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Aqueous medium toxicity assessment by *Daphnia magna* swimming activity change

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ABSTRACT

This paper presents toxicity evaluation data for the water containing various substances of known concentrations of various substances by *Daphnia magna* swimming activity change. The toxicity of the following substances was evaluated: potassium dichromate, zinc sulphate, pesticide esfenvalerate and cyanobacterial toxin of microcystin-LR. The swimming activity was determined using a computer vision system under normal conditions and after the toxicant introduction. It has been shown that at exposure time of 30 minutes, the median swimming speed of Daphnia changes. This fact may be used for the rapid assessment of aquatic toxicity, as well as for the development of the biological early warning systems for the contamination presence.

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INTRODUCTION

The water pollution is one of the most pressing environmental problems [1]. In order to assess the impact level on aquatic ecosystems, the biological testing is actively used along with the chemical analysis methods as an integral assessment of the environment toxic pollution [2]. This is due to the fact that the chemical analysis, in many cases, does not allow estimating the true danger of pollutants introduction into the environment, to predict the consequences of their effects on living organisms. For this reason, the methods of the water integrated assessment are applied. The main of these methods is the biological testing. Among the methods of biological testing the determination of the environment toxicity using lower crustaceans plays an important role, primarily using *Daphnia magna* Straus, 1820. The methods of daphnia biological testing are widely used for the environmental monitoring, both in Russia and abroad. The crustaceans mortality is mainly used as a test reaction, and the observations of fertility and offspring quality changes are performed when the chronic toxic effects are revealed [2-6]. The list of reactions may be significantly extended if the additional information on the test object is used based on its functional performance, including behavioral responses. This would allow to assess the quality of the aqueous samples more quickly.

At the present stage of technological development, the sensitive determination of toxic substances presence in the water is possible according to the behavioral characteristics of test objects by the means of their digital image computer analysis. The first works related to the environment toxicity assessment appeared in the 90-ies of XX century (e.g., according to daphniids of 1998 [7,8], by flagellates of 1999 [9]). In particular, the daphnia experiments assumed the exposure of test objects in a toxic environment (about a day) and the comparison of the obtained data with control ones by the motion rates. Due to the low computational power of computers, the researchers were limited by sequence analysis of several dozen images.

In some cases, this approach is used in practice for the biological early warning systems development concerning the presence of toxic substances in the environment (biological early warning systems). The fish and crustaceans is mainly used as biological indicators. Thus, the commercial system "Daphnia Toximeter" (bbe Moldaenke, Germany) in a continuous flow chamber (0.5-2 l/h) implements the monitoring of daphnia swimming activity (12 pcs.). The average speed, the distance between daphnia, their size, and some other parameters are controlled. Registration of water quality deterioration is carried out quite operatively [10]. The disadvantages of this method is the inability to manage the intersection of daphnia swimming in the space, i.e. no tracking of individual test organisms is carried out (average assessment made). In addition, the test uses filtered water only, while the European Water Framework Directive recommends the use of unfiltered water for

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testing procedures, as filtering can remove toxic substances adsorbed on particulate matter [11]. Another significant drawback is the high cost of "Daphnia Toximeter", which is about 65 thous. Euros in its standard configuration. It is suggested to solve some deficiencies of "Daphnia Toximeter" system with the use of a six-channel system "Grid Counter", performing tracking of individual daphnia [12]. Russia produces an instrument complex "BioLaT" to assess toxicity by infusoria. Based on computer analysis of the sequence of image pairs, the number of test objects and their displacement is determined, whereby the conclusion about their viability is made. An undying interest in the use of computer vision techniques in ecotoxicology [11, 13, 14] shows the perspective of the said method in toxicity assessment.

MATERIALS AND METHODS

This paper deals with the toxicity evaluation of water containing various substances of known concentrations of various substances by *Daphnia magna* swimming activity change. Swimming activity was assessed with the use of the hardware and software developed by the author for detecting and registering behavioral responses of test objects - "Toxicity Analyzer "TrackTox", implementing computer vision algorithms. In particular such as: receiving video, applying filters to eliminate "noise", converting the color system, determining the boundary values for the video thresholding, producing a binary video stream, its single-shot processing to find the contours and define centers of their mass, calculating the coordinates of an object, tracking the object to the original video.

The hardware of the complex (see Fig. 1) consists of a sealed container with clamps for mounting the test chambers with the test organism (volume up to 30 ml). The inner part of the container is illuminated with LED illumination (2500 lux). The front camera provides registration of behavior of the test organism (recommended resolution 640x360 pixels, possible recording rate - up to 30 frames/s). Power can be supplied from both the network (220 V) and the accumulator battery, which provides portability of the device.

