Tr. J. of Zoology
23 (1999) 275-284

The Effects of Oil Pollution on Free–living Ciliates

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Received: 04.09.1997

Abstract: For the first time the effects of oil pollution on free–living ciliates at community level and also at cellular level were studied at the same time. The effects of various oil concentrations from two oil fields (Sangachal and Guneshli) on psammophile and periphyton ciliates were studied. As a result, the parameters using the oil pollution biotesting with the help of these test systems were determined. Oil from the Guneshli oil field was determined to be more toxic.

The effects of oil pollution on the physiological parameters of 4 ciliate species – *Frontonia marina, Oxytricha fallax, Strombidium conicum,* and *Euplotes harpa* were studied also. The sensitivity of *Strombidium conicum* to oil pollution was determined and such species were suggested as new reliable sensitive species.

Key Words: Psammophile and periphyton ciliates, Biotesting.

Petrol Kirliliğinin Serbest Yaşayan Siliyatlara Etkisi

Özet: Bu çalışmada, petrolün değişik konsantrasyonlarının serbest yaşayan siliyatlara toksik etkisi gerek topluluk gerekse hücre düzeyinde ilk kez araştırılmıştır. Denemelerde iki lokalitede (Sangaçal ve Güneşli) işlenen petrolden yararlanılmıştır. Güneşli petrolünün Sangaçal petrolüne oranla daha toksik olduğu belirlenmiştir. Sonuç olarak petrol kirliliğini biyolojik açıdan test etmede, psammofil ve perifiton siliyat topluluklarını esas olan yeni parametreler tespit edilmiştir.

Petrol kirliliğinin etkileri 4 siliyat türü (*Frontonia marina, Oxytricha fallax, Strombidium conicum* ve *Euplotes harpa*)'ne ait fizyolojik parametreler esas alınarak araştırılmıştır. *Strombidium conicum*'un petrol kirliliğine duyarlı olduğu belirlenmiş ve bu türün petrol kirliliği açısından yeni ve uygun bir indikatör olarak alınabileceği ileri sürülmüştür.

Anahtar Sözcükler: Psammofil ve perifiton siliyatlar, Biyolojik Test.

Introduction

In spite of the measures undertaken, the pollution of the seas and oceans by off shore oil production in steadily increasing. Oil contamination is particularly dangerous for inland bodies of water such as the Caspian Sea. At present many sections of the Caspian Sea area are "dead zones" as a result of oil or other kinds of pollution. These sections of the Caspian Sea are characterized by the complete absence of hydrobionts.

The toxic effects of oil pollution on hydrobionts have been confirmed by the large number of experiments carried out mainly on multicellular organisms (fishes, molluscs and crustaceans).

However the majority of investigations were carried out on individual animal species in the middle or upper sections of the food chain for marine biocenoses. Naturally such data, in spite of their scientific value, can not give an idea of the effects of various degrees of oil pollution on water communities as a whole. Studies on the effects of oil on organisms at the base of the food pyramid in the marine biocenosis, i.e. on unicellular organisms are of great interest.

The use of free–living ciliates in biological monitoring is widespread in many countries including Azerbaijan. However, most of these investigations were carried out on a cellular level until recently, i.e. the effects of toxicants were registed directly on the physiological parameters of ciliates.

Biotesting at the community level was recognized only at the end of the 80's and now it is widely used in Canada, England, Spain, Austria etc. (1–4).

The possibility of rehabilitation of communities after a single contamination by various amounts of oil is of great practical interest. It should be noted that such investigations have not been carried out previously.

Materials and Method

During the period 1991–1996 samples of psammophile and periphyton ciliates were taken from sections of the Absheron coastline with various degrees of oil contamination. During the collection of the benthic samples, the soil layer were taken with water gathered with a plastic jar from the seabed. Periphyton samples were collected by means of marine growth scrape off from the various things under the water. All collected samples were taken to the laboratory and treated immediately. Immediate sample treatment is extremely important in the quantitative counting of ciliates, since correlation of various species in samples very widely when ambient temperature changes. Live samples were counted in a Bogorov tray under a binocular microscope with several portions of 3 ml each 5 to 10 times. Then the mean quantity was recounted in 1 dm^2 for each species.

Different methods of silver impregnation of the kineties (5, 6) were applied for the detailed qualitative treatment of the collected material. Colouring with Mayer's hemalum and the Feulgen reaction on DNA in simplified modification was applied for the detailed study of the nucleus apparatus.

Experiments studying the effects of crude oil on ciliates were undertaken in two ways. The first consisted of experiments on the model level of communities of ciliates, which studied their structural changes under the effects of different concentrations of crude oil. The second consisted of experiments on the cell level, which studied the effects of crude oil on the various physiological parameters of ciliates.

In order to obtain more accurate results were combined the experiments on the cell level and community level.

We used oil from the Sangachal and Guneshli fields which have different characteristics of toxicity on hydrobionts.

