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Assessment of the Toxicity of the Natural and Technogenic Environment for Motor Activity of *Daphnia magna*

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ABSTRACT
The *Daphnia magna* Straus (1820) crustaceans are used in many countries to assess the quality of the environment. Researchers are guided more often by the mortality of individuals. However, the sublethal effects contribute to the development of biological early warning systems (BEWS). A visual method for recording the motor activity of *D. magna* was proposed. This method has advantages over automated analogs. It is simple, accessible to performers and does not require the use of special instruments. The method was tested under conditions of modeling the lethal and sublethal effects of heavy metal salts (Cu and Zn). The diagnosis of lethal doses is possible after 1 hour of exposure, whereas the death of crustaceans can occur in 3–4 days of the experiment. The effect of sublethal doses of heavy metals becomes statistically significant after 24 ($p < 0.05$). The sensitivity of the method was confirmed in the studies of the aqueous extracts from the soils contaminated with various heavy metals (heavy metal processing area). The method showed good results in assessing the toxic effect of the waters anthropogenically contaminated with the mineral forms of nitrogen ($NH_4^+$and NO_3^-) in the region of fertilizer production. The maximum inverse correlation between the motor activity and the concentration of ammonium ions in water (-0.83) was shown after 24 hours. Thus, the method of visual assessment of motor activity can be used in environmental monitoring.

Keywords: ammonium ions, bioassay, *Daphnia magna*, heavy metal, methods of bioassay, motor activity.

INTRODUCTION

Various types of lower crustaceans have been used in toxicological studies for more than 100 years. The most common protocols are bioassay using *Daphnia magna* Straus, 1820 [ISO 6341:2012 2012; Environment Canada 1996; FR.1.39.2007.03222 2007]. Scientists proposed to evaluate the toxic effects not only on the death of daphnia, but also on the changes in the trophic activity [Kovács et al. 2012; Shashkova, and Grigor'ev 2013], heart rate [Jeong 2018], and biochemical markers [Parolini et al. 2018; Domingues et al., 2018]. According to our observations, one of the first responses of *D. magna* to toxic stress is a change in the motor activity of crustaceans [Olkova, 2017].

The test function "motor activity" was estimated by scientists in *Oncorhynchus mykis*s [Osswald et al. 2013], *Artemia franciscana* [Morgana et al. 2018], honeybees [Aliouane et al. 2009], *Gammarus lawrencianus* [Wallace and Estephan 2004]. These researchers noted the high information content and speed of this physiological reaction.

Scientific studies aimed at evaluating the motor activity of *D. magna* are also conducted. A team of researchers developed a method for assessing the motor activity of crustaceans with the definition of the speed of movement, the distance between daphnia, counting their number and determining the toxicity index. A specially developed software product is used for this purpose [Lechelt et al. 2000]. Shimizu and co-authors suggest evaluating the motion trajectory of *D. magna* to determine the acute toxicity of the polluted media [Shimizu at al. 2002].

The purpose of this work is a visual assessment of the motor activity of lower crustaceans *D. magna* under the conditions of toxic stress, as well as the determination of the possibility of instant diagnosis of water pollution with this method of bioassay.

MATERIAL AND METHODS

The study used a laboratory synchronized culture of *Daphnia magna* Straus. The cultivation of individuals and further experiments took place in a climate chamber: the temperature was 20–22°C, the lighting was 600 Lux, the lighting period lasted 12 hours. For the experiment juvenile crustaceans were selected at the age of 6–24 hours.

The quantitative assessment of the motor activity of the body was made by visual counting of the number of daphnia intersections with the conventional lines of the observed field of view. Optimal cells of the pallets (5×5 mm) were chosen. The counting time was 5 minutes. The parameter was expressed in the number of intersections of the pallet lines (IPL).

