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Diversity and Distribution of Microfungi in Tuz Lake, Turkey

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Abstract: The aim of this study is to help illuminate the microfungus diversity in Tuz Lake using traditional and molecular diagnostic data and to correlate it with population dynamics. Microfungi were isolated from a total of 24 samples taken from three stations seasonally by membrane filtration method using two types of media. When using DRBC medium, colony counts were 68 CFU / 100 ml in saltpan water and 86 CFU / ml in lake water. On the DRBC17 medium, the number of colonies in both types of water samples was found to be the same 21 CFU / 100 ml. While 52 different species belonging to 19 genera have been isolated on DRBC medium, 30 different species belonging to 11 genera were detected on DRBC17. In total, 54 different species belonging to 21 genera were isolated. According to the variety of species, the leading genera are *Penicillium* (15 spp), *Aspergillus* (8 spp), *Alternaria* (8 spp), *Cladosporium* (5 spp), *Arthrinium* (2 spp). This study showed that Tuz Lake mycobiota has a high diversity and most of the isolated species are adapted to saline environments.

Key words: Fungal diversity, Hypersaline, Saltern, Abundance, Frequency

Tuz Gölü (Türkiye) Mikrofungus Çeşitliliği ve Dağılımı

Öz: Bu çalışmanın amacı, geleneksel ve moleküler tanılama verileri kullanılarak Tuz Gölü'ndeki mikrofungus çeşitliliğinin aydınlatılması ve popülasyon dinamikleriyle ilişkilendirilmesidir. Mikrofunguslar, mevsimsel olarak üç istasyondan alınan toplam 24 örnekten, iki tip ortam kullanılarak membran filtrasyon yöntemi ile izole edilmiştir. DRBC besiyerinin kullanıldığında koloni sayıları tuzla havuz suyunda 68 CFU / 100 ml ve göl suyunda 86 CFU / ml olmuştur; DRBC17 besiyerinde ise her iki tip su örneğinde de koloni sayısı aynı 21 CFU / 100 ml bulunmuştur. DRBC ortamında 19 cinse ait 52 farklı tür izole edilirken, DRBC17 üzerinde 11 cinse ait 30 farklı tür tespit edilmiştir. Toplamda 21 cinse ait 54 farklı tür izole edilmiştir. Tür çeşitliliğine göre önde gelen cinsler *Penicillium* (15 tür), *Aspergillus* (8 tür), *Alternaria* (8 tür), *Cladosporium* (5 tür), *Arthrinium* (2 tür) 'dur. Bu çalışma, Tuz Gölü mikobiyotasının yüksek bir çeşitliliğe sahip olduğunu ve izole edilen türlerin büyük bir kısmının tuzlu ortamlara adapte olmuş türler olduğunu göstermiştir.

Anahtar kelimeler: Mantar çeşitliliği, Hipersalin, Tuzlu su, Bolluk, Frekans

Introduction

The fact that eukaryotic microorganisms are believed to be unable to survive in extreme environments for many years is considered to be the reason for the limited findings related to the biodiversity of such habitats. The hypersaline environments first have become the focus of interest for prokaryotic diversity, and then studies examining eukaryotic organisms in extreme environments have increased. Because of their unique nature and ability to survive in a wide variety of environments and since they could be found in nearly each layer of the earth and widely spread in the ecosystem, the fungi have always been a point of interest for researchers.

Although many previous studies have been conducted on the isolation and identification of halotolerant/halophilic fungi, the first comprehensive study suggesting that fungi are active members of hypersaline environments was carried out by Gunde-Cimerman et al. (2000). Previously Cronin and Post

(1977) have studied on the isolation and description of a halophilic filamentous fungus *Cladosporium* sp. from the Great Salt Lake, Utah, a hypersaline lake. In addition the isolation and identification of three fungal species from Dead Sea water samples carried out by Buchalo et al. (1998) is noteworthy. Many halotolerant/halophilic fungi isolated from various sources such as salted food, sea water and arid soils have been reported (Andrews and Pitt, 1987; Asan, 2004). The fungi, which are capable of developing in low water activity (a_w), have also been isolated from foods with high levels of salt or sugar as preservatives. Although they have being considered as food contaminants halotolerant/halophilic fungi have been discovered in hypersaline environments studied around the world (Gunde-Cimerman et al., 2009).

Natural salty lakes, solar salterns, salty drainage waters, seashore and wellsprings located within salt deposits underground are common hypersaline, thalassohaline/athalassohaline waters. Examples of such environments comprehensively examined in terms of fungal diversity are the well-recognized Great Salt Lake in Utah, the Cabo Rojo Solar Salterns set on the southwest coast of Puerto Rico and Dead Sea in Israel (Cronin and Post, 1977; Kis-Papo et al., 2001; Kis-Papo et al., 2003; Nazareth et al., 2012). Studies to date have shown that halotolerant/halophilic fungi are a component of microbial populations in hypersaline environments.

The Tuz Lake (Turkey), a Special Environmental Protection Area (SEPA), is amongst the most saline lakes in the world after Dead Sea. All of the lake surface and surrounding include waterbeds and some of the important neighboring steppe areas. The area is a unique ecosystem in terms of natural life in Turkey. The emergence of a rich plant and microbial diversity in the Salt Lake and its basin is the result of arid and extreme salty conditions. 279 plant species and 120 halophylic bacteria have been reported in the Tuz Lake ecosystem (UNESCO). So far, the Tuz Lake microbiota has mainly been studied in terms of prokaryotic diversity (Birbir et al., 2007; Mutlu et al., 2008). There are few studies supporting the Tuz Lake mycobiota, and most of them are on soil-borne fungi and include fungal species isolated for biotechnological use.

The aim of this study is to characterize the spatiotemporal species composition of the fungal community of the Tuz Lake and for this purpose it has been investigated whether the diversity and composition of fungal communities varies in the saltpans and brine of Tuz Lake. Another aim of the research has been to determine the significance of salt in the medium used for the isolation of microfungi from hypersaline environments. In addition, the frequency and relative abundances of the isolated microfungi have been calculated and the fungal diversity in the Tuz Lake has been evaluated through the Simpson and Shannon biodiversity.

Material and Metod

Site description: The Tuz Lake, second largest in Turkey, is about 1.831 km^2 and is located between the borders of three cities, Ankara, Aksaray, and Konya. It lies at an altitude of 905 m above the sea level. It is a shallow lake with a tectonic origin whose depth is below 0.5 m. While most of the lake is completely dry in summer, its size changes with a maximum depth of 1.5 m in spring (Dengiz et al., 2010) (Fig 1).