Then species composition and quantity changes of individual species were estimated in comparison with the controls. The estimation was made daily for the first 2 days, then once every 5 days. The experiments were repeated 5 times for each concentration. The experiment lasted 30 days.

The following indices were used to estimate the changes in the communities:

1. The coefficient of similarity (S) between two different samples (7).

$$S = \frac{2C}{A + B}$$

where A is the number of species in sample A; B is the number of species in sample B;

and C is the number of species in common.

2. Dominance index (C) (7)

$$C = \Sigma \left(\frac{n_i}{N}\right)^2$$

where n_i is the number of cells in each species, and N is the total number individuals in the community.

3. The index of species diversity (d) (7)

$$d = \frac{S - 1}{\ln N}$$

where S is the number of species in community, and N is the total number of the community species.

4 species were used (*Frontonia marina, Oxytricha fallax, Strombidium conicum, Euplotes harpa*).

Cultures of these species were isolated from the natural samples of sea water and kept in petri dishes at room temperature. Physiological parameters such as multiplication rate, motion activity, phagocytosis and contractile vacuoles pulsation velocity were taken as toxicity indices. In addition, the general test survival criterion was applied.

Effects of Crude Oil on Model Communities of Ciliates

It is known that one of the striking indices characterizing the qualitative distinction of compared biosenosis is the coefficient of similarity of their species composition. Before the toxicological experiments, we carried out preliminary tests to estimate the community coefficient (depending on vessel volume) of species composition of isolated model communities of psammophile and periphyton ciliates.

As table 1 shows, the coefficients of similarity of model communities of psammophile ciliates placed in vessels with volume of 1.5 l are characterized by high significance. It is possible to distinguish two periods in the development of model communities depending on period. The first period is the environment stabilization ending on the 3rd day with practically identical composition similarity coefficient in the model communities and control communities.

The second one begins approximately after the 20th day and is characterized by a decrease in the model communities' similarity coefficient which becomes significantly noticeable on the 30th day. In our opinion the second period is conditioned by the so–called "bag effects" caused by the changes in the model communities at the expanse of the small capacity of their environment.

Table 1. The coefficient of similarity (SJ of isolated (capacity–1.51) model communities of psammophile ciliates (in % to control).

Day of experiment	Coefficient of similarity (S) of model communities of psammophile ciliates									
1st	81	82	79	84	80	81	82	79	85	83
3rd	97	99	95	100	98	98	99	93	99	95
7th	98	100	97	100	98	98	100	98	100	99
15th	98	100	98	100	95	96	100	96	100	99
20th	93	87	95	95	97	93	98	89	94	98
30th	75	73	81	78	83	76	80	78	74	86

For the comparison, analogous experiments were carried out on model communities of psammophile ciliates placed in petri dishes, i.e. with a lower volume of environment. The results showed that the stabilization period of the environment in the model communities of psammophile ciliates in petri dishes is practically not available (because of small volume), but on the other hand the second period characterized by the decrease in coefficient of similarity began on the 15th day (K–from 71% to 85%), but by the 30th day the coefficient of similarity with the control did not exceed 47%. The difference in coefficients between separate model communities in petri dishes was 15% by the 30 th day.

Similar preliminary experiments were carried out with model communities of periphyton ciliates as well (Table 2).

As the data in table 2 shows, the coefficient of similarity of model communities of periphyton ciliates in the 1.5 1 volumes increased much more slowly than in the communities of psammophilous ciliates over time. The period of initial stability of the model community of periphyton ciliates covered the first weeks of the experiment and only after 15 days did the coefficient of similarity approach the control indices. The absence of the second period in the periphyton model communities which is typical for the communities of psammophilous ciliates should be noted. The possible absence of "bag effects" in the periphyton ciliate communities is explained by fewer, individual species in them and less species variety which allow periphyton communities to be provided by fodder organisms and the other necessary conditions for the development over a long period of time (up to 75 days according to our data).

It was determined during the investigation on model communities of periphyton ciliates placed in small capacities (petri dishes), that the initial period of stabilization occupied only the first 48 hours in this case after which the similarity coefficient of their species composition increased from 87% to 100%, i.e. approached the control indices. At the same time, during the second period a decrease in the similarity coefficient was observed, beginning on the 30th day. The similarity coefficient of periphyton ciliate communities from the separate volumes (petri dishes) differed from each other by no more than 7%, i.e. it was rather high for reliable toxicological experiments.

Summarizing all the results of the preliminary tests we concluded that it is possible to get the most reliable data on the chronic effects of small concentrations of oil on ciliate communities if the isolated (1.51 volume) model communities of psammophile ciliates are used after the 3rd day of their stabilization and the same model communities of periphyton ciliates after the 7–10th day of stabilisation. It is preferable to use the model communities isolated in petri dishes to carry out acute experiments in a relatively short experiment. The results of tests on the effects of various concentrations of oil in the Sangachal oil field on model communities of

Table 2. The coefficient of similarity (S) of model communities of periphyton ciliates (in % to control).

