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Toxicity of Oil and Products of its Refinement to *Daphnia magna*: Time and Temperature Dependences

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Abstract: This study was designed to investigate the effect of oil and products of its refinement on crustacean cladoceran *Daphnia magna*. In acute experiments (t°=23°C), a clear dependence between *D. magna* lifetime and concentration of crude oil was seen only at high concentrations. In chronic toxicity experiments, crude oil at level of 3 admissible concentration limits (t°=23°C) reduced lifetime and fertility of *D. magna.* There was no dependence between rectification of crude oil and its initial concentration (at 10-40 mg/L; at 23 and 28°C). Toxic effect on *D. magna* is reducing in the following direction: diesel oil>crude oil>benzine A-76>aqueous extract from benzine A-93>aqueous extract from benzine A-76. Narcoanesthesia in *D. magna* was connected with soluble fractions while nonreversible toxic effects – with hard oil fractions.

Key words: Daphnia magna · Oil · Diesel oil · Benzine · Toxicity

INTRODUCTION

Oil and products of its refinement are main toxicants in areas with intensive crude oil output and developed industry. There is no unitary opinion on the toxicity of various oil fractions on aquatic organisms. Some authors [1, 2] suggest that level of toxicity and cumulative effect of oil and oil products depend on degree of its aqueous solubility. Other investigators [3] consider that namely insoluble oil fractions have more pronounced toxic effects. Toxicity of oil to aquatic organisms is mediated by insoluble aromatic fraction with high temperature of bubbling [4]. So, the lack of collective opinion motivated us to study the effect of oil and products of its refinement on crustacean cladoceran *Daphnia magna.* Time and temperature dependences were also taken into account.

MATERIALS AND METHODS

Daphnia magna Straus, 1820, were obtained from the Department of Zoology (Kazan State University, Kazan, Russia). The culture of genetically homogeneous organisms were reproduced from a single ancestor and was maintained in a 10 L aquaria with dechlorinated aerated tap water at room temperature (22±2°C).

The crude oil of from Romashkinskoe oil field (Tatarstan, Russia), benzine (A-76 and A-93 type) and diesel oil were entered in 200 mL flasks with water at chosen concentrations. To estimate the fractional composition of oil, the latter was isolated using extraction with carbon tetrachloride, chromatographic separation in a thin layer of aluminium oxide in a system of organic solvents consisting of petroleum-ether: carbon tetrachloride: acetic acid (70: 30: 2) [5]. "EKO" fluorimeter was used to determine the quantitative content of each fraction.

RESULTS AND DISCUSSION

In acute toxicity experiments (at 23°C), the following concentrations of the crude oil did not cause mortality in test animals: 0.5 mg/L, 1.0 mg/L, 4.0 mg/L and 10 mg/L. At the initial concentration of the crude oil of 40 mg/L and 60 mg/L, by the end of experiment 77% and 97% (respectively) of test animals died (Fig. 1). After increasing temperature till 28°C, mortality of test animals were elevated during the first day of experiment. Chronic toxic action of oil (in the concentration area of 1 to 10 mg/L) resulted in reduction of the amount of *D. magna* female with eggs (Table 1). At concentration of

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Table 1: The influence of crude oil on *Daphnia magna* reproduction at 23°C. Note: C – control; * - eggs were not produced; ** - eggs were produced but not spawned; *** 50% mortality was absent; L_{50} = lethality of 50% of animals; A = absent

	Parent females				1st generation				2nd generation			
		oil (mg/L)				oil (mg/L)				oil (mg/L)		
Parameters	C	1	4	10	C	1	4	10	C	1	4	10
Time of eggs production (daphnia age in days)	5	5	5	5	6	7	6	7	5	*	5	*
Time of the 1st generation appearance (daphnia age in days)	8	8	8	8	8	9	9	9	6	**	6	**
Number of offsprings per 1 female for 13 days	13	10.8	11.9	15	9	5.8	5.4	3.4	3.1	A	2.1	A
Number of egg layings for 13 days	3	2	4	3	4	2	2	3	2	A	2	A
L_{50} (days)	10	***	11	11	13	11	10	10	6	3	5	4