Although salinity levels change with regard to seasonal fluctuations, the water of the lake is extremely saline with a salt content of 32%. The lake and its surroundings cover approximately 70% of the annual salt demand of Turkey and have a large share in salt exports (Dengiz et al., 2010).

Sampling, isolation and enumeration of fungi: The water samples were compositely taken from three main stations, Kayacık, Yavşan and Kaldırım salterns, at Tuz Lake in June, November 2012 and February, May 2013. For each station, the water samples were taken from the salterns (Saltern Water-SW) and the lake (Lake Water-LW) (Fig 1). All the samples taken have been analyzed in terms of such variables as temperature (°C), pH, and salinity (%) (Table 1).

To isolate and enumerate the fungal species from water, 20 ml of each sample has been filtered through the sterile Cellulose Nitrate Membrane Filters (pore size 0.45µm, Ø 47 mm Sartorius) and placed onto the Petri plates containing DRBC (aw 1.0) and DRBC17 (DRBC+17% NaCI, aw 0.89) media with chloramphenicol (100 mg/L) (King Jr et al., 1979). For DRBC17 medium, untreated salt harvested from the Tuz Lake salterns has been used. The plates have been incubated for 5 weeks at 25°C. Fungal colony forming units (CFUs) were counted on 3rd, 5th, 7th, 14th and 30th days of incubation, and subcultures were made of all of the morphologically distinct colonies from each sample on Malt Extract Agar (Merck) slants and kept at 4°C. Individual pure strains have been deposited in the culture collection of the Department of Biology, Eskisehir Osmangazi University (Turkey).

Figure 1. Tuz Lake and the stations (Kaldırım, Kayacık and Yavşan Salterns

Identification of fungi: For identification Potato Dextrose Agar (PDA), Czapek-Dox agar (CZ), Czapek Yeast autolysate Agar (CYA), 25% Glycerol Nitrate agar (G25N) and Malt Extract Agar (MEA) media have been used. Identifications of fungal specimens have been based on their micro and macro morphologies and upper and lower surface colorations on CYA, CY20S, CZ, G25N, MEA and PDA. Genus identifications have been carried out following Barnett and Hunter (1999) and the identifications of species have been executed complying with related literatures (Ellis, 1965; Klich, 2002; Pitt, 1979; Pitt and Hocking, 2009; Samson et al., 2004a).

Table 1. Analyzed environmental variables for each station in the Tuz Lake. The saltern number refer to Fig 1.

Salterns		**Samples type**	**Temp. (°C)** SU/FA/WI/SP	**pH** SU/FA/WI/SP	**Salinity (%)** SU/FA/WI/SP	**Coordinates**
Kayacık	1	Saltpan water	26/12/5/27	6.18/7.13/7.75/7.78	30/28/26/28	38°49'50.57"N 33°25'34.83"E
	2	Lake water	26/12/5/27	5.94/7.15/7.71/7.59	33/29/26/28	38°50'12.95"N 33°24'14.05"E
Kaldırım	3	Saltpan water	28/13/5/29	6.36/7.25/7.79/7.69	30/28/25/29	38°59'58.41"N 33°24'06.25"E
	4	Lake water	30/12/6/29	6.40/7.18/7.64/7.66	28/28/26/28	39°02'59.08"N 33°23'42.04"E
Yavsan	5	Saltpan water	29/20/7/28	6.50/7.35/7.62/7.79	28/27/25/28	38°45'50.38"N 33°10'27.33"E
	6	Lake water	30/20/6/28	6.36/7.32/7.97/7.76	30/29/20/28	38°47'07.42"N 33°12'09.06"E

SU, Summer; FA, Fall; WI, Winter; SP, Spring

Water samples of three aliquots have been filtered in parallel and the average number of colonies has been calculated as CFUs/100ml. Water activities of the media have been determined using the water activity meter (Aqualab, Decagon Devices, USA).

After all these processes, their identity has been confirmed using the analysis of internal transcribed spacer regions (ITS1and ITS2) of the ribosomal DNA operon (including the 5.8S gene) of the filamentous fungi. For the molecular study all isolates have been grown in

Malt Extract broth (Merck) of 2 mL of in 15 mL tubes and incubated at 25ºC for 7 days in darkness. (Samson et al., 2004b). Genomic DNA of selected pure cultures has been extracted using CTAB following the related protocol (Graham et al., 1994; Murray and Thompson, 1980). The extracted DNAs have been stored at -20ºC in a freezer. ITS region has been amplified with the universal primers forward ITS1 (5′-TCCGTAGGTGAACCTGCG G-3′) and reverse ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Purification and sequencing analyses of amplicons have been performed by BMLabosis (Ankara, Turkey). The sequences were compared with those deposited in the NCBI GenBank Database via BLAST searches (Altschul et al., 1997; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The closest Blast results are reported for each taxon.

For phylogenetic analysis, the sequence alignments were performed using the Muscle in MEGA X (Kumar et al., 2018) software package, together with the other sequences of morphologically and phylogenetically related species that were obtained from NCBI GenBank. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993) with 1000 bootstrap replications. *Allomyces arbusculus* HQ888729 (Not listed species on "The Checklist of Fungi of Turkey") was used as the out-group. The obtained sequence data have been deposited in GenBank with accession numbers (Table 2).

Data analysis: For each microfungus species, the frequency of occurrence (%) and relative abundance (%) have been calculated. The frequency of occurrence of fungi has been artificially grouped according to the percentage occurrence of fungi as very frequent (>20%), frequent (10-20%), and infrequent (<10%) (Maria and Sridhar, 2003; Sarma and Hyde, 2001).

The diversity of fungi at stations has been assessed based on two diversity indices, Simpson and Shannon. Being one of the most common methods, Simpson's Indice use the relative abundance of different species to estimate the diversity. Another index is the Shannon-Wiener Diversity index (H) that is commonly used to characterize species diversity in a community. The Shannon Index gives a value between 0 and 5. However, the results between 1.5 and 3.5 are considered as statistically significant. If the result of the calculation is closer to 5, it is inferred that the diversity is high (Magurran, 2013).

