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Title: Combined toxic effect of potassium butyl xanthate and oil on duckweed (*Lemna minor*)

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duckweed, oil, potassium butyl xanthate, biotesting, regression equation

Potassium butyl xanthate (PBX) is widely used as a reagent in ore beneficiation by flotation. PBX is present in the wastewater of these industries [Goryachev, 2014; Ignatkina, 2014]. Petroleum products are found in a wide variety of waste waters. They, like flotation reagents, have a depressing effect on aquatic organisms.

Biotesting methods allow an integral assessment of the quality of the environment. The study of the combined action of oil and flotation reagent by biotesting methods in a multifactor experiment makes it possible to reveal the nature of this interaction (additive, antagonistic or synergistic) [Zholdakova, 2012; Gelashvili, 2016].

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The purpose of this work is to study the isolated and combined effect of potassium butyl xanthate and oil on an aquatic plant - duckweed (*Lemna minor*) by changing the growth of fronds.

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In experiments on biotesting, we used plant cultures of duckweed (Lemna minor) grown in a medium of constant composition at a certain temperature and illumination regime. The growth medium was one of the modifications of the Sternberg medium.

Concentrations of PBX ($C_5H_9OS_2K$) and oil in experiments with isolated action on small duckweed were 10.0; 50.0; 100.0; 500.0; 1000.0 mg/l. The toxicity of the samples was assessed by the increase in the number of L. minor fronds in the samples [GOST 32426-2013]. The study was carried out in three independent experiments, three replicates each.

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The toxicity of the samples was assessed by the level of growth of L. minor in the samples. The number of fronds on the third, fifth and seventh days was counted and the growth rate (r) of duckweed was determined by the formula:

$$r = \frac{(lnN_t - lnN_0)}{t},$$

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:

$$T = \frac{(r_к - r_{токс.})}{r_{к.}} * 100\%$$

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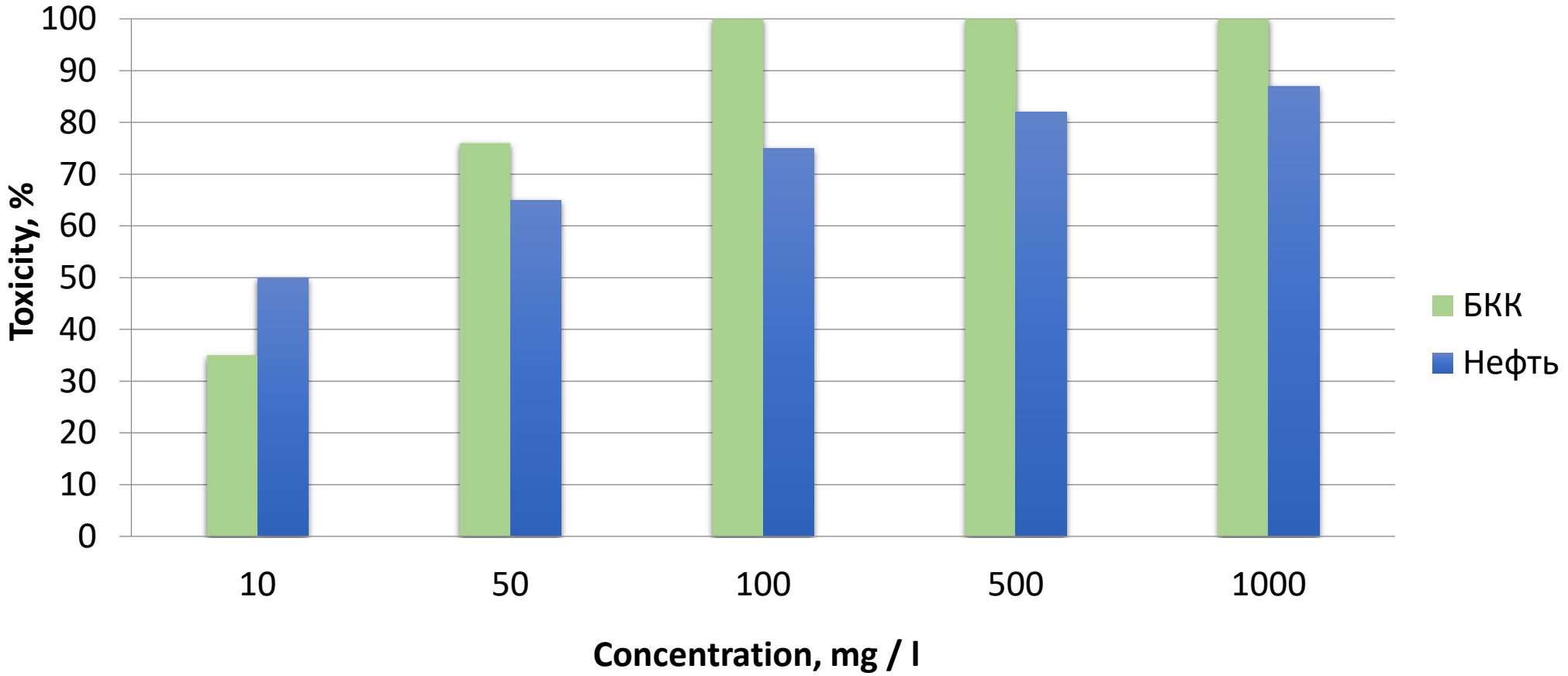
The results of the isolated toxic effect of PBX and oil on the increase in the number of duckweed fronds are shown in Figure 1.



