

Investigation of the Microfungal Flora of the Bird Paradise National Park in Bandırma, Balıkesir (Turkey)

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Abstract: Fifteen soil samples taken from the Bird Paradise National Park in Bandırma were investigated using the soil dilution plate and soil washing methods. Thirty-three species and four different sterile microfungal taxa were identified. Twenty-eight of these species belonged to the Hyphomycetes and 5 to the Mucorales. The genus with the highest species diversity found in the study area was *Penicillium*, represented by 16 species. Some chemical properties of the soil samples were also established.

Quantitative analysis based on the soil dilution plate method revealed a statistically significant difference between soil samples subjected to the rising water level of the lake (21,500 microfungi propagules per gram oven-dried soil) and samples not influenced by fluctuating water levels (314,000 microfungi propagules).

Key Words: Soil, Microfungi, Bird Paradise, Turkey

Bandırma Kuş Cenneti İçinde Kalan Toprakların Mikrofungus Florası Üzerine Bir Araştırma

Özet: Bu araştırmada Bandırma Kuş Cenneti Milli Park alanından alınan 15 toprak örneğinin toprağı sulandırma ve toprağı yıkama metodları ile incelenmiş ve 33 ayrı tür ve varyete ile 4 farklı steril mikrofungus elde edilmiştir. Ayrıca toprakların bazı kimyasal özellikleri de belirlenmiştir. Elde edilen taksonlardan 28 tanesi Hyphomycetes, 5 tanesi ise Mucorales takımlarına aittir. Tür sayısı bakımından en zengin takson *Penicillium*'dur.

Kantitatif analiz sonucu toprağı sulandırma metoduna göre 1g fırın kuru toprağa karşılık gelen taze toprakta göl sularının mevsimsel yükselmesinden fazla etkilenen topraklarda 21500 , suların fazla etkilenmeyen topraklarda ise ortalama 314000 mikrofungus propagülü elde edilmiştir. Bu farklılık istatistiksel olarak önemli bulunmuştur.

Anahtar Sözcükler: Toprak, mikrofungus, Kuş Cenneti, Türkiye

Description of Study Area

Situated on the north-eastern shore of Kuşgölü (Bird Lake), about 18 km from the Bandırma district of Balıkesir province, Bird Paradise National Park is of significant environmental interest to the scientific community in Turkey and abroad. Bird Paradise was originally designated a National Park because of its crucial role in sheltering vast bird populations. Despite its relatively small size (64 hectares), this area acts as a refuge for 2-3 million birds, representing to date 258 different species. As a consequence of this massive natural diversity and proper conservation, the park was awarded a Class A European Diploma by the Council of Europe on March 15, 1976. This was renewed on a five-year basis in 1981, 1986, 1991 and 1996 (1), until being suspended in 2001.

The study area is located in Lake Manyas, at 37° 27'N latitude, 32° 10'E longitude. Lake Manyas is situated

south-east of the Sea of Marmara and west of the city of Bursa. Administratively, the lake is located within the borders of Bandırma and Manyas, both of which form part of Balıkesir province (Figure 1).

Introduction

Environmental pollution is an important problem that can profoundly influence the biology of soil microorganisms, as well as of all living organisms. Since soil microfungi play an important role in the decomposition of organic matter, pollution can have detrimental implications for soil fertility and eventually alter the ecological balance.

In Turkey, as well as in many other industrialised regions of the world, industrial plants do not generally address, let alone solve, the potential problems resulting from unsatisfactory sewage treatment. In many cases

