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Oil-Oxidizing Bacteria of Zaporozhskoye Reservoir

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ABSTRACT. The study of oil-oxidizing bacteria number and activity has been carried out for the first time in contaminated with oil products and relatively clean areas of Zaporozhskoye reservoir in spring 2014. The research showed high potential oxidative capacity (POC) of water microflora that probably corresponds to the level of water pollution with oil products. During spring season the spatial distribution of oil bacteria as well as dependence on sampling time and water temperature were studied. Analysis of spatial distribution of oil-oxidizing bacteria numbers allowed to perform ranking of the sites with increasing bacteria numbers in direction from the site "Near Kodaki water draw-off" to "Monastyrskiy island". Distribution of oil-oxidizing bacteria observed during the period of study reflects pollution and self-cleaning state of the sites. Using correlation analysis conclusion about connection of oil-oxidizing bacteria with diatoms "bloom" in spring has been made that shows importance of "bloom" for the realization of bacteria role in the reservoir self-cleaning.

The results are important for understanding the mechanisms of maintaining Zaporozhskoye reservoir ecosystem stability as well as for forecasting of pollution and self-purification processes and in general for the development and implementation of natural and artificial ecosystems sustainable development.

1. INTRODUCTION

Zaporozhskoye reservoir is subjected to enhanced anthropogenic pressure. Among various agents of anthropogenic impact chemical contamination with oil products, heavy metals, pesticides and other toxicants is the most significant part [1, 2]. Water pollution by oil products is especially hazardous because of its very high level in Zaporozhskoye reservoir holding the first place among Dnieper cascade reservoirs by oil products concentration (60 $MAC_{fishery}$, $MAC_{fishery} = 50\ mg/dm^3$; MAC is maximum admissible concentration) [2, 3, 4]. Petroleum and its derivatives being in the aquatic environment are divided into the most unstable light fractions that form the surface film, medium fractions which are distributed in the water, creating an emulsion and heavy fractions that are mostly delayed in the ecosystem as they quickly deposit to the bottom forming a film on it, incorporating into the bottom deposits and thus creating the possibility of the secondary water pollution.

Impact of oil and products of oil decomposing which can be even more toxic and very hazardous for aquatic fauna occurs in several ways [4, 5]. Superficial film of oil delays diffusion of gases from the atmosphere into water and disturb gas exchange in reservoir causing a shortage of oxygen. Oily substance covering gill surface of fishes with a thin film disturb gas exchange that lead to asphyxiation. Water-soluble oil compounds easily penetrate into body of fishes causing poisoning. Oil bottom sediments undermine food base of water-bodies and absorb oxygen from water.

Self-cleaning mechanism decreases level of pollution in the reservoir and includes basic physical, chemical and biotic processes [5]. Central and regulatory role in self-cleaning system belongs to biological factors especially microflora [6, 7]. Due to vast metabolizm types and natural "physiological flexibility" bacteria take the first place among other hydrobionts in destruction of toxic substances such as oil and others [6, 8, 9]. Even Voroshilova and Dianova showed that such bacteria genera as *Corinebacterium*, *Actinomyces, Serratia, Aspergillus, Penicillum* oxidize oil [10]. Modern researchers found some patterns including oil-oxidizing bacteria activity dependency on the concentration of oil hydrocarbons [9, 11]. However in conditions of Zaporozhskoye reservoir very actual study of oil bacteria hasn't been conducted previously.

Thus an actual and urgent task is to elucidate the natural mechanisms of self-purification and maintaining of ecosystem stability of Zaporozhskoye reservoir greatly contaminated with oily wastes and especially to find out the role of oil bacteria for stable development of the reservoir ecosystem.

2. MATERIALS AND METHODS

To assess the role of oil-oxidizing bacteria in self-purification process in conditions of Zaporozhskoye reservoir wherein the highest rates of oil products pollution in the Dnieper cascade, the research of oil-oxidizing bacteria number and activity has been carried out for the first time using modern methods of water microbiology and statistical analysis.