Day of experiment	Coefficient of similarity (S) of model communities of periphyton ciliates									
1st	19	15	12	17	13	19	11	18	15	21
3rd	27	26	31	28	29	26	29	29	29	27
7th	64	59	68	66	69	68	59	68	68	70
15th	88	82	89	85	88	83	86	89	87	88
20th	95	97	98	93	99	97	92	97	91	94
30th	95	96	98	96	97	95	94	97	94	98

psammophile ciliates are shown in figure 1. The minimum concentration (0.01 ml/l) of crude oil practically exerted no toxic effect on communities, as the similarity coefficient was close to the control in this case and it did not decrease to less than 95%. A decrease in the similarity coefficient was observed with 0.05 ml/l concentration only on the 15th day (89%), but by the end of the experiment (30th day) this index had fallen to 86%. With 0.1 ml/l concentration of crude oil the toxic effects were observed on the 7th day (84%), then by the 15th day the coefficient of similarity had fallen to 77%, but by the end of the experiment it had fallen to 75%.

The noticeable toxic effects on the model community of psammophile ciliates were observed with 1 ml/l concentration of Sangachal oil from the 15th day of exposure, but by the end of the experiment the coefficient of similarity had fallen to 74%.

With 5 ml/l concentration the toxic effects on modeL communities were noticed on the 7th day (65%) without its subsequent strong changes. The toxic effects of 10 ml/l concentration of oil were strikingly observed after 7 days (60%), then the coefficient of similarity remained almost unchanged until the 15th day of exposure and it had fallen to 57% by the end of the test. If the effects of maximum concentration (25 ml/l) differed slightly from the previous one (53%) in terms of quantity in the first week of the experiment, then the similarity coefficient fell to 44% after 15 days, but by the 30th day it had fallen sharply to 22%, which indicates strong degradation of model communities under the effects of oil.

Figure 2 summarises the data on the effects on the different concentration of oil from Sangachal on model communities of periphyton ciliates. With concentrations (0.01 ml/l – 0.1 ml/l) of oil the coefficient of similarity underwent approximately the same insignificant change as in the experiments described above. Changes in the similarity coefficient of great significance with 0.5 ml/l concentration attract attention. Thus this index was 75% in the first week, it had fallen, to 69% by the 15th day, but by the end of the experiment it had reached 60%. The effects of stronger concentrations of oil from Sangachal (1–25 ml/l) on model communities of periphyton ciliates were approximately analogous to those on communities of psammophile ciliates, but the changes in the coefficient of similarity were almost parallel.

Figure 3 summarises the data on changes in the similarity coefficient of psammophile ciliates model communities under the effects of different oil concentrations from the Guneshli oil field. Only with the minimum concentration – 0.01 ml/l was the coefficient of similarity of model communities of psammophile ciliates close to the control (97% after 7 days, 94% after 15 days and 95% after 30 days). With 0.05 ml/l

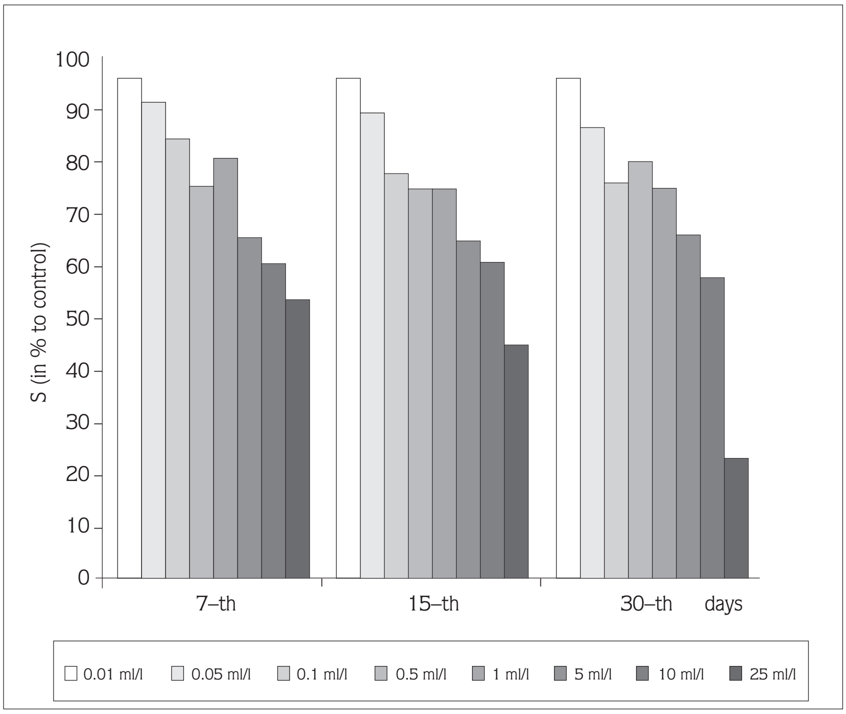

Figure 1. The effect of different concentrations of oil from Sangachal on coefficient of similarity (S) of model communities of psammophile ciliates.

