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# Impact of Oil Sludge from the Ilyinsky Oil Sludge Pits on the Locomotor Activity of *Daphnia magna*: Seasonal Variations and Toxic Effects

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This article examines the toxic effects of oil sludge from the Ilyinsky oil sludge pits on the locomotor activity of *Daphnia magna* Straus, depending on seasonal factors. Oil sludge resulting from the accumulation of petroleum products poses a serious environmental threat due to its high hydrocarbon content and toxicity. Experiments were conducted using a visual method to record the locomotor activity of *D. magna,* which allowed us to establish the dependence of the organisms' responses on contamination level with various samples from four monitoring points. A study of the dynamics of locomotor activity revealed that during the summer and fall, a suppression of functions was observed at points with high levels of contamination, particularly at sites No. 3 and No. 4. The results of the experiments confirmed that in summer, *D. magna* exhibited a significant decrease in activity and metabolic slowdown in response to exposure to toxic substances, accompanied by short-term episodes of hyperactivity. More pronounced toxic reactions were observed in the fall, indicating the need for careful monitoring of these ecologically hazardous areas. Analysis of chemical parameters of oil sludge, such as hydrocarbons, mechanical impurities, and chlorides, also indicated a potential hazard to aquatic ecosystems. Statistical analysis showed that the motility of daphnia was a more sensitive indicator of toxicant exposure than mortality rates: reactions to chemical contaminants were recorded after just 1–3 hours, while mortality assessment required prolonged exposure of 96 hours. The article highlights the importance of ecosystem monitoring, as the response of *D. magna* to toxic substances is significantly more informative than mortality rates, making bioindication an important tool for assessing the ecological situation in the studied water bodies.

*Key words*: *Daphnia magna* Straus, toxicity, oil sludge, environmental hazard, bioindication, ecosystem monitoring, pollution, temperature.

## INTRODUCTION

The Ilyinsky oil sludge pits, located approximately 20 kilometers southwest of Astrakhan, represent a complex of 19 open reservoirs historically used for the accumulation of various petroleum products. These include fuel oils, oil sludges, residues from the cleaning of oil tankers, drilling fluids, and wastes from oilfield operations. The total area occupied by these sludge pits amounts to 103 hectares, making this site a significant object in the context of ecological vulnerability and the urgent need for environmental protection measures.

In the studies of many researchers (Surikova, 2013; Nasyrova, 2024), the indicator of motor activity of test objects is used to assess the toxicity of the environment. Researches show that daphnia respond to changing environmental conditions, including pollution levels and toxicity, making them reliable bioindicators. Assessing daphnia movement allows the authors to identify negative changes in the aquatic environment caused by exposure to oil sludge. Given the environmental vulnerability of the Ilyinsky oil sludge field and its large area, monitoring daphnia responses to the presence of various toxicants is crucial for understanding the environmental impacts and developing effective water conservation strategies.

The purpose of our research was to assess the toxic impact of oil sludge from the Ilyinsky oil sludge pits on the locomotor activity of *D. magna* Straus as a bioindicator, and to establish the dependence of this impact on seasonal factors.

## MATERIALS AND METHODS

The study utilized an emulsion of petroleum products from the Ilyinsky oil pits (Astrakhan Region), containing various classes of petroleum products, including crude oil, fuel oil, and light fractions. The sample is a water-cut petroleum product and it is classified as *D. magna* Straus.

For this study, five monitoring points located in the immediate vicinity of the Ilyinsky oil pits were selected:

1) upstream of the oil pits;
2) the beginning of the oil pits;
3) between the oil pits;
4) the end of the oil pits (Fig. 1*a*).

The locomotor activity of *D. magna* (Fig. 1*b*) was assessed using a visual method of relative observation, for which observation chambers of an optimal size of 5×5 mm were selected. The parameter serving as the indicator of the studied phenomenon was the number of line crossings, (NLC). In each experiment, three Daphnia individuals were sequentially placed into a 15 ml test


Fig. 1. Locations of monitoring points (*a*), test organism (*b*) and sample of oil sludge from the Ilyinsky pits (*c*)

medium, with all experiments repeated three times. The age of the test organisms did not exceed 24 hours, ensuring sample homogeneity. A binocular microscope of the MBS type was used for the observations, enabling precise measurements.