The study was conducted in spring 2014 within Dnepropetrovsk city. Water samples were taken at 5 sites of upper part of the reservoir (fig. 1). Based on the long-term data of Laboratory of hydrobiology, ichthyology and radiobiology Research Institute of Biology, Oles Honchar DNU upper part of the reservoir has been under intense technologically pressure impact, including the sites of largest oil pollution [1, 2, 3].

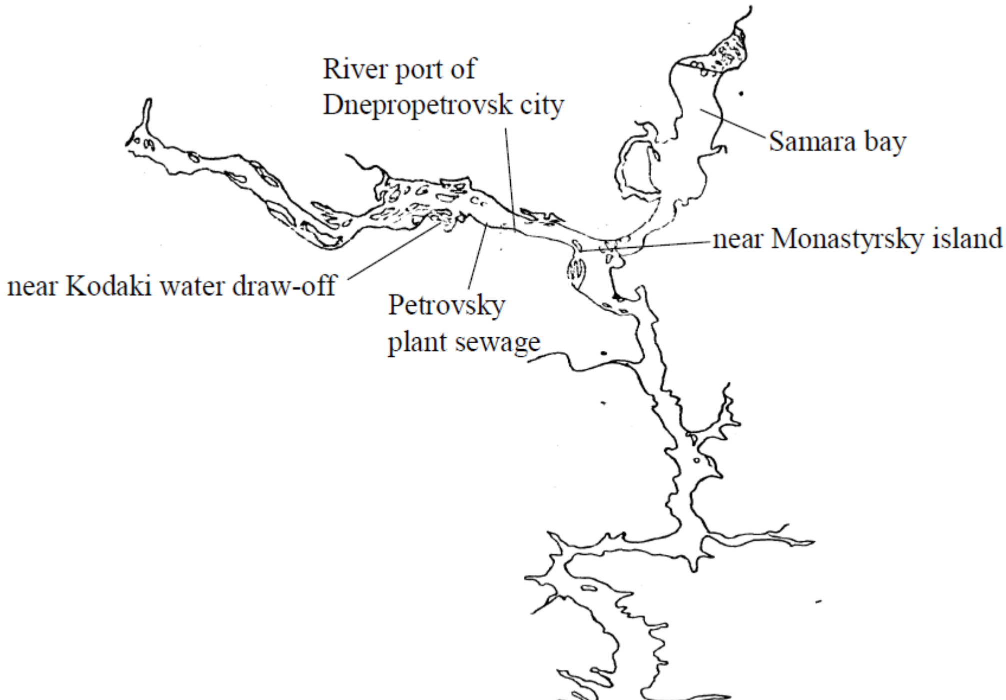

Figure 1. The sites of oil-oxidizing bacteria sampling along Zaporozhskoye reservoir (47°57′36″N, 35°06′52″E)

Distribution of oil bacteria has been studied by sampled at 5 sites: 1) Near Kodaki water draw-off, 2) Petrovsky plant, 3) River port of Dnepropetrovsk city, 4) near Monastyrsky island, 5) Samara bay. Relatively clean station "Near Kodaki water draw-off" served as the site for conparison. At each site the samples were taken at the double replication accordingly to the recommendations [12]. The total number of samples was equal to 40.

To estimate oil bacteria activity Winkler method was used [7, 13]. Studing of oil bacteria numbers was performed using sterilized freshly prepared Dianova - Voroshilova medium by limiting dilution way according to the method [13] in modification [9] (Fig. 2).

Figure 2. Research of oil bacteria numbers in water sample by limiting dilution way

As oil substrate diesel oil was used. 7 bacterial inoculation with volume from 10^{-1} to 10^{-7} cm^3 was performed for each sample. According to the methodology evaluation of the results obtained was conducted on the 3rd, 7th and 14th day of incubation at 30 °C upon condition of presence of precipitate, bacterial slimes, turbidity, color change. Based on the need to adapt the methodology because of high level of oil-oxidizing bacteria activity the final result was registered compared with the control on the 7th day at maximum titer at which changes were observed.