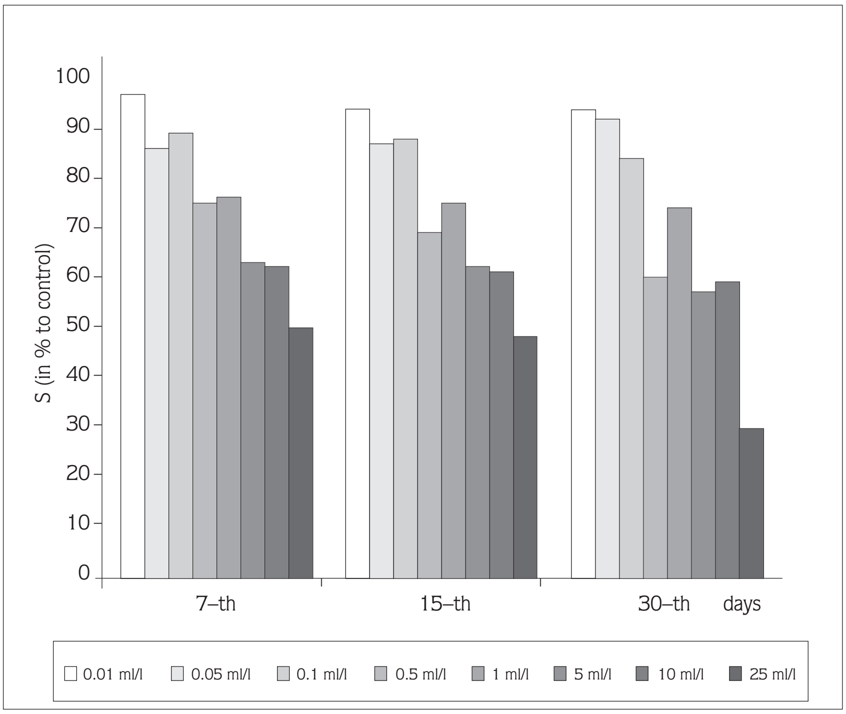

Figure 2. The effect of different concentrations of oil from Sangachal on coefficient of similarity (S) of model communities of periphyton ciliates.

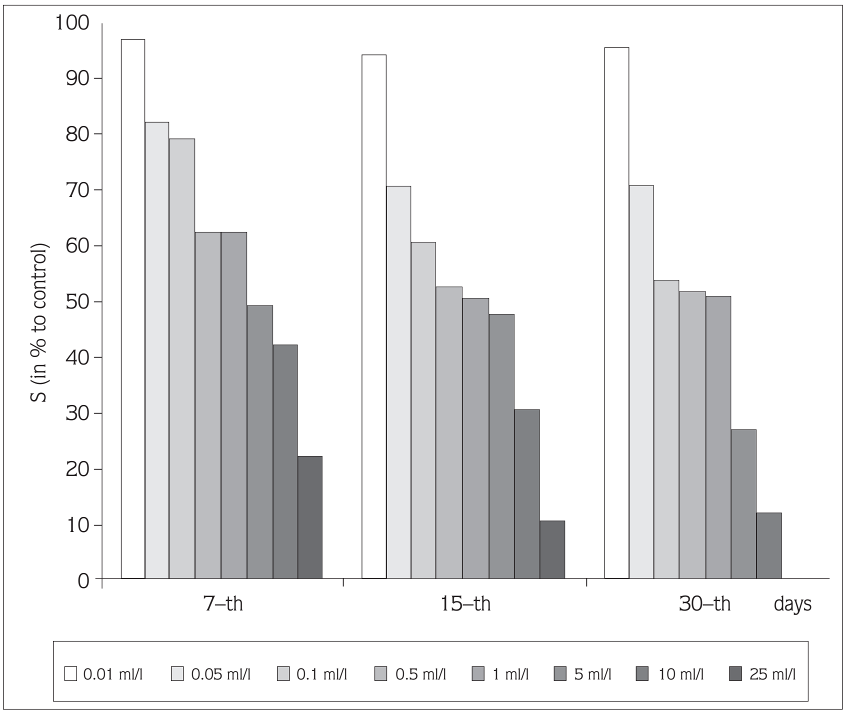

Figure 3. The effect of different concentrations of oil from Guneshli on coefficient of similarity (S) of model communities of psammophile ciliates.

concentration of oil a noticeable decrease in this index was observed on the 7th day of exposure (82%) with a decrease by the 15th day to 70%.