Before counting the motor activity, the individuals were in the control and test environments. The exposure was from 1 to 96 hours (see "Results"). The experiment was carried out in three replications, each containing 3 *D. magna* individuals. Then, each of the 9 daphnia used in the experiment was alternately placed in a test medium of 15 ml each. The criterion of toxicity was a mathematically significant difference in the motor activity index of *D. magna* in a sample that does not contain toxic substances (control) and in the test sample (experiment).

For approbation of the method, three series of experiments were carried out.

RESULTS AND DISCUSSION

The influence of the Copper and Zinc Ions on the motor activity of *D. magna*

Copper sulfate ($CuSO_4 \cdot 5H_2O$) was used to model the water contamination. The lethal concentrations of copper (1, 0.5, 0.1 mg/dm^3), as well as the sublethal concentrations of the toxicant (0.01, 0.005, 0.001 mg/dm^3) were tested. Acute toxicity for 96 hours (lethal effect) was established before the main experiment. The results are presented in Table 1.

The sublethal doses of copper after an hour of exposure led to an unreliable tendency to increase the motor activity of the crustaceans. The shift of the effect towards stimulation in a given range of copper concentrations is quite natural, since copper in micro doses is an element necessary for most animals [Egorova and Ananikov 2017]. A day later, in a variant with a maximum sublethal dose, a significant oppression related to the activity of the crustaceans was established. Consequently, according to the "mortality" test function, the toxicity of this variant was not diagnosed, and it was possible to do this according to the "motor activity" test function.

The lethal doses, equal to 0.5 and 1 mg/dm^3, had a significant inhibitory effect on daphnia after 1 hour of exposure. These individuals died in 5 and in 2 hours, respectively, however, the developing toxic effect was established earlier due to the evaluation of motor activity. For other substances, the time of manifestation of sublethal and lethal effects may differ more significantly (see below).

The copper addition equal to 0.1 mg/dm^3 caused a significant stimulating effect on the motor activity of test organisms. The death of all experimental individuals occurred after 48 hours of exposure. In this case, the stimulation of the sublethal test function was a manifestation of the initial stage of the toxic process. This phenomenon is known as hormesis. This is a biphasic dose–response phenomenon characterized by low-dose stimulation and high-dose inhibition [Calabrese 2008].

Table 1. The influence of sublethal and lethal doses of the copper ions on the motor activity of *D. magna*

Variant		Motor activity (IPL)	
		1 hour	24 hours
Control values for sublethal concentrations		161.8±17.4	140.6±10.1
Sublethal doses (mg/dm^3)	0.001	164.3±16.3	169.0±20.4
	0.005	172.1±20.4	139.0±8.7
	0.01	172.0±28.5	119.7±5.5*
Control values for lethal concentrations		190.3±11,6	139.5±9.8
Lethal doses (mg/dm^3)	0.1	176.0±23.3	181.0±15.6** death in 48 hr.
	0.5	149.3±17.6*	death in 5 hr.
	1	153.6±4.2*	death in 2 hr.

Notes: * – a significant decrease in the indicator as compared to the control values ($p < 0.05$); ** – significant increase in the indicator as compared to the control values ($p < 0.05$).

Table 2. Motor activity of *D. magna* in extracts from soils contaminated with heavy metals

Exposure, (hours)	Variant / motor activity (IPL)			
	Control	No. 1	No. 2	No. 3
1	129.1±6.9	125.7±3.1	123.7±6.8	133.4±5.8
3	132.8±6.9	125.7±4.6	105.4±7.5*	136.1±7.5
24	130.9±7.1	124.2±4.8	104.8±5.8*	138.4±8.6
48	140.0±6.7	128.0±4.4*	100.1±6.2*	139.8±7.7
72	148.3±5.7	128.7±5.5*	101.2±8.4*	146.8±5.0
96	156.1±2.3	131.3±4.8*	102.1±3.9*	143.7±3.9

Notes: * – the values differ significantly from the control ones ($p < 0.05$).

Approbation of the method with the addition of copper sulfate showed that the assessment of motor activity allows predicting a dangerous concentration of the toxicant after 1 hour of the experiment.

