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Oil Hydrocarbon Degradation Capability of Bacterial Strains Isolated from the Sapanca Lake, Turkey

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ABSTRACT

In this study, the analyses were carried out to determine oil-degradation capacity and oil-resistance levels of bacteria isolated from water surface (0-30 cm) of nine stations of the Sapanca Lake from September of 2008 to May of 2010. Twenty-seven bacteria species belonging three classes and six families total of eighty-five wild bacteria were identified and screened against crude oil with respect to Minimum Inhibitory Concentration (MIC). Bacterial isolates showing resistance against crude oil were chosen for Emulsification Index (E_{24}) test. Isolates displaying higher E_{24} values were selected for further degradation tests regarding pH and oil thickness values in experimental setups. Fifty bacterial strains of eighty-five isolates were recorded to be resistant against oil hydrocarbon. Positive reactions percentages of the isolates against crude oil were detected in variable ranges between 25% and 100%. The results of the emulsification index test for crude oil indicated that the isolates of S49-*Stenotrophomonas maltophila*, S50-*Aeromonas hydrophila*, S59-*E. coli*1, S38-*Aeromonas hydrophila* and S43-*Enterobacter cloaceae* have higher emulsification percentages. The results of the degradation test showed that isolate of S59-*E. coli*1 has higher degradation ability than all isolates tested. The bacteria screened against crude oil were detected to be sensitive against crude oil during the first year of the study. Presence of petroleum-resistant bacteria in subsequent tests was associated with the unexpected oil spill occurred in the Sapanca Lake at the time. Detected crude oil resistant bacteria isolates were stocked for a possible use in upcoming bioremediation related studies.

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Sapanca Gölü'nden İzole Edilen Bakteri Suşlarının Petrol Hidrokarbonlarını Parçalama Yetenekleri

ÖZET

Bu çalışmada, Eylül 2008 ile Mayıs 2010 tarihleri arasında Sapanca Gölü'nde belirlenen dokuz istasyonda yüzey sularından (0-30 cm) izole edilen bakterilerin petrol parçalama kapasiteleri ve direnç düzeylerini belirlemek amacıyla analizler yapılmıştır. Üç sınıf yedi familyaya ait yirmi yedi bakteri türünden oluşan toplam seksen beş doğal bakteri izolatı tanımlanmış ve bu izolatların hepsi Minimum İnhibitör Konsantrasyon (MİK) açısından ham petrole karşı taranmıştır. Emülsifikasyon indeksi (E_{24}) testi için ham petrole karşı direnç gösteren bakteri izolatları seçilmiştir. E_{24} testlerinde yüksek değerler gösteren izolatlar ileri parçalama testlerinde pH ve petrol katman kalınlığı değerleri kaydedilmek üzere seçilmiştir. Seksen beş izolattan elli tanesi petrol hidrokarbonuna karşı dirençli olarak kaydedilmiştir. Ham petrole karşı pozitif reaksiyon gösteren izolatların yüzdeleri %25 ile %100 arasında değişiklik göstermiştir. S49-*Stenotrophomonas maltophila*, S50-*Aeromonas hydrophila*, S59-*E. coli*1, S38-*Aeromonas hydrophila* and S43-*Enterobacter cloaceae* suşları en yüksek Emülsifikasyon İndeksi yüzdeleri gösteren izolatlar

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Anahtar Kelimeler
Ham petrol,
doğal bakteri izolatları,
minimum inhibisyon konsantrasyonu,
Sapanca Gölü

Araştırma Makalesi

olarak kaydedilmiştir. Parçalanma testinin sonuçları, S59-*E coli*1 izolatının test edilen tüm izolatlardan daha yüksek parçalama kabiliyetine sahip olduğunu göstermiştir. Çalışmanın ilk yılında yapılan ham petrole karşı dirençlilik testlerinde taranan bakterilerin tümünün duyarlı olduğu kaydedilmiştir. Daha sonraki testlerde petrole karşı direnç gösteren bakterilerin tespit edilmesi aynı dönemde Sapanca Gölü'nde meydana gelen beklenmedik petrol sızıntısı ile ilişkilendirilmiştir. Ham petrole dirençli izolatlar, aday bakteriler olarak belirlenmiş ve ileride olası biyoremediasyon çalışmalarında kullanılmak amacıyla stoklanmıştır.

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INTRODUCTION

The biogeochemical importance of bacteria in freshwater ecosystems was first recognized in 1940s, since this early recognition of the critical role of bacteria in regenerating and mobilizing nutrients in freshwater food webs, it has become clear that aquatic bacteria drive transformations and the cycling of most biologically active elements in these ecosystems (Cole, 1988; Cotner, 2002; Newton et al., 2011).

