



**TOXICITY ASSESSMENT OF OIL-CONTAMINATED SEDIMENTS ON OSTRACODS
(*HETEROCYPRIS INCONGRUENS*) IN LABORATORY BIOASSAYS
AND IN FIELD MESOCOSM EXPERIMENT**

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Abstract

The present study demonstrates toxicity assessment of model sediments, spiked by crude oil in different concentrations, by examining the native macroinvertebrate taxa that colonized sediments in field-based mesocosm condition and in laboratory bioassays. The results of two months exposure in natural river (Kazanka river, Republic of Tatarstan, Russia) showed that density of benthos community especially Insecta taxa were decreasing in vessels with total hydrocarbons content at the level of 210 mg kg⁻¹. High level correlations were showed between total number, biomass of Oligochaeta taxa and total hydrocarbons content. Toxicity assessment with ostracods (*Heterocypris incongruens*) revealed survival decreasing since concentration of total hydrocarbons content at the level of 250 mg kg⁻¹. Therefore, total hydrocarbons content in sediments in concentration of 250-300 mg kg⁻¹ can cause toxic effect that can lead to degradation of the most sensitive taxa of benthic community.

Keywords: Toxicity assessment; Bioassay; Crude oil; Sediments; Mesocosm; *Heterocypris incongruens*

Introduction

Currently, of one of the main components of water object pollution are oil and petroleum products in oil producing regions [1]. According to the results of experimental and field observations, generalized by S.A. Pat in (2001), the lethal effect of oil on marine benthic organisms is manifested at its content of 1000-7000 mg kg⁻¹ in bottom sediments, while sub lethal and threshold effects (eating, behavior, physiological and biochemical function disorders, etc.) as well as pathological changes in organs and tissues (including carcinogenic tumors) appear usually within the concentration range of 100 - 1000 mg kg⁻¹ [2]. For the most toxic components and petroleum fractions, particularly for polycyclic aromatic hydrocarbons (PAHs), similar effects are possible at lower levels - from 10 to 100 mg kg⁻¹ [3, 4] also for the freshwater and benthos and bottom feeders. The core of sediment quality criteria should be represented

by the indicators that take into account the level of zoobenthos quantitative development. The soils with the signs of oil contamination are characterized by poor composition of species at a high abundance and biomass of the forms resistant to pollution, and at a strong chronic pollution the inhibition of the entire community is observed, including resistant forms [5, 6]. The aim of this study was the evaluation of oil toxic effect at the level of the most common concentrations in the sediments of Tatarstan rivers (Russia) on ostracods (*Heterocypris incongruens*) and in mesocosm conditions.

Study object. Materials and methods

The study object involved bottom sediments (BS), containing organic substances and fine fractions (<50 μm) at the level of values, typical for the Republic of Tatarstan river bottom sediments (about 2%) [7]. The model bottom sediments were contaminated with oil within the range of concentrations corresponding to the most common content level in the bottom sediments of the Republic of Tatarstan rivers [1]. The preparation of model samples for bottom sediments was performed as follows: the sand, selected in an ecologically clean region (the Yushut River on the territory of the National Park Mari Chodra, the Republic of Mari El, Russia) and peat, sifted through a sieve with a mesh size of 1 mm were mixed to the concentration of organic substances in a mixture (losses during calcination) at the level of 2%. Prepared BS model at the weight of 10 kg were placed in a bucket, 50 mL of crude oil from Tatarstan New Suksinsky oil field were added and mixed thoroughly. After a day BS were filled with water. The excess of oil not absorbed in the bottom ground and concentrated on the surface of the water was drained, and BS were re-filled with water. For 3 weeks BS contaminated with oil each day were washed with water before the moment when an oil film started to develop on a water surface. The residual content of oil products was measured in the dried BS contaminated with oil and it made 1,5 g kg^{-1} . Based on this content, adding uncontaminated BS samples they prepared the samples with oil product content in the range of their average content in the BS of natural water objects: 25, 50, 100, 250 mg kg^{-1} . The lowest limit of oil product content (25 mg kg^{-1}) was determined by the value recommended as a standard in the works written by Mikhailova and Isachenko Baume [4], Stepanova et al. [1]. The upper limit (250 mg kg^{-1}) is the most common value of oil product content in the Republic of Tatarstan rivers [7]. They put prepared BS samples with different oil product content into the plastic containers of 1 L. The experiment was performed in 3 replicates. The containers were placed in the coastal area of the Kazanka River near the settlement Kulseitovo (55°54'42.2" N 49°12'39.9" E). The exposure time made 2 months (June 18 - August 25, 2014), after which a residual oil product content in BS was determined by IR spectroscopy method using AN-2

analyzer (Neftehimavtomatika-SPb, Russia [8]), the toxic effects on a test object (ostracods *Heterocypris incongruens*, Ostracodtoxkit F, MicroBioTests Inc., Belgium)) were evaluated in vitro using a 6-day contact chronic test [9], which shows a number of advantages in comparison with commonly used methods [10], the population of the containers by main benthic taxa in mesocosm environment were assessed after the flushing of bottom sediment through a sieve and the analysis of biological samples [11, 12]. They used t_d reliability criterion and compared it with the Student's criterion for the level of significance $p = 0,95$ to perform the statistical analysis of results. The significance of differences was tested using one-factor analysis of ANOVA variance in AtteStat program.

