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Communities of Halotolerant Micromycetes from the Areas of Natural Salinity

E. V. Smolyanyuk¹ and E. N. Bilanenko

Moscow State University, Moscow, 119991 Russia

Abstract—The halophilic community of natural hypersaline soils has been isolated. Species of the genera *Penicillium*, *Aspergillus*, *Cladosporium*, as well as dark-colored yeast-like organisms, are most frequently isolated on selective media from all saline soil samples that we have studied. It has been shown that the community of micromycetes of hypersaline habitats is less dependent on geographical position than on the physico-chemical parameters of habitat.

Keywords: saline soil, halophilic and halotolerant micromycetes.

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Habitats with high and extremely high sodium chloride concentrations are represented in nature by hypersaline water bodies, saline arid soils (salt marshes, solonchaks, etc.), and places of natural salting. High salt concentrations in the environment cause stress associated with low water availability. The prokaryotic community of hypersaline habitats has been studied in detail [1–4], whereas fungi have been studied in food microbiology as contaminants of salt-preserved foods. The term “halophilic” implies that an organism demands significant NaCl concentrations (1.0–1.5 M) for intensive growth, while halotolerants can grow on the media with high salt concentrations but do not need them for development [5]. Halotolerant organisms grow better at NaCl concentrations in the medium below 0.2 M. Intensive research into biodiversity, peculiarities of physiology, and mechanisms of adaptation of halotolerant and halophilic fungi of natural hypersaline habitats has been carried out in the recent decade [6–12]. By now it is known that halotolerance is widespread within the orders *Capnodiales*, *Dothideales*, *Eurotiales* (*Ascomycota*), and *Wallemiales* (*Basidiomycota*), whereas the fungal community of natural hypersaline habitats is limited by a few genera. These are dark-pigmented yeast-like fungi *Hortaea werneckii*, *Phaeothea triangularis*, *Trimmatostroma salinum* and halotolerant strains of *Aureobasidium pullulans*, species of the large genera *Aspergillus* and *Penicillium*, dark-colored species of the genera *Cladosporium*, *Scopulariopsis*, *Alternaria*; among basidiomycetes, this is the genus *Wallemia* [8, 9].

MATERIALS AND METHODS

Samples were taken in three geographical points in areas of natural salinity. Lake Pomorie is located near the town of Pomorie in Bulgaria (42°33'24"N, 27°38'43"E). Water salinity in the Pomorie lagoon increases to more than 80‰; its shore is characterized by formation of natural hypersaline soils with near-neutral pH. The Dead Sea (Israel) is one of the saltiest lakes on Earth (salinity ~340‰). The activity of undiluted Dead Sea water is about 0.669 [13]. Samples were taken not far from the town of Ein Bokek. Also samples were taken quite near the water of Lake Baskunchak (48°10'N and 46°53'E). Its salinity is about 300‰. Samples were taken quite near the water edge from the upper soil horizon (1–5 cm deep) with a sterile rod, put into sterile closed vials, and kept in a refrigerator at 8–10°C until the analysis.

The method of soil extracts was used to determine the concentrations of the most important ions in soil samples (Table 1). The samples were analyzed by the method of dilutions. Soils samples from the Black Sea coast of Bulgaria were cultured in three repeats from 1 : 10, 1 : 100 and 1 : 1000 dilutions in the following nutrient media: wort agar (WA, pH 5.5), wort agar with 5, 10, 15, and 20% NaCl (pH 5.5), and alkaline agar (AA, pH 10.5). Other samples were cultured on the selective nutrient media for isolation of halotolerant micromycetes: wort agar with 5, 10, 15, and 20% NaCl (pH 5.5). Petri dishes were incubated at 25°C for two months. Pure cultures were isolated in test tubes with wort agar.

Identification was performed by the morphological and cultural characters using domestic and foreign markers [14–25].