Data obtained were analyzed statistically using the Microsoft Excel program for Windows 2007.

3. RESULTS AND DISCUSSIONS

3.1. Evaluation of oil-oxidizing bacteria activity in Zaporozhskoye reservoir

Conduction of trial experiment was necessary to set new previously not used methods of estimation of oil-oxidizing bacteria number and activity. For this purpose oil-oxidizing bacteria was sampled in March 2014 at monitoring site – Monastyrsky island where significant oil pollution was noted accordind to results of long-term observations [1, 2]. Incubation was conducted by the method [13], in 100 ml sterilized freshly prepared Dianova - Voroshilova medium and 5 ml (5%) of oil with 2 ml of water analysed(bottle 1) or with same volume of distilled water and 5 ml (5%) of oil (bottle 2) for 14 days at 30 °C; it showed the results of oil-oxidizing bacteria activity (fig. 3).

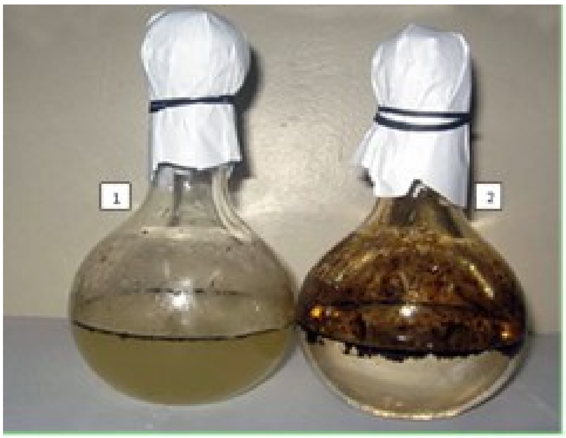

Figure 3. Results of trial experiment indicate the presence of oil-oxidizing bacteria activity in water of Zaporozhskoye reservoir 1 - research bulb; 2 - control bulb.

Quantitative study of oil-oxidizing bacteria by the level of dissolved oxygen by Winkler method was a next step [7, 13]. Estimation of oil bacteria activity was conducted in water of the site "Monastyrskiy island" (March 2014). It was found that potential oxidative capacity (POC) of water microflora under destruction of oil hydrocarbons in early spring averaged 1.05 mgO_2/dm^3/day. This figure was very high - much higher than in Gorky reservoir (0.8 mgO_2/dm^3 / day), Kuibyshev and Volgograd reservoirs (0.96 mgO_2/dm^3/day) [14], that may reflect high levels of water pollution with oil products up to 60 fishery MAC [1, 2, 8] in early spring.

3.2. Spatial distribution of oil-oxidizing bacteria number

Based on presence of expressed oil-oxidizing activity we studied oil-oxidizing bacteria number for the first time in Zaporozhskoye reservoir. To study the spatial distribution of heterotrophic bacteria which can use oil as a source of carbon sampling was carried out 4 times in five sites of Zaporozhskoye reservoir upper part in spring 2014.

The first sampling was carried out in early spring at a temperature 3 °C (Table 1).

According to the method any changes in dilutions which was incubated at 30 °C condidered as indication of bacterial activity. The data obtained showed that expressed oil-oxidizing activity appeared at all investigated sites already on the third day of water samples incubation on diesel oil. The lowest number of bacteria (by number of dilution) was marked at the site "River port of Dnepropetrovsk city" and the largest was marked at the site "Samara Bay". However this does not necessarily indicate an appropriate degree of contamination of these sites. According to modern concepts presence of oil-oxidizing bacteria in water does not mean presence of antropogenic origin oil (that is specific for Samara Bay) whereas plants and bacteria continuously synthesize hydrocarbons which are similar to oil products. Moreover oil-oxidizing bacteria as heterotrophs can digest primarily easily oxidable organic matter [4, 11] which concentration is usually high in Samara Bay [1, 2].