**RESULTS AND DISCUSSION**

Polycyclic aromatic hydrocarbons (PAHs), including phenanthrene, are identified as highly toxic compounds. The established standard for phenanthrene in fishery water bodies is 40,000 ng/L, and actual measurements specified that its concentration at the study sites remained below the maximum permissible concentration (MPC). Nevertheless, it is important to note that fluctuations in PAH concentrations can significantly affect aquatic ecosystems, particularly given the toxicity of compounds such as benzo[a]pyrene, which exhibits the highest carcinogenic activity among the PAHs mentioned, with a toxicity index of 1. The presence of heavier PAHs, such as benzo[k]fluoranthene and benzo[a]pyrene, may indicate the accumulation of toxic compounds and potential risks to ecosystem health.

The oil sludge from the Ilyinsky pits is characterized by a dense, viscous consistency with a noticeable water separation.

The studies revealed that oil sludges contain a substantial proportion of hydrocarbon components (Table 1). The analyzed sample is a water-saturated petroleum product and belongs to the class of "water-in-oil" emulsions (Fig. 1*c*). The emulsion is characterized by moderate mobility, with a noticeable process of water separation, while the petroleum product itself exhibits low fluidity at room temperature.

*Table 1*

Physicochemical characteristics of the studied oil sludge

| Indicator of oil sludge | Value |
|---|---|
| Hydrocarbon phase, wt.% | 85.85 |
| Water content, wt.% | 9.40 |
| Mechanical impurities content, wt.% | 4.75 |
| Chloride salts content, mg/L | 200.00 |
| Paraffin content, wt.% | 5.00 |
| Resin content, wt.% | 7.00 |
| Asphaltene content, wt.% | 3.05 |

The water content in the studied oil sludge sample reached 9.40 % by weight. This parameter may slow down the migration of hydrocarbons into the aquatic environment; however, the presence of moisture also implies the potential leaching of toxic substances, thereby creating an additional risk for aquatic organisms and their habitats.

The content of mechanical impurities was found to be 4.75 %. This parameter is significant, as mechanical impurities such as sand or silt can deteriorate the sanitary state of water and be embedded within organic matrices, hindering the biodegradation of hydrocarbons by microorganisms and complicating ecosystem recovery.

The analysis of chloride salts revealed their presence at the level of 200 mg/L. This may indicate potential toxicity for aquatic organisms, particularly under long-term exposure. Chloride compounds can exert harmful effects on flora and fauna in ecologically sensitive zones, such as coastal ecosystems.

The study demonstrated that the contents of paraffins, resins, and asphaltenes in the oil sludge amounted to 5.00 %, 7.00 %, and 3.05 % of the total mass, respectively. These values reflect the complexity of decomposing these components, which may lead to persistent contamination of the aquatic environment. Paraffins and resins, due to their high viscosity and stability, can persist in the environment for extended periods, thereby impeding the natural self-purification processes of aquatic ecosystems.

*Table 2*

Characteristics of the hydrocarbon fraction of averaged samples from the Ilyinsky oil sludge pits

| Indicator | Value |
|---|---|
| Density at 20 °C, ρ $20^4$ | 0.910 kg/m³ |
| Viscosity at 80 °C, ν (80) | 61 mm²/s |
| Emulsified water content | 0.05 % |
| Mechanical impurities content | 0.67 % |

The characteristics of the hydrocarbon phase obtained after sedimentation are presented in Table 2. A comparative analysis of the properties of the hydrocarbon phase with those of petroleum products suggests that the hydrocarbon component of this sample primarily consists of fuel oil or heavy petroleum fractions. It is characterized by high density, which may be associated with the prolonged influence of climatic factors on the oil sludge, including elevated temperatures (Dubovtsev, 2019).

Table 3 presents the fractional composition of the Ilyinsky oil sludges, characterized by different boiling point ranges under residual and atmospheric pressures, as well as by the mass fractions of the components.

*Table 3*

Fractional composition of the Ilyinsky oil sludges

| Fraction | Boiling temperature at residual pressure, °C | Boiling temperature at atmospheric pressure, °C, recalculated | Mass fraction, wt. % |
|---|---|---|---|
| Water | – | 100 | 9.4 |
| 1st fraction | 30–150 | 30–150 | 45.0 |
| 2nd fraction | 150–300 | 150–300 | 25.0 |
| Residue | >300 | >300 | 15.0 |
| Losses | – | – | 5.6 |

The aqueous phase accounted for 9.4 %, indicating the presence of moisture in the oil sludge and potential risks upon interaction with the environment. The first fraction, with a boiling range of 30–150 °C and comprising 45.0 %, consists of light hydrocarbons that may easily evaporate, leading to the formation of volatile organic compounds. This creates the possibility of air pollution and adverse effects on human health and ecosystems.

