from Wadi El-Natrun lakes revealed that pH was highly alkaline in different lakes ranging from 8.4 in El-Zugm to 9.5 in Fasida (Table 1). This is in agreement with previous results obtained by Taher (1999) and Moussa et al. (2009) in Wadi El-Natrun Lakes (pH 8.51-9.45) and by Steiman et al. (2004) of Mono Lake of California (pH 9.4-9.8); however it is remarkably different from that of the Dead Sea water which is acidic (pH 6.6) (Steiman et al. 1995). Total soluble salts (TSS%) was much higher in water than those recorded in both soil and mud samples (Gouda 2009), ranging from 50% in Al-Gaar to 87% in Fasida; however higher amounts were recorded in salt samples (Gouda 2009). In the study of Taher (1999) the concentration of the total soluble salts (TSS) ranged from 283 g/l in Khadra to 557 g/l in Al-Beida Lake. However she reported the lowest salt contents of water in spring (97 g/l), a very low value compared to our results in spring. The higher values of total soluble salts in water of all lakes are due to the high concentrations of both sodium and chloride ions, which is similary reported by Taher (1999). Although the Dead Sea (Steiman et al. 1995) and the Great Salt lake (Utah Geological survey, 2001) have similar salinities to those of Wadi El-Natrun lakes water, but their chemical composition is different. Sodium cations (mg/ml) ranged from 13.0 in Fasida to 44.0 in El-Zugm Lake; results agreed with those reported by Moussa et al. (2009) who found nearly similar composition of sodium (954.0 mmol/l = 41.5 mg/ml), however, lower than those (30.2 - 295.2 g/l) reported by Taher (1999).

Chloride anions exhibited the highest value in Umm Risha (23.0 mg/ml) and the lowest in Al-Beida (10.5). In agreement with our results Moussa et al. (2009) recorded almost similar concentrations of chlorides in Wadi El-Natrun water (920.0 mmol/l = 25.6 mg/ml). However higher concentrations (12.7- 125.7 g/l) were reported by Taher (1999). Also Steiman et al. (2004) and Mason (1967) recorded 1.4-11.4 and 17.5 g/l in Mono Lake water respectively. However, far highest amounts of chlorides were obtained from the Dead Sea water (223.3 g/l) (Steiman et al. 1997) and in the Great Salt Lake (54.51) (Utah Geological survey, 2001). Potassium cations (K⁺, 0.1-0.3 mg/ml), carbonates (CO3⁻², 0.2- 2.0), bicarbonates (HCO3⁻, 0.1-1.0), calcium cations (Ca⁺², 0.01-0.5) and magnesium cations (Mg^{+2} , 0.02-0.4) showed lower values

Lake	Al-Gaar	Khadra	Al-Beida	El-Zugm	Hamra	Rosetta	Umm-Risha	Fasida
pН	8.8	9	9.0	8.4	9	9.1	9	9.5
OM	0.05	0.1	1.0	0.1	0.07	0.08	0.05	0.1
TSS	50	80	65	80.2	80	67	70.2	87
Na ⁺	17	22	22	44	40	20	40	13
K ⁺	0.2	0.3	0.2	0.2	0.3	0.3	0.1	0.2
CO_{3}^{-2}	0.2	1.1	1.3	0.2	0.5	0.3	1.0	0.2
HCO ₃ ⁻	0.22	2	0.23	0.2	1.0	0.11	1.2	0.19
Ca ⁺²	0.02	0.04	0.1	0.5	0.01	0.1	0.01	0.1
Mg^{+2}	0.03	0.05	0.02	0.4	0.03	0.3	0.03	0.09
Cl	12	14	10.5	22	20.1	12.5	23	15.2

Table 1: Chemical analysis of water samples collected from Wadi El-Natrun lakes.

Figures were obtained during spring 2007 of study; OM and TSS are calculated as % of the water samples analyzed; Na^+ , K^+ , CO_3^{-2} , HCO_3^{-} , Ca^{+2} , Mg^{+2} and Cl^- are calculated as mg/ml water.

than their respectives in different lakes ranging from 0.01 in calcium to 2.0 in carbonates. In agreement with our results, Moussa *et al.* (2009) found almost similar concentrations of calcium (6.32 mmol/l = 0.15 mg/ml), magnesium (8.76 mmol/l = 0.3 mg/ml), potassium (21.48 mmol/l = 0.6 mg/ml) and bicarbonates (98.5 mmol/l = 1.6 mg/ml).