The fate of spilled oil in the aquatic environments depends on a number of factors such as weathering, evaporation, oxidation, biodegradation and emulsification. Oil pollutants in aquatic ecosystems are biodegraded primarily by bacteria, yeast, and fungi. Understanding the degradation capability of bacteria and selecting of the most suitable oil degrading bacteria are important for bioremediation process (Atlas, 1995; Çiftçi and Altuğ, 2010; Das and Chandran, 2011; Marchand *et al.* 2017). The possible uses of bacteria to obtain more efficiency for degradation of petroleum have become an important topic for bioremediation studies (Le-Petit and Barthelemy, 1968; Ezikpe et al., 2009).

Bacteria can develop tolerance and resistance against some compounds such as oil hydrocarbons, heavy metals and antibiotics, depending on environmental conditions in natural aquatic media. Due to the fact that bacteria play a crucial role in the fate of pollutants, biodegradation of petroleum hydrocarbons in aquatic environments by microorganisms is important (Farrington, 1980; Okoh, 2006).

Lake ecosystems are more sheltered water mass than marine ecosystems. For instance, in Lake Eric (USA), the highest levels of PAHs were found in urbanized harbors in cities (DeBruyn et al., 2009). PAH contamination in lake sediment is widespread. The lakes were located in urbanized watersheds. The study concluded that rapid urbanization in Turkey contributed to this trend. Indeed, heavy industrialization and increased populations in the last decade contributed to the sediment contamination and threatened water supply for nearby areas. Also marine ecosystems have more intense petroleum transporting activities. However, the Sapanca Lake has different character due to the fact that NATO pipeline with respect to potential oil pollution risks.

The Sapanca Lake is one of the major drinking water resources of the Northwestern Turkey. The basin is surrounded by motorways (TEM, Trans-European Motorways) and a railway connecting Asia to Europe (Tanık et al., 1998). Additionally, the NATO pipeline is located to the south of the Sapanca Lake. Accordingly, the Sapanca Lake is under the potential threat of possible oil pollution.

It was documented that Sapanca Lake is under the influence of chemical and biological pollution due to agricultural and industrial activities (Akçaalan et al., 2014; Akkoyunlu and Akiner, 2012; Morkoç 2008; Duman et al., 2007a; 2007b; Anon 1984; 1998). However, there is no data related to oil hydrocarbon and resistant bacteria or biotechnological potential of the bacterial isolates in the region.

In this study, the bacteria isolated from the Sapanca Lake were investigated regarding the minimum inhibitory concentration of crude oil, capability of producing biosurfactants and pH variables with an aim to detect oil degradation capability and biotechnological potential of the isolates.

MATERIALS and METHODS

Study Area

The water samples were taken from water surface (0-30 cm) in the nine stations from the Sapanca Lake. The samples collected monthly from September of 2008 to May of 2010. The sampling stations are shown in Figure 1.

The surface water samples were taken to the sterile bottles under aseptic conditions and transported daily with cold chain (APHA, 2000). The samples were analyzed at the Aquatic Microbial Ecology Laboratory of Faculty of Aquatic Sciences of Istanbul University.