The second fraction, with a boiling range of 150–300 °C and a mass fraction of 25.0 %, contains heavier hydrocarbons that are less prone to evaporation. However, in the event of spills or improper disposal, these compounds can exert significant toxic effects. The residual portion (15.0 %) includes resins and asphaltenes, indicating potential ecological hazards upon contact with these components, as they are highly persistent and contribute to long-term environmental contamination.

The losses, amounting to 5.6 %, suggest the possibility of leaks or evaporation of volatile components during the handling of oil sludges. Consequently, the fractional composition of the Ilyinsky oil sludges identifies them as potentially hazardous to the ecological state of the environment.

Our study further elucidated seasonal dynamics of the locomotor activity of *D. magna*. In summer, the daphnids exposed to water from monitoring point No. 1 exhibited virtually no response during the first 24 hours; their movements were so slow and minimal that they could hardly be interpreted as active. Subsequently, a gradual increase in locomotor activity was observed, as the daphnids began to spend more time in the upper water layers, where they typically reside, instead of remaining motionless at the bottom. A similar response was recorded in the sample from point No. 2 (Table 4).

*Table 4*

Locomotor activity of *D. magna* in summer water samples

| Monitoring points | Exposure / Locomotor activity, NLC (number of line crossings). | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 3 h | 1 day | 2 day | 3 day | 4 day |
| Control | 130.0±5.0 | 130.5±4.0 | 131.0±5.0 | 132.0±5.5 | 133.0±5.0 | 134.0±4.5 |
| No. 1 | 131.1±5.9 | 131.1±5.9 | 131.1±5.9 | 132.6±6.5 | 133.7±4.3 | 135.2±4.8 |
| No. 2 | 132.6±3.2 | 133.4±3.1 | 134.8±5.7 | 139±4.2 | 141.2±6.4 | 143.7±6.3 |
| No. 3 | 127.8±7.1 | 125.3±6.2 | 121.3±7.2 | 119.1±6.2 | 120.2±8.3 | 121.2±4.5 |
| No. 4 | 126.3±2.3 | 127.1±3.8 | 128.4±6.2 | 129.7±5.1 | 130.1±5.9 | 130.9±6.4 |

Note. * – values are significantly different from the control ($p<0.05$).

A pronounced response was observed to the water from monitoring point No. 3 (which, according to previously obtained results, was distinguished by elevated contamination): here, suppression of locomotor function began within the first 3 hours and persisted for three days, after which recovery of function commenced. In this case, an “inverse” response was observed – namely, a sharp increase in locomotor activity accompanied by chaotic movements, spinning in place.

Overall, the response to water from monitoring points No. 3 and No. 4 during the summer period, which are considered the most contaminated with petroleum products (up to 6 MPC), was diverse yet expected. This is consistent with earlier findings, according to which the higher the temperature, the more toxic the oil sludges contaminating monitoring sites No. 3 and No. 4 become. Consequently, the response of daphnids to water from these sites was more pronounced and differed markedly from the responses to water from points No. 1 and No. 2.

In autumn, the response of daphnids to water from the same monitoring points changed. For example, while in summer there was no response to water from point No. 1 during the first 24 hours, followed by increased locomotor activity, in autumn suppression of locomotor function was recorded within the first 3 hours and lasted for three days. This suppression could be evaluated through reduced movement frequency, less active use of swimming appendages, and a general decline in metabolic levels. In particular, the daphnids exhibited increased periods of immobility and reduced reactivity to external stimuli, indicating intoxication or oxygen deficiency contributing to a decline in functional activity, after which recovery began.

The response to water from point No. 2 was entirely different: periodic suppression and recovery of locomotor function were observed, with a zigzag dynamic. This reaction persisted during the first 24 hours, after which gradual recovery of locomotor activity occurred.

The response to water from points No. 3 and No. 4 also differed substantially. In the former case, there was a sharp surge in locomotor activity within the first three-hour period, followed by functional suppression lasting up to the third day of observations; recovery began on the fourth day. The response to water from point No. 4 followed the same dynamics, although with different numerical values.

Table 5 presents detailed data on the locomotor activity of *D. magna* in water from the four monitoring points studied within the study.