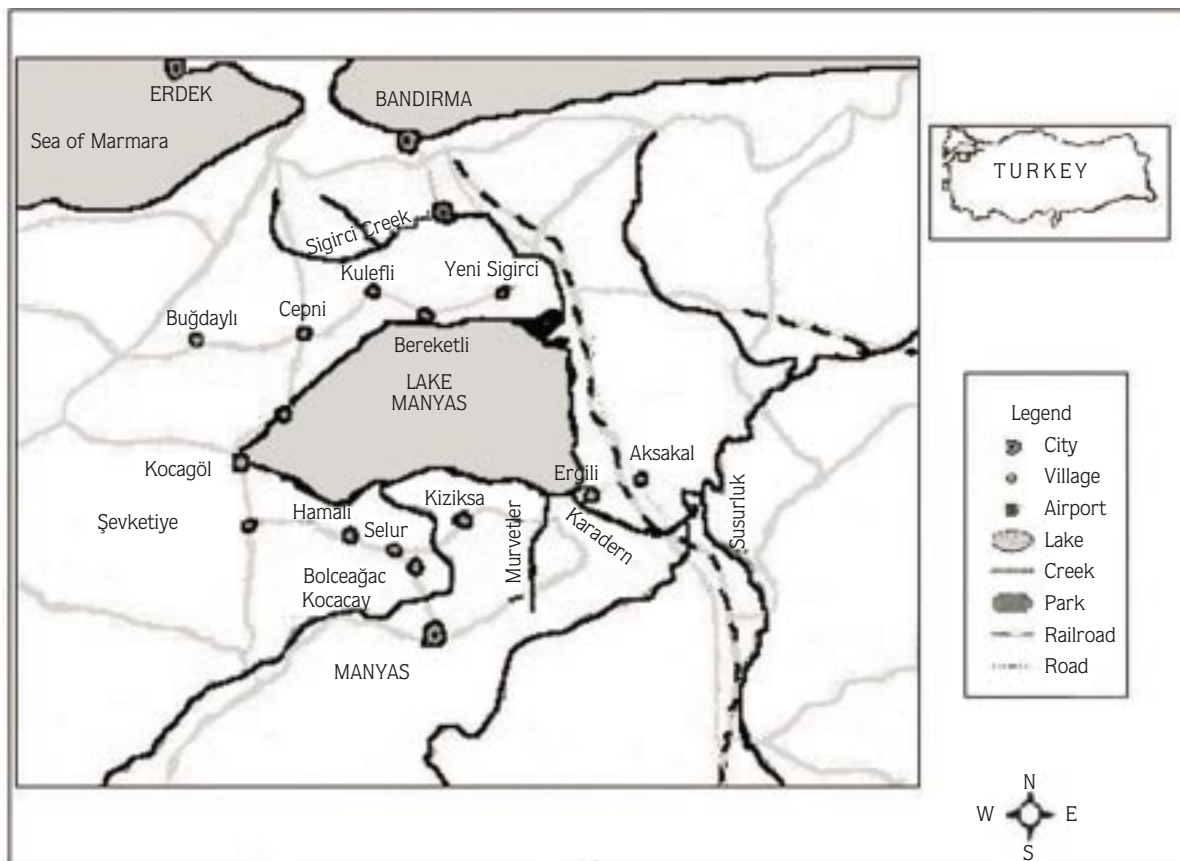


Fig. 1. Map of study area.

water sources and soils in the immediate vicinity of such industries are heavily polluted and the ecological balance is irrecoverably destroyed.

The negative effects of Lake Manyas on Bird Paradise include water level fluctuations and lake pollution. Water quantities and levels in Lake Manyas are both particularly important for Bird Paradise, and maintaining the right balance between these parameters is crucial for the flora and fauna of the district. For this reason, the drainage of excessive water in Lake Manyas without causing flooding or disturbing the hydrological balance between ingoing and outgoing water has to be carefully monitored (Table 1) (1).

Studies on soil mycology in Turkey have primarily been concentrated on Northeast Anatolia (2-4) and western Anatolia (5- 13).

Many industries around Lake Manyas discharge their sewage into the lake via various creeks, Sığirci Creek in particular serving this purpose. Although several studies have addressed the pollution parameters in Lake Manyas

and their effects on the fauna and flora, as well as the influences of Lake Manyas on cultural structure and conservation (14-16), the soil microbiology in the river basin has not yet been the subject of research.

Table 1. Ideal monthly lake water levels for Bird Paradise required by General Directorate of National Parks, Hunting and Wild Life.