In our opinion the data in figure 4 are very interesting. This index rapidly decreased on the 15th day even with the minimum concentration of oil – 0.01 ml/l,

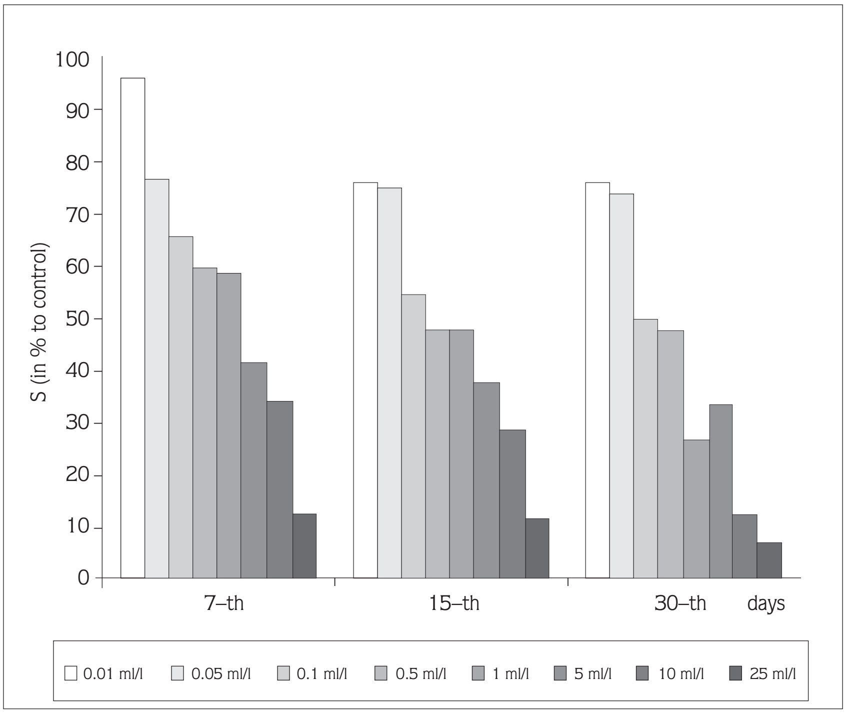

Figure 4. The effect of different concentrations of oil from Guneshli on coefficient of similarity (S) of model communities of periphyton ciliates.

which clearly shows the negative effects on model communities of periphyton ciliates. The negative effects of oil from the Guneshli oil field on model community steadily increased with the rise in concentration to maximum judging by the decreasing similarity coefficient, with maximum concentrations (10–25 ml/l) (12% and 7% respectively after 30 days).

Thus, the preliminary analysis of data on changes in the coefficient of similarity of model communities of psammophile and periphyton ciliates shows the rather high sensitivity of these test systems to oil pollution.

Analogous experiments with model communities of psammophile ciliates were carried out to obtain more objective data. However, in this case the structural changes in communities under the effects of different oil concentrations were taken into consideration according to a dominance index change (7) and to an index of species diversity (7).

It is known that the more unfavourable the environmental conditions are, the fewer dominant species become in the community. Therefore, the closer the dominance index is to unity, the worse the environmental conditions are. If there is only a single species in the community, then the dominance index is equal to 1. The index of species diversity is reflected in the number of species making up the community. Thus, the higher the value of this index, the more varied the coefficient of similarity of model community, which reflects its prosperity degree.

Figure 5 summarises the results of experiments on the effects of different concentrations of oil from the Sangachal oil field on changes in the dominance index of model community of psammophile ciliates. Even the minimum concentration of oil – 0.01 ml/l the dominance index of model community of psammophile ciliates differed from the control (0.25 and 0.1 respectively) after one week of exposure. In the following period this index increased and by the 15^{th} day was equal to 0.30, but by the end of the test it had not exceeded 0.15. With higher concentrations of oil there was a similar tendency of the dominance index to increase in all cases. The decrease in this index with 0.1 ml/l concentration after the 15^{th} day was an exception. This fact can be explained by the mass development of phytoplankton organisms in the community, that in turn led to larger numbers of some phytophagouse ciliates species.

However, as previously mentioned there was a clear correlation between oil concentration and the dominance index in the rest of the cases.

The data in figure 6 on changes in the species diversity index of psammophile ciliates model community under the effects of different concentraitons of oil from the