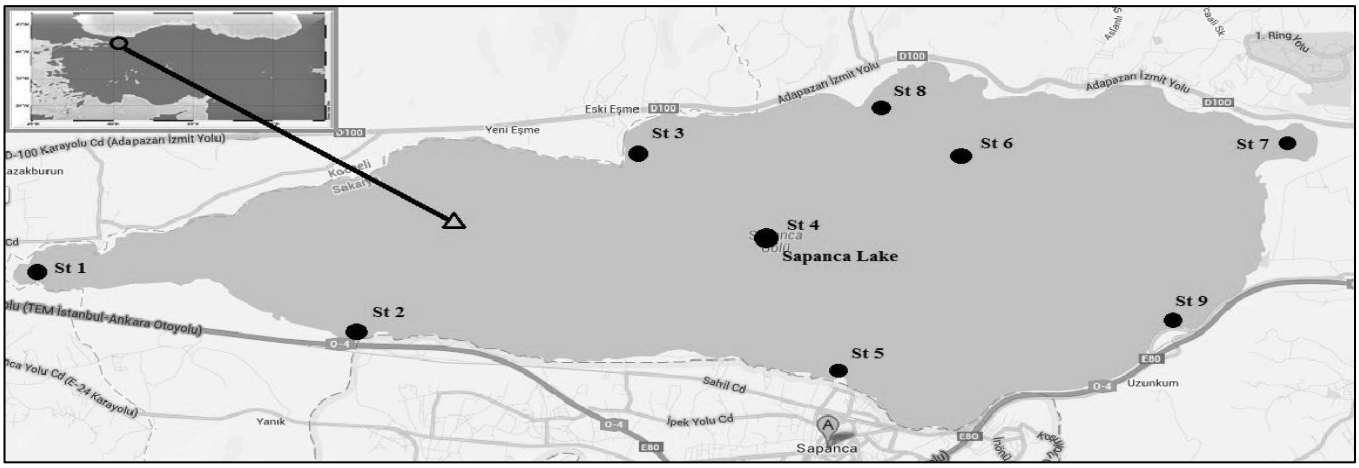

Figure 1. Sampling stations. St 1: Masukiye Stream, St 2: Yanık Stream, St 3: Eşme Stream, St 4: Midpoint of the Lake, St 5: Mahmudiye Stream, St 6: Off the Adasu Water Pump System, St 7: Çark Stream, St 8: Adasu Water Pump System, St 9: Sarp Stream

Bacterial Identification Analyses

The samples were enriched by Nutrient Broth initially and spread to Pseudomonas agar, TCBS agar, Endo agar, SS agar and Cetrimide agar and were incubated 24 hours at 37±0.1°C. Following incubation, different colonies were picked and were recorded according to the colony type, shape and color, and then selected colonies were sub-cultured. The pure isolates were identified using API 20E (Biomérieux). API 20 E is a standardized biochemical identification system for Gram negative rods (Holmes et al., 1978).

Micro Dilution Analyses

The bacterial suspension cultures, incubated overnight in a mineral salt medium (MSM), were diluted to $3x10^8$ cfu/ml by using McFarland No1 standard solution. A volume of 50 µl of bacterial suspension and 50 µl of MSM (except control cell) was then added into 96 well micro-titer plates. Crude oil was added to 12 unit micro-wells in decreasing amounts ranging from 100 µg/ml to 0.09 µg/ml. The first micro-well (without MSM and containing only crude oil and bacteria) was used to detect bacteria that could survive in media containing crude oil as the only carbon source. The last micro–well without crude oil (bacterial suspension and MSM) was used as a positive growth control (Table 1). After incubation at 37 °C for 18 h, the petroleum concentration of the well without turbidity was accepted as the minimum inhibitory concentration (MIC) (ANON, 1997).

Table 1. The ratios (µl) of crude oil, MSM and bacterial suspension cultures used for micro-dilution assay

Micro-wells	1	2	3	4	5	6	7	8	9	10	11	12
Crude oil (µl)	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	*
MSM (µl)	**	50	50	50	50	50	50	50	50	50	50	50
Bacterial suspension (µl)	50	50	50	50	50	50	50	50	50	50	50	50

*micro–well without crude oil ** micro–well without MSM

Emulsification Index Analyses (E_{24})

For determination of the Emulsification Index of the isolates, 2 ml of a crude oil were added into each 2 ml bacterial culture and the samples were incubated at room temperature for 24h after a thorough mix using a vortex for 2 min. The E_{24} index was determined as the ratio of the height of the emulsified layer (mm) to the total liquid column (mm) (Tabatabaee et al., 2005; Sarubbo, 2006).

Bacterial Biodegradation Capability Analyses

For degradation tests, minor adjustments were made for each bacterial sample by taking MIC values into consideration. A total of 100 ml MSM, 25 ml crude oil and 25 ml bacterial suspension (McFarland No:1 standard solution: $3x\ 10^8$ cfu/ml) were added to the sterile flasks. The selected bacterial strains were incubated in batch cultures of 250-ml flasks containing 100 ml of MSM supplemented with 25 ml crude oil as the sole carbon source. Following the addition of a 25 ml bacterial suspension (McFarland No:1 standard solution: $3x\ 10^8$ cfu/ml) to the flasks, the samples were incubated for 30 days at 25 °C on a shaker (150 rpm) (Rahman et al., 2002). The individual strains were used for degradation studies. The changes of pH values and oil layers' thickness in the test flasks were recorded at 72 hours' intervals.

RESULTS and DISCUSSION

The numbers of the isolated bacteria according to the sampling stations were presented on the Table 2.

Eighty-five bacterial strains were isolated from the samples taken from the surface water of Sapanca Lake. In this study twenty-seven bacteria species belonging to three classes (81.5% Gammaproteobacteria, 14.8% Bacilli and 3.7% Flavobacteria) were identified (data not shown; Altuğ et al., 2018, submitted).

The MIC values of the bacterial isolates were shown on Table 3.

The strains showing resistance against crude oil by MIC tests results were applied to the E_{24} tests.

The results of E_{24} tests were shown in Figure 2.