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Using probit analysis, the concentrations of PBX and oil were found that inhibit the studied reaction parameters by 25% (ICR_{25}), 50% (ICR_{50}), 75% (ICR_{75}) (Table 1).

Table 1

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

Compounds, mg / l	Percentage of inhibition		
	25%	50%	75%
PBX	3,8	21,9	46,8
OIL	9,4	53,6	500,0

These concentrations served as three levels of variation of the PBX -oil factors in terms of a full factorial experiment.

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When designating the concentrations of the two substances included in the combination as x1 and x2, the toxic effect on small duckweed under the combined effect of the mixture was determined by the results of nine experiments, the actual Yi (Table 2.), which made it possible to formulate the regression equation:

$$Y=b_0+b_1x_1+b_2x_2+b_{11}x_1^2+ b_{22}x_2^2+b_{12}x_1x_2$$

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Table 2
Plan, actual and calculated results of the acute combined effect of oil and PBX on the growth of duckweed fronds.

№ experience	Factors		results		Error, $\Delta = Y_{pi} - Y_i$
	X_1	X_2	Actual, Y_i	Estimated, Y_{pi}	
1	-1	-1	27,8	31,7	3,9
2	1	-1	76,9	74,2	2,7
3	-1	1	84,1	87,7	3,6
4	1	1	95,8	92,7	3,1
5	0	0	70,0	71,64	1,6
6	0	1	87,9	87,4	0,5
7	0	-1	51,4	50,2	1,,2
8	1	0	80,4	86,2	5,8
9	-1	0	70,0	62,5	7,5

x_1 – oil 25%(-1) 50%(0) 75%(1)

x_2 – PBX 75 25%(-1) 50%(0) 75%(1)

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According to the results Y_i the coefficients of the regression equation were calculated:

$$Y=71,64+11,87x_1+18,62x_2+2,73x_1^2-2,82x_2^2-9,35x_1x_2$$

The values of the factors were substituted into the equation, the calculated Y_{pi} was obtained and compared with the actual and experimental ones.

The average error is Δ = 3.3%, which is significantly less than the experimental error (= 9%).

This approximation accuracy allows us to consider the obtained regression equation as a mathematical model of the combined effect of oil and potassium butyl xanthate on small duckweed.

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Analysis of the equation made it possible to conclude that the action of substances is unidirectional, interdependent ($b_{12} \neq 0$), and the interaction is expressed quite strongly. Coefficients b_1 and b_2 indicate that the impact of factor X_2 (PBX) is greater than factor X_1 (oil). Isolated injection of oil with an increase in concentration from ICR_{50} to ICR_{75} causes an increase in the toxic effect by 15%, and PBX - by 16%, while with combined action - by 21%.

Thus, it can be concluded that the nature of the combined action of PBX and oil is interdependent, less than additive.

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Conclusions

The toxic nature of the combined action of potassium butyl xanthate and oil are interdependent, antagonistic (less than additive).

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Thank you for your attention!

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