Combined Action of Sodium Dodecyl Sulfate, Tween-85 and Oil on Duckweed (*Lemna Minor*)

M N Saksonov¹, A B Kupchinsky², D I Stom¹²³

¹Irkutsk State University, st. Karl Marx 1, Irkutsk, 664003, Russia
²Baikal museum of the Irkutsk Scientific Center of the Siberian Branch of the Russian Academy of Science, Akademicheskaya Street 1, Listvyanka 664520, Russia
³Irkutsk National Research Technical University, st. Lermontov 83, Irkutsk, 664074, Russia

E-mail: stomd@mail.ru

Abstract. Surfactants (surfactants) are found in large quantities in industrial and domestic wastewater. Along with them, there are also oil products. In a certain concentration, they have a depressing effect on aquatic organisms. In recent decades, along with physicochemical methods for assessing the quality of water bodies, biodiagnostics - biotesting and bioindication - has been actively used. Depending on a number of factors, the effect of the combined action of substances on the test organism can be additive, antagonistic, or synergistic. Biotesting in combination with a multifactorial experiment makes it possible to identify the final effect and the nature of the combined toxic effect of pollutants. The study investigated the isolated and combined effect of oil and some surfactants on the duckweed. The criterion for the influence of pollutants was the growth dynamics of fronds of this aquatic plant. During biotesting, the most widely used anionic detergent, sodium dodecyl sulfate (SDS), was used. In addition, we studied the effect of the nonionic surfactant Tween-85 on this test object. The toxicity of the effect of certain substances on the duckweed was assessed, and this made it possible to calculate the concentrations that cause a certain toxic effect. Then a full two-factor experiment was carried out with the combined action of two pairs of substances: oil - SDS, oil - Tween-85. Regression equations for two pairs of substances are compiled. The action of the investigated compounds is interdependent. The nature of the combined toxic effect turned out to be less than additive.

1. Introduction

Surfactants are one of the most common components of polluted waters. They are found in large quantities in domestic and industrial wastewater. By their scale, oil and oil products have long been recognized as the main pollutants of the environment. Pollution with oil and oil products can occur both at the stages of oil production, transportation, and at the stage of oil refining [1, 2]. Often surfactants, oil products and oil are present in the same wastewater.

Surfactants are widely used to eliminate emergency oil spills and to rehabilitate contaminated soils and water bodies [3-6]. Currently, the creation of new analytical systems for assessing the state of the environment is of paramount importance. The existing methods of analytical chemistry are mainly focused on the quantitative determination of individual compounds [7-9]. Therefore, more and more important place is given to biotesting methods. The value of the latter lies in the fact that they provide...
an integral assessment of the quality of the environment. The methods of physical and chemical analysis do not allow this.

There is a large number of works on the study of the toxic effect of certain oil products and surfactants on hydrobionts. On the other hand, insufficient attention is paid to studies of joint action [10-12].

However, the combined environmental impact of these pollutants on organisms is not a simple sum. These pollutants interact with each other. In this case, the resulting products can have a higher toxicity or neutralize each other [13-16].

The study of the combined action of oil products and surfactants, biotesting of mixtures of these substances in combinations of various concentrations in a multifactorial experiment allows us to reveal the nature of this interaction – additive, antagonistic, or synergistic [17-19]. The latter option can be quite dangerous. SDS is one of the most widely used anionic detergents used in industry as a cleaning and wetting agent, in the production of most detergents and shampoos. Tween-85 is used as an emulsifier, solubilizer, lubricant stabilizer. Duckweed is a fairly highly sensitive aquatic animal to the action of chemicals of various classes. It is often used as a test organism in water biotesting [20].

Based on the foregoing, the study investigated the isolated and combined effect of oil and some surfactants on the increase in the number of duckweed fronds.

2. Objects and research methods
An aquatic plant *Lemna minor* was used as a test organism. The following compounds were taken as pollutants:
- Surfactant: sodium dodecyl sulfate (Reakhim), Tween-85 (Sigma-Aldrich).
- Oil (Markovskoye field).

The concentration of toxicants in experiments with an isolated effect on duckweed was 10.0; 50.0; 100.0; 500.0; 1000.0 mg / l.

For the experiment, 10 fronds of the plant were placed in each Petri dish filled with a solution of 20 ml of the test compound. All samples were incubated in a thermoluminostat at a temperature of 24 (± 1) °C and illumination 6000 lx - 6500 lx.

The toxicity of the samples was assessed by the increase in the number of *Lemna minor* fronds in the samples [20, 21]. The number of fronds on the seventh day was counted and the growth rate (r) of duckweed was determined by the formula:

\[ r = \frac{\ln N_t - \ln N_0}{t} \]

where \( N_0 \) is the initial number of duckweed fronds; \( N_t \) is the average number of fronds after incubation time \( t \) (in days).

And then they found the indicator of toxic action in relation to the control sample:

\[ T = \frac{r_{контр.} - r_{токс}}{r_{контр.}} \times 100\% \]

For each concentration, two independent experiments were performed with three analytical replicates in each. Statistical data processing was performed using the Microsoft Office software package.

3. Results and discussion
At the first stage, the toxic effect of individual substances on the increase in the number of duckweed fronds was assessed. All compounds had a different toxic effect on duckweed. Concentrations of oil, SDS and Tween-85 causing inhibition of the increase in the number of fronds by 50% - 53.6 mg / l; 85.1mg / l; 1122 mg / l, respectively (Fig. 1).
Figure 1. Influence of surfactants and oil on germination of new duckweed fronds (■ - sodium dodecyl sulfate, ■ - tween-85, ■ - oil).