The highest E_{24} value was determined to be 60% for S49- *A. hydrophila* and S50- *A. Hydrophila.* Additionally, S59-*E. coli*1, S38-*A. hydrophila* and S43-*E. cloaceae* sustained the E_{24} values of 54.73%, 50.26% and 50.06 %, respectively.

The thickness of the oil layers and pH values in the test flasks were recorded at 72 hours' intervals for 30 days. The results of oil layer thickness and pH values were shown in the Figure 3, 4 and Table 4.

Table 2. Distribution of the isolates according to the sampling stations

Stations	Sampling Locations	Number of the Isolates
St 1	Masukiye Stream	9
St 2	Yanık Stream	9
St 3	Eşme Stream	9
St 4	Midpoint of the Lake	9
St 5	Mahmudiye Stream	13
St 6	Off the Adasu Water Pump System	9
St 7	Çark Stream	9
St 8	Adasu Water Pump System	9
St 9	Sarp Stream	9
	Total Number of the Isolates	85

Table 3. The bacterial strains that showed MIC values equal or more than 25 µl against the crude oil.

	Crude oil µl											
Isolates	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	0
S1	-	-	-	-	-	-	-	-	-	-	-	-
S2	-	-	-	-	-	-	-	-	-	-	-	-
S3	-	-	-	-	-	-	-	-	-	-	-	-
S4	-	-	-	-	-	-	-	-	-	-	-	-
S5	-	-	-	-	-	-	-	-	-	-	-	-
S6	-	-	-	-	-	-	-	-	-	-	-	-
S7	-	-	-	-	-	-	-	-	-	-	-	-
S8	-	-	-	-	-	-	-	-	-	-	-	-
S9	-	-	-	-	-	-	-	-	-	-	-	-
S10	-	-	-	-	-	-	-	-	-	-	-	-
S11	-	-	-	-	-	-	-	-	-	-	-	-
S12	-	-	-	-	-	-	-	-	-	-	-	-
S13	-	-	-	-	-	-	-	-	-	-	-	-
S14	-	-	-	-	-	-	-	-	-	-	-	-
S15	-	-	-	-	-	-	-	-	-	-	-	-
S16	-	-	-	-	-	-	-	-	-	-	-	-
S17	-	-	-	-	-	-	-	-	-	-	-	-
S18	-	-	-	-	-	-	-	-	-	-	-	-
S19	-	-	-	-	-	-	-	-	-	-	-	-
S20	-	-	-	-	-	-	-	-	-	-	-	-
S21	-	-	-	-	-	-	-	-	-	-	-	-
S22	-	-	-	-	-	-	-	-	-	-	-	-
S23	-	-	-	-	-	-	-	-	-	-	-	-
S24	-	-	-	-	-	-	-	-	-	-	-	-
S25	-	-	-	-	-	-	-	-	-	-	-	-
S26	-	-	-	-	-	-	-	-	-	-	-	-

	Crude oil µl											
Isolates	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	0
S27	-	-	-	-	-	-	-	-	-	-	-	-
S28	-	-	-	-	-	-	-	-	-	-	-	-
S29	-	-	-	-	-	-	-	-	-	-	-	-
S30	-	-	-	-	-	-	-	-	-	-	-	-
S31	-	-	-	-	-	-	-	-	-	-	-	-
S32	-	-	-	-	-	-	-	-	-	-	-	-
S33	-	-	-	-	-	-	-	-	-	-	-	-
S34	-	-	-	-	-	-	-	-	-	-	-	-
S35	-	-	-	-	-	-	-	-	-	-	-	-
S36	-	-	-	-	-	-	-	-	-	-	-	-
S37	-	+	+	+	+	+	+	+	+	+	+	+
S38	-	+	+	+	+	+	+	+	+	+	+	+
S39	-	+	+	+	+	+	+	+	+	+	+	+
S40	-	+	+	+	+	+	+	+	+	+	+	+
S42	-	+	+	+	+	+	+	+	+	+	+	+
S43	-	+	+	+	+	+	+	+	+	+	+	+
S44	-	+	+	+	+	+	+	+	+	+	+	+
S45	-	+	+	+	+	+	+	+	+	+	+	+
S46	-	+	+	+	+	+	+	+	+	+	+	+
S47	-	-	+	+	+	+	+	+	+	+	+	+
S48	-	+	+	+	+	+	+	+	+	+	+	+
S49	-	+	+	+	+	+	+	+	+	+	+	+
S50	-	+	+	+	+	+	+	+	+	+	+	+
S51	+	+	+	+	+	+	+	+	+	+	+	+
S52	-	+	+	+	+	+	+	+	+	+	+	+
S53	-	+	+	+	+	+	+	+	+	+	+	+
S54	-	+	+	+	+	+	+	+	+	+	+	+
S55	+	+	+	+	+	+	+	+	+	+	+	+
S56	-	+	+	+	+	+	+	+	+	+	+	+
S57	-	+	+	+	+	+	+	+	+	+	+	+
S58	-	+	+	+	+	+	+	+	+	+	+	+
S59	-	+	+	+	+	+	+	+	+	+	+	+
S60	-	+	+	+	+	+	+	+	+	+	+	+
S61	-	+	+	+	+	+	+	+	+	+	+	+
S62	-	+	+	+	+	+	+	+	+	+	+	+
S63	-	+	+	+	+	+	+	+	+	+	+	+
S64	-	+	+	+	+	+	+	+	+	+	+	+
S65	-	+	+	+	+	+	+	+	+	+	+	+
S66	-	+	+	+	+	+	+	+	+	+	+	+
S67	-	+	+	+	+	+	+	+	+	+	+	+
S68	-	+	+	+	+	+	+	+	+	+	+	+
S69	-	+	+	+	+	+	+	+	+	+	+	+
S70	+	+	+	+	+	+	+	+	+	+	+	+
S71	-	+	+	+	+	+	+	+	+	+	+	+
S72	-	+	+	+	+	+	+	+	+	+	+	+
S73	-	+	+	+	+	+	+	+	+	+	+	+
S74	+	+	+	+	+	+	+	+	+	+	+	+
S75	+	+	+	+	+	+	+	+	+	+	+	+
S76	-	+	+	+	+	+	+	+	+	+	+	+
S77	-	+	+	+	+	+	+	+	+	+	+	+
S78	-	+	+	+	+	+	+	+	+	+	+	+
S79	-	+	+	+	+	+	+	+	+	+	+	+