Month	Maximum (metre)	Average (metre)	Minimum (metre)
January	16.00	15.50	15.00
February	16.50	16.00	15.50
March	17.00	16.50	16.00
April	18.00	17.50	17.00
May	17.25	16.75	16.25
June	16.25	15.75	15.25
July	15.50	15.00	14.50
August	15.00	14.50	14.00

Materials and Methods

Soil samples were collected in September 2000. Prior to sampling the length of time during which the soil had been submerged was estimated by consulting local guides.

The stations from which samples were taken were randomly chosen. During sampling, a soil profile was first extracted and then the surface of the profile cleaned (17). Subsampling was achieved by slicing the sediment profile with a disinfected spatula into 10 cm depth horizons. The samples were stored in a large sterilised and cooled thermos flask during transportation to the laboratory. Samples were subsequently processed using the soil dilution plate (18) and soil washing methods (19). In applying the soil dilution plate method, the moisture content of a certain amount of soil was determined and fresh soil quantities corresponding to 25 g oven-dried soil calculated (20). Subsampling was attained by diluting the original samples to 1/10,000 of the initial concentration (21). Prior to the settling of organic matter and soil particles (22), 1 mL of these solutions was inoculated on ready-made Peptone Dextrose agar plates (23). A total of 10 petri dishes were prepared for each sample.

Twenty gram of fresh soil was placed in a glass funnel lined with muslin for isolation using the soil washing technique. The pore size of the muslin was 0.5 mm. The soil samples were first washed with 2 L of tap water and the outflow was collected in a funnel. The procedure was then repeated using 2 L of sterile water. After this treatment, the muslin and its contents were transferred into a sterile petri dish with the same water containing streptomycin. Organic particles floating on the surface of the water and the washed soil particles were extracted with a loop and forceps and transferred on to plates of Peptone Dextrose agar containing Rose bengal. These plates were incubated at 25 °C for ten days (24). In order to suppress bacterial growth and restrict the colony size, 30 mg/L streptomycin and 30 mg/L Rose bengal respectively were added to the isolation medium, (25).

The colonies that developed on the petri plates were carefully counted, and individual colonies were identified with the aid of a stereomicroscope and transferred to a separate agar plate. The isolates of the genera *Aspergillus* Mich ex Fr. and *Penicillium* Link ex Gray were transferred to Czapek Dox agar and Malt Extract agar, and the others to Malt Extract agar. Identification was undertaken

following the Smith method (1971) (26). For this purpose, pure colonies of isolates were obtained in Czapek Dox and Malt Extract agar. Developing colonies were regularly examined both macroscopically (developing degree of cultures, colour of colonies and changes in colour, colour of colony reverse and changes in colour, colour changes of medium, texture of colony surface, presence of odour, presence of exudates and if so the situation) and microscopically (habit of hifa and its combination, development of fructification, colour, dimension and form of fructification, details of structure and all details of spores) to mate the final identifications.

Identification of the isolates was carried out according to (27-37).

The lime content of the soil samples used in the research was determined to be CaCO₃ equivalent by a Scheibler calcimeter (38), the content of organic matter was determined by the Smith-Weldon method (39), (pH) 1:25 rate of soil and water mixture by pH meter, the phosphorus content by the molybdophosphoric blue colour method and the total nitrogen content by the micro Kjeldahl method (39), subjecting soil samples to a mixture of sulphuric acid and salt.

Average quantitative values obtained from an analysis of individual soil zones were statistically compared using the t-test (SPSS Inc.). Citations of the authorities presented were standardised according to Kirk and Ansell (1992) (40).

Discussion and Conclusion

One hundred and eighty-nine microfungi isolates were obtained by examining 15 soil samples taken from Bandirma's Bird Paradise National Park by the soil dilution plate and washing methods.

Thirty-seven different species representing 12 genera and four different sterile microfungi were identified. Thirty-two of the taxa belonged to the Hyphomycetes and the remaining five to the Mucorales. The genus with the highest species diversity found in the study area was *Penicillium*, represented by 16 species (Tables 2 and 3).

Considering that the number of microfungi propagules in 1 g oven-dried soil equivalent to fresh soil is on average 400,000, we may say that the soils in the study area are quantitatively quite poor in comparison to

Table 2. Numbers of colonies and isolates for individual genera.