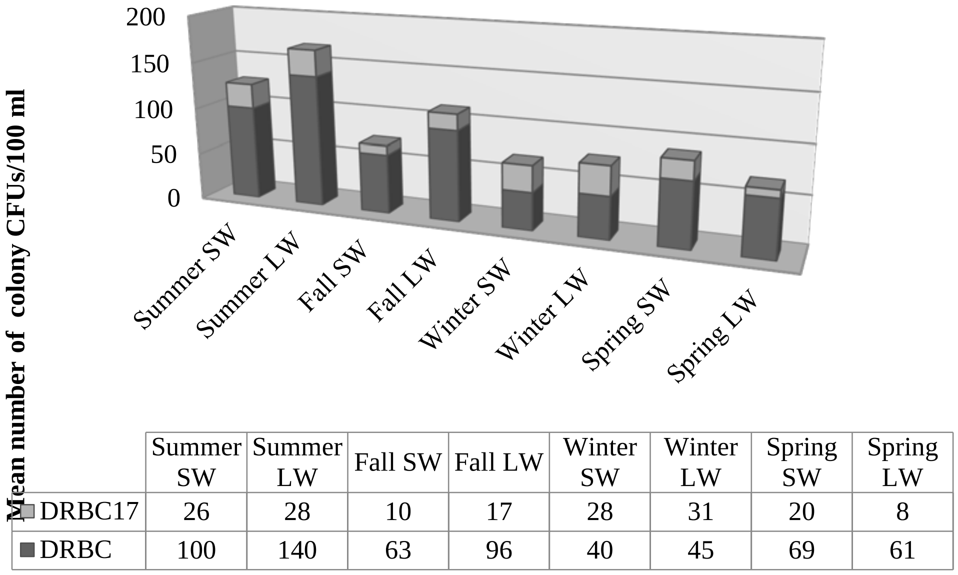
Results

In order to represent the Tuz Lake, a total of 24 water samples have been taken seasonally both from the three saltpans and the lake. During the year, the temperature was between 5℃ and 30°C. The average temperature was 28°C in summer, 15°C in the autumn, 5.5°C in winter and 28°C in spring. The pHs of the water samples at the stations were between 5.94 and 7.97. The mean pH (6.29) in summer was lower than those in autumn, winter, and spring (7.23, 7.74, 7.71), respectively. Salinity at the locations where the water samples were taken ranged between 20% and 30% according to the seasons and sampling points. The rate of salinity especially at the first station, reached a remarkable point of 33% in summer. The average pH, temperature and salinity of water samples have been presented in Table 1.

Enumeration and identification of fungi: With the culture-dependent counting method, fungal isolates have been enumerated and isolated using two different media having low (DRBC17, a_w 0.89) and high water activity (DRBC a_w 1.0). Then the dynamics of microfungi inhabited in hypersaline waters have been estimated. During isolation from water samples of the fungal species, when 20 ml of each sample has been filtered, optimal distribution of colonies on medium in petri dishes after incubation has been observed.

In this research, a total of 2486 fungal isolates have been recovered from 24 water samples using DRBC and DRBC17 media. While 79 % of these fungal isolates has been isolated from the DRBC, 21% of isolates has been recovered from the DRBC17 plates. For all samples, the CFU numbers on DRBC17 medium have been significantly lower than that of on the DRBC. The CFU numbers obtained on both media have reached the highest numbers in summer (195 CFUs/100ml LW on DRBC; 69 CFUs/100 ml LW on DRBC17). The mean colony numbers have been 68 CFUs/100ml and 21 CFUs/100ml for saltpan water on DRBC and DRBC17, respectively. The mean colony numbers for lake water 86 CFUs/100 ml and 21 CFUs/100ml on DRBC and DRBC17 medium, respectively (Fig 2).

Distribution of fungi identified, based on seasons and stations, has been organized by alphabetic order and presented in Table 2. While 52 different species belonging to 19 genera have been isolated on DRBC medium 28 different species belonging to 11 genera on DRBC17 have been isolated. Within the isolation process with DRBC and DRBC17 media, 54 different species in total belonging to 21 genera have been isolated. The discovered species have distributed among nine ordo: *Hypocreales* Lindau (3 spp), *Pleosporales* Luttr. ex M.E. Barr (15 spp), *Xylariales* Nannf. (3 spp), *Eurotiales* G.W. Martin ex Benny and Kimbr (24 spp), *Capnodiales* Woron (5 spp), *Dothideales* Lindau (2 spp), *Mucorales* Fr. (1 sp), *Sordariales* Chadef. ex D. Hawksw. and O.E. Erikss (1 sp). The genera according to species diversity, *Penicillium* (15 spp), *Aspergillus* (8 spp), *Alternaria* (8 spp), *Cladosporium* (5 spp) and *Arthrinium* (2 spp) have been represented by more than one species. However, *Acremonium*, *Beauveria*, *Botrytis*, *Chalastospora*, *Curvularia*, *Epicoccum*, *Fusarium*, *Leptospora*, *Mucor*, *Nigrospora*, *Phoma*, *Pringsheimia*, *Pithomyces*, *Sordaria*, *Stemphylium*, and *Talaromyces* genera have been represented by one species. Using their morphological characteristics as well as ITS data, the isolates have been identified to species level, and only sequence matches above 98.5% have been regarded as meaningful. The phylogenetic tree created is presented in Figure 3.



	Summer SW	Summer LW	Fall SW	Fall LW	Winter SW	Winter LW	Spring SW	Spring LW
▨ DRBC17	26	28	10	17	28	31	20	8
■ DRBC	100	140	63	96	40	45	69	61

Figure 2. The mean number of colony of fungi seasonally isolated from Saltpan Water (SW) samples and Lake Water (LW) samples of Tuz Lake.

Dinamics of Fungal Population: The estimates for DRBC medium have showed that a total of 20 species including *Alternaria infectoria* E.G. Simmons (Meşeküfü), *A. tenuissima* (Nees) Wiltshire (Narinküf), *Arthrinium arundinis* (Corda) Dyko and B. Sutton (Saz kütlesi), *Aspergillus flavus* Link (Cıbıl asper), *A. chevalieri* (Mangin) Thom. and Church (Bey asper), *A. neoniveus* Samson, S.W. Peterson, Frisvad and Varga (Akça asper), *A. repens* (Corda) Sacc (Vuran asper), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (Karakökküfü), *C. herbarum* (Pers.) Link (Yaygınkurutan), *C. sphaerospermum* Penz. (Güllekurutan), *C. uredinicola* Speg. (Heybekurutan), *Curvularia inaequalis* (Shear) Boedijn (Çok eğiksispor), *Penicillium atrovenetum* G. Sm. (Kıro penisilyum), *P. brevicompactum* Dierckx (Sağlam penisilyum), *P. citrinum* Thom (Limon penisilyum), *P. commune* Thom (Zonlu penisilyum), *P. solitum* Westling (Bir penisilyum), *P. decumbens* Thom (Sabun penisilyum), *P. hordei* Stolk (Arpa penisilyum), and *P. polonicum* K.W. Zalessky (Leh penisilyum) have been very frequent (>20%). In DRBC17, total of 7 species including *A. amstelodami* (Mangin L.) Thom and Church (El asperi), *A. flavus*, *A. repens*, *C. sphaerospermum*, *P. commune*, P. polonicum, and *Mucor racemosus* Fresen. (Salkımküf) have showed very frequent occurrence. The species with very frequent occurrence for both media were *A. flavus*, *A. repens*, *C. sphaerospermum*, and *P. polonicum*.