Results and its discussion

After 2 months of exposure within mesocosm conditions they found out that oil product content increased in the first three containers as compared with the initial concentrations of 25, 50 and 100 mg kg⁻¹, and became almost equal having reached the content of 210-250 mg kg⁻¹. This increase is related apparently to the additional supply of oil products with water and suspended solids, on which the particles of encapsulated oil were sorbed. Kazanka River experiences an anthropogenic load, which leads to the contamination of its water and soil by oil products. The content of oil products in the control point (above the container installation site) increased up to 119 mg kg⁻¹, which reveals the additional quantitative supply of oil products from upstream areas. The results of ostracod toxicological studies in a chronic contact test showed that the survival of crustaceans in the variants 2-4 decreased to 67-80% (Fig. 1). There were no significant changes of ostracod finite size as compared to control group in all experiment variants.

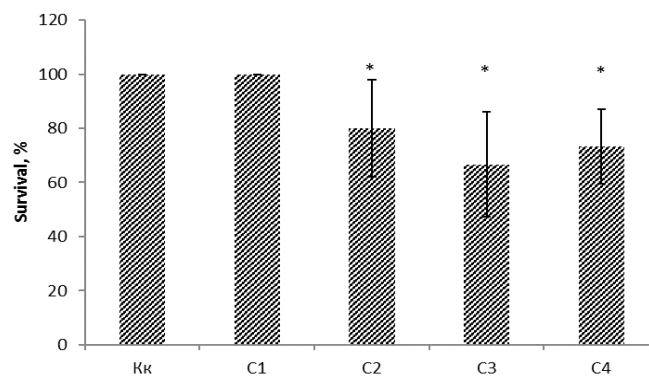


Fig. 1. The survival of ostracods in containers with different variants of oil product content (1 - 25/210; 2 - 50/240; 3 - 100/250; 4 - 250/300 (numerator - primary oil content, denominator - final oil content in mg kg⁻¹).

The significance of * $p < 0.05$ by a t-test.

Thus, the results of the toxicological study showed that oil products at the level of 240-300 mg kg⁻¹ may have a toxic effect on epibenthic organisms. The analysis of experimental container occupancy containing BS contaminated by petroleum products was conducted by major taxa (Table 1).

Table 1. Number/Biomass (ind./mg per container) of the main taxa in model bottom sediments after the exposure in natural conditions.

№	Taxa	Control (Kk)	C1	C2	C3	C4
1	Nematoda	0.0/0.0	0.0/0.0	0.3/1.4	0.0/0.0	0.0/0.0
2	Oligochaeta	3.7/6.1	5.7/11.0	26.0/103.3	17.3/31.7	22.0/99.5
3	Hirudinea	0.0/0.0	0.0/0.0	0.0/0.0	0.7/6.0	0.5/2.1
	species 1	0.0/0.0	0.0/0.0	0.0/0.0	0.3/1.4	0.5/2.1
	species 2	0.0/0.0	0.0/0.0	0.0/0.0	0.3/4.6	0.0/0.0
4	Mollusca	1.3/161.9	2.3/29.0	2.7/45.4	0.3/79.4	3.0/82.4
	Gastropoda	1.0/34.1	2.3/29.0	2.7/45.4	0.3/79.4	3.0/82.4
	species 1	1.0/34.1	2.0/25.6	1.7/30.5	0.0/0.0	1.0/12.8
	species 2	0.0/0.0	0.3/3.4	0.7/6.8	0.0/0.0	2.0/69.6
	species 3 (Planorbidae)	0.0/0.0	0.0/0.0	0.3/8.1	0.0/0.0	0.0/0.0
	species 4 (<i>L.ovata</i>)	0.0/0.0	0.0/0.0	0.0/0.0	0.3/79.4	0.0/0.0
	Bivalvia	0.3/127.8	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
5	Crustacea	0.0/0.0	0.3/7.1	0.0/0.0	1.7/12.4	3.0/7.6
	Isopoda (Asellidae)	0.0/0.0	0.3/7.1	0.0/0.0	1.7/12.4	3.0/7.6
6	Insecta	9.0/92.6	2.7/101.7	3.0/83.7	0.7/11.4	1.5/40.7
	Megaloptera (Sialidae)	2.3/77.3	2.3/92.5	3.0/83.7	0.3/10.7	1.5/40.7
	Diptera (Tabanidae)	0.0/0.0	0.3/9.2	0.0/0.0	0.0/0.0	0.0/0.0
	Diptera (Chironomidae)	6.7/15.3	0.0/0.0	0.0/0.0	0.3/0.6	0.0/0.0
	TOTAL:	14.0/260.7	11.0/148.7	32.0/233.8	20.7/140.8	30.0/232.3

The analysis of abundance and biomass indicators according to such taxa as Insecta and Oligochaeta reveal the dependence on the level of oil product concentration (Fig. 2). The oligochaete resistance to the contamination by organic substances is known, including petroleum products [13]. According to the terms of numbers and biomass, a general trend to their increase is revealed at the growth of oil product content. The reduction of other taxa number provides the advantages for the development of oligochaetes, which start to occupy the vacant ecological niche.