	Crude oil µl										
Isolates	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	0
S80	-	+	+	+	+	+	+	+	+	+	+	+
S81	-	+	+	+	+	+	+	+	+	+	+	+
S82	-	+	+	+	+	+	+	+	+	+	+	+
S83	-	+	+	+	+	+	+	+	+	+	+	+
S84	-	+	+	+	+	+	+	+	+	+	+	+
S85	-	+	+	+	+	+	+	+	+	+	+	+

(+) Bacterial growth was observed (-) Bacterial growth was not observed

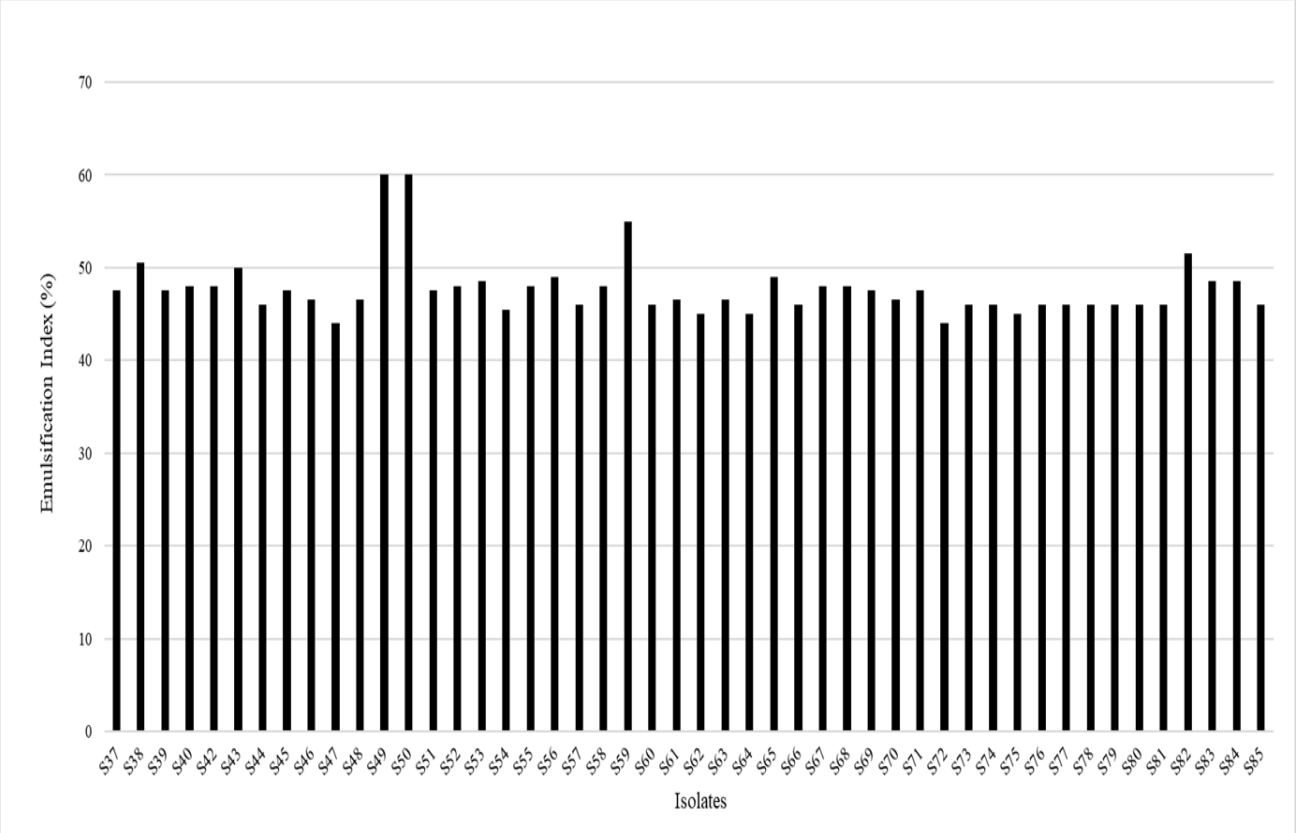