The influence of water extracts from soils contaminated with heavy metals on *D. magna*

Approbation of the method was continued in the study of the integrated toxicity of urban wastes, selected in the area of a metallurgical enterprise in Vladikavkaz (Russia). The soil samples were characterized by a high level of anthropogenic contamination with heavy metals. For example, the multiplicity of the excess of Russian standards for total copper forms was 2 to 7 times (from 100.5 ± 0.8 to 383.8 ± 1.8 mg/dm^3), for zinc – from 12 to 37.5 times (from 1165 ± 12 to 3750 ± 90 mg/dm^3). Water extracts were prepared from the soil samples. The ratio of solid and liquid phases was 1:4. Later, a bioassay of the extracts was carried out. The estimation of motor activity was carried out more often than in the model experiments (Table 2).

The first toxic effect was noted after three hours (sample No. 2). In the extract from Sample No. 3, the effect was detected only on the fourth day. It was noted that the reaction remains stable throughout the experiment: the first recorded oppression of the motor function was also noted in subsequent measurements of the test function.

The studied soil samples did not exert an acute toxic effect on *D. magna* in the bioassay, taking into account the mortality of individuals. This is probably due to the buffer properties of the soil, which reduce the bioavailability of toxicants. Such processes are known for a long time [Benedetti et al. 1995]. However, the evaluation of the sublethal test function allowed establishing a toxic effect.

In order to characterize the integral degree of contamination of soil samples by heavy metal compounds, the complex pollution index (Z_C) was calculated by means of the equation (1):

$$Z_C = \sum K_к - (n - 1) \quad (1)$$

where: K_K – is the concentration factor: the multiplicity of the excess of the heavy metal content in the monitoring section over its average content in the background area; n – is the number of chemical elements with a concentration in the monitoring section exceeding the background values.

In order to analyze the sensitivity of the method, see Table 3.

Table 3 shows that as the total contamination with heavy metals increases, the oppression of *D. magna's* motor activity increases and the effect comes faster. Therefore, the method for assessing

Table 3. The manifestation of test functions of mortality and motor activity *D. magna*, depending on the degree of contamination of the sample

Variant	Mortality *D. magna* (%)	The first recording of oppression of motor activity	Z_c (CPI)*
Control	0	-	-
Sample №2	6.7±1.5	in 3 hours	57.6
Sample №1	0	in 2 days	16.1
Sample №3	0	in 4 days	1.1

Notes: * – the complex pollution index.

the motor activity is informative in the study of soil contamination.

The influence of surface waters contaminated with mineral forms of nitrogen on the motor activity of *D. magna*

The third part of approbation was a bioassay of samples of surface water taken from the technogenic landscapes of ponds in the area of chemical enterprises "Uralchem" (Russia, Kirov region, Kirovo-Chepetsk). The company "Uralchem" produces mineral fertilizers, and some water reservoirs near it are contaminated with the nitrate ions and ammonium ions. The content of these pollutants was determined by means of the chromatographic method, then compared with the reaction of *D. magna*. The motor activity was assessed after 1 hour and 24 hours of exposure. The samples were taken at different times; therefore, each control value corresponds to its control value (Table 4).

According to the tables, it is seen that statistically significant oppression of motor activity in most samples is observed after an hour of exposure. In a day, all the tests reduce the ability of the daphnia to move actively. Attention was drawn to sample No. 8, in which the ammonium ion content exceeds the Russian standard by 5646 times, and the nitrate ions by 218 times. This test has a lethal effect on *D. magna*, but after 48 hours. Assessment of the motor activity allowed drawing a conclusion about its toxicity after 1 hour.

The oppression of the motor activity after 1 hour of exposure was minimal, but sufficient for the conclusion about the toxic effect, as the criterion was a mathematically significant difference from the control values. After a day of exposure, the slowing movements of daphnia became more significant. The maximum decrease in the indicator is observed for the most polluted sample No. 8.