Table 1. Number of oil-oxidizing bacteria according to the data obtained during sampling 11.03.2014

	Dilution №	Near Kodaki water draw-off	Petrovsky plant sewage	River port of Dnepropetrovsk city	Monastyrskiy island	Samara bay
Third day	1	+	++	+	++	+-
	2	+	+	+-	+	-++
	3	-+	+	-	-+	-+
	4	-	-	-	-+	-+
	5	-	-	-	-	-+
	6	-	-	-	-	-
	7	-	-	-	-	-
Seventh day	1	-+ precipitate, turbidity	+- turbidity, darkening, precipitate	+- putrefactive smell, slime	+- precipitate, turbidity	-+ precipitate
	2	+- precipitate, turbidity	+- putrefactive smell	+- slime	+- precipitate, turbidity	+- darkening slime, precipitate
	3	+- precipitate, turbidity	-+ putrefactive smell, precipitate	-+ turbidity, precipitate, slime	+- turbidity, precipitate, slime	-+ slime, darkening
	4	+- turbidity, putrefactive smell, precipitate	-	+ turbidity, precipitate, darkening	+- turbidity, precipitate	+- slime, precipitate, darkening
	5	-	-	- darkening	+- turbidity, precipitate, slime	-+ darkening slime, precipitate
	6	-	-	- darkening	+- precipitate, slime	-+ turbidity slime, precipitate
	7	-	-	-	-	-
Result: titer, concentration		T=10^{-4} cm^3 or C =10000 cells/cm^3	T=10^{-3} cm^3 or C =1000 cells/cm^3	T=10^{-4} cm^3 or C =10000 cells/cm^3	T=10^{-6} cm^3 or C = 1000000 cells/cm^3	T=10^{-6} cm^3 or C = 1000000 cells/cm^3

Note: Dilutions № from 1 to 7 correspond to the volume of bacterial inoculation from 10^{-1} to 10^{-7} cm^3; ++ intensive, + medium, +- noticeable, -+ low level of indication expression; - not registered

Further increasing of bacterial oil-oxidizing activity was noted on the seventh day: near Monastyrskiy island and near Samara Bay titer of oil-oxidizing bacteria reached T = 10^{-6} cm^3. On the other hand in Petrovsky plant sewage bacteria titer was noted to decrease from 10^{-7} cm^3 to 10^{-3} cm^3. That may be caused by oil-oxidizing bacteria activity inhibition because these bacteria was active-responsed and quick-responsed but unstable. Putrefactive smell in the samples indicates bacterial decay.

Although in the samples from other sites oil-oxidizing bacteria showed more resistance on the 14th day (data wasn't shown in the table) nonetheless putrefactive smell became common feature for all samples from the sites investigated except Monastyrskiy island. Near the island high level of oil pollution has been constantly registered and presence of oil-oxidizing bacteria is very important for self-purification of this site. Probably shift of optimum and dependence on time (and therefore registration) of bacteria activity to an earlier period is possible for reservoir with high oil pollution level and increased oil-oxidizing bacteria activity. Taking into account the need for appropriate modification of activity evaluation methodology data obtained on 7th day were taken as a result.

The second sampling was performed 2 weeks after the first sampling at a temperature +7,2 °C during diatoms bloom. Unlike the first sampling the second sampling showed much fewer oil-oxidizing bacteria number on the third day at all the sites except "River port of Dnepropetrovsk city". Decreasing of oil-oxidizing bacteria number may be caused by general pollution restriction of the reservoir in the spring due to self-cleaning process in which diatom algae play active role. Diatoms bloom takes place at temperature from +2 to +8 °C in the spring.