Figure 2. The percentage of emulsification index (E24) of the bacterial isolates that was able to use crude oil

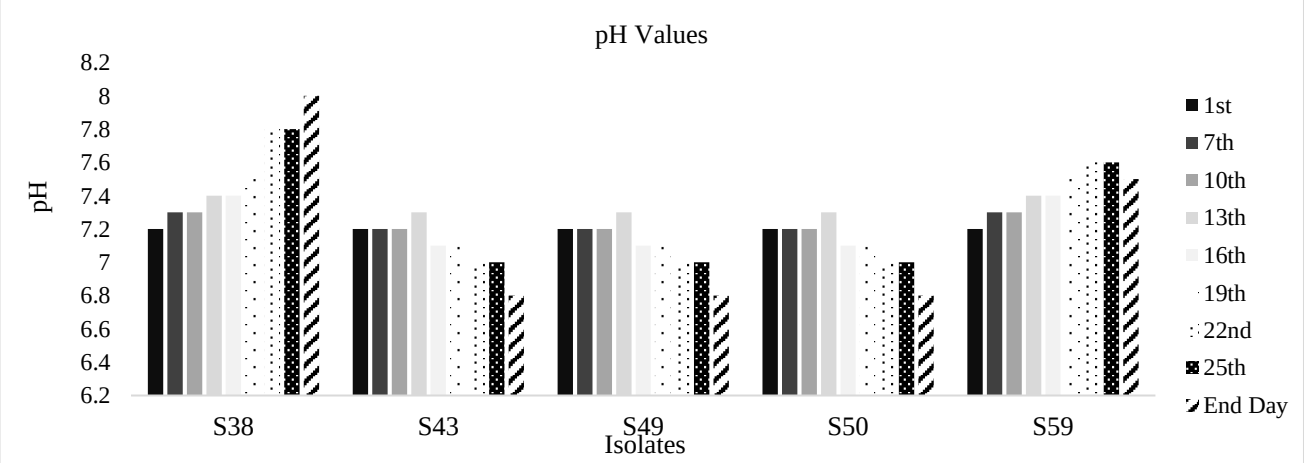

Figure 3. Recorded pH values in the flask containing crude oil and bacteria.

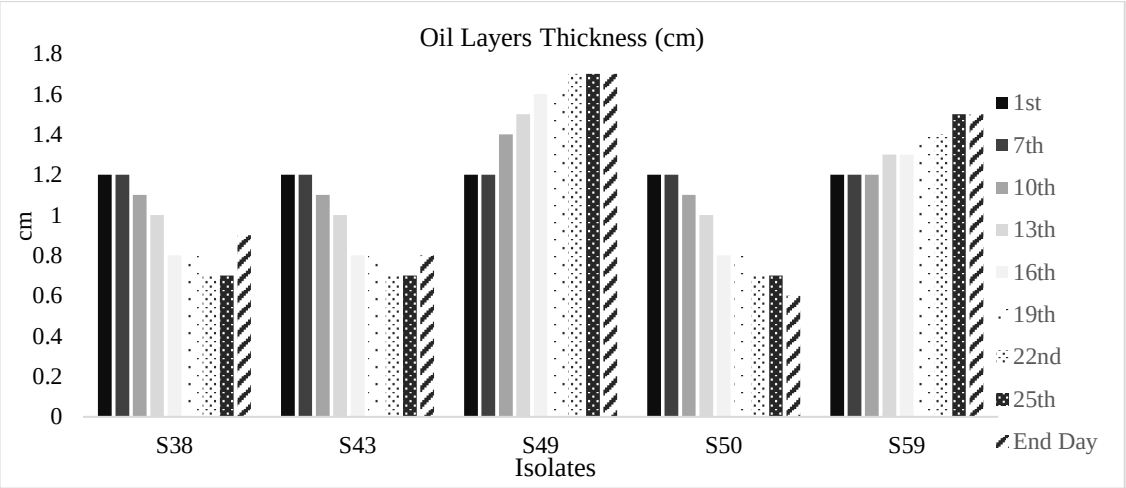

Figure 4. Oil layer thickness (cm) obtained from individual bacterial isolates

Table 4. Recorded pH values and oil layers thickness (cm) in the flask containing crude oil and bacterial isolates