General frequency was calculated as a ratio of the number of samples, where a species (genus) occurs (when isolated in all types of media), to the total num-

¹ Corresponding author; e-mail: esmol@yandex.ru

Table 1. Physicochemical parameters of soil samples

Index	Ca ²⁺ , %	Mg ²⁺ , %	K ⁺ , %	Na ⁺ , %	OH ⁻ , %	SO ₄ ²⁻ , %	Cl ⁻ , %	pH
1*	0.25	0.22	0.1	2.14	0.03	1.02	2.38	8.81
2*	0.08	0.17	0.03	0.18	0.01	0.02	0.65	8.54
3*	0.23	0.24	0.1	1.01	0.01	0.06	29.87	10.6

Note: 1*, Black Sea coast of Bulgaria; 2*, Dead Sea shore; 3*, Lake Baskunchak shore.

ber of samples under study expressed in percentage. Specific abundance was the number of diaspores of a given species (genus) expressed in percentage of the total number of diaspores.

RESULTS AND DISCUSSION

Seventy six micromycete isolates belonging to 39 species (Table 2) were isolated from saline soil samples taken in places of natural salt-mining on the Pomorie Lake shore. The phylum *Ascomycota* is represented by 3 species: *Chaetomium atrobrunneum*, *Sordaria fimicola*, and *Sporormiella intermedia*. The phylum *Basidiomycota* is represented by yeasts of the genus *Rhodospiridium* and by sterile mycelia. Other isolates belonged to the anamorphic species of ascomycetes. More than one third (13 species) of the anamorphic species are of the class *Dothideomycetes*. In addition, 9 light-colored and 8 dark-colored sterile mycelia were isolated. The species *Alternaria alternata*, *Cladosporium halotolerans*, *Penicillium aurantio-*

griseum, and *P. chrysogenum* demonstrate the highest frequency of occurrence (Table 2, Fig. 1).

Twenty four isolates obtained on selective media with high NaCl concentrations (10, 15, 20%) were referred to 15 species: *Aspergillus halophilicus*, *A. repens*, *Camarosporium obiones*, *Cladosporium halotolerans*, *C. salinae*, *Monodictys levis*, *M. paradoxa*, *Paecilomyces* sp., *Penicillium aurantiogriseum*, *P. thomii*, *Phaeotheca triangularis*, *Scolecobasidium salinum*, *Trimmatostroma salinum*, *Mycelia sterilia* (dark-colored), and *M. sterilia* (light-colored). The highest values of specific abundance index during the growth on the above media were demonstrated by the following species: *C. halotolerans*, *M. sterilia* (dark-colored), and *P. triangularis* (Fig. 4a). Only some of them were shown to grow on the nutrient medium containing 20% NaCl: *C. halotolerans*, *P. chrysogenum*, *T. salinum*, *P. triangularis*, and some representatives of the *M. sterilia* group.

Eight species of micromycetes were isolated on selective media from soil samples taken near the town of Ein Bokek (Israel) on the Dead Sea shore (Table 3).