Wadi El-Natrun Lake water contained extremely lower concentrations of magnesium (0.4 mg/ml) and calcium (0.1 mg/ml) than those reported in the Dead Sea (22.0 and 42.4 for calcium and magnesium respectively) (Steiman et al. 1995) and the Great Salt Lake (0.2 for calcium, 3.3 for magnesium g/l) (Utah Geological Survey, 2001). However lower concentrations of calcium and magnesium were reported in Mono Lake water (Mason, 1967; Steiman et al. 2004). Comparison of Wadi El Natrun lake water analysis in the current study and the studies of Taher (1999) and Moussa et al. (2009) with that of Sothern African lakes studied by Steiman et al. (1991) revealed that the latters were much less saline (mostly <50 g/l) and with pH varies between 9.2 and 10.4.

Terrestrial Fungi recovered from water samples

Fifteen species related to 7 genera were recovered on Czapek Dox agar (as control medium) from water collected from the 8 lakes during the 3 seasons of study. *Aspergillus* (5 species), *Acremonium* (3), *Penicillium* (3) were the most common genera. They accounted for 49.0%, 30.2% and 9.9% of total fungi respectively (Table 6).

Aspergillus (with A. terreus being the most dominant species, 24.1% of total fungi) was reported from all lakes during winter and spring seasons only. The peak of Aspergillus count was recorded from Khadra during spring 2007. The remaining Aspergillus species were recorded

from 3 lakes: A. niger and A. flavus (El-Zugm, Umm-Risha and Al-Gaar), or one lake: A. fumigatus (Al-Beida, winter 2007) and A. parasiticus (Al-Gaar). The current results agree with those reported by Faryal and Hameed (2005) who isolated A. niger, A. fumigatus and A. flavus from water samples with pH values between 8.06 to 12.44 in Peshawar Road, Rawalpindi. Also, Aspergillus candidus, A. melleus, A. niger, A. ochraceus, Chaetomium globosum, Cladosporum cladosporioides, C. sphaerospermum, Hortaea werneckii, Myrothecium roridum, Penicillium citrinum and P. chrysogenum, have been reported in hypersaline waters from temperate or tropical regions (Butinar et al. 2005a, b, Gunde-Cimerman et al. 2004 and Kis-Papo et al. 2001, 2003). Acremonium and its dominant species Acremonium furcatum were isolated from Rosetta and Khadra lakes or from Rosetta only (A. kiliense, A. strictum) during winter 2007. Acremonium persicinum and A. terricola were isolated from Dead Sea water (Buchalo et al. 1999, 2000 a,b and Kis-Papo et al. 2001).

Penicillium was recorded from 3 lakes (Al-Beida, El-Zugm and Al-Gaar). *P. chrysogenum* and *P. puberulum* were recovered each from 2 lakes during winter or spring season while *P. oxalicum* was recovered from one lake (Al-Gaar, winter 2007). Also, Cantrell *et al.* (2006) isolated *Penicillium citrinum*, *P. chrysogenum*, *P. oxalicum* and *P. variabile* from water of salt ponds in hypersaline environments of solar salterns in Puerto Rico.

Fusarium (represented only by *F. solani*) was recorded from 5 lakes (Hamra, Al-Beida, El-Zugm, Rosetta and Umm-Risha) during winter and spring 2007. The remaining 3 species were isolated from 3 lakes: *Trichoderma* sp. (El-Zugm, Umm-Risha and Fasida during winter), or 2 lakes: *Cladosporium cladosporioides* (Hamra and Fasida during autumn and winter

seasons) and *Cochliobolus tuberculatus* (El-Zugm, Al-Gaar, winter 2007 during winter). In this respect, *Cladosporium cladosporioides* was reported from Dead Sea water (Buchalo *et al.* 1999, 2000 a, b and Kis-Papo *et al.* 2001). *C. cladosporioides, C. oxysporum, C. sphaerospermum* and *Emericella nidulans* were isolated from the water of the salt ponds in hypersaline environments in Puerto Rico (Cantrell *et al.* 2006).

