Assessment of Toxicity of Technical Detergents Using Complex of Bioassay Methods

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Abstract. The purpose of this work is to compare the sensitivity of biotesting techniques based on the change in bioluminescence intensity of the "Ecolum" test system, the survival rate and the variation in the trophic activity of daphnia in determining the toxicity of waters containing "TOR-LOCOMOTIVE" and "Chemitek Polypro". "Tor-locomotive" and "Chemitek Polypro" – highly effective technical means for removing complex contaminants of various nature, for washing the main diesel locomotives and electric locomotives. The trophic activity of the crustaceans was estimated by decreasing the fluorescence intensity of chlorophyll of algal cells. It was revealed that "TOR-LOCOMOTIVE" and "Chemitek Polypro" have an inhibitory effect on the ability of plankton filter-daphnia to extract algae from water during their filtration activity. A number of sensitivity of test-functions of biotests in relation to the detergents studied is constructed: trophic activity of crustaceans > survival of daphnia > bioluminescence "Ecolum". The most sensitive method of biotesting is to change the trophic activity of daphnia, and the most rapid one is to quench the luminescence of the luminous bacteria. The danger of getting these detergents into the water bodies is associated with the violation of ecological processes essential for self-purification of water, including Lake Baikal.

1. Introduction
 "Tor-locomotive" and "Chemitek Polypro" modern highly effective technical means for removing complex contaminants, for washing main diesel locomotives and electric locomotives. These substances contain fairly high concentrations of surfactants. Many surfactants are highly toxic for various groups of hydrobionts: microorganisms, algae, invertebrates [1, 2]. Therefore, it is important to assess the toxicity of aqueous solutions of "TOP-LOCOMOTIVE" and «Chemitek Polypro» Due to the simplicity of operability and accessibility, biotesting is widely used to assess water quality [3, 4]. The most common test organism used for toxicological studies is Daphnia magna. The D. magna crust serves as a calibration standard for other methods for biotesting liquid media. In the literature, the variation in the trophic activity of the branching crustaceans in response to the effects of pollutants of different nature has been described [5, 6]. High sensitivity to pollutants, rapidity and reproducibility determine the promise of a method based on changes in the intensity of bioluminescence of luminous bacteria [7]. A number of studies have shown the suppression of the filtration activity of aquatic organisms by certain surfactants [8, 9, 10].
The suppression of their filtration activity by surfactants is an additional hazard. This can weaken the regulatory function of zooplankton on many parameters of the aquatic ecosystem, which is one of the causes of anthropogenic eutrophication of water bodies with the growth of chemical pollution of the aquatic environment [11, 12, 13].

The purpose of the work is to compare the sensitivity of biotesting methods based on the change in bioluminescence intensity of the EcoLum test system, the survival rate and the variation of trophic activity of daphnia in determining the toxicity of waters containing "TOP-LOCOMOTIVE" and "Chemitek Polypro".

2. Materials and equipment

"Chemitek Polypro" is a concentrated liquid low-foam alkaline agent for removing industrial contaminants. Means "Chemitek Polypro" is recommended for washing bodies and running gear of locomotives. Composition: non-ionic surfactants 5-15 %, alkali 15-30 %, inorganic salt < 5 %, water. "TOR-LOCOMOTIVE" is a tool intended for washing and cleaning of the exterior and interior surfaces of the bodies of passenger and traction rolling stock from fat- and oil operational pollution. Ingredients: alkali, various alkali salts, synthetic surfactants, complexing agents. The concentration of working solutions of these detergents is 1 %.

The toxicity of the test preparations was evaluated according to the methods recommended for state environmental monitoring of the mortality of daphnia (D. magna) [14]; change in the intensity of bioluminescence of the preparation "Ecolum" [15], trophic activity of daphnia. Incubation of used test organisms in solutions of the test solutions corresponded to the requirements of the methods: daphnia - 4 days, preparation "Ecolum" – 30 minutes. In determining the toxicity by changing the intensity of bacterial bioluminescence, the preparation of luminescent bacteria "Ecolum" (CJSC "NVO Immunotech") was used. The latter is a lyophilized culture of Escherichia coli bacteria with the cloned lux-operon Photobacterium leiognathi. Measurement of bioluminescence intensity of the preparation "Ecolum" was carried out on a biomagnetometer "Biotox-10M. The quantitative evaluation of the test responses was expressed by the relative difference between the control and trial samples (in %) using the toxicity index (T):

$$T = \frac{X_k - X_o}{X_k} \times 100\%$$

(1)

where \(X_k\) is the average value of the test parameter in the control, \(X_o\) is the average value of the test parameter in the experiment at a fixed exposure time.