However until the seventh day significant increase of oil-oxidizing bacteria numbers was observed at all sites. The most remarkable change took place in Samara bay – from no reaction of oil-oxidizing bacteria to its significant expression in 6 dilutions. This may be a consequence of latent phase in which oil-oxidizing bacteria prefer to consume readily oxidizable organic matter at its elevated levels in the sample. Zaporozhskoye reservoir especially Samara Bay is rich in dissolved organic matter, which concentration increases in spring due to excretion of diatoms *Melosira islandica*. In general compared with the first sampling at all sites except Monastyrskiy island the second sampling showed increase of oil-oxidizing bacteria numbers 17.65% in average.

The following third sampling was conducted in mid-April (15.04.2014) at temperature + 8,5 °C. Analyzing of the data obtained showed that the distribution of bacterial titer was very similar to the second sampling results with "slow start" at the beginning of first week and explosive type at the end. This similarity apparently is caused by the temperature range in second and third sampling in which diatoms bloom takes place. Concordance of microbial processes and phytoplankton development V. I. Shcherbak explained by leading role of phytoplankton in plankton community and in ecosystem as a whole [15].

Conspicuous is the fact that on the third day like in previous cases samples from the sites "Petrovsky plant sewage" and "Monastyrskiy island" showed expressed activity of oil-oxidizing bacteria. At these sites high levels of water contamination by oil products usually observed, in other words populations of oil-oxidizing bacteria are "rapid reaction units" at the sites and provide especially in spring oil self-cleaning of reservoir water. On the seventh day figures along the sites was more uniform and average increasing of oil-oxidizing bacteria titer compared with the second sampling was 8%.

The fourth sampling during the spring was at the end of May first decade when water temperature reached 14.4 °C. The results of oil-oxidizing bacteria assessment in nature are very similar to the results observed in the first sampling. Activity of oil-oxidizing bacteria began to be manifested from the beginning of incubation at all sites but in the end the results became different – from 10^{-4} cm^3 at the sites "Near Kodaki water draw-off" and "River port of Dnepropetrovsk city" to 10^{-7} cm^3 at "Monastyrskiy island". Maximal figures of oil-oxidizing bacteria numbers were noted at the sites of strong permanent oil pollution. The exception was Samara bay where process of eutrophication stimulated heterotrophic microorganisms development.

3.3. Ranking of surveyed sites

The results of the sampling are summarized in Table 2 and presented in commonly used in microbiology index -lgT which facilitates statistical and graphical data processing.

Table 2. Figures of oil bacteria titer at reservoir sites according to the results of fourth sampling

Data	Near Kodaki water draw-off		Petrovsky plant sewage		River port of Dnepropetrovsk city		Monastyrskiy island		Samara bay	
	T	-lgT	T	-lgT	T	-lgT	T	-lgT	T	-lgT
11.03.2014	10^{-4}	4	10^{-3}	3	10^{-4}	4	10^{-6}	6	10^{-6}	6
25.03.2014	10^{-5}	5	10^{-4}	4	10^{-5}	5	10^{-5}	5	10^{-6}	6
15.04.2014	10^{-5}	5	10^{-5}	5	10^{-5}	5	10^{-6}	6	10^{-6}	6
09.05.2014	10^{-4}	4	10^{-6}	6	10^{-4}	4	10^{-7}	7	10^{-6}	6
In average	$10^{-4,5}$	4,5±0,5	$10^{-4,5}$	4,5±0,5	$10^{-4,5}$	4,5±0,5	$10^{-5,8}$	5,8±0,8	10^{-6}	6±0
Average number	55000 cells/cm^3		277750 cells/cm^3		55000 cells/cm^3		2440000 cells/cm^3		1000000 cells/cm^3	

Analysis of spatial distribution of oil-oxidizing bacteria population revealed that at the sites "Near Kodaki water draw-off", "Petrovsky plant sewage" and "River port of Dnepropetrovsk city" average index of titer was the same (-4.5), indicating common conditions at these sites that caused by strong speed of current as well as low square and density of macrophytes beds. At the station "Monastyrskiy island" and "Samara Bay" index of titer was very close (respectively -5.8 and -6) also due to common conditions (reverse current) and interinfluence between these stations. Since Samara Bay and Monastyrskiy island are situated very close the results obtained may reflect the circulation of water masses.