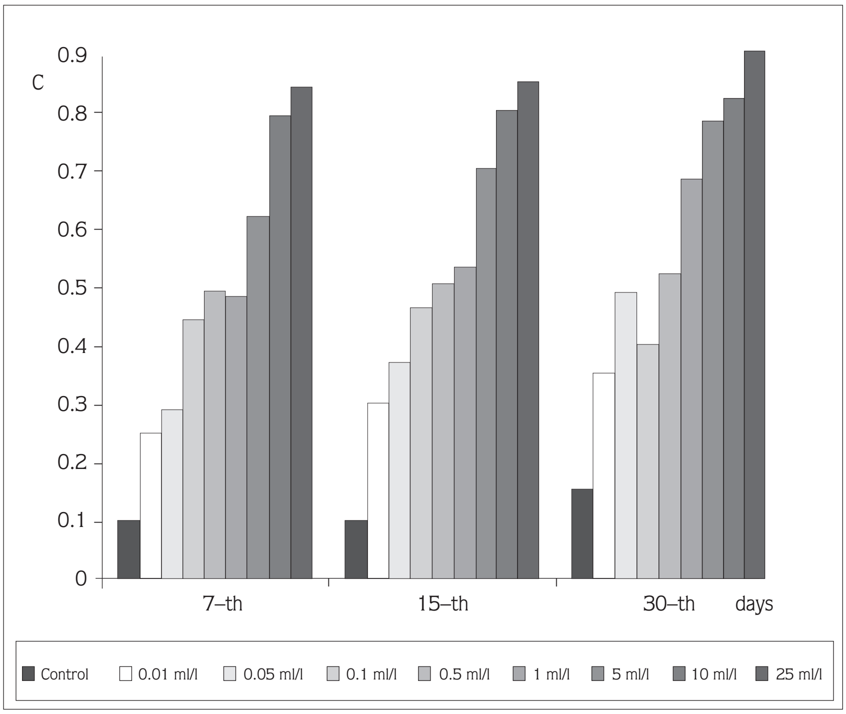

Figure 5. The effect of different concentrations of oil from Sangachal on dominance index (C) of model communities of psammophile ciliates.

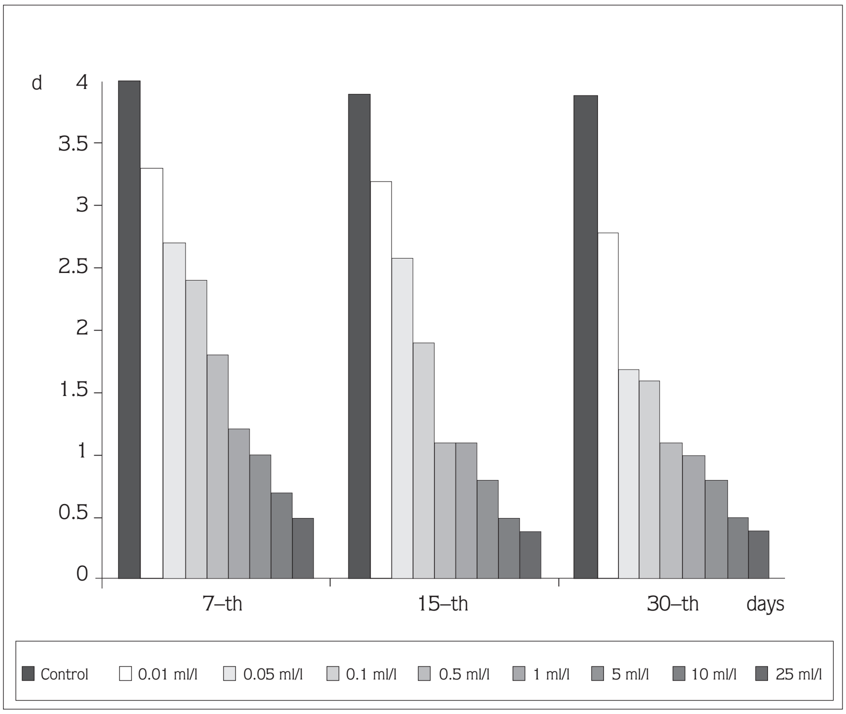

Figure 6. The effect of different concentrations of oil from Sangachal on index of species diversity (d) of model communities of psammophile ciliates.

Sangachal oil field confirm the above mentioned data. The toxic effects of oil on the model community was noticeable by the 7^{th} day. By the 15^{th} day, and particularly by the end of the experiment (30^{th} day) the species diversity index had decreased still further. With higher concentrations of oil the species diversity of model communities had decreased reaching the minimum (0.75) with the maximum concentration – 25 ml/l, by the end of the test.

The results of analogous experiments on changes in the dominance index and species diversity in model communities of psammophile ciliates under effects of different concentrations of oil from the Guneshli oil field are given in figures 7 and 8. As figure 7 shows, the dominance index differs very much from the control values even with minimum concentrations of oil and it is