It was specified that daphnids exhibited differences in their responses to toxicant exposure, underscoring the diversity of adaptive strategies among these organisms. Some individuals reacted to carcinogenic compounds with a sharp increase in locomotor activity, manifested by chaotic movements and spinning in place. At the same time, other daphnids demonstrated the opposite response, expressed as reduced locomotor activity. These behavioral changes may result in the accumulation of substances within the organism, which is an important aspect for understanding the impact of toxicants on ecosystems and population structure.

Consistent with prior hydrochemical data, monitoring points No. 3 and No. 4 were confirmed as the most ecologically unfavorable across both seasons.

*Table 5*

Locomotor activity of *D. magna* in autumn water samples

| Monitoring points | Exposure / Locomotor activity, NLC (number of line crossings). | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 3 h | 1 day | 2 day | 3 day | 4 day |
| Control | 135.0±4.0 | 134.5±3.5 | 133.0±3.0 | 132.0±2.5 | 131.0±2.0 | 130.0±2.5 |
| No. 1 | 128.4±4.9 | 126.6±5.7 | 123.2±3.9 | 121.2±3.2 | 120.1±5.4 | 123.7±6.9 |
| No. 2 | 129.3±6.4 | 130.2±5.1 | 129.5±6.9 | 131±3.6 | 132.4±5.4 | 135.8±6.6 |
| No. 3 | 129.3±4.8 | 130.5±4.2 | 128.7±3.5 | 126.9±3.8 | 125.3±5.6 | 126.1±5.7 |
| No. 4 | 128.5±3.1 | 131.8±7.3 | 130.1±2.1 | 129.4±4.5 | 127.6±2.1 | 128.3±5.5 |

Note. * – values are significantly different from the control ($p < 0.05$).

Let us compare the obtained results with the locomotor activity of *D. magna* during the winter and spring periods (Tables 6 and 7).

*Table 6*

Locomotor activity of *D. magna* in winter water samples

| Monitoring points | Exposure / Locomotor activity, NLC (number of line crossings). | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 3 h | 1 day | 2 day | 3 day | 4 day |
| Control | 120.0±4.0 | 121.0±4.5 | 124.0±4.0 | 127.0±5.0 | 129.0±5.5 | 130.0±5.0 |
| No. 1 | 119.7±5.5 | 120.2±5.5 | 123.5±5.4 | 127.8±4.5 | 130±5.9 | 130.2±5.9 |
| No. 2 | 124.1±4.5 | 124.9±5.4 | 124.1±4.7 | 129±4.1 | 131.6±4.5 | 137.1±5.1 |
| No. 3 | 128.3±6.2 | 131.6±5.5 | 132.9±4.2 | 133.1±5.1 | 134.2±6.3 | 135±5.5 |
| No. 4 | 134.3±5.1 | 131.4±6.3 | 132.1±6.5 | 132.1±5.6 | 136.5±7.6 | 140.7±5.5 |

Note. * – values are significantly different from the control ($p < 0.05$).

In winter, the locomotor activity of *Daphnia magna* decreases regardless of other factors, reflecting the influence of seasonal cycles on the organisms.

In water from monitoring point No. 1, a gradual increase in locomotor activity was observed - it is important to note that the differences in LC values across each analyzed time interval were insignificant. A similar lack of significant variation was observed in the response to water from point No. 2; however, unlike the dynamics observed in water from point No. 1, here a slight increase in locomotor activity during the first 24 hours was followed by suppression of locomotor function, after which recovery of locomotor activity was recorded.

In water from point No. 3, locomotor activity showed only an upward trend, whereas in water from point No. 4 an initial slight suppression was recorded during the first three hours, after which recovery of the locomotor function of *D. magna* commenced.

*Table 7*

Locomotor activity of *D. magna* in spring water samples

| Monitoring points | Exposure / Locomotor activity, NLC (number of line crossings). | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 3 h | 1 day | 2 day | 3 day | 4 day |
| Control | 130.0±5.0 | 128.0±4.5 | 126.5±4.0 | 125.5±4.5 | 124.5±5.0 | 125.5±4.5 |
| No. 1 | 124.5±6.7 | 106.8±6.5 | 105.3±5.3 | 103.2±6.3 | 105.9±6.4 | 106.7±4.8 |
| No. 2 | 125.7±4.8 | 123.9±4.7 | 128±4.3 | 128.6±5.5 | 131.3±4.7 | 125.6±4.5 |
| No. 3 | 129.1±4.9 | 132.7±4.9 | 130.8±5 | 139.9±5.7 | 138.3±4.7 | 139±4.2 |
| No. 4 | 138.9±4.9 | 137.1±7.4 | 138.4±8.5 | 139.7±7.1 | 133.4±5.7 | 139.8±4.4 |

Note. * – values are significantly different from the control ($p<0.05$).