Osmophilic and osmotolerant terrestrial fungi from water samples

Six genera represented by 12 species of osmophilic and osmotolerant fungi were recovered from water samples collected from Wadi El-Natrun lakes during 3 seasons on Czapek Dox agar supplemented with 40% sucrose compared to 7 genera and 15 species on the control medium (Table 3).

Aspergillus (5 species), Penicillium (2) and Acremonium (1) were the most common genera on 40% sucrose medium accounting for 65.8%, 15.0% and 2.8% of total fungi respectively (Table 6).

Aspergillus and its dominant species A. terreus (37.1%) were recovered from all lakes during the 3 seasons of study. Their count peaks were recorded from El-Zugm during spring 2007. The remaining Aspergillus species were isolated from 3 lakes: A. niger (El-Zugm, Khadra and Fasida during the 3 seasons), 2 lakes: A. flavus (Al-Gaar and Fasida during winter and spring 2007), or one lake: A. ochraceus (Fasida during autumn 2006 and spring 2007) and Aspergillus sp. (Umm-Risha during spring 2007). In this respect, A. flavus and Aspergillus sp. were reported from the water of the salt ponds collected from hypersaline environments in Puerto Rico (Cantrell et al., 2006) and A. niger from swash zone interstitial water during June and August 2005 on a Mediterranean beach, Genoa, Italy (Vezzulli et al. 2009). Also, Faryal and Hameed (2005) isolated A. niger, A. fumigatus and A. flavus from water samples at pH values between 8.06 to 12.44 in Peshawar Road, Rawalpindi.

Penicillium was recovered from 5 lakes during 2 seasons of study. It had the highest CFUs in water of Al-Beida Lake. *P. chrysogenum* was recovered from 4 lakes during winter and spring 2007 while *P. puberulum* was recorded from 3 lakes during winter 2007 (Hamra, Al-Beida and Umm-Risha). *P. chrysogenum* was also recovered from water of salt ponds in hypersaline environments of Puerto Rico (Cantrell *et al.* 2006) and from Dead Sea water (Buchalo *et al.* 1999, 2000 a, b and Kis-Papo *et al.* 2001).

Acremonium furcatum was recovered from water of Rosetta and Khadra lakes during winter 2007. Other species of Acremonium (A. persicinum and A. terricola) were isolated from Dead Sea water by Buchalo *et al.* (1999, 2000 a,b) and Kis-Papo *et al.* (2001).

Fusarium (represented by 2 species) was recorded from water of 4 lakes during winter and spring 2007. The peak of Fusarium count was recorded from Rosetta during spring 2007. F. solani was encountered during 2 seasons from 3 lakes (Hamra, El-Zugum and Rosetta) while F. subglutinans was isolated only during winter 2007 from Umm-Risha Lake water. Other remaining 2 species were recorded either from 2 lakes: Cladosporium cladosporioides (Umm-Risha and Al-Gaar during spring 2007), or one lake: Emericella quadrilineata (Al-Beida during winter 2007). Also, C. cladosporioides, C. oxysporum, C. sphaerospermum and Emericella nidulans were isolated from the water of the salt ponds in hypersaline environments in Puerto Rico (Cantrell et al. 2006). C. cladosporioides was also reported from Dead sea water by Buchalo et al. (1999, 2000 a, b) and Kis-Papo et al. (2001), and Cladosporium spp. on medium containing 50% and 70% sugar from hypersaline waters throughout the whole season in Sečovlje salterns in the south east part of the Piran bay, at the delta of the Drag-onja river, at the border between Slovenia and Croatia (Gunde-Cimerman et al. 2000).

Halophilic and halotolerant terrestrial fungi recovered from water samples

Using Czapek Dox agar supplemented with 10% NaCl, only 3 species related to *Scopulariopsis* (*S. halophilica* and *S. brumptii*) and *Acremonium* (*A. hyalinulum*) were isolated from water samples during one season only. In this respect, *Acremonium persicinum* and *A. terricola* were isolated from Dead sea water (Buchalo *et al.* 1999, 2000 a, b and Kis-Papo *et al.* 2001).