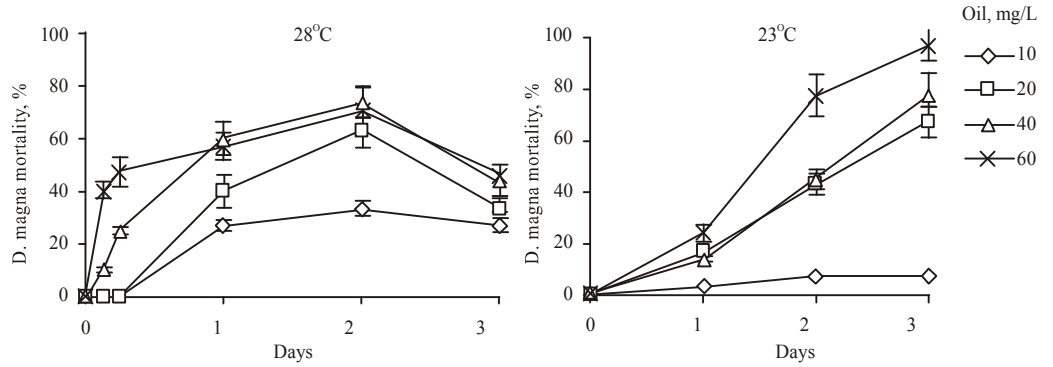

Fig. 1: Influence of crude oil on *D. magna* mortality at 28°C and 23°C (in relation to control)

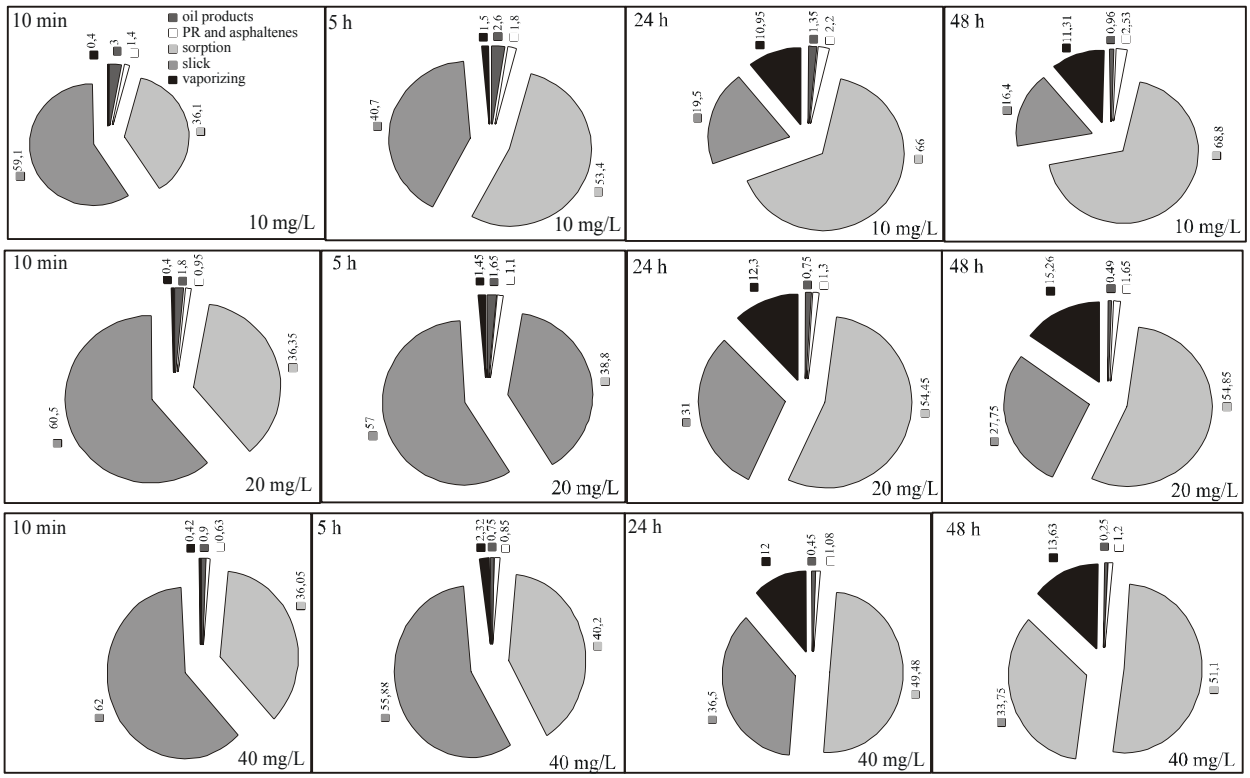

Fig. 2: Distribution of oil fractions in water medium at 23°C (% to initial concentration is indicated)