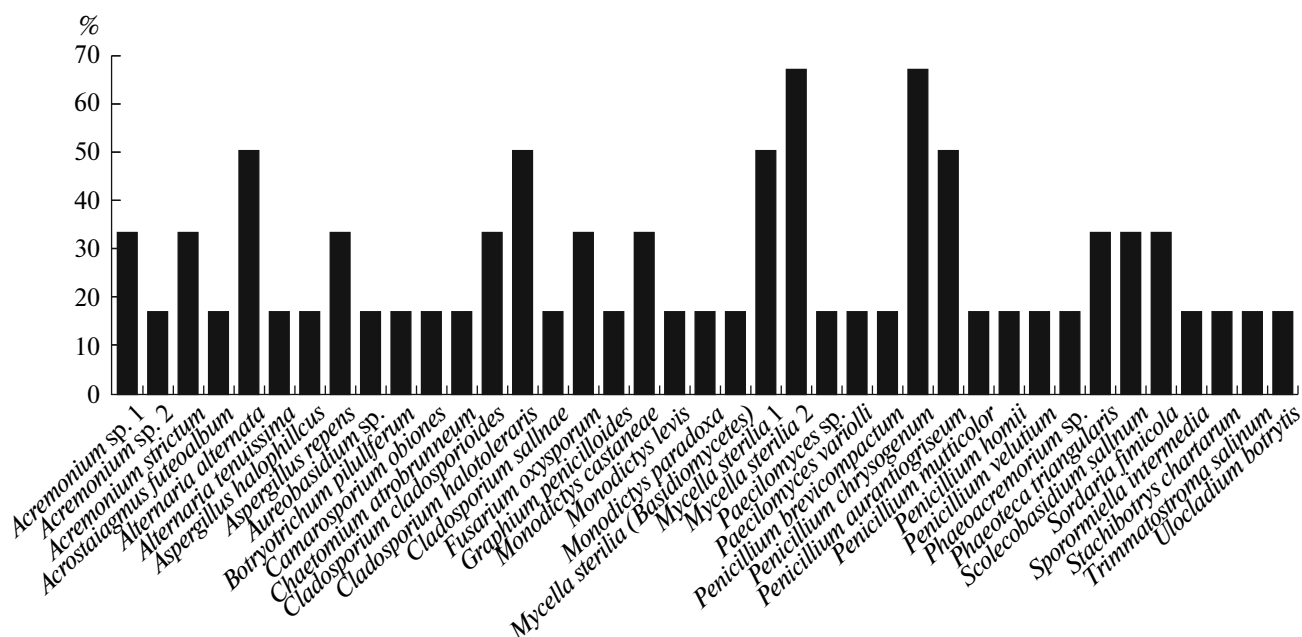


Fig. 1. The Frequency of occurrence of micromycetous species isolated from soil samples taken on the Black Sea coast of Bulgaria.

Table 2. Fungal species isolated from soil samples taken on the Black Sea coast of Bulgaria, their occurrence and specific abundance indices