To compare the sensitivity of biotests graphically with the use of probit analysis [14], the following values were calculated:

- harmless concentrations (HC), causing a 10 % or less change in the survival of daphnia and 20% or less the intensity of bioluminescence of luminous bacteria compared to the control.

- Inhibitory concentrations (IC50), causing a decrease in the value of the test functions by 50 % or more, compared with the control.

The determination of trophic activity was carried out according to the methods of [16, 17]. In control samples, the crustaceans were placed in a suspension of algae Scenedesmus quadricauda G.M. Smith without the addition of toxicants. In addition, samples of algae were prepared in toxicant solutions without crustaceans. This is to check the effect of the test substances on the fluorescence of chlorophyll cells of algae. The fluorescence intensity of chlorophyll of algal cells was measured on the Fluorimeter "Qubit-2.0". The calculation of trophic activity of daphnia was carried out according to the Formula 2:

$$TA = \frac{F_{hl} - F_{h} + P}{F_{hl}} \times 100\%$$

(2)

where TA is trophic activity; \(F_{hl}\) – the indicator of fluorescence of chlorophyll of a suspension of cells of seaweed; \(F_{h}\) + p – the indicator of the chlorophyll fluorescence of a suspension of algal cells with crustaceans.
All experiments were carried out in at least 3 independent experiments with 3 parallel measurements in each. For statistical processing of the received data, the Microsoft Excel software package was used. The reliability of the difference in the results was determined with the help of the Student's test for the probability of an error-free prediction \( p \geq 0.95 \).

3. Results and discussions

From the toxicity values obtained by Formula 1, harmless inhibitory concentrations of technical detergents were calculated (Table 1.).

| Detergents            | Mortality \( D. \) magna | Bioluminescence “Ecolum” |
|-----------------------|---------------------------|---------------------------|
|                       | \( HC_{10-96} \), %      | \( IC_{50-96} \), %       |
| «TOP-LOCOMOTIVE»      | 0.005                     | 0.014                     |
| «Chemitek Polypro»    | 0.006                     | 0.014                     |

It is necessary to dilute the working solution 200 times to achieve a harmless concentration for the survival of \( D. \) magna. A slight difference between \( HC_{10-96} \) and \( IC_{50-96} \) indicates a rather high toxicity of detergents. Daphnia were somewhat more sensitive to the toxic effects of technical detergents than luminous bacteria.

To increase the sensitivity of the bioluminescent method, further developed studies can be used to develop the so-called "chronic" bioluminescent test. In this embodiment, the decrease in the luminescence intensity is determined not after 30 minutes of incubation, as in the standard test, but after 24 hours [18]. The multiplicity of the dilution of water extracts of technical detergents, in which no harmful effect, corresponds to the third class of danger for the environment [19]. It was shown in [2] that the test objects are characterized by different sensitivity to each of the surfactant compounds studied, which is determined both by the specificity of the test reaction of the test object itself and by the various properties of the compounds.

To study the effect of technical detergents on the trophic activity of daphnia, harmless concentrations of these compounds were used to survive daphnia (\( K_{10-96} \)) (Figure 1. and Figure 2.)

![Figure 1. The influence of "TOP-LOCOMOTIVE" on the trophic activity of daphnia.](image)
We emphasize that in the control samples containing algal suspensions in solutions of toxicants, without crustaceans, the fluorescence of chlorophyll of algae did not change in the three-hour experiment. "TOP-LOCOMOTIVE" at a concentration of 0.005 % does not cause daphnia death (100 % survival) for four days. While a drop in trophic activity was observed by almost 50 % compared with the control. Therefore, according to this indicator, the effect of "TOP-LOCOMOTIVE" in this concentration is acutely toxic.

"Chemitek Polypro" concentration of 0.01 % causes the death of 50 % of daphnia in 96 hours. This concentration suppresses the trophic activity of daphnia for three hours by 65 % compared to the control. At a concentration of 0.006 % with an exposure of 96 hours, 100 % of daphnia survive and their trophic activity, for three hours, at the control level.

4. Conclusion
"TOP-LOCOMOTIVE" had a stronger toxic effect on the survival and trophic activity of daphnia, bioluminescence of luminous bacteria than "Chemitek Polypro". The most sensitive to the test technical detergents – a method based on the trophic activity of daphnia.

"TOP-LOCOMOTIVE" has a significant inhibitory effect on the trophic activity of daphnia in concentrations considered harmless in the survival of daphnia. The concentration of 0.005% of this pollutant does not affect the survival of daphnia in a four-day trial. At the same time, trophic activity drops twofold during the three-hour experiment.

The toxicity rating of "TOP-LOCOMOTIVE" is determined by mortality of daphnia and changes in the bioluminescence of luminous bacteria are clearly underestimated. Therefore, in determining the toxicity of detergents, along with other methods of biotesting, it is necessary to apply tests to change the trophic activity of hydrobionts.

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