The species with frequencies of occurrence higher than 20% in both medium and also with high abundance in DRBC17 have been *A. repens* (21.23%), *C. sphaerospermum* (9.72%), and *A. flavus* (9.52%). Whereas the frequent ones in DRBC have been *C. cladosporioides* (15.05%) *P. commune* (9.34%), *P. polonicum* (7.71%), and *C. sphaerospermum* (4.51%). Interestingly, *A. amstelodami* has only been isolated in DRBC17 and has been the species that has showed both very frequent occurrence (>20%) and high abundance (9.13%) (Table 2 and Fig 4). It has been isolated from the samples of saltpan and lake water of two stations in autumn and winter.

Table 2. Dynamics of the fungal taxa isolated from three salterns at Tuz Lake /(on two media DRBC and DRBC17). Frequency of occurance ***very frequent /(>20%), **frequent /(10-20%), and *infrequent /(<10%)

Taxa	**NCBI ACCESSION**	**Relative abundance (%) /Frequency of occurance (*)**	
		DRBC	**DRBC17**
Acremonium potronii Vuill. (x)	**KY458479**	-	0.60 /(*)
Alternaria alternata (Fr.) Keissl. (Astımküfü)	**KY439023**	2.12 /(**)	0.99 /(*)
A. arborescens E.G. Simmons (Tasküfü)	**KY458470**	0.49 /(*)	-
A. brassicae (Berk.) Sacc. (Lahanaküfü)		1.52 /(*)	-
A. consortialis /(Thüm.) J.W. Groves and S. Hughes (x)	**KY458467**	1.88 /(**)	-
A. infectoria	**KY458469**	1.41 /(***)	1.19 /(*)
A. tenuissima	**KY439017**	1.41 /(***)	0.20 /(*)
A. tellustris (E.G. Simmons) Woudenb. and Crous (x)	**KY439021**	0.27 /(**)	-
A. phragmospora Emden (x)	**KY458487**	0.16 /(*)	-
Arthrinium arundinis	**KY458464**	1.20 /(***)	0.99 /(**)
A. phaeospermum (Corda) M.B. Ellis (Odun kütlesi)	**KY439016**	0.87 /(**)	-
Aspergillus alliaceus Thom and Church (Tembel asper)		0.60 /(**)	-
A. amstelodami	**KY439027**	-	9.13 /(***)
A. chevalieri	**KY439032**	2.55 /(***)	0.99 /(**)
A. flavus	**KY458486**	4.24 /(***)	9.52 /(***)
A. nidulans (Eidam) G. Winter (Çıplak asper)	**KY439029**	0.05 /(*)	-
A. neoniveus	**KY439030**	2.01 /(***)	0.40 /(*)
A. penicillioides Speg. (Çarpık asper)	**KY458475**	0.11 /(*)	1.98 /(*)
A. repens.		0.38 /(***)	21.23 /(***)
Beauveria bassiana (Bals.-Criv.) Vuill. (Böcekküfü)	**KY439035**	0.16 /(*)	-
Botrytis cinerea Pers (Kurşuniküf)	**KY439019**	0.11 /(*)	-
Chalastospora gosspii E.G. Simmons (İncekatar)	**KY458477**	0.38 /(**)	0.99 /(*)
Cladosporium cladosporioides	**KY458490**	15.05/(***)	2.38 /(**)
C. herbarum	**KY458497**	4.35 /(***)	-
C. ossifragi (Rostr.) U. Braun and K. Schub. (x)	**KY458493**	5.98 /(**)	6.75 /(*)
C. sphaerospermum	**KY458492**	4.51 /(***)	9.72 /(***)
C. uredinicola	**KY439020**	1.09 /(***)	-
Curvularia inaequalis	**KY439028**	2.55 /(***)	-
Epicoccum sp. Link (Boyalıküf)	**KY458494**	0.65 /(**)	0.20 /(*)
Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach and Nirenberg (Delik solduran)		1.30 /(**)	-
Leptospora galii N.L. de Silva and K.D. Hyde (x)	**KY458465**	0.38 /(*)	-
Mucor racemosus	**KY458466**	0.43 /(**)	2.58 /(***)
Nigrospora oryzae (Berk. and Broome) Petch (Çeltik karasporu)	**KY458482**	0.05 /(*)	-
Penicillium atrovenetum	**KY439033**	2.23 /(***)	4.96 /(**)
P. aurantiogriseum Dierckx (Çok penisilyum)	**KY439026**	0.16 /(*)	-
P. brevicompactum	**KY439015**	0.76 /(***)	1.98 /(*)
P. canescens Sopp (Verimli penisilyum)	**KY458474**	0.49 /(**)	-
P. citrinum	**KY439022**	7.50 /(***)	8.53 /(**)
P. commune	**KY458471**	9.34 /(***)	2.58 /(***)
P. solitum		3.20 /(***)	2.78 /(*)
P. decumbens	**KY458484**	2.66 /(***)	-
P. expansum Link (Geniş penisilyum)	**KY439037**	0.33 /(**)	-
P. granulatum Bainier (Yamuk penisilyum)	**KY439025**	0.87 /(**)	0.20 /(*)
P. hordei	**KY458472**	0.49 /(***)	0.99 /(*)
P. nalgiovense Laxa (Bolsulu penisilyum)	**KY439024**	0.05 /(*)	0.99 /(*)
P. polonicum	**KY458478**	7.71 /(***)	4.96 /(***)
P. raistrickii G. Sm. (Pütürlü penisilyum)	**KY439018**	0.54 /(**)	0.60 /(*)

Taxa	NCBI ACCESSION	Relative abundance (%) /Frequency of occurance (*)	
		DRBC	**DRBC17**
P. spinulosum Thom (İğneli penisilyum)	**KY439036**	0.22 /(**)	0.40 /(*)
Pleospora bjoerlingii Byford (Gömükömür)		0.76 /(*)	0.40 /(*)
Pseudopithomyces chartarum (Berk. and M.A. Curtis) Jin F. Li, Ariyaw. and K.D. Hyde (Benekliküf)	**KY458473**	0.11 /(*)	-
Pringsheimia euphorbia Froid. (x)	**KY458476**	0.05 /(*)	-
Sordaria sp. Ces. and De Not. (Keneküf)	**KY458496**	2.01 /(*)	-
Stemphylium solani G.F. Weber (Küt durusefil)	**KY458468**	0.76 /(**)	0.60 /(*)
Talaromyces flavus (Klöcker) Stolk and Samson (Sarı süpürgen)		0.22 /(**)	-
Unidentified isolates		2.17	-