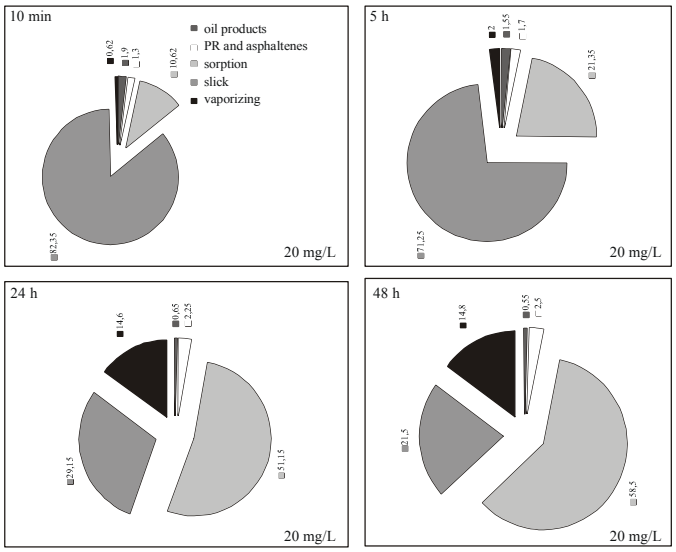

Fig. 3: Ditribution of oil fractions in water medium at 28°C (% to initial concentration is indicated).
Note: PR – polyester resins

4 mg/L and 10 mg/L, the values were 64.5% and 57.5%, respectively. A tendency to reduction of offsprings in terms of one female. In the first generation, one-day delay in egg laying, reduction of lifetime and overall fertility were observed. In the second generation, the aberrations had more pronounced character.

Laboratory modelling of oil evaporation showed that during the first minutes after contact between water and oil the bigger part of the latter (from 59 till 62%) was distributed at water surface (Fig. 2). During the first days, there was a main redistribution of oil fractions in water phase. This confirms the beginning the natural autopurification processes. It is known from the literature that insoluble and slightly soluble fractions are exposed to sorption that are difficult to destruct [2]. The other process of autopurification – eveporation – also intensively was going during the first days. By the first 5 h and 24 h, 1.5 – 2.0% and 10-15% (respectively) of oil evaporated (irrespective of initial concentration) (Fig. 2). The amount of dissolved oil products (at initial oil concentration of 10, 20 and 40 mg/L) was 0.30, 0.36 and 0.36 mg/L in first minutes while after 2 days the value was equal to 0.10 mg/L. This fact confirms that water enrichment with soluble fractions is not depended on initial oil concentration but is governed by solubility itself. The presence of hard oil fractions in the oil – resins and asphaltenes – increased in course of time in all experiment cases (Figure 2). The obtained data confirm the fact of independence between character of oil rectification and initial concentration.

With the aim to simulate oil evaporation in cooling ponds, we investigated dynamics of oil rectification in water at higher temperatures (28°C, Fig. 3). The obtained results were similar to those found at 23°C: dynamics of oil rectification was analogous but performed more intensively.

To investigate what oil fraction is responsible for nonreversible toxic effect, we performed acute experiments at various stages of oil evaporation. We found that maximal mortality of *D. magna* (88.0 %) was found by 4th day at initial oil concentration of 40 mg/L with 48 h exposure before the beginning the experiment (Fig. 3). At initial oil concentration of 20 mg/L, maximal mortality of test animals was detected in cases with 24 h exposure before the beginning the experiment. The obtained results demonstrated increase of toxic effect in time and concentration dynamics (Fig. 4).

It is clear from the data presented in Table 2 that acute toxic action of A-76 benzine and diesel oil was seen when concentration of the chemical were significantly increased. At that, the acting concentration of A-76 benzine was higher than those for diesel oil. During the first hours of experiment, narcoanesthesia in *D. magna* was detected due to action of A-76 benzine. Concentration of 67 mg/L caused a paralysis which lasted for 2 days. At the initial concentration of 145 mg/L, motor performance in the test animals was restored fractionally after 2 days. At the initial concentration of 700 mg/L, only 27% of test animals restored slightly motor performance after 3 days. But after 4 days, all of these died.