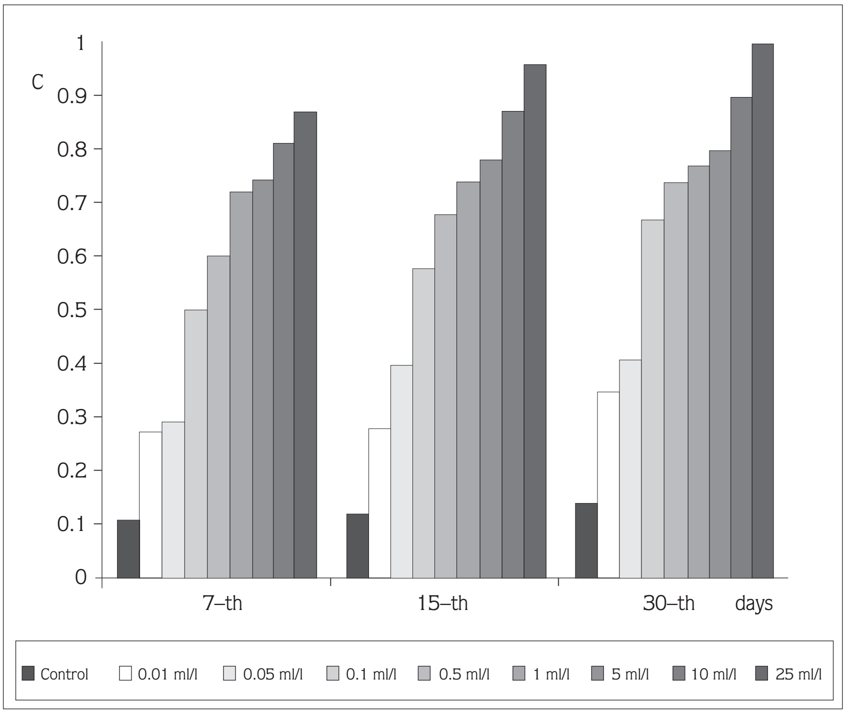

Figure 7. The effect of different concentrations of oil from Guneshli on dominance index (C) of model communities of psammophile ciliates.

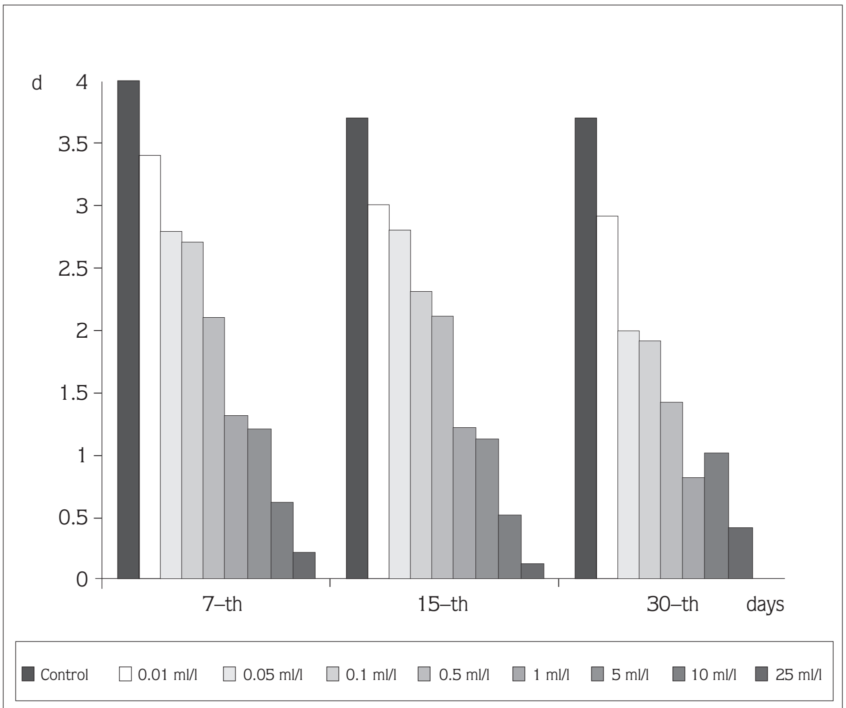

Figure 8. The effect of different concentrations of oil from Guneshli on index of species diversity (d) of model communities of psammophile ciliates.

directly dependent on the concentration of the toxicant. The maximum concentrations of oil from the Guneshli oil field (10–25 ml/l) led to degradation and complete elimination of the psammophile ciliate model community by the end of the experiment (after the 30th day the dominance index was equal to 1 with 25 ml/l concentration).

Data on change in the species diversity index (Fig. 8) confirm the above mentioned results.

The analysis of all findings leads to certain conclusions. The use of such test systems as the model communities of psammophile and periphyton ciliates for biotesting of the toxicity of crude oil is justified and expedient in view of the availability and low cost of such experiments. The use of ciliate model communities for biotesting and analysis of structural changes in them allows the effects of changes weak doses of toxicant to be measured during the earliest stages. It is necessary to analyse the structural changes in the model communities according to all 3 principal parameters: coefficient of similarity, dominance index and species diversity index. It increase the reliability of the results much more. In addition, higher sensitivity of the latter 2 indices should be noted in comparison with the similarity coefficient which is usually used by specialists. As all the above mentioned results showed, generally the toxic effects of oil from the Sangachal oil field were rather weaker than oil from the Guneshli oil field. If the similarity coefficient of Sangachal oil was close to the control figures with the minimum concentration of oil (0.01 ml/l), then in Guneshli this index was significantly lower than the control. Probably, this is because of different percentages of fractions and naphthenic acids in them, and it determines the level of oil toxicity from the various oil fields (8).