(x) Not listed species on "The Checklist of Fungi of Turkey" (Güner et al., 2020)
The species names have been updated according to the indexfungorum.org website (Index Fungorum, 2021)

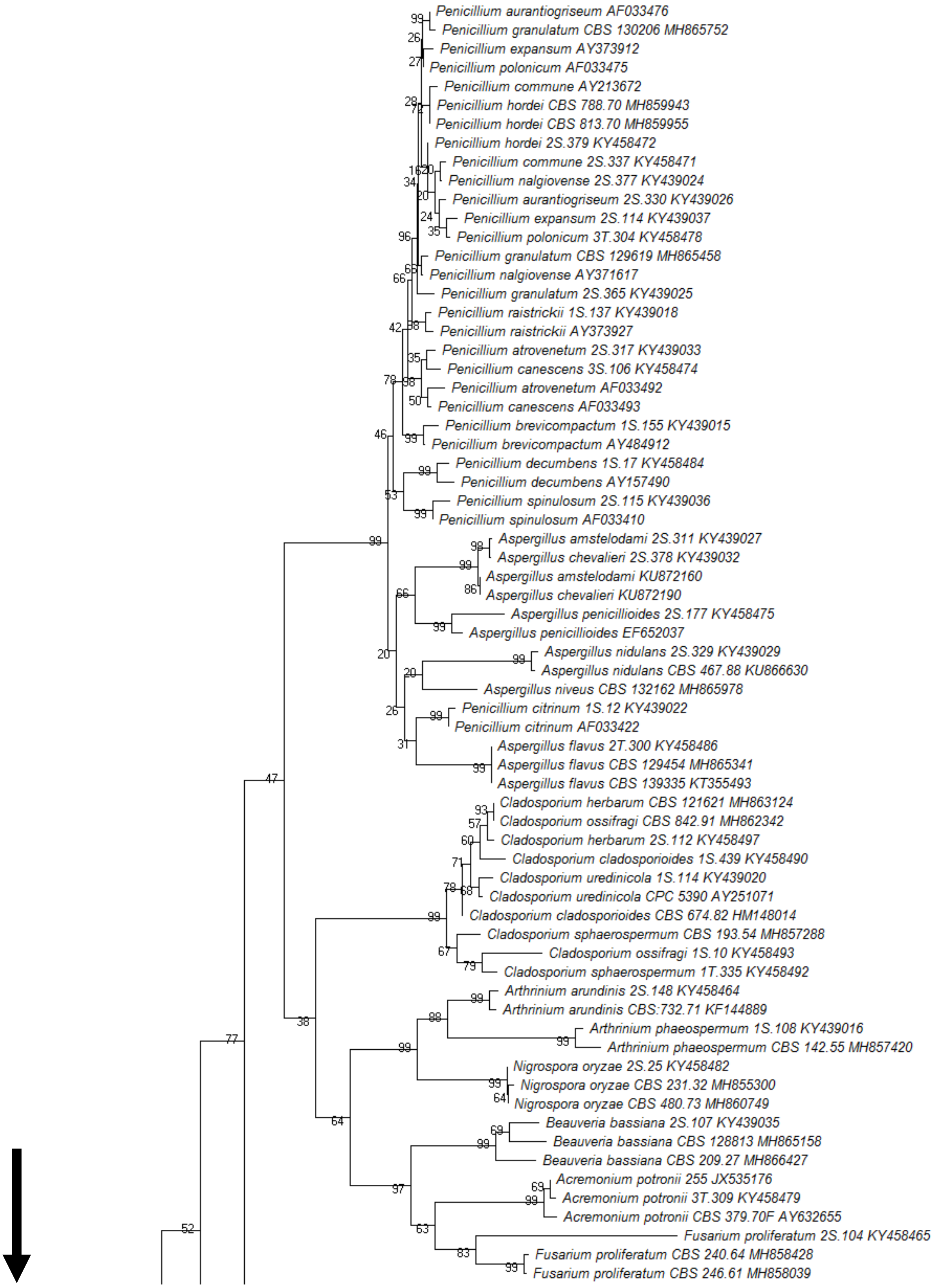
The tree with the highest log likelihood (-20250.71) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 100 nucleotide sequences. There were a total of 1916 positions in the final dataset.

The number of species in the stations have varied from 26 to 32 on DRBC, while on DRBC17 has showed a lower number between 7-17. The values of Simpson Diversity Index and Shannon Diversity Index have varied between 0.653 and 0.908, 1.370 and 2.721 respectively. According to these indexes, no significant difference has been observed in Tuz Lake salterns and lake water in terms of diversity (Table 3).

Discussions

The oceans are the largest bodies of saline water on earth with average salinities of 3.5 % (35 g/L, 0.6 M). Hypersaline environments, denoted thalassohalines, are similar to sea water in terms of salt composition but they have a salinity of 35%, which is 10 times higher than that of seawater and this rate increases as a consequence of evaporation of nearby seawaters or salt-water lakes (DasSarma and DasSarma, 2012). In such waters including Tuz Lake, sodium and potassium ions are present at higher concentrations than calcium and magnesium ions. Due to the high temperature in summer as evaporation of seawater proceeds, the crystalline NaCl ratio increases. As a result, sodium ions in water decreases while chlorides of Mg, Ca, and K become dominant and pH tends to be slightly acidic (Oren, 2016; Uygun and Şen, 1978). This remarkable result supports to the fact that the water samples collected in summer are slightly acidic and close to saturated salinity (Table 1).

The fungi growing below a_w 0.85 have been characterized as xerophilic/halophilic (Pitt and Hocking, 2009). Therefore, we have used DRBC medium with high salt concentration of salt (17% NaCl) for selective isolation of halotolerant and halophilic fungi. Halotolerant and halophilic fungi have been described as active inhabitants of hypersaline environments. The mycobiota that inhabit in these natural hypersaline environments consists of phylogenetically unrelated halotolerant, extremely halotolerant and halophilic fungi. Dominant representatives are different species of black yeast-like and related melanized fungi of the genus *Cladosporium*, different species belonging to the genus *Aspergillus* and *Penicillium*, some non-melanized yeast species and *Wallemia* spp.(Gunde-Cimerman and Zalar, 2014). So far, the presence of these fungi in different salterns and salt lakes around the world has been reported. Tuz Lake, which are among the few salt lakes in the world, have not been the subject of a detailed study which is carried out to determine the fungal biodiversity so far. Within the scope of this study, the fungal species that are isolated from saltpans and lake water samples seasonally taken are given in Table 2.