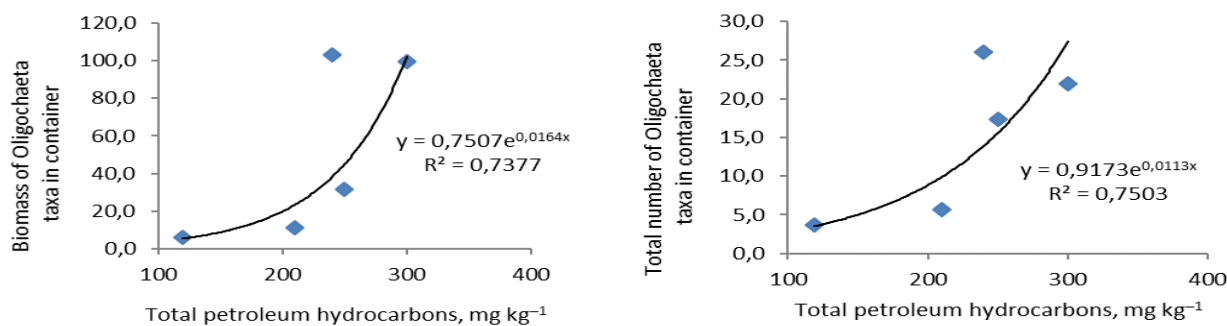


Fig. 2. Correlations between biomass (left), total number (right) of Oligochaeta taxa and total petroleum hydrocarbons content.

Another major taxon - Insecta - the organisms of which are more sensitive to a higher content of oil products showed the reduction of this group of organisms with an increasing content of petroleum products in the model BS (Figure 3).

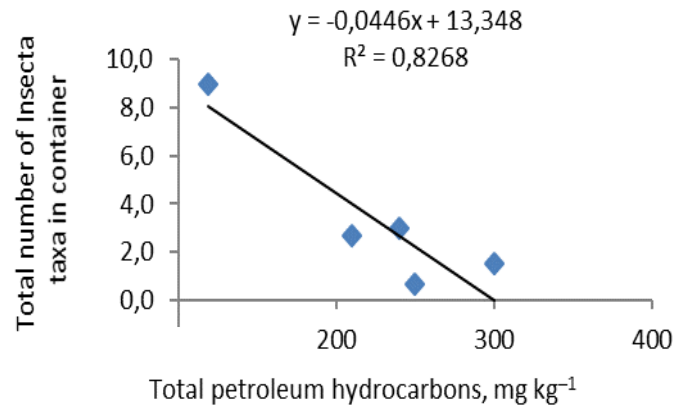


Fig.3. Correlations between total number of Insecta taxa and total petroleum hydrocarbons content.

Summary

The performed study with the introduced petroleum products into the model bottom sediments showed that the selected for the exposure body of water - the river Kazanka near the settlement Kulseitovo - is contained with oil, which resulted in their accumulation within the experimental containers. This may be related to the inflow of oil products with a surface runoff, as well as by the population use of small vessels. The analysis of occupancy by native benthic organisms of model bottom sediments with different levels of oil product introduction showed that the representatives of Sialidae group - alderflies - the family of insects from the order Megaloptera are the most sensitive ones. Their abundance and biomass begin to decline when the content of petroleum products makes 210 mg kg⁻¹ or more. At the same time the increase of Oligochaeta number and biomass is observed. Oligochaeta acquire the benefits of development due to the oppression of other groups, starting with the concentrations of 250-300 mg kg⁻¹.

The laboratory toxicological study revealed the presence of toxic effect, which manifests itself in the form of reduced survival of ostracods *Heterocypris incongruens* during 6-day contact test, starting with the concentration of oil products at the level of 250 mg kg⁻¹. Therefore, oil products can cause toxic effects that can lead to inhibition of the most sensitive species and reduce the biodiversity of benthic community at the concentration 250-300 mg kg⁻¹.

The results of this research can be used in the development of measures for water body improvement in the locations of oil production and transportation, as well as for the bioindication of water ecosystems exposed to the impact of petroleum products.

Conclusions

The toxic effect of petroleum products in the composition of model bottom sediments, starting with the concentration of 250 mg kg⁻¹, is shown in the form of ostracods *Heterocypris incongruens* reduced survival within the laboratory bioassay.

Two-month exhibition of model bottom sediments under mesocosm conditions showed that during the concentration of petroleum products at the level of 210 mg kg⁻¹ and above, the abundance and biomass number reduction of the most sensitive taxa, such as alderflies, and the increase of sustainable taxon Oligochaeta biological indicators are observed.

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