Isolates	pH Values								
	Days								
	1st	7th	10th	13th	16th	19th	22nd	25th	30th
S38	7.2±0.01	7.3±0.01	7.3±0.02	7.4±0.06	7.4±0.06	7.5±0.08	7.8±0.15	7.8±0.15	8±0,21
S43	7.2±0.01	7.2±0.01	7.2±0.02	7.3±0.06	7.1±0.06	7.1±0.08	7.0±0.15	7±0.15	**6,8±0,21**
S49	7.2±0.01	7.2±0.01	7.2±0.02	7.3±0.06	7.1±0.06	7.1±0.08	7.0±0.15	7±0.15	6,9±0,21
S50	7.2±0.01	7.2±0.01	7.2±0.02	7.3±0.06	7.1±0.06	7.1±0.08	7.0±0.15	7±0.15	**6,8±0,21**
S59	7.2±0.01	7.3±0.01	7.3±0.02	7.4±0.06	7.4±0.06	7.5±0.08	7.6±0.15	7,6±0.15	7.5±0,21
Isolates	Oil Layers Thickness (cm)								
	Days								
	1st	7th	10th	13th	16th	19th	22nd	25th	End Day
S38	1.2±0.01	1.2±0.01	1.1±0.05	1.0±0.09	0.8±0.14	0.8±0.15	0.7±0.19	0.7±0.19	0.9±0.19
S43	1.2±0.01	1.2±0.01	1.1±0.05	1.0±0.09	0.8±0.14	0.8±0.15	0.7±0.19	0.7±0.19	**0.8±0.19**
S49	1.2±0.01	1.2±0.01	1.4±0.05	1.5±0.09	1.6±0.14	1.6±0.15	1.7±0.19	1.7±0.19	1.7±0.19
S50	1.2±0.01	1.2±0.01	1.1±0.05	1.0±0.09	0.8±0.14	0.8±0.15	0.7±0.19	0.7±0.19	**0.6±0.19**
S59	1.2±0.01	1.2±0.01	1.2±0.05	1.3±0.09	1.3±0.14	1.4±0.15	1.4±0.19	1.5±0.19	1.5±0.19

Data are means (n=5)±SD

The thickness of oil layers was recorded lower (0.7 cm) in the medium of S59-*E. coli*1 as compared to the reference medium (1.2 cm) without any bacterial strains. This situation implied that oil degradation rate affected by S59-*E. coli*1 during the incubation period.

Throughout the 30-day incubation period, recorded maximum and minimum pH values were 8 and 6.8, respectively.

Oil pollution is an environmental problem of increasing importance. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in oil-containing environments, have an important role in the biological treatment of this pollution. The biodegradation of many components of petroleum hydrocarbons by bacteria has been reported in a variety of terrestrial and marine ecosystems (Atlas, 1995; Pritchard and Costa, 1991; Ron and Rosenberg, 2002; Ciftci and Altug, 2010; Altug et al., 2011; Altug et al., 2012).

The degradation of complex molecules in the nature can be accelerated by human activities with biological and environmental changes. Since each bacteria shows higher productivity in its own adaptive ecosystem, identification of bacterial community based on each geographical areas are important for understanding of bacterial remediation occurrence potential of the regions.

In this study Sapanca Lake was selected as a study area due to being an important water source and was surrounded by motorways (TEM, Trans-European Motorways) and a railway connecting Asia to Europe (Tanık et al., 1998). Locating of NATO pipeline at the

south of this lake also made this area even better study site.

Since, Sapanca Lake is under the potential threat of oil pollution, to identify of the local bacteria as a possible bioremediation agent is important. Correspondingly, in the MIC test it was detected that the first thirty-six strains were displayed negative results against crude oil in 2008. However, 37th strain and followers were found to be resistant to crude oil after May of 2009. These results were associated with the unexpected oil spillage happened in the Sapanca Lake at that time. This situation showed that the bacteria did not meet with the petroleum-derived contaminants during this period. NATO oil pipeline leaked in May 2009 and then positive results have been received in MIC tests which were carried out after on this date. As a result, the bacteria isolated from Sapanca Lake displayed different metabolically behaviors with respect to crude oil resistance after the unexpected accident of the region.