Species	Total frequency of occurrence, %	Specific abundance, %					
		1*	2*	3*	4*	5*	6*
<i>Acremonium</i> sp. 1	33.33		2.96		2.47		
<i>Acremonium</i> sp. 2	16.67		2.63	0.33			
<i>Acremonium strictum</i> W. Gams	33.33	1.15	6.25				
<i>Acrostalagmus luteoalbus</i> (Link) Zare, W. Gams et Schroers	16.67			0.49			
<i>Alternaria alternata</i> (Fr.) Keissl	50.00		0.49	0.99			
<i>Alternaria tenuissima</i> (Kunze) Wiltshire	16.67		1.97				
<i>Aspergillus halophilicus</i> M. Chr., Papav. et C.R. Benj	16.67				0.16		
<i>Aspergillus repens</i> (Corda) Sacc.	33.33				0.33	0.66	
<i>Aureobasidium</i> sp.	16.67		0.49				
<i>Botryotrichum piluliferum</i> Sacc. et Marchal	16.67	0.49	0.49				
<i>Camarosporium obiones</i> Jaap	16.67				0.33		
<i>Chaetomium atrobrunneum</i> L.M. Ames	16.67		0.16				
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	33.33		0.99	0.66			
<i>Cladosporium halotolerans</i> Zalar, de Hoog, Gunde-Cim	50.00	0.49			5.76	0.33	0.66
<i>Cladosporium salinae</i> Zalar, de Hoog et Gunde-Cim.	16.67					1.64	
<i>Fusarium oxysporum</i> Schltdl.	33.33			2.47			
<i>Graphium penicillioides</i> Corda	16.67	0.16					
<i>Monodictys castaneae</i> (Wallr.) S. Hughes	33.33		0.82				
<i>Monodictys levis</i> (Wiltshire) S. Hughes	16.67		0.33		1.97		
<i>Monodictys paradoxa</i> (Corda) S. Hughes	16.67		2.30		0.99		
<i>Mycelia sterilia</i> (Basidiomycetes)	16.67	1.64	0.16				
<i>Mycelia sterilia</i> 1**	50.00	0.16	0.82	4.77	3.45	0.66	1.15
<i>Mycelia sterilia</i> 2**	66.67	0.82	4.28	6.09	2.47		
<i>Paecilomyces</i> sp.	16.67				0.16		
<i>Paecilomyces variotii</i> Bainier	16.67			0.33			
<i>Penicillium aurantiogriseum</i> Westling	50.00	0.16	5.77	4.76	0.49		
<i>Penicillium brevicompactum</i> Dierckx	16.67			0.82			
<i>Penicillium multicolor</i> Grig.-Man. et Porad.	16.67		0.16				
<i>Penicillium thomii</i> Maire	16.67		0.66		0.33		
<i>Penicillium velutinum</i> Terui et Shibas.	16.67		0.16				
<i>Phaeoacremonium</i> sp.	16.67			0.16			
<i>Phaeotheca triangularis</i> de Hoog et Beguin	33.33					5.76	0.99
<i>Rhodospiridium</i> sp.	33.33	5.76					
<i>Scolecobasidium salinum</i> (G.K. Sutherl.)	33.33			5.76	0.99		
<i>Sordaria fimicola</i> (Roberge ex Desm.) Ces. et De Not.	33.33	0.16	0.82				
<i>Sporormiella intermedia</i> (Auerswald) S.I. Ahmed et Cain ex Kobayasi	16.67		0.99				
<i>Stachybotrys chartarum</i> (Ehrenb.) S. Hughes	16.67	0.16	0.66				
<i>Trimmatostroma salinum</i> Zalar, De Hoog et Gunde-Cimerman	16.67					1.15	1.48
<i>Ulocladium botrytis</i> Preuss	16.67	0.33					
Total number of species		12	22	12	13	6	4

Note: 1*, alkaline agar; 2*, wort agar; 3*, wort agar with 5% NaCl; 4* wort agar with 10% NaCl; 5*, wort agar with 15% NaCl; 6*, wort agar with 20% NaCl; 1**, light-colored; 2**, dark-colored.

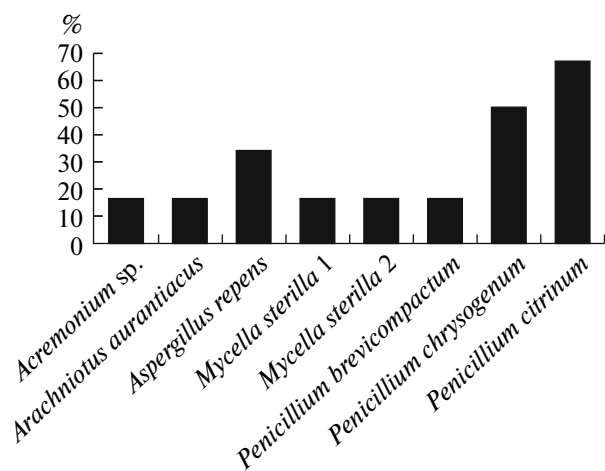


Fig. 2. The Frequency of occurrence of micromycetous species isolated from soil samples taken on the Dead Sea shore.

The phylum *Ascomycota* was represented by a single species: *Arachniotus aurantiacus*. Other isolates belonged to the anamorphic species of ascomycetes, three of them being representatives of the genus *Penicillium*. The species *P. chrysogenum*, *A. aurantiacus*, *P. citrinum*, and *Aspergillus repens* have the highest specific abundance index. At the same time, the index of specific abundance for *P. chrysogenum* is 72.5% (Fig. 4b). Occurrence is maximal for the species *A. repens*, *P. chrysogenum*, and *P. citrinum* (Table 3, Fig. 2).