The obtained Pearson correlation coefficients (r) confirm the informativeness of the method in the study of waters with mineral contamination. After 1 hour, the coefficient r between the concentration of nitrate ions and the decrease in motor activity of daphnia was (-0.53), between the content of ammonium ions and the oppression of the test function (-0.74). The negative relationship between the increase in the content of ammonium ions and the oppression of the *D. magna* mobility intensified in a day (-0.83). A similar calculation for nitrate ions showed a decrease in the dependence to (-0.23). These results indicate a predominant contribution of ammonium ions in comparison with the nitrate ions in the formation of toxic effects in their joint presence.

Thus, the method for assessing toxicity from the change in motor activity of *D. magna* was effective in controlling the pollution of natural waters that are regularly contaminated with mineral forms of nitrogen.

Nowadays, rapid and sensitive instruments for ecotoxicological research are developed around the world that fill the gap in the necessary "early warning" signals. The behavioral reactions are among the most promising in this area [Hellou 2011]. The change in many biochemical reactions quickly leads to a change in the behavior of organisms [Castro et al. 2004; Gravato and Guilhermino 2009].

Table 4. The results of the investigation of surface waters of anthropogenic landscape around the city of Kirovo-Chepetsk

Sample No.	Motor activity (IPL)				Content of mineral forms of nitrogen (mg/dm³)	
	in 1 hour		in 24 hours		NH_4^+	NO_3^-
	control	experiment	control	experiment		
1	155.5±7.3	146.8±5.2*	172.7±6.2	143.5±3.2*	0.74±0.26	29.7±3.6
2	158.7±7.7	151.1±4.5	171.6±3.8	136.0±3.3*	3.8±0.8	3.9±0.5
3	159.0±6.2	151.1±5.9	177.0±4.8	128.2±7.5*	5.9±1.2	8.5±1.0
4	154.6±10.0	150.0±5.8	179.3±3.4	128.5±5.4*	6.1±1.3	62±7
5	159.5±6.1	151.6±4.3*	170.0±4.5	132.0±3.5*	8.4±1.8	54±6
6	165.8±6.1	142.3±5.2*	175.6±6.8	147.7±4.8*	43±9	111±13
7	162.6±8.5	141.6±5.3*	173.7±5.6	144.1±4.4*	44±9	380±50
8	148.8±4.1	135.5±4.3*	175.1±4.6	106.1±4.7*	10900±2300	9800±1200

Notes: * – values differ significantly from control ($p < 0.05$); the Russian norm of the maintenance (NH_4^+) counting on nitrogen 1.5 mg/dm³, for (NO_3^-) – 45 mg/dm³.

The obtained data show that the "mortality" test function of *D. magna* often fails to diagnose toxicity even if high levels of contamination are observed. At the same time, the motor activity of the crustaceans allows us to make an adequate conclusion about the toxicity, and in a short period of time. It should be especially noted that the low information content of *D. magna* in the case of determining the mortality of individuals is due to the buffer properties of soils and natural waters, rather than the low sensitivity of the test organism as a whole. Selective sorption of pollutant ions by solid soil particles and organic matter of water, as well as their binding during complex formation, leads to a significant decrease in the bioavailability of many elements, including heavy metals [Toro et al. 2001; Thorslund et al., 2017]. This process, in turn, leads to an underestimation of toxicity. Therefore, the development of the bioassay methods using sublethal effects is promising.

We agree with the authors of the study [Parolini et al. 2018], which combines the evaluation of sublethal effects (motor activity and changes in biochemical parameters of *D. magna*) with the measurement of chronic effects (changes in the fecundity of individuals). This approach makes it possible to study the effects of pollution on the environment better.

CONCLUSION

The conducted studies showed that the test function of the motor activity of daphnia is effective for assessing the degree of toxicity of water environments, including extracts from soils. The advantages of the proposed method include a simple bioassay algorithm, rapid measurement of the parameter (5 minutes), and high sensitivity in comparison with the procedures for assessing the toxicity leading to the mortality of crustaceans.

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