Penicillium aurantiogriseum AF033476
Penicillium granulatum CBS 130206 MH865752
Penicillium expansum AY373912
Penicillium polonicum AF033475
Penicillium commune AY213672
Penicillium hordei CBS 788.70 MH859943
Penicillium hordei CBS 813.70 MH859955
Penicillium hordei 2S.379 KY458472
Penicillium commune 2S.337 KY458471
Penicillium nalgiovense 2S.377 KY439024
Penicillium aurantiogriseum 2S.330 KY439026
Penicillium expansum 2S.114 KY439037
Penicillium polonicum 3T.304 KY458478
Penicillium granulatum CBS 129619 MH865458
Penicillium nalgiovense AY371617
Penicillium granulatum 2S.365 KY439025
Penicillium raistrickii 1S.137 KY439018
Penicillium raistrickii AY373927
Penicillium atrovenetum 2S.317 KY439033
Penicillium canescens 3S.106 KY458474
Penicillium atrovenetum AF033492
Penicillium canescens AF033493
Penicillium brevicompactum 1S.155 KY439015
Penicillium brevicompactum AY484912
Penicillium decumbens 1S.17 KY458484
Penicillium decumbens AY157490
Penicillium spinulosum 2S.115 KY439036
Penicillium spinulosum AF033410
Aspergillus amstelodami 2S.311 KY439027
Aspergillus chevalieri 2S.378 KY439032
Aspergillus amstelodami KU872160
Aspergillus chevalieri KU872190
Aspergillus penicillioides 2S.177 KY458475
Aspergillus penicillioides EF652037
Aspergillus nidulans 2S.329 KY439029
Aspergillus nidulans CBS 467.88 KU866630
Aspergillus niveus CBS 132162 MH865978
Penicillium citrinum 1S.12 KY439022
Penicillium citrinum AF033422
Aspergillus flavus 2T.300 KY458486
Aspergillus flavus CBS 129454 MH865341
Aspergillus flavus CBS 139335 KT355493
Cladosporium herbarum CBS 121621 MH863124
Cladosporium ossifragi CBS 842.91 MH862342
Cladosporium herbarum 2S.112 KY458497
Cladosporium cladosporioides 1S.439 KY458490
Cladosporium uredinicola 1S.114 KY439020
Cladosporium uredinicola CPC 5390 AY251071
Cladosporium cladosporioides CBS 674.82 HM148014
Cladosporium sphaerospermum CBS 193.54 MH857288
Cladosporium ossifragi 1S.10 KY458493
Cladosporium sphaerospermum 1T.335 KY458492
Arthrinium arundinis 2S.148 KY458464
Arthrinium arundinis CBS:732.71 KF144889
Arthrinium phaeospermum 1S.108 KY439016
Arthrinium phaeospermum CBS 142.55 MH857420
Nigrospora oryzae 2S.25 KY458482
Nigrospora oryzae CBS 231.32 MH855300
Nigrospora oryzae CBS 480.73 MH860749
Beauveria bassiana 2S.107 KY439035
Beauveria bassiana CBS 128813 MH865158
Beauveria bassiana CBS 209.27 MH866427
Acremonium potronii 255 JX535176
Acremonium potronii 3T.309 KY458479
Acremonium potronii CBS 379.70F AY632655
Fusarium proliferatum 2S.104 KY458465
Fusarium proliferatum CBS 240.64 MH858428
Fusarium proliferatum CBS 246.61 MH858039
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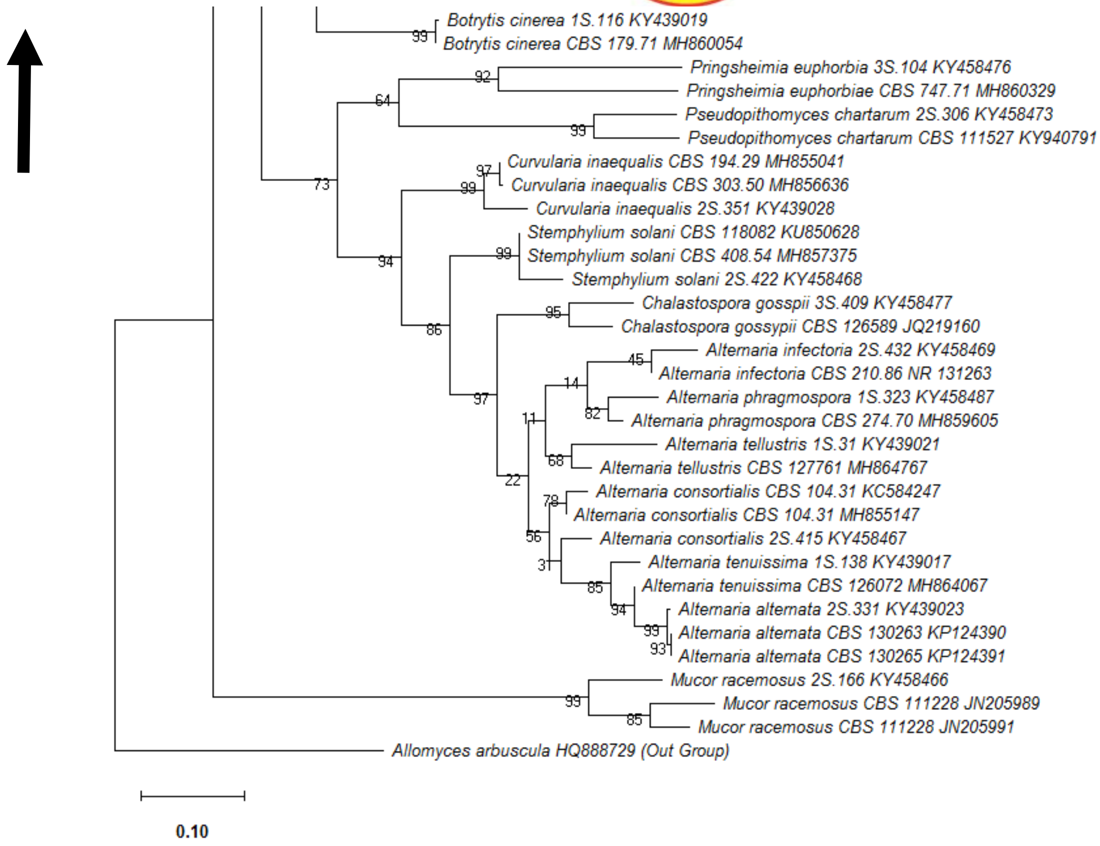