Fifty-four strains were isolated on selective media from soil samples taken on the Lake Baskunchak shore and referred to 14 anamorphic species of the following genera: *Aspergillus*, *Penicillium*, *Ulocladium*. Only

4 species were able to grow on the nutrient medium with 20% NaCl: *A. repens*, *P. lanosum*, *P. miczynskii*, and *P. rugulosum* (Table 4). The following species of the genus *Penicillium* demonstrate the highest values of specific abundance index: *P. dierckxii*, *P. miczynskii*, *P. oxalicum*, *P. rugulosum*, and *P. sp.* (Fig. 4c). The following species of the genus *Penicillium* also demonstrate the highest values of occurrence index: *P. dierckxii*, *P. oxalicum*, *P. rugulosum*, *Penicillium sp.*; for *P. dierckxii* and *P. rugulosum*, these values are 100% (Table 4, Fig. 3).

Natural hypersaline water bodies may have different conditions depending on the anion and cation composition [5]. The analyzed samples had pH values of 8.5 to 10.6 (Table 1). Analysis of the samples from the Lake Pomorie shore showed that most of the species (22) were isolated on the standard CA medium, 12 species were isolated on AA, 6 to 13 species were isolated on the media with NaCl concentrations of 5–15%, and only 4 species were isolated on the medium with 20% NaCl (Table 2). It seems that the community of micromycetes includes the primordial fungal species with different resistance to high salt concentrations and pH values.

The objective of this research was to isolate a community of halotolerant fungal species; therefore, further isolation was carried out on the media with NaCl concentrations of 10–20%. The species most frequently isolated on selective media from all studied samples of saline soils belong to the genera *Penicillium* (*P. rugulosum*, *P. miczynskii*, *P. citrinum*, *P. lanosum*), *Aspergillus* (mainly from the *Glaucus* group), *Cladosporium* (*C. halotolerans*, *C. salinae*), and dark-colored yeast-like species *Phaeotheca triangularis* and *Trimmatostroma salinum*. Such species composition of saline soils and water bodies in different regions was

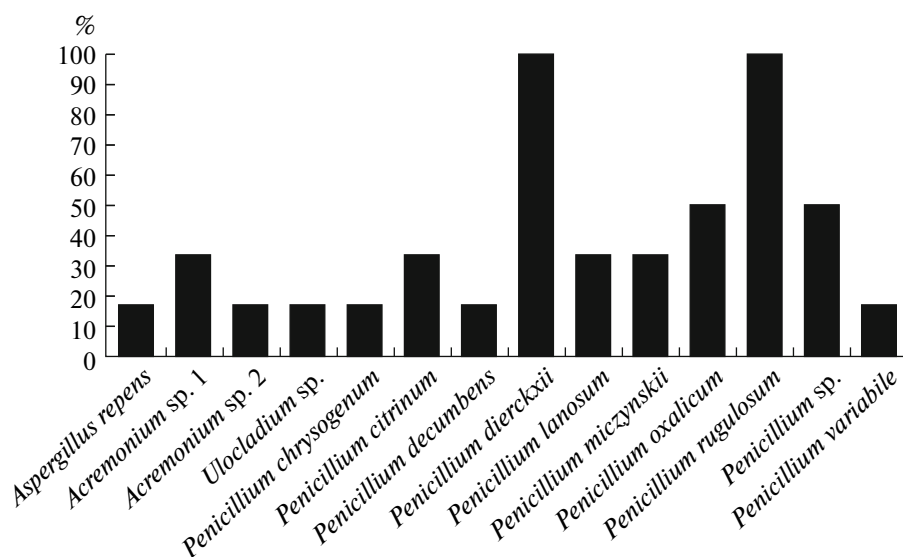


Fig. 3. The Frequency of occurrence of micromycetous species isolated from soil samples taken on the Lake Baskunchak shore.