Fungal taxa	Hamra	Al-	El-	Rosetta	Umm-	Al-	Khadra	Fasida
		Beida	Zugum		Reisha	Gaar		
Acremonium W. Gams				2			1, 2, 3	
A. furcatum Moreau &				2			1, 2, 3	
Moreau ex Gams								
A. kiliense Gütz				2				
A. strictum W. Gams				2				
Aspergillus P. Micheli ex Link	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3
A. flavus Link			3		2	2, 3		
A. fumigatus Fresenius		2						
A. niger van Tieghem			2, 3		2	2		
A. parasiticus Speare						2		
A. terreus Thom	3	3	2, 3	2, 3	3		3	2, 3
Cladosporium cladosprioides	2							1
(Fresenius) de Vries								
Cochliobolus tuberculatus			2			2		
Sivanesan								
Fusarium solani (Martius)	2	2	2	3	2			
Saccardo								
Penicillium Link		2, 3	2			2		
<i>P. chrysogenum</i> Thom		2				2		
P. oxalicum Currie & Thom						2		
P. puberulum Bainier		2, 3	2					
Trichoderma sp.			2		2			2
No. of genera (7)	3	3	5	3	3	3	2	1
No. of species (15)	3	5	7	5	5	6	4	4

Table 2: Seasons of isolation of terrestrial fungi on control medium from water of different lakes of Wadi El-Natrun.

1 = autumn 2006, 2 = winter 2007 and 3 = spring 2007

Table 3: Seasons of isolation of osmophilic and osmotolerant fungi from water of different lakes of Wadi El-Natrun.

Fungal taxa	Hamra	Al- Beida	El- Zugum	Rosetta	Umm- Reisha	Al- Gaar	Khadra	Fasida
Acremonium furcatum				2			2	
Aspergillus	3	3	2, 3	2	2, 3	2	2, 3	1, 2, 3
A. flavus						2, 3		3
A. niger			1, 2				2	1, 3
A. ochraceus Wilhelm								1, 3
A. terreus	3	3	2, 3	2	2, 3	2	2, 3	1, 2, 3
Aspergillus sp.					3			
Cladosporium cladosporioides					3	3		
<i>Emericella quadrilineata</i> (Thom & Raper) Benjamin		2						
Fusarium	2		2	3	2			
F. solani	2		2	3				
<i>F. subglutinans</i> (Wollenweber & Reinking) Nelson, Toussoun & Marasas					2			
Penicillium	2	2			2, 3	2, 3	2	
P. chrysogenum		2			3	2, 3	2	
P. puberulum	2	2			2			
No. of genera (6)	3	3	2	3	4	3	3	1
No. of species (12)	3	4	3	3	6	4	4	4

The same legends as below Table 2

Acidiphilic and aciditolerant terrestial fungi recovered from water samples

A total of twenty-one species related to 10 genera of acidiphilic and aciditolerant fungi were collected at pH4, 12 species and 6 genera and at pH5, 13 spp. and 6 genera, compared to 15 spp. and 7 genera on Czapek Dox agar (as a control medium, pH 7) (Table 4).

Aspergillus (7 species), Acremonium (3) and Penicillium (5) comprised the major proportions of the total propagules at both pHs. They accounted for 87% and 65.4%, 8.4%, 28.8%, and 1.5% and 1.0% of total fungi on media adjusted at pH 4 and pH 5 respectively (Table 6). Aspergillus was the most common fungus in water from the 8 lakes investigated on both acidic media. The peak of Aspergillus was recorded from Al-Beida during winter 2007 at both pHs. Aspergillus and its dominant species A. terreus (57.4%, 42.2% of total fungi) were recorded from all lakes during winter and spring seasons only. A. ochraceus was recovered from 5 lakes while A. *flavus* from 4 lakes, each during 2 seasons. The remaining Aspergillus species were recovered from water of 3 lakes: A. niger (Al-Beida, Al-Gaar and Fasida during winter 2007), or one lake: A. fumigatus, A. sydowii (both from Al-Beida) and A. ustus (Khadra). In this respect, Aspergillus candidus, A. melleus, A. niger, A. ochraceus, Chaetomium globosum, cladosporioides, Cladosporum С. sphaerospermum, Hortaea werneckii, Myrothecium roridum, Penicillium citrinum and P. chrysogenum, have been reported in hypersaline waters from temperate to tropical regions (Butinar et al. 2005a, b, Gunde-Cimerman et al. 2004 and Kis-Papo et al. 2001, 2003a, b). Also, Khallil and Abdel-Sater (1992) found that the water and submerged mud directly exposed to industrial effluents of Mankabad superphosphate factory (highly acidic, low content of oxygen and relatively high contents of total soluble salts, phosphate, sulphate, calcium and magnesium) were poor in terrestrial fungi. They also found Aspergillus fumigatus, A. niger and A. flavus, Alternaria alternata. Fusarium verticillioides and Stachybotrys chartarum were the most prevalent terrestrial fungi.