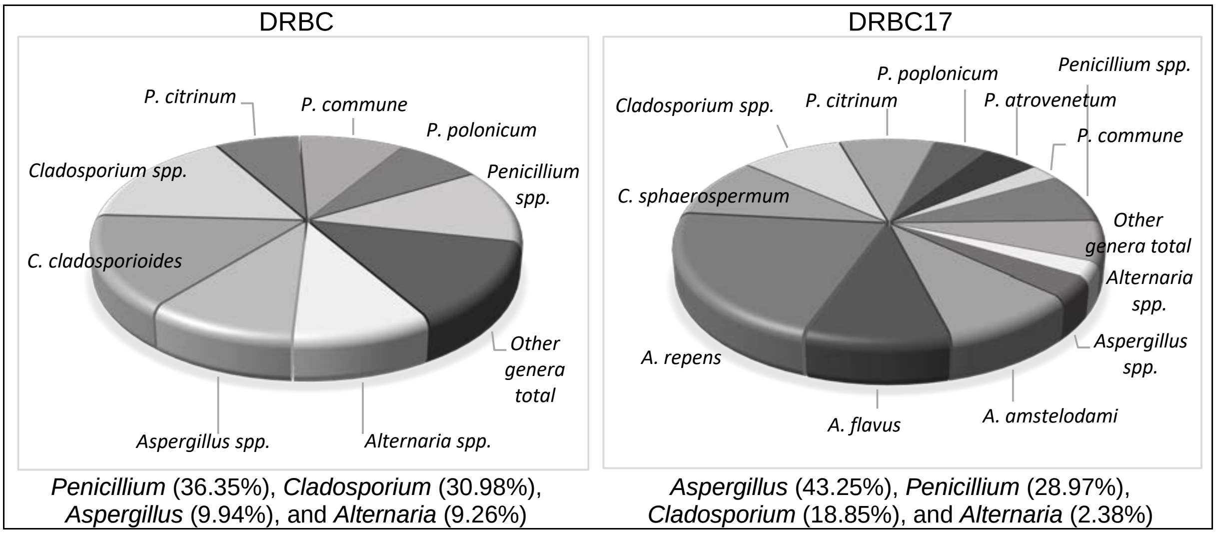
Figure 3. Best-scoring maximum likelihood tree based on ITS sequences of isolates showing the relationships of the newly generated sequences in this study with previously known taxa in the NCBI GenBank. The tree is rooted with *Allomyces arbusculus* (HQ888729) (bootstrap 1000).

The fact that most of the species isolated using DRBC medium have not followed a consistent spatial or temporal pattern indicates that most species are not unique to Tuz Lake brine. However, it is throught that species that have been isolated during at least three seasons and have a high frequency of occurrence are probably specific to Tuz Lake. These were *A. infectoria*, *A. tenuissima, A. flavus, C. cladosporioides, C. herbarum, C. sphaerospermum, C. uredinicola, Curvularia inaequalis, P. atrovenetum, P. citrinum, P. commune, P. solitum, P. hordei,* and *P. polonicum*. The species isolated using DRBC17 medium are *A. amstelodami, A. flavus, A. repens, P. commune, P. polonicum* and *M. racemosus* and they all have been followed by a consistent spatial and temporal pattern (Table 2).

Some of the non-prominent species in DRBC are actually the species inhabiting in the Tuz Lake and some of them have been supported by the data obtained with DRBC17. For example, *A. potronii* and *A. amstelodami* (previously known as *Eurotium amstelodami*) have only been isolated on DRBC17 medium. *A. potronii* seems to be an external contamination because of its low frequency of occurrence (<10%). However, the growth of *Acremonium* species in DRBC17 medium and the fact that it has been previously reported to be a natural members of hypersaline waters reveal that it is not an external contamination (Cantrell and Baez-Félix, 2010; Cantrell et al., 2013).

Aspergillus, *Penicillium* and *Talaromyces* are diverse genera which belong to the Order *Eurotiales* and contain a large number of species possessing a worldwide distribution and a huge range of ecological habitats. Some members are able to grow in extreme environments such as those with high/low temperatures, high salt/sugar concentrations, low acidities or low oxygen levels (Tsang et al., 2018). *Aspergillus* section *Restricti* together with sister section *Aspergillus* (formerly *Eurotium*) comprises xerophilic species, that are able to grow on substrates with low water activity and in extreme environments (Sklenář et al., 2017). Butinar et al. (2005b) have reported that *A. amstelodami* was detected most consistently, at salinities above 17% NaCl. The fact that

A. amstelodami was isolated in high frequency (> 20%) using DRBC17 (a_w 0.89) medium rather than in salt-free DRBC medium according to the results of present study supports its salt requirement or low water activity demand. In addition, the same study has reported that *A. repens* (formerly *Eurotium repens*) and *A. herbariorum* (formerly *E. herbariorum*), *A. rubrum* (formerly *E. rubrum*), *A. chevalieri* (formerly *E. chevalieri*) have repeatedly been isolated in a mycodiversity study of hypersaline waters (Butinar, et al., 2005b). *A. chevalieri* and *A. repens* have been isolated from Tuz Lake with both media but *A. repens* have been found to have higher frequency (> 20%) and abundance (21.23%) in DRBC17 than in DRBC medium. As seen in *A. amstelodami* and *A. repens*, the use of selective media with high salt concentration or low water activity (17%, a_w 0.89) in fungal isolations is effective in detecting fungal diversity.



Penicillium (36.35%), *Cladosporium* (30.98%), *Aspergillus* (9.94%), and *Alternaria* (9.26%)

Aspergillus (43.25%), *Penicillium* (28.97%), *Cladosporium* (18.85%), and *Alternaria* (2.38%)

Figure 4. The abundances of dominant fungal taxa isolated from Tuz Lake on DRBC and DRBC17.

Other *Aspergillus* species isolated from Tuz Lake salterns and lake water have been *A. alliaceus, A. flavus, A. nidulans* (formerly *Emericella nidulans*), *A. neoniveus, A. penicillioides*. Among them, *A. flavus* has same frequency of occurrence in both medium while it has been isolated at higher abundance (9.52%) in the medium with low water activity (DRBC17). The others have formed only a small number of colonies on DRBC medium or on both media at low frequency (Table 2). *A. penicillioides* has been found only in autumn with low frequency of occurrence and rarity (0.11% on DRBC and 1.98% on DRBC17). It is a true halophile, present in polyhaline systems, and in different geographical locations (Nazareth and Gonsalves, 2014a). It has been found in diverse habitats with low a_w, such as the Dead Sea, solar salterns, mangroves, estuary (Ali et al., 2013; Gonsalves et al., 2012; Nayak et al., 2012; Nazareth and Gonsalves, 2014b).