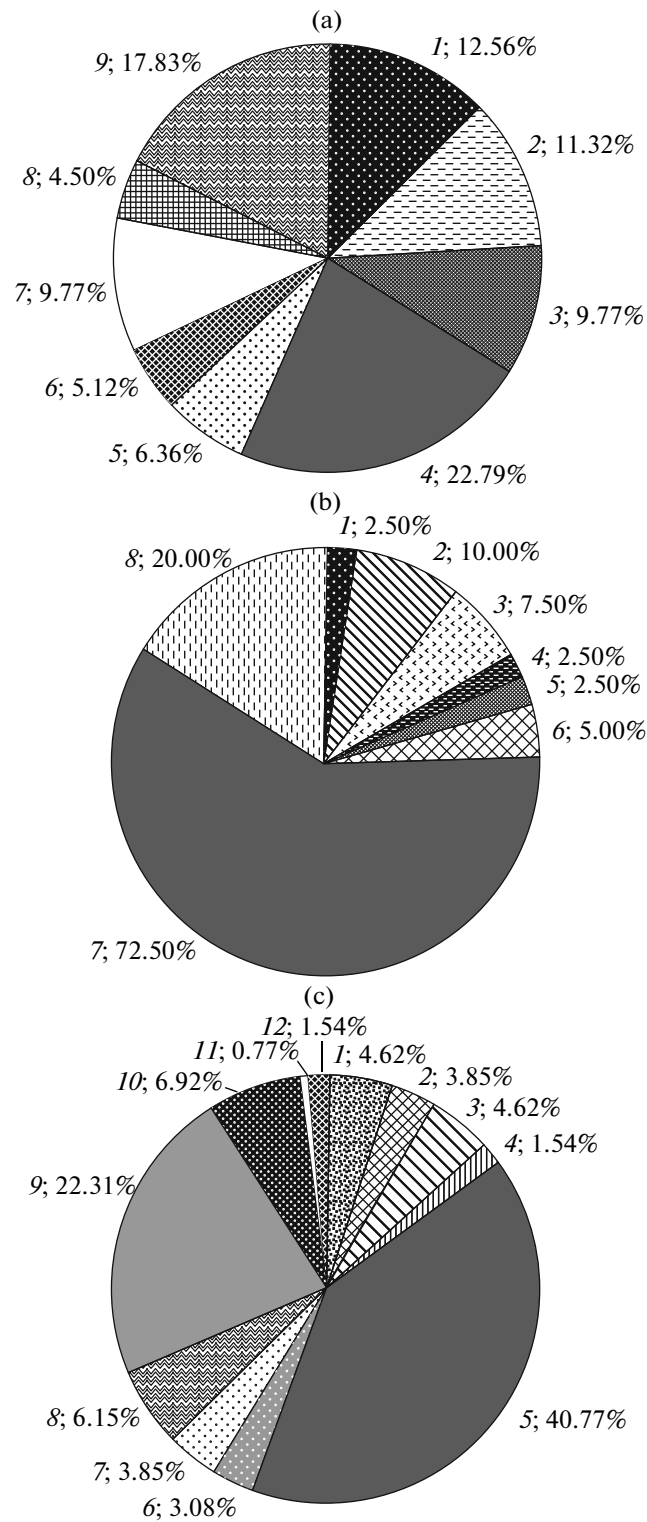


Fig. 4. Specific abundance indices of the species and genera of micromycetes isolated on selective media from soils samples: (a) taken on the Black Sea coast of Bulgaria: 1, *Acremonium* sp.; 2, *Mycelia sterilia* 1; 3, *Mycelia sterilia* 2; 4, *Penicillium* sp.; 5, *Scolecobasidium salinum*; 6, *Alternaria* sp.; 7, *Cladosporium* sp.; 8, *Phaeotheca triangularis*; 9, other; (b) taken on the Dead Sea shore: 1, *Acremonium* sp.; 2, *Arachniotus aurantiacus*; 3, *Aspergillus repens*; 4, *Mycelia sterilia* 1; 5, *Mycelia sterilia* 2; 6, *Penicillium brevicompactum*; 7, *Penicillium citrinum*; (c) taken on the Lake Baskunchak shore: 1, *Aspergillus* sp.; 2, *Penicillium chrysogenum*; 3, *P. citrinum*; 4, *P. decumbens*; 5, *P. dierckxii*; 6, *P. lanosum*; 7, *P. miczynskii*; 8, *P. oxalicum*; 9, *P. rugulosum*; 10, *Penicillium* sp.; 11, *P. variable*; 12, *Ulocladium* sp.

Table 3. The species isolated from soil samples taken on the Dead Sea shore, their specific abundance and occurrence indices

Species	Total frequency of occurrence, %	Specific abundance, %		
		1*	2*	3*
<i>Acremonium</i> sp.	16.67	2.00		
<i>Arachniotus aurantiacus</i> (Kamyschko) Arx	16.67	8.00		
<i>Aspergillus repens</i> (Corda) Sacc.	33.33	8.00		
<i>Mycelia sterilia</i> 1**	16.67	2.00		
<i>Mycelia sterilia</i> 2**	16.67	2.00		
<i>Penicillium brevicompactum</i> Dierckx	16.67	4.00		
<i>Penicillium chrysogenum</i> Thom	50.00	58.00		
<i>Penicillium citrinum</i> Thom	66.67	14.00	2.00	
Total number of species		8	1	0

Note: 1*, wort agar with 10% NaCl; 2*, wort agar with 15% NaCl; 3*, wort agar with 20% NaCl; 1**, light-colored; 2**, dark-colored.