Acremonium was recovered in 3 lakes during winter 2007 only. The peak of Acremonium was recorded from Umm-Risha Lake at both pHs. Acremonium furcatum was identified from 2 lakes (El-Zugm and Umm-Risha) while an unidentified Acremonium species was recovered from Hamra Lake. In this respect, Acremonium persicinum and A. terricola were isolated from Dead Sea water by Buchalo *et al.* (1999, 2000 a, b) and Kis-Papo *et al.* (2001).

Pencillium and its dominant species P. puberulum were recorded from 5 lakes during winter and spring seasons. The peak was recorded from Al-Beida at pH4 and from El-Zugm at pH5. The remaining Pencillium species were recorded from water of either 3 lakes: P. chrysogenum (Al-Beida, El-Zugm and Al-Gaar), or 2 lakes during winter 2007 only: P. aurantiogriseum (Al-Beida and El-Zugm), or one lake: P. expansum (El-Zugum). In this respect, Cantrell et al. (2006) reported several species of Penicillium: P. citrinum, P. chrysogenum, P. oxalicum and P. variabile from the water of the salt ponds in hypersaline environments of solar salterns in Puerto Rico, most of them are halotolerant.

The remaining fungal species were isolated during winter 2007 from water of either 3 lakes: Cochliobolus tuberculatus (El-Zugm, Umm-Risha and Al-Gaar), 2 lakes: Scopulariopsis brumptii (Rosetta and Khadra), or one lake: Paecilomyces Alternaria tenuissima, sp., (from Al-Beida), Ulocladium botrytis Staphylotrichum coccosporum (from Umm-Risha), Trichoderma sp. (Fasida). A wide variety of fungi have been reported from water samples of Korangi Creek and Clifton areas of Karachi, Pakistan and these were Aureobasidium pullulans, Bispora sp., **Botrytis** sp., Cladosporium sp., Fusarium solani, Humicola sp., Mucor sp., Penicillium sp., P. expansum, P. brefeldianum, Phoma sp., Pythium sp., and Rhizopus sp. (Mehdi and Saifullah 1992) and from deep-sea water samples from different geographical locations (Cladosporium spp., Alternaria spp., Aspergillus sydowii, Nigrospora spp., and Penicilliun solitum) (Roth et al. 1964 and Raghukumar et al. 1992).

Alkaliphilic and alkalitolerant terrestial fungi recovered from water samples

Seventeen species related to 8 genera of alkaliphilic and alkalitolerant fungi were recovered from water samples collected from Wadi El-Natrun lakes on Czapek Dox agar adjusted at pH10 (13 species related to 5 genera) and pH13 (7 species related to 3 genera) (Table 5).

Aspergillus (6 species), Acremonium (2) and Penicillium (5) were the most common genera at both pHs accounting for 64.1%, 31.2% and 1.2%, and 65.6%, 32.4% and 1.4% of total fungi at pH 10 and pH 13 respectively. However, no fungi were isolated from water samples collected from the Mono Lake in California, an alkaline, hypersaline and closed