In spring, as noted earlier, the locomotor function of the organisms was higher than during the winter period.

The response of daphnids in water from monitoring point No. 1 was rather noteworthy: during the first three-hour interval, a sharp decline in locomotor activity was recorded. Suppression of locomotor function persisted for two consecutive days, after which a gradual and very slow recovery began. In water from point No. 2, the response was different: although a decline was also observed during the first three-hour interval, recovery of locomotor function started within the first 24 hours. However, on the fourth day, suppression occurred again, and this time the decline was more pronounced than during the initial three hours.

In water from point No. 3, the dynamics were zigzag-shaped: a pattern similar to the response of daphnids in water from monitoring point No. 2 during the autumn period.

In water from point No. 4, the response of the daphnids was the “inverse”: initially, locomotor activity increased, accompanied by chaotic movements and spinning in place. This lasted for two days; on the third day a slight decline in locomotor activity occurred, but by the fourth day the activity increased again, marking the onset of recovery of locomotor function in the test organisms, *D. magna*.

In summary, the previously obtained results and formulated conclusions are confirmed: in terms of both mortality and locomotor activity, the warm season is unfavorable for *D. magna*. Monitoring points No. 3 and No. 4, as noted earlier, are the most polluted and therefore more toxic to the organisms compared with points No. 1 and No. 2.

This result is consistent with the study of L. A. Lesnikov (1971), who concluded that the higher the temperature, the greater the toxicity of oil sludges. The increase in toxicity during the warm season had a negative effect on both mortality and locomotor activity of *D. magna*.

It should also be emphasized that identifying toxicity through the locomotor activity index is significantly faster than through mortality: in the latter case, at least 96 hours (the life cycle of daphnids) are required, whereas suppression of locomotor activity in response to pollution occurs within 1–3 hours.

**CONCLUSION**

Thus, bioindication using *D. magna* revealed spatiotemporal heterogeneity of toxic effects: the most unfavorable sites were points No. 3 and No. 4. The locomotor activity of *D. magna* demonstrated reproducible patterns: suppression, “zigzag” phase changes, or short-term hyperactivity with chaotic movements, reflecting acute neurotoxic and stress effects of mixed contamination. The seasonal factor proved to be significant: toxicity was higher in the warm period, as evidenced by more pronounced suppression of locomotor activity and increased mortality; in the cold period, the baseline activity was lower, but the dynamics of responses were smoother. Evaluation based on locomotor activity is more informative than mortality at early exposure stages, as the behavioral response is recorded within the first 1–3 hours, whereas mortality requires long-term exposure. The combination of physicochemical characteristics of the sludges, PAH composition, and bioassay results unequivocally indicates the high ecological hazard of the complex, with peaks of toxic impact in the warm seasons and maximum risk localized at sites No. 3 and No. 4.

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**Нгуен Н. А. Влияние Ильинских нефтешламов на двигательную активность *Daphnia magna* Straus: сезонные колебания и токсические эффекты** // Экосистемы. 2025. Т. 44. С. 143–150.

В настоящей работе проведена оценка токсического воздействия нефтяных шламов на двигательную активность *D. magna* с учетом сезонной изменчивости. Выявлено, что поведенческая биоиндикация по изменению двигательной активности является высокочувствительным методом, позволяющим регистрировать реакции на загрязнение в течение 1–3 часов, в отличие от оценки летальности, требующей 96-часовой экспозиции. Принципиально важно, что в теплый период (лето – осень) наблюдаются более выраженные токсические эффекты, проявляющиеся в супрессии функций, эпизодах гиперактивности и «зигзагообразной» динамике на участках с повышенным уровнем загрязнения (№ 3 и № 4). Зимой общий уровень активности *D. magna* снижена, тогда как весной регистрируются контрастные паттерны ответа, включающие кратковременную угнетение с последующим восстановлением. Анализ физико-химических характеристик нефтяных шламов, включая фракционный состав, высокое содержание полициклических ароматических углеводородов (ПАУ) и смол, убедительно свидетельствует об их потенциальной экологической опасности. Полученные данные подчеркивают кумулятивный риск и синергизм токсического действия загрязняющих веществ.

*Ключевые слова*: дафния большая, токсичность, нефтешламы, нефтеямы, экологическая опасность, биоиндикация, мониторинг экосистем, загрязнение, температура.

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