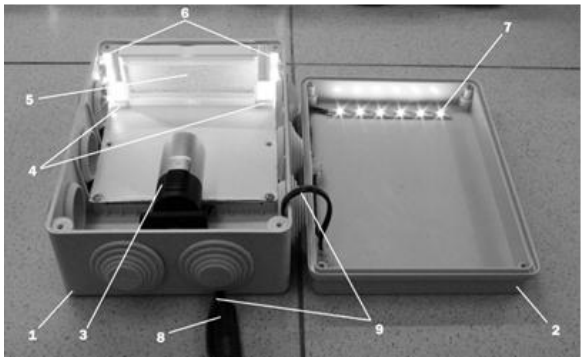

Fig. 1: Hardware and software complex "Toxicity Analyzer "TrackTox". 1) the device housing; 2) cover; 3) video-camera; 4) clamps of the test chamber; 5) the test chamber; 6) side LED illuminators (optional); 7) upper LED illuminators; 8) USB-cable for transmitting video-signal to a computer; 9) the power cable.

Processing and analysis of the data obtained is performed by a management station, consisting of the connected computer and a program for tracking the test objects "TrackTox" [15]. The following parameters shall be determined: the current coordinates in two-dimensional space, swimming speed, height of the test object location in the chamber, the current and the total distance covered, the size of the test object and its orientation in space.

We used in our experiments a laboratory monoculture of *D. magna*, grown in the laboratory incubator "V4" (Energolab, Moscow, Russia) [16]. To analyze the behavioral activity, we used data on the swimming speed in this study. The experimental scheme is shown in Fig. 2.

An individual daphnia is transferred quickly and accurately with the use of micropipette from a stock culture in a clear plastic test chamber (100 mm × 45 mm × 10 mm) with 25 ml of the cultivation water (1) which is transferred to the toxicity analyzer (2) disposed in a thermostatic environment (20 ± 2°C). Daphnia are kept for 10 minutes in a chamber for acclimatization, after which their swimming activity is measured during next 20 minutes, the so-called "control" (3). To reduce the excessive sensitivity, the processing is carried out at a lower rate (5 frames). To study the impact of a single toxicant, it is placed in the test chamber in an amount necessary to achieve the concentration required (4). There is another variant of experiment implementation, when daphnia is transferred to another container with the already prepared toxicant solution. After 10 minutes of exposure, the swimming activity is measured again during 20 minutes, the so-called "experiment" (3). Thus, the total time of exposure to the toxicant solution is 30 min. During the experiment, the data are visualized on the screen (5) and at the end of the experiment data on daphnia swimming activity (~ 6000 measurements in

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control and experiment) are transmitted to a text file available for subsequent processing and statistical analysis (6).

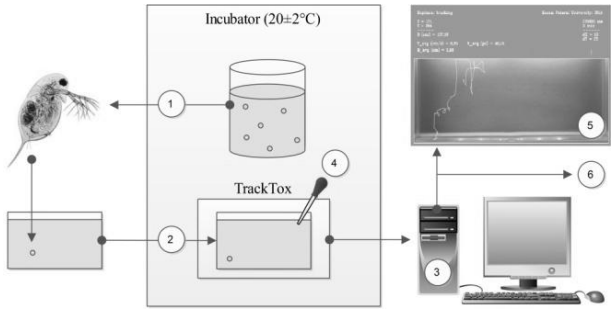

Fig. 2: Determination scheme of daphnia swimming activity in the experiment.

Statistical processing was performed using the software package STATISTICA 8.0 (StatSoft, Tulsa, USA). Data compliance with the normal distribution was checked visually on the speed frequency distribution diagram, as well as using the Kolmogorov-Smirnov criterion.

The toxicity was assessed by the change of test-function - the swimming speed of daphnia (toxicity index A, %) according to the formula:

$$A,\% = 100 \cdot \frac{X_c - X_e}{X_c}$$

where Xc and Xe - the test function values obtained in the control and experiment, respectively.

RESULTS AND DISCUSSION

Based on the above scheme we have determined the toxicity of aqueous solutions containing the following individual toxicants: potassium dichromate (2.0 mg/l), zinc sulphate heptahydrate (1.5 mg/l), pesticide esfenvalerate (3 µg/l), cyanobacterial toxin of microcystin-LR (0.15 µg/l).