Fungal taxa	Hamra	Al- Beida	El- Zugum	Rosetta	Umm- Reisha	Al- Gaar	Khadra	Fasida
Acremonium	2		2		2			
A. blochii (Matruchot) W. Gams								
A. furcatum			2		2			
Acremonium sp.	2							
Alternaria tenuissima (Kunze: Fries) Wiltshire		2						
Aspergillus	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3
A. flavus		2, 3	2, 3			2, 3		2
A. fumigatus		2						
A. niger		2				2		2
A. ochraceus		2	2, 3	2		2		2
A. sydowii (Bainier & Sartory) Thom & Church		2, 3						
A. terreus	2, 3	3	2, 3	3	2, 3	2	2, 3	2, 3
<i>A. ustus</i> (Bainier) Thom & Church							2	
Cochliobolus tuberculatus			2		2	2		
Paecilomyces sp.		2						
Penicillium		2, 3	2, 3	2	2	2		
P. aurantiogriseum		2	2					
P. chrysogenum		2	2			2		
<i>P. expansum</i> Link			2					
P. puberulum		2, 3	2, 3	2	2	2		
Scopulariopsis brumptii Salvanet-Duval				2			2	
<i>Staphylotrichum coccosporum</i> Meyer & Nicot					2			
<i>Trichoderma</i> sp.								2
Ulocladium botrytis Preuss		2						
No. of genera (10)	2	5	4	3	5	3	2	2
No. of species (21)	2	12	9	4	5	7	3	5

Table 4: Seasons	of isolation of	of acidiphilic	and acidit	olerant	terrestrial	fungi	from	water in	n different	lakes o	f Wadi
El-Natrun.											

The same legends as below Table 2

basin (Steimen et al. 2004) as well as from the Dead Sea water (Guiraud et al. 1995 and Steimen et al. 1995). These authors stated that this may be attributed to the high salt levels (mostly sodium and potassium) in both waters combined with alkaline pH (9.4-9.8) in the Mono Lake or acidic pH (6.6) in the Dead Sea, which usually not favorable to fungal life. On the other hand, halophilic and halotolerant fungal species can live in some hypersaline waters (Zalar et al. 1999, Buchalo et al. 1998a, b and Grunde-Cimerman et al. 2000). Aspergillus was the most common fungus in water of the 8 lakes investigated on both alkaline media and from 6 out of 8 lakes on the control medium. The peak of Aspergillus count was recorded from Al Beida during winter 2007 at both pHs.

The total CFUs of Aspergillus collected from the 8 lakes during the 3 seasons of study was higher at pH10 than at pH13. Aspergillus and its dominant species A. terreus (47.2% and 43.4% of total fungi on both pHs) were recovered from all lakes during winter and spring seasons. A. flavus was found in 4 lakes (Al-Beida, El-Zugm, Umm-Risha and Al-Gaar). The remaining Aspergillus species were recorded either from 3 lakes: A. niger (Al-Beida, Al-Gaar and Fasida), 2 lakes: A. ochraceus (El-Zugm and Rosetta), A. ustus (Al-Gaar and Khadra), or from Al-Beida Lake only (A. fumigatus). In this respect, several species of Aspergillus such as A. candidus, A. caespitosus, A. flavus, A. flavipes, A. melleus, A. nidulans, A. ochraceus, A. penicillioides and A. unguis most of them are halotolerant were recovered from the water of the salt ponds in hypersaline environments of solar salterns in Puerto Rico (Cantrell et al. 2006). Also, Faryal and Hameed (2005) found that the fungi from water samples of textile industry effluent (pH 8.06 to 12.44) included species of Rhizopus, Aspergillus, Penicillium, Candida, Drechslera, and Rhodotorula with species of Aspergillus (A. niger, A. fumigatus and A. flavus) and Rhizopus spp. being the most predominant, and Drechslera spp. showing the lowest incidence. Acremonium was isolated from water collected from 4 lakes during winter 2007 only. It had the highest CFUs in water samples collected from Umm Risha Lake at both pHs. A. hvalinulum was isolated in Al-Beida, El-Zugm and Umm-Risha lakes and the unidentified Acremonium species was isolated from Khadra Lake. Other species of Acremonium were reported from Dead Sea water (Buchalo et al. 1999, 2000 a, b and Kis-Papo et al. 2001).

Penicillium (with *P. puberulum* being the most common) was recorded from 6 lakes. The remaining *Penicillium* species were recorded either from 2 lakes: *P. aurantiogriseum* (Al-Beida and Rosetta), or one lake during winter 2007: *P. chrysogenum* and *P. expansum* (Al-Beida) and *P. verrucosum* (Hamra). In this respect, Cantrell *et al.* (2006) reported *Penicillium citrinum*, *P. chrysogenum*, *P. oxalicum* and *P. variabile* from the water of the salt ponds in hypersaline environments of solar salterns in Puerto Rico.