It has been well documented that various crude oil tolerant microorganisms including bacteria (*Serratia marcescens, Pseudomonas aeruginosa, Enterobacter aerogenes* and *Escherichia coli* etc.) yeast and fungi were isolated from natural environments such as soil, marine and fresh water (Yakimov et al., 2007; Zarate et al 2014). They tolerate high concentrations of the hydrocarbons and have a high capability for their degradation. Most of which belong to Gammaproteobacteria (Okoh and Trejo-Hernandez, 2006; Jacques et al., 2008; Onbasili et al., 2011). In this study, while S59-*E. coli* and *A. hydrophila* gave the best results, the bacteria species displayed high performance against crude oil were detected to be S49-*A. hydrophila,* S50- *A. hydrophila,* S59-*E. coli*1, S38-*A. hydrophila* and S43-*E. cloaceae.*

It was reported that pH values affect the emulsion capacity and stable activity of rhamnolipid-type biosurfactants' (Prieto et al., 2008). The decreases in pH values observed during incubation period was positively associated with degradation rate and this situation reported as an indicator to utilization of crude oil by microbes as an energy source in media (Anon 1991; Udo and Fayemi, 1995; Head et al., 2006). Similarly, in this study observed decreases in pH values were associated with positive efficiency of bacteria on degradation of crude oil. During the study the pH varied between 6 and 8 values (Table 4). The initial pH values were adjusted to 7.2 in in experimental setups. The most extreme drop in pH to a value 6.8 was observed the 30th day in S43-*E. cloacea* and S50-*E. coli*. A decrease of 6.8 in the pH values was observed especially in the case of thinning in the petroleum layer. This situation implies that utilization of crude oil as an energy source by S43-E. *cloacea* and S50-*E. coli* was higher than the others isolates.

Principally, the decrease of oil layer thickness during incubation period was also positively associated with degradation rate. In this study, oil layer thickness in the experimental set up, containing different bacterial media, displayed variables throughout the incubation period.

Despite of the observed decreases in oil layer thickness in the experimental set up, some increases was also recorded. For instance, the highest thickness of the oil layer was recorded in S49- and S59 strains. According to Altuğ *et al.* (2011) and Dean *et al.* (2001) many hydrocarbon degrading bacteria produced bio surfactants that assist in hydrocarbon association with the substrate. In this study, it can be assumed that the increasing of the oil layer thickness in the media containing S49- and S59 strains is due to production of the biosurfactants by bacteria. In this study E_{24} values recorded also showed that these isolates have emulsification potential for produce biosurfactants.

Biosurfactants can promote the growth of oil-degrading bacteria and improve their capacity to utilize hydrocarbons as carbon source. Some bacteria, remarkably, produce waxes after degrading crude oil (Ishige et al., 2003). Emulsification (E_{24}) value was accepted as an indicator to determinate the candidate bacterial species which was resistant to crude oil. Due to bioemulsifiers apply to stimulate the bioremediation process in fields as a stabilizer, to understand E_{24} value of the candidate bacteria is important for bioremediation studies. In this study, E_{24} values of the isolates were between 60% and 44.06%. The results showed that the bacterial strains tested in this study may be considered as bioemulsifier-overproducing bacteria for possible use in bioremediation studies to increase the solubility and bioavailability of hydrocarbons in natural environments.

It is not surprising that hydrocarbons are considered to be contaminants distributed in large areas in nature. The abilities of bacteria in bioremediation arena is being studied extensively because bacterial isolates are easily cultivatable, better suited to molecular biology techniques. Also, they metabolize chlorinated organic substances, mineralize such chemical substances, and use carbon as a source of energy (Bouwer ve Zehnder, 1993).

Altuğ et al. (2011, 2012) reported that bacterial strains isolated from the northern Marmara Sea and Istanbul Strait (Turkey) sustained high MIC values. In this study, though, the bacteria isolated from Sapanca Lake displayed lower MIC values than those of Marmara Sea. Both results offer opportunities for further possible remediation studies.

CONCLUSION

The use of domestic bacteria in the removal of petroleum hydrocarbons from the environment is

extremely important in terms of end-point acceleration and recommended method. In this study oil degrading bacteria in Sapanca Lake were investigated for the first time.

The strain of S59-*E. coli*1 showed the highest degradation ability among five selected strains and it determined as potential candidates for detailed studies. We suggest that natural bacterial isolates from oil polluted areas are more suitable candidates as oil-degrading bacterial strains for the rehabilitation of oil polluted aquatic ecosystems.

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