Table 3. Diversity parameters of the microfungi isolated from water samples on DRBC and DRBC17 media.

		STATION NAME					
	Indices	**KAY-SW**	**KAY-LW**	**KAL-SW**	**KAL-LW**	**YAV-SW**	**YAV-LW**
DRBC	**(*S*)**	31	27	32	29	27	26
	(*n*)	337	366	270	416	223	244
	Simpson	0.901	0.890	0.879	0.856	0.877	0.824
	Shannon	2.721	2.656	2.680	2.503	2.500	2.367
	Evenness	0.49	0.53	0.46	0.42	0.45	0.41
DRBC17	**(*S*)**	14	11	7	17	7	10
	(*n*)	168	141	59	74	24	36
	Simpson	0.865	0.792	0.653	0.908	0.816	0.838
	Shannon	2.221	1.843	1.370	2.558	1.813	2.038
	Evenness	0.66	0.57	0.56	0.76	0.88	0.77

(*S*): Number of Taxa, (*n*) Total number of Individuals, KAY: Kayacık, KAL:Kaldırım, YAV:Yavşan Salterns, SW: Saltpan Water, LW: Lake Water

In addition to *Aspergillus* spp., the *Penicillium* species have been isolated most frequently from diverse polar ecological niches to various saline environments (Gunde-Cimerman et al., 2005). In this study, fifteen different species of the genus *Penicillium* have been identified by being isolated from saltpans and Tuz Lake water samples. Among them, *P. atrovenetum, P. brevicompactum, P. citrinum, P. commune, P. solitum, P. granulatum, P. hordei, P. nalgiovense, P. polonicum, P. raistrickii,* and *P. spinulosum* have been isolated in both media. The other species have developed only a small number of colonies on DRBC medium at low frequency (Table 2). *P. decumbens* has been isolated only in DRBC at high frequency of occurrence (> 20%) and abundance (2.66%). Among the species with high frequency of occurrence (> 20%), *P. commune* (9. %), *P. polonicum* (7.71%) and *P. citrinum* (7.50%) have been the leading species in terms of abundance. Most of *Penicillium* species isolated from Tuz Lake, primarily *P. brevicompactum, P. citrinum, P. solitum* and *P. decumbens* have been reported in various saline environments (Cantrell et al., 2006; Gonsalves et al., 2012; Gunde-Cimerman et al., 2009).

An important group of extremophilic fungi within *Capnodiales* are members of the genus *Cladosporium*. The halophilic and halotolerant mycobiota from hypersaline aqueous habitats worldwide frequently contain these fungi (Butinar et al., 2005a; Gunde-Cimerman et al., 2000; Zalar et al., 2007). Five species belonging to the genus *Cladosporium*, which are reported as dominant representatives of the hypersaline environments, have been isolated from the Tuz Lake. *C. cladosporioides, C. sphaerospermum,* and *C. herbarum* have been found as prominent species in terms of frequency of occurrence (>20% for all) and abundance (15.05%, 4.51%, 4.35% respectively) in DRBC medium (Table 2). In contrast to the salt-free medium (DRBC), the abundance of *C. sphaerospermum* (9.72%) in the medium containing 17% salt (DRBC17) has been higher than that of *C. cladosporioides*. This is noteworthy, Hocking et al. (1994) have reported that *C. cladosporioides* and *C. sphaerospermum* have germinated at a minimum a_w of 0.815. *C. sphaerospermum* have also grown at this a_w, but germinating conidia of *C. cladosporioides* have not produce microcolonies. Although it has been reported from various habitats including osmotically stress-free niches, this apparent osmotolerance supports that *C. spherospermum* prefers osmotic stressed environments and therefore, it could be inferred that it is a permanent member of the hypersaline environments. A similar situation could be said for *C. ossifragi*, although its frequency of occurrence and relative abundance in DRBC (<10-20%, 5.98% respectively) and DRBC17 medium (<10%, 6.75% respectively) are not high.

Three of the eight *Alternaria* spp. isolated in this study, *A. alternata, A. infectoria,* and *A. tenuissima*, have grown on both media. Although their relative abundance and frequency of occurrence are lower, the growth of these three species in DRBC17 is a sign that they have a tolerance against high salt concentrations. *A. alternata* is the most frequently isolated species from hypersaline environmens (Gunde-Cimerman et al., 2005; Özgök and İlhan, 2020) and it has been discovered in various saline environments including in hypersaline stream water in România (Diguță et al., 2018). In addition to *Alternaria* members, *Chalastospora gosspii* and *Stemphylium solani* isolated from water samples within this study belong to the three genera included in the *Alternaria* complex (Woudenberg et al., 2013). They have a low frequency and are rare species in water samples of Tuz Lake.

Diversity index values showed that fungal diversity is high in Tuz Lake saltpans and lake water and species that cause diversity between stations were quite different species.

In this study, we have determined the fungus species found in the Tuz Lake water and salterns which represents one of the extreme environments believed to be too harsh for the growth of fungi. Our results support the fact that such organisms can survive in extreme environments with low water activity, high salt concentration, and UV light from the sun, and the results obtained in this study generally comply with those of similar studies of fungi isolated from different hypersaline environments.

This study has showed that the mycobiota of the Tuz Lake has a high diversity and that the majority of the isolated species are permanent members of the saline environments. It has been found out that the adapted species to the hypersaline environments belonged to the genus *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria*. The species highly represented in the mycobiota of the Tuz Lake are *P. citrinum, P. polonicum, P. atrovenetum, P. commune, A. repens, A. amstelodami, A. flavus, C. sphaerospermum, C. cladosporioides,* and *A. alternata.*

Some of the filamentous fungi species which contaminated by environmental sources constitute the temporary inhabitants of Tuz Gölü and they survive for short periods in salt water. On the other hand, it is becoming increasingly clear that Tuz Lake has an indigenous mycobiota that has adapted to long-term survival and possibly even vegetative growth under specific conditions. The species that are isolated repeatedly in every season and in different locations, in other words, are the species with a high spatiotemporal distribution and adapted best to the harsh conditions of the lake. Studies on salt tolerance and genetic structure on these species will contribute to the understanding of true inhabitants.

The fungal diversity of hypersaline environments could be best studied with a combination of traditional and modern techniques. Therefore, in the identification of fungal isolates obtained with culture-dependent approach, the use of molecular techniques has been complementary in cases where the traditional methods are insufficient. Furthermore, it has been revealed that the use of selective media with different salt concentration in fungal isolations is effective in detecting fungal diversity.

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