The remaining fungi were recorded from either 2 lakes: *Trichoderma* sp. (Rosetta and Fasida), or from one lake during winter 2007 only: *Humicola grisea, Paecilomyces* sp., yeasts (from Khadra) and *Eurotium chevalieri* (Al-Beida). In this respect, Mehdi and Saifullah (1992) isolated *Humicola* sp. from water samples of Clifton, Pakistan. Butinar *et al.* (2005c) isolated six species of *Eurotium* from hypersaline waters of salterns and determined *in vitro* the adaptive ability of propagules to survive prolonged exposure to hypersaline conditions indicating that *E. amstelodami, E. herbariorum* and *E. repens* contribute to the indigenous fungal community in hypersaline water environments, while *E. rubrum, E. chevalieri* and *E. halotolerans* are only temporal inhabitants of brine at lower salinities. Moreover, some species were also isolated from surface and deep waters of the Dead Sea: Aspergillus phoenicis, Chaetomium nigricolor, *Emericella nidulans, Gymnascella marismortui, Paecilomyces farinosus, Penicillium variabile, P. westlingii* and Acremonium sp., Stachybotrys chartarum and Ulocladium chlamydosporum (Buchalo et al., 1998a, b, 1999, 2000a).

In conclusion: chemical analysis revealed that waters of Wadi El-Natrun lakes were highly alkaline and were high in total soluble salts, chloride, sodium and potassium ions. Water collected from El-Zugm Lake showed the highest levels of organic matter, sodium, calcium, magnesium and chloride among all lakes. On the other hand, the other parameters showed their peak in other lakes e.g. pH and total soluble salts in Fasida. A total number of genera (16) and species (33) were recorded from water samples collected from all lakes and the widest spectrum of species was recorded on the control (14) and the lowest on 10% NaCl media Aspergillus, Acremonium followed by (3). Penicillium were the most dominant genera possessing the highest proportions of propagules on all isolation media except on 10 % NaCl. Only species of the genera Scopulariopsis and Acremonium were isolated on 10% NaCl medium. From Aspergillus, A. terreus followed by A. flavus and A. niger were the most common on all isolation media. On the other hand, A. ochraceus was dominant on acidic media only. Other most commonly encountered species, Penicillium chrysogenum and P. puberulum were encountered on all media but not on 10 % NaCl medium. Some species were isolated on one medium but not on the others.

Table 5: Seasons of isolation of alkaliphilic and alkalitolerant terrestrial fungi from water in different lakes of Wadi El–Natrun.

Fungal taxa	Hamra	Al- Beida	El-Zugum	Rosetta	Umm- Reisha	Al- Gaar	Khadra	Fasida
Acremonium		2	2		2		2	
A. hyalinulum (Saccardo) W. Gams		2	5		2			
Acremonium sp.							2	
Aspergillus	3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3
A. flavus		2, 3	3		3	2, 3		
A. fumigatus		2						
A. niger		2				2		2

A. ochraceus			2, 3	2				
A. terreus	3	3	2, 3	2, 3	2, 3	3	2, 3	2, 3
A. ustus						2	2	
Eurotium chevalieri		2						
Mangin								
Humicola grisea							2	
Traaen								
Paecilomyces sp.							2	
Penicillium	2	2	2, 3	2	2	2		
P. aurantiogriseum		2		2				
P. chrysogenum		2						
P. expansum		2						
P. puberulum	2	2	2, 3		2	2		
P. verrucosum	2							
Peyronel								
Trichoderma sp.				2				2
Yeasts							2	
No. of genera (8)	2	4	3	3	3	2	5	2
No. of species (17)	3	10	5	4	4	5	6	3

The same legends as below Table 2

Table 6: Percentage counts of the most common fungi (of the total) recovered from water samples collected from Wadi El-Natrun lakes on different isolation media.

Fungal taxa	Cz %	pH4 %	pH5	pH10	pH13	40% S	10%
							Nacl
Aspergillus	49.0	87	65.4	64.1	65.6	65.8	0.0
Acremonium	30.2	8.4	28.8	31.2	32.4	2.8	40
Penicillium	9.9	1.5	1	1.2	1.4	15.0	0.0

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