	Soil Dilution Plate Method		Soil Washing Method	
	Colony Number		Isolate Number	
	A	B	A	B
MUCORALES				
<i>Absidia</i> van Tieghem	-	4	4	9
<i>Mucor</i> Mich ex Fr.	-	6	10	5
HYPHOMYCETES				
<i>Acremonium</i> Link ex Fr.	15	54	6	3
<i>Aspergillus</i> Mich ex Fr.	3	4	1	2
<i>Beauveria</i> Vuill.	7	-	-	-
<i>Cladosporium</i> Link ex Fr.; Link	2	23	-	-
<i>Fusarium</i> Link ex Fr.	3	58	-	-
<i>Glocladium</i> Corda	3	32	-	-
<i>Penicillium</i> Link ex Gray	54	830	11	5
<i>Phymatotrichopsis</i> Hennebert	7	-	1	-
<i>Trichoderma</i> Pers. ex Fr.	4	2	3	-
<i>Ulocladium</i> Preuss	-	1	-	-

A: The area which is more influenced by the fluctuating lake water.

B: The area which is less influenced by the fluctuating lake water.

fertile soils. This paucity is even more extreme in soils which are more influenced by the rising level of the lake waters. In these soils the microfungal abundance is significantly lower, averaging approximately 21,500 propagules per gram oven-dried soil equivalent to fresh soil. The fact that the soil is submerged by polluted water may inhibit aerobic microfungal growth. Conversely, soils not subjected to fluctuating water levels displayed much higher microfungal densities, averaging 314,000 propagules per gram oven-dried soil. Not many varieties of plants were observed in soils that had been exposed to water for a sustained period of time. On the other hand, the fact that willows and herbaceous plants are continually drying out is indicative of the potential problems in this area. In addition, *Phymatotrichopsis omnivora* Hennebert, a pathogen in living plant roots, was isolated in the same zone.

Another feature of this zone of the research area is that it is situated at a lower level than the rest of the national park. Consequently, organic particles are easily accumulated from the rising lake waters, a phenomenon substantiated by the high concentrations of organic matter usually found in this zone (3.0-5.0%) (41).

Another parameter is soil pH, which may affect the results of quantitative and qualitative analyses. In general, microfungi prefer acidic conditions for optimal growth (pH 5-6) (42). However, the pH measured in the sampled soils was over 7.5. Lime content, which effects in soil pH neutralisation, was usually low to medium-high. Nitrogen, which is essential for successful micro-organism growth, was high and phosphorus generally at a very high level (41) (Table 4).

Comparison with the tabulated results above demonstrates that the number of microfungal propagules in 1 g soil is lower in soils subjected to overlying polluted water, but that the values for soils that are influenced to a lesser extent by rising water levels generally relate well to those reported from other localities examined in Turkey. The actual number of species, however, is very low in both subjected and non-subjected zones which is conceivably a result of direct or indirect pollution effects. This can be interpreted as circumstantial evidence that the ecological conditions are damaged and that soil micro-organisms are thus influenced, both qualitatively and quantitatively.

Table 3. Numbers of colonies and isolates for all taxa.