At recouning of oil-oxidizing bacteria average number in spring for each site appeared that minimal figures (55,000 cells / cm^3) was at the sites "Near Kodaki water draw-off" and "River port of Dnepropetrovsk city" and maximal figures (2,440,000 cells / cm^3) near Monastyrskiy island, indicating corresponding pollution rates. Despite the fact that contamination level at the station "Petrovsky plant sewage" is similar with contamination near Monastyrskiy island the average oil-oxidizing bacteria numbers is 9 times lower compared with maximal at these sites that can be explained by inhibiting influence of other pollutants and by speed of current.

Ranking of the sites surveyed by increasing of average bacterial titer provided the following line:

Near Kodaki water draw-off < River port of Dnepropetrovsk city < Petrovsky plant sewage < Monastyrskiy island < Samara bay. Ranking of the sites by increasing of average oil-oxidizing bacteria number provided slightly different line:

Near Kodaki water draw-off < River port of Dnepropetrovsk city < Petrovsky plant sewage < Samara bay < Monastyrskiy island.

Concerning relatively high oil-oxidizing bacteria number in Samara Bay (1000000 cells/cm^3) and taking into account low level of oil products pollution at this site such high numbers can be explained by favourable conditions for this group of heterotrophic bacteria in expecially vast macrophytes beds, slow speed of current and great volumes of organic matter enrichment (eutrophication effect). Stimulating of oil-oxidizing bacteria development by the factors listed above causes high ability for water self-purification in conditions of Samara Bay.

3.4. Time dependency of oil-oxidizing bacteria number

Studies of time dependency of oil-oxidizing bacteria numbers showed that the lowest numbers was noted on 3.11.2014 and maximal on 05.09.2014. The number of oil-oxidizing bacteria in Samara bay was constant. In Petrovsky plant sewage number of oil-oxidizing bacteria gradually increased with temperature rising. At the sites "Near Kodaki water draw-off" and "River port of Dnepropetrovsk city" number of oil-oxidizing bacteria varied with maximum in early April during diatoms "bloom" because excretions of these algae promote development of heterotrophic bacteria. At the station "Monastyrskiy island" fluctuations of bacteria number were also observed with maximum on 09.05 that can be caused not only by temperature regime but also increased oil pollution in connection with beginning of shipment and recreational period as well as with rainwater runoffs.

3.5. Dependence of oil-oxidizing bacteria numbers on temperature

By graphical and correlation analysis of the results obtained certain dependence between oil-oxidizing bacteria numbers and temperature was found (fig. 4).