Potassium dichromate ($K_2Cr_2O_7$) is a substance, standardly used to assess levels of physiological activity of daphnia laboratory cultures (the selected concentration is the upper limit of a range of concentrations at which we should observe the acute toxicity for Daphnia [16]. Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$) is a standard toxicant in biotest for bacterial bioluminescence in the test system "Ekolum" (selected concentration is also the limit of the test system response [17]. Esfenvalerate ($C_{25}H_{22}ClNO_3$) is a representative of a group of synthetic pyrethroids, one of the most produced and used preparations to control pests of plants (selected concentration is MAC of the pesticide in water). Microcystins ($C_{49}H_{74}N_{10}O_{12}$) - one of the most famous and widespread cyanobacterial toxins produced by some species of cyanobacteria during the "bloom" of water bodies (the selected concentration - the detection limit of many quantitative analysis methods, such as ELISA, HPLC).

Daphnia swimming activity parameters obtained in experiments are shown in the Table below. It should be noted that many biological early warning systems, built on the speed analysis of the test objects, use parametric statistics, excluding the nature of the data distribution. In our case, it was found that the distribution of daphnia swimming speeds is different in all cases from the normal one, with positive asymmetry coefficient. Therefore, it is better to use nonparametric statistics (median, etc.), moreover, they are more robust in the presence of emissions than parametric statistics.

Table. Daphnia swimming activity parameters (median speed) under normal conditions (Vc) and with addition of toxicants (Ve); Median toxicity index value in series of N experiments (A, %).

Substance	N	Vc, cm/s	Ve, cm/s	A, %
Potassium dichromate	4	0.36	0.33	11
Zinc sulphate heptahydrate	4	0.21	0.12	39
Esfenvalerate	4	0.18	0.40	-119
Microcystin-LR	10	0.29	0.37	-82

The data presented show that the inhibition of daphnia swimming activity was observed in experiments with potassium dichromate and zinc sulphate. Median speed varied in the range of 0.21-0.36 cm/s under control conditions, reducing to 0.33 and 0.12 cm/s after adding the potassium dichromate and zinc sulphate, respectively. The difference in the swimming speeds under control conditions is explained by some differences in the size of daphnia taken for biotest (2.4 mm for $K_2Cr_2O_7$ and 2.1 mm for $ZnSO_4 \cdot 7H_2O$). The median value of the toxicity index was 11 and 39%, respectively.

The median speed of daphnia significantly increased in the experiment with the addition of an insecticide from 0.18 to 0.49 cm/s, and the increase in the spread of data was also observed. Toxicity index was -119%,

which indicates a significant stimulation of swimming activity of the test object with the addition of esfenvalerate at low exposure time (30 min.).

In the experiment with cyanotoxins the median speed varied from 0.11 to 0.47 cm/s under control conditions and from 0.20 to 0.92 cm/s after adding microcystin-LR. The group medians velocity, in general, increased from 0.29 to 0.37 cm/s after adding cyanotoxin, the median of test function change was -82%.

The results obtained are generally consistent with the data on daphnia swimming activity known from the literature. In particular, the inhibition of swimming activity in the presence of zinc and chromium ions is consistent with the behavior of *D. magna* with other metals (Cd [8], Cu [18]), described in the literature. Studies by Zein *et al*. [19] dealt with the effect of acetylcholinesterase inhibitor (as a component of many pesticides) on *D. pulex*, and showed that low concentrations cause the same increase in swimming speed of daphnia. There are also studies [20], which studied the behavior of *D. magna* in the presence of some non-hazardous cyanobacteria with the help of "Daphnia Toximeter". It was shown that the presence of the cyanobacterium *Cylindrospermopsis raciborskii* promotes an increase in the daphnia swimming speed, however, a warning was not sounded. It should be noted that information on the effects of substances, considered in this paper, on the swimming activity of daphnia have been obtained for the first time.

CONCLUSIONS

Under the conditions of operating biotest (30 min.) the presence of metal ions (Cr and Zn) causes suppression of daphnia swimming activity, while the presence of organic substances (pesticide esfenvalerate and cyanotoxin microcystin-LR), increases it, conversely. The presence of the daphnia reaction expressed to the presence of toxicants in water suggests the possibility of the application of this bioassay in detecting low concentrations of pesticides and cyanotoxins for both the rapid assessment of aquatic toxicity and the development of the biological early warning systems for the contamination presence.

SUMMARY

The investigation of the effect of potassium dichromate, zinc sulphate, pesticide esfenvalerate and cyanobacterial toxin of microcystin-LR on daphnia swimming activity was performed for the first time. It has been shown that at exposure time of 30 minutes, the median swimming speed of daphnia changes, the toxicity index depending on the substance is: 11, 39, -119 and -39% respectively.

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