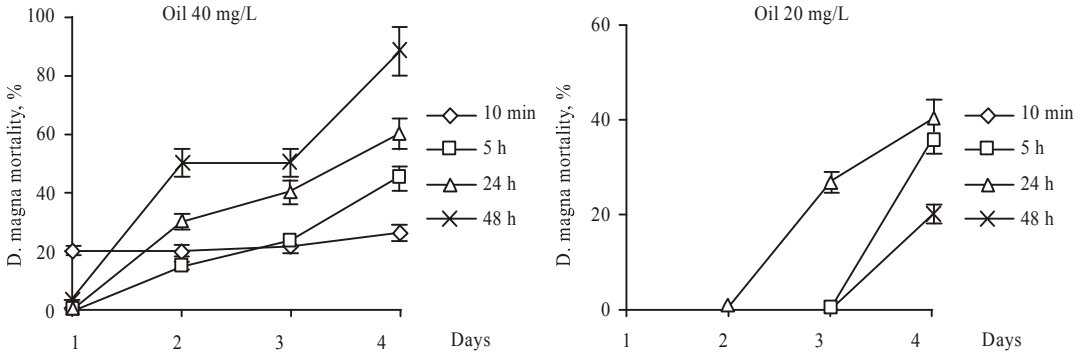

Fig. 4: Acute toxicity of crude oil on *D. magna* in dynamics of vaporizing (in relation to control)

Table 2: Acute toxicity of A-76 benzine and diesel oil on *D. magna* at 23°C (in relation to control, in %). Note: *activity of alive daphnia as in control; **activity of alive daphnia is reduced

	D. magna mortality, %			
Concentration, mg/L	2 h	24 h	48 h	72 h
benzine A-76				
67	Paralysis	Paralysis	0*	13±1.0*
145	Paralysis	Paralysis	27±2.2*	30±2.7*
700	Paralysis	Paralysis	Paralysis	73±7.5**
diesel oil				
32	100	100	100	100

Table 3: Chronic toxicity of diesel oil (4 mg/L) on *D. magna* at 23°C (in relation to control, in %). Note: ND = not detected

	Days				
Type of effect	1	3	4	13	16
Mortality of parent animals	23.3±1.9	ND	26.7±2.2	26.7±2.4	26.7±2.1
Mortality of the 1st generation	50±4.7	70±7.1	100	ND	ND

Table 4: Chronic toxicity of benzine (type A-76) on *D. magna* at 23°C (in relation to control, in %)

Concentration, mg/L	Mortality for 12 days
67	70±6.8
145	73±7.6

Table 5: The influence of oil and oil products on motor performance of *D. magna*. Note: "-" activity is absent; "±" activity is very weak; "+" average activity; "++" activity is the same as in control

	Time of observation							
Concentration, g/L	10 min	30 min	1 h	2 h	5 h	24 h	48 h	72 h
benzine (type A-93)								
1.0	±	–	–	–	–	+	++	++
0.5	±	±	±	±	±	+	++	++
benzine (type A-76)								
1.0	++	–	–	–	±	+	++	++
diesel oil								
1.0	++	++	++	++	++	++	++	++
24.0	++	++	++	++	++	++	++	+
crude oil								
1.0	++	++	++	++	++	++	++	++
0.5	++	++	++	++	++	++	++	++

After transportation of test animals after 2 hours (in the case of initial concentration of 700 mg/L) to pure water, motor performance was gradually restored; *D. magna* continued to live and reproduce.

In chronic toxicity experiments, the acting concentraion of diesel oil was similar to acting concentration of the crude oil and lower in comparison with benzine (data are presented in Tables 3 and 4). In the first generation by the 4th day there was 100 mortality while among parent generation only 26.7% animals died at concentration of 4 mg/L of diesel oil. The obtained results allowed us to position of the xenobiotics into following lane (decrease in toxicity): diesel oil>crude oil>A-76 benzine. The obtained results are in agreement with conception of physical-chemical and structural features of the oil products [2]. The amount of soluble fractions is little in diesel oil, bigger in crude oil and maximal in A-93 benzine. Results from Table 5 suggest that water extracts of diesel oil (even at initial concentration of 24.0 g/L) and the crude oil (at 0.5 and 1 g/L) did not result in inhibition of vital activity in the test animals in acute experiments. Water extracts from A-76 and A-93 benzines during the first hours caused acute narcotic effects but after 2 days the vital activity was restored.

Therefore, it is reasonable to conclude that nonreversible toxic effect on *D. magna* was connected with action of insoluble and slightly soluble fractions of oil and oil products. The toxic effect of the oil fractions may be connected with abberation in neurility, distortion of water and gas exchange, filtration processes in crustaceans [6, 7].

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