Using probit analysis, concentrations were found that inhibit the studied reaction parameter by 25% (IC\textsubscript{25}), 50% (IC\textsubscript{50}), 75% (IC\textsubscript{75}) for the compounds used in the assessment of combined action (Table 1).

Table 1. Concentrations of surfactant solutions (mg / l) and oil (mg / l), causing an inhibitory effect on the increase in the number of duckweed fronds by 25%, 50% and 75%.

| Compounds, mg / l | Inhibition (%) |
|------------------|----------------|
|                  | 25%     | 50%      | 75%      |
| tween-85         | 355     | 1122     | 1905     |
| sodium dodecyl sulfate | 29,5    | 85,1     | 224,0    |
| oil              | 9,35    | 53,6     | 500,0    |

According to the plan of the experiment to study the combined effect of two factors (Table 2), mixtures of pollutants were prepared and the toxic effect of these mixtures on duckweed was determined, as described above.

Table 2. Experiment design to study the combined effects of two factors.

| Experience number | Factors |
|------------------|---------|
|                  | $x_1$   | $x_2$   |
| 1                | -1      | -1      |
| 2                | 1       | -1      |
| 3                | -1      | 1       |
| 4                | 1       | 1       |
| 5                | 0       | 0       |
| 6                | 0       | 1       |
| 7                | 0       | -1      |
| 8                | 1       | 0       |
| 9                | -1      | 0       |

Note: concentrations that inhibit the increase in the number of duckweed fronds by 25% (IC\textsubscript{25}) were coded as {-1}; 50% (IC\textsubscript{50}) as {0} and 75% (IC\textsubscript{75}) as {1}.
These concentrations served as three levels of variation of the oil-surfactant factors in terms of a full factorial experiment.

When the concentrations of the two substances included in the combination are designated as \(x_1\) and \(x_2\), the toxic effect on *Lemma minor* under the combined effect of the mixture is determined from the results of nine experiments as the dependence \(y = f(x_1, x_2)\) and is expressed as a second-order polynomial:

\[
y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2
\]

where \(b_0\) – is the coefficient corresponding to the value of the studied indicator with the average value of both factors (with the introduction of both substances in medium doses);

\(b_1, b_{11}\) – coefficients reflecting the linear and nonlinear components of the first factor effect,

\(b_2, b_{22}\) – coefficients reflecting the linear and nonlinear components of the second factor effect;

\(b_{12}\) – coefficient characterizing the influence of one factor depending on the level of another, i.e. reflecting the overall effect of the interaction of factors.

Coefficients \(b\) are calculated using the formulas given in [19].

When characterizing the types of combined action of chemicals, the following main ones are distinguished. Additive action is a type of combined action of chemicals in which its joint effect is equal to the sum of the effects of each of the substances with an isolated effect on the body. With synergism (more than an additive effect), the effect of the combined effect exceeds the sum of the effects of each of the substances. Antagonism (less than additive action) - the combined effect is less than the sum of the effects of each of their substances.

As a result of experiments according to the plan (Table 2.), regression equations were obtained.

**Oil – Sodium dodecyl sulfate**

\[
y = 80.51 + 8.98x_1 + 23.43x_2 - 0.03x_1^2 - 15.85x_2^2 - 4.89x_1x_2
\]

The toxic effect of oil and SDS turned out to be interdependent – the coefficient \(b_{12}\) in the last term of the equation is not equal to 0.

Isolated injection of oil with an increase in concentration from IC\(_{50}\) {0} to IC\(_{75}\) {1} causes an increase in the toxic effect by 8.95% (\(b_1-b_{11} = 8.98-0.03\)). The isolated effect of SDS – by 7.58% (\(b_2-b_{22} = 23.43-15.85\)). In case of joint action (instead of \(x_1\) and \(x_2\) we put their codes \{1\}) – by 11.64%.

The sum of isolated toxic effects is 8.95 + 7.58 = 16.53%> than the toxic effect with a combined action of 11.64%. Thus, we can conclude that the effect of the combined action is less than additive (antagonistic).

**Oil – Tween-85**

\[
y = 34.79 + 15.50x_1 + 5.37x_2 + 8.76x_1^2 + 7.10x_2^2 - 2.07x_1x_2
\]

The effects of oil and Tween-85 are interdependent (\(b_{12} \neq 0\)).

Isolated injection of oil with an increase in concentration from IC\(_{50}\) to IC\(_{75}\) causes an increase in the toxic effect by 24.26%, and Tween-85 – by 12.47%. In this case, the sum of the effects with an isolated increase in individual toxicants is 36.73%. With the combined action, the toxic effect is 34.66%.

The nature of the combined action of oil and Tween-85 is less than additive (antagonistic).

**4. Conclusion**

The influence of SDS, Tween-85 and oil on the increase in the number of duckweed fronds was studied. On the basis of the obtained data, the following series of toxicity of the studied compounds for duckweed small oil > SDS > Tween-85 was established. Concentrations of oil, SDS and Tween-85 causing inhibition of the increase in the number of fronds by 50% - 53.6 mg / l; 85.1mg / l; 1122 mg / l, respectively.

When performing a full two-factor experiment with the combined action of oil and surfactants, regression equations were obtained. In the equations oil – SDS and oil – Tween-85, it is shown that the
toxic effects of the compounds under study are interdependent. This interdependence is weakly expressed, especially for the oil-Tween-85 pair. The nature of the combined effect of the studied pairs of substances on the growth of duckweed fronds was less than additive.

5. References

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