	Soil Dilution Plate Method		Soil Washing Method	
	Colony Number		Isolate Number	
	A	B	A	B
MUCORALES				
<i>Absidia cylindrospora</i> Hagem	-	4	4	8
<i>Absidia repens</i> Tiegh.	-	-	-	1
<i>Mucor circinelloides</i> Tiegh.				
<i>Mucor hiemalis</i> f. <i>hiemalis</i> Wehmer	-	6	10	4
<i>Mucor</i> sp. 1	-	6	-	3
HYPHOMYCETES				
<i>Acremonium furcatum</i> Moreau & R. Moreau ex Gams	3	-	-	-
<i>Acremonium strictum</i> W. Gams	12	40	2	-
<i>Acremonium</i> sp. 1	-	-	3	3
<i>Acremonium</i> sp. 2	-	14	1	-
<i>Aspergillus niger</i> Tiegh.	1	4	1	2
<i>Aspergillus terricola</i> Marchal & E.J. Marchal	2	-	-	-
<i>Beauveria bassiana</i> (Bals.-Criv.) Vuill.	7	-	-	-
<i>Cladosporium cladosporoides</i> (Fresen) G. A. de Viries	2	4	-	-
<i>Cladosporium sphaerospermum</i> Penz.	-	19	-	-
<i>Fusarium</i> sp. 1	3	44	-	-
<i>Fusarium</i> sp. 2	-	14	-	-
<i>Giocladium roseum</i> Bainier	1	27	-	-
<i>Giocladium</i> sp. 1	2	5	-	-
<i>Penicillium brevicompactum</i> Dierckx	7	147	-	-
<i>P. canescens</i> Sopp	5	55	4	-
<i>P. chermesinum</i> Biourge	3	34	-	-
<i>P. chrysogenum</i> Thom	-	-	2	-
<i>P. citrinum</i> Thom	-	33	3	1
<i>P. clavigerum</i> Demelius	-	-	1	2
<i>P. corylophilum</i> Dierckx	-	-	-	1
<i>P. diversum</i> Raper & Fennel	-	93	-	-
<i>P. expansum</i> Link	2	119	1	1
<i>P. frequentans</i> Westling	11	-	-	-
<i>P. jenseni</i> Zalewski	1	76	-	-
<i>P. lanosum</i> Westling	3	-	-	-
<i>P. raciborskii</i> Zalesky	21	123	-	-
<i>P. steckii</i> Zalesky	1	-	-	-
<i>P. sublateritium</i> Biourge	-	3	-	-
<i>P. variable</i> Wehmer	-	147	-	-
<i>Phymatotrichopsis omnivora</i> Hennebert	7	-	1	-
<i>Trichoderma harzianum</i> Rifai	4	2	3	-
<i>Ulocladium atrum</i> Preuss	-	1	-	-
Sterile 1	1	2	1	2
Sterile 2	3	12	3	-
Sterile 3	1	7	2	-
Sterile 4	-	3	-	-

Soil sample	CaCO ₃ (%)	pH	Organic matter	N (%)	P (ppm)
1	1.23	7.70	3.513	0.218	137.3
2	1.07	7.61	3.252	0.345	163.2
3	0.08	7.06	2.862	0.325	124.3
4	6.23	7.59	2.082	0.227	93.39
5	11.64	7.86	2.212	0.167	32.81
6	4.42	7.46	5.074	0.406	118.1
7	4.10	7.43	2.602	0.579	69.89
8	10.09	7.83	2.732	0.246	88.20
9	6.56	7.82	2.082	0.114	21.52
10	0.49	7.86	3.122	0.190	23.80
11	1.72	7.78	3.252	0.261	68.67
12	0.65	7.56	2.797	0.256	71.11
13	3.77	7.75	3.773	0.231	18.31
14	3.94	7.91	3.383	0.271	25.48
15	3.53	7.75	3.643	0.395	79.96

Table 4. Chemical characteristics of the study area.

Table 5. Densities of microfungal propagules (1 gr oven-dried soil equivalent to fresh soil) obtained from previous soil mycology studies in Turkey are summarised below.

Author	Number of microfungal propagules obtained	Research area	Material
Hasenekoğlu (1982) (2)	134,600	Erzurum	Polluted soil around meat plant
Hasenekoğlu (1985) (43)	400,000	Sarıkamış/Kars	Grass and field soils
Hasenekoğlu and Azaz (1991) (3)	183,720	Sarıkamış/Kars	Clear-cut forest soil
	287,160	Sarıkamış/Kars	Non-clear-cut forest soil
Asan (1992) (12)	187,564	Edirne	Soils of Edirne province
Azaz and Hasenekoğlu (1999) (44)	41,300 (winter)	Artvin/ Murgul (Göktaş)	Soils around the copper factory, where higher plant flora were destroyed.
	129,750 (summer)		
	52,400 (winter)		
263,090 (summer)	Artvin/ Murgul (Göktaş)	Soils with bush flora	
	58,200 (winter)		
286,830 (summer)	Artvin/ Murgul (Göktaş)	Soils with tree flora	

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