Toxicity assessment of polluted sediments using swimming behavior alteration test with Daphnia magna

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Abstract. Recently behavioral responses of organisms are increasingly used as a reliable and sensitive tool in aquatic toxicology. Behavior-related endpoints allow efficiently studying the effects of sub-lethal exposure to contaminants. At present behavioural parameters frequently are determined with the use of digital analysis of video recording by computer vision technology. However, most studies evaluate the toxicity of aqueous solutions. Due to methodological difficulties associated with sample preparation not a lot of examples of the studies related to the assessment of toxicity of other environmental objects (wastes, sewage sludges, soils, sediments etc.) by computer vision technology. This paper presents the results of assessment of the swimming behavior alterations of *Daphnia magna* in elutriates from both uncontaminated natural and artificially chromium-contaminated bottom sediments. It was shown, that in elutriate from chromium contaminated bottom sediments (chromium concentration 115±5.7 μg l^{-1}) the swimming speed of daphnids was decreases from 0.61 cm s^{-1} (median speed over the period) to 0.50 cm s^{-1} (median speed at the last minute of the experiment). The relocation of Daphnia from the culture medium to the extract from the non-polluted sediments does not essential changes the swimming activity.

1. Introduction

Various bioassay methods are actively used for assess the quality of natural waters and other environmental objects as well as the safety of existing or new artificially created chemical substances. In the tests, the evaluation is performed on the basis of observations and experiments with "living sensors" – organisms whose vital activity is disturbed in the toxic conditions [1–3]. Despite the fact that the range of organisms used as tests is very wide, there is no organism that is universally sensitive to all existing toxicants. Therefore, tend to use batteries of tests organisms [4, 5].

Cladocera (an order of minute branchiopod crustaceans) are one of the most frequently used organisms in ecotoxicological studies [6]. A feature of most of them is the filtration method of feeding, in which considerable amounts of water are passed over a specialized filtering structure. Cladocera are able to extract from the water not only the necessary food but also dissolved and suspended solids including the components of contaminants.

In most cases, as test objects used crustaceans *Daphnia magna*