It was revealed in the course of the experiments, that not all the species of the ciliate communities had similar responses to the various degrees of oil pollution. For example, with oil concentrations of 0.5 to 5 ml/l the numbers of *T. prenanti* rapidly decreased in the first 7 days and later (from the 15th to 30th day) only single specimens were found. *Pelagohalteria* and *Strombidium* species were sensitive even to weak doses (0.01 – 0.5 ml/l). They disappeared from the communities in 3 days with concentrations higher than 0.05 ml/l. At the same time all *Peritrichida* were very steady to oil pollution and were found with maximum concentrations of oil – 25 ml/l. *Diophrys* and *Uronychia* species have the same ecological plasticity.

Effects of Crude Oil on Physiological Parameters of Ciliates

During the investigation on oil pollution biotesting at the level of model ciliate communities we had a great deal of material on the degree of sensitivity of many species to different oil concentrations. These results served as the basis for the selection of ciliate species as test objects. In addition, the possibility of keeping of these cultures under laboratory conditions over a long period of time was taken into consideration. We selected 4 species of ciliates to be analysed for biotesting: *Frontonia marina, Oxytricha fallax, Strombidium conicum,* and *Euplotes harpa*. In addition to the widely used criterion of organism survival at various concentrations of toxicant, we considered such physiological parameters as change in the rate of multiplication, phagocytosis and time of filling and the following contraction of the contractile vacuole.

The division rate is one of the important indices of the physiological state of unicellular organisms. This criterion allows the tracing of the chronic effects of toxicants on ciliata reproduction during ecotoxicological investigations. The findings are of particular interest, because they make it possible to judge the adaptation possibilities of test object organisms to toxic effects.

Phagocytosis, like rate of division, is one of the most important criteria of the physiological state of unicellular organisms. Its intensity, manifested in more of fewer food vacuoles, changes depending on changes in the environmental conditions and the presence of different doses of toxicants also. Thus, it is expedient to also apply the rate of phagocytosis as a test criterion to estimate the effects of the different toxic substances and oil on ciliates.

The functioning of the contractile vacuole in unicellular organisms is connected closely with the osmoregulating eliminative functions of the organisms. Biotesting to change this physiological parameter allows a general view of the degree of toxicity of either substance.

Tablo 3 shows the average data of the effects of the different concentrations of oil from Sangachal and Guneshli on the survival and physiological parameters of 4 ciliate species. The data of biotesting of oil on cellular level were consistent with the results of biotesting at community level. It is necessary to note the high sensitivity of *S. conicum* to oil pollution. It was the only specimen from the 4 we selected, to exhibit considerable deviations from the controL by all the test criteria with minimum oil concentrations of 0.01 ml/l.

At the same time, analysis of the average data presented in table 3 confirm our earlier conclusion on the

Table 3. The effects of various oil concentrations on physiological parameters of some ciliate species (in % to control) n = 25. A–survival; B–division rate; C–number of food vacuoles; D–contractile vacuoles activity.

Physiol. parameters	Oil from Sangachal Concentrations (ml/l)								Oil from Guneshli Concentrations (ml/l)							
	.01	.05	.1	.5	1	5	10	25	.01	.05	.1	.5	1	5	10	25
F. marina																
A	100	100	93	78	71	62	60	43	100	85	75	61	56	48	39	31
B	100	100	100	65	66	48	43	22	100	92	61	52	41	34	30	18
C	100	100	100	94	81	60	38	19	100	98	92	71	51	32	20	15
D	98	95	95	71	54	48	45	43	92	90	85	58	51	38	35	30
O. fallax																
A	100	100	100	91	90	89	85	78	100	90	79	76	72	64	58	53
B	100	100	100	97	95	90	81	68	100	100	92	83	80	61	52	38
C	100	100	93	90	85	80	71	70	100	92	86	82	70	64	60	36
D	95	90	89	78	62	54	50	42	90	85	82	70	57	46	40	34
S. conicum																
A	96	82	76	50	44	33	18	–	91	70	66	47	34	12	–	–
B	90	71	61	58	42	21	10	–	81	65	53	38	22	–	–	–
C	88	72	56	48	40	18	–	–	76	60	47	34	11	–	–	–
D	67	65	60	56	50	37	–	–	52	24	11	–	–	–	–	–
E. harpa																
A	100	100	95	81	75	60	58	44	100	80	71	59	51	39	33	25
B	100	100	91	75	68	61	54	41	100	91	82	70	60	54	40	32
C	100	89	73	64	58	50	42	36	93	72	61	54	45	31	27	20
D	87	80	78	75	70	65	59	48	81	72	65	60	54	50	38	30

- A – survival
- B – division rate
- C – number of food vacuoles
- D – contractile vacuoles activity

basis of results on biotesting at community level, about the significantly higher toxicity of Guneshli oil compared with Sangachal oil. Apparently in the near future, after the beginning of development of this oil field it will be necessary for the nature conservation agencies in Azerbaijan to take into consideration the higher toxicancy of oil from the Guneshli oil field.

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