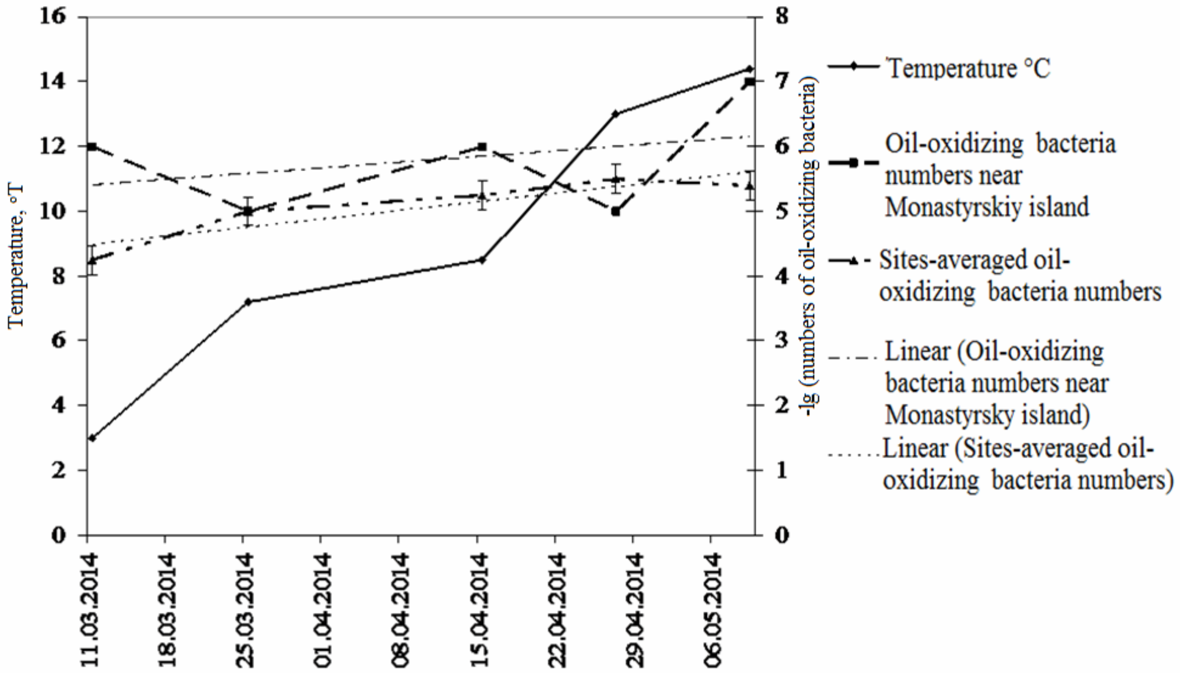

Figure 4. Dependence of oil-oxidizing bacteria numbers on water temperature

Analysis of the graphs shows that the general trend is an increasing of oil-oxidizing bacteria number together with rising water temperatures, especially in the period from the end of March to May. This was confirmed by significant correlation coefficient between bacteria number and temperature ($r = 0.5$). The hypothesis that phytoplankton "bloom" may indirectly affect on correlation between bacteria numbers and temperature in the spring was confirmed. The correlation coefficient between oil-oxidizing bacteria number and index of toxicity (IT) that depends on spring diatoms "bloom" was $r = -0.5$ for the period March - April 2014 (toxicity index was determined in parallel with number of oil-oxidizing bacteria in the same samples, number of samples for correlation analysis was $n = 20$). The data obtained suggest that increasing of water temperature in spring in conditions of diatoms "bloom" and decay stimulated development of oil-oxidizing bacteria.

4. CONCLUSION

For the first time study of oil-oxidizing bacteria numbers and activity has been carried out in Zaporozhskoye reservoir in contaminated with oil products and relatively clean sites in spring 2014. The study showed high potential oxidative capacity of water bacterioflora in reservoir that corresponds to high level of oil pollution.

Analysis of spatial distribution of oil-oxidizing bacteria number allowed to perform ranking of the sites surveyed by bacteria number increasing in direction from the site "Near Kodaki water draw-off" to "Monastyrskiy island" that reflects both pollution and self-cleaning state of the sites. Using correlation analysis connection between oil-oxidizing bacteria development and diatoms "bloom" in spring was concluded that may reflect adaptation of reservoir ecosystem for intake of pollution with spring floods. Stimulation of heterotrophic oil-oxidizing microorganisms by phytoplankton development is probably a common adaptation or trigger self-cleaning mechanism for self-cleaning processes initiation in water-bodies [15, 16] and that is very important at the beginning of vegetative season.

Thus self-purification processes especially due to microflora counteract contamination by oil and its derivatives in Zaporozhskoye reservoir. Based on the results obtained monitoring of oil-oxidizing bacteria activity as well as further clarification of conditions and factors affecting on expression of their activity is an actual problem because these bacteria are effective factor of self-cleaning process in conditions of the reservoir.

The results obtained are important for understanding of stability maintenance mechanisms in reservoir ecosystems in order to forecast pollution and purification processes and in general for planning and realization of sustainable development conception of natural and artificial ecosystems.

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