noted by researchers, which is evidence of the relative constancy of species composition irrespective of the geographical position of habitat [6, 8, 26–31]. Similar composition is demonstrated by the communities of

micromycetes from the surface of rocks [32–34], arid and permafrost soils, glaciers [7, 35, 36], and saline waters [31]. All of these habitats are characterized by the ionic and osmotic stresses associated with low water availability.

Thus, the community of micromycetes of hypersaline habitats is less dependent on geographical position than on the physicochemical parameters of habitat and has many species in common with the fungal communities of other habitats with low water activity indices.

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Table 4. The species isolated from soil samples taken on the Lake Baskunchak shore, their specific abundance and occurrence indices

Species	Total frequency of occurrence, %	Specific abundance, %		
		1*	2*	3*
<i>Aspergillus repens</i> (Corda) Sacc.	16.67	1.57		
<i>Aspergillus</i> sp. 1	33.33	1.57		
<i>Aspergillus</i> sp. 2	16.67			1.57
<i>Penicillium chrysogenum</i> Thom	16.67	1.57		
<i>Penicillium citrinum</i> Thom	33.33		2.36	
<i>Penicillium decumbens</i> Thom	16.67		1.57	
<i>Penicillium dierckxii</i> Biourge	100.00	31.5	10.24	
<i>Penicillium lanosum</i> Westling	33.33		1.57	1.57
<i>Penicillium miczynskii</i> K.M. Zalessky	33.33		0.79	3.15
<i>Penicillium oxalicum</i> Currie et Thom	50.00	6.30	0.79	
<i>Penicillium rugulosum</i> Thom	100.00		0.79	22.05
<i>Penicillium</i> sp.	50.00	2.36	5.51	
<i>Penicillium variabile</i> Sopp	16.67	0.79		
<i>Ulocladium</i> sp.	50.00	2.36		
Total number of species		8	8	4

Note: 1*, alkaline agar; 2*, wort agar; 3*, wort agar with 5% NaCl; 4* wort agar with 10% NaCl; 5*, wort agar with 15% NaCl; 6*, wort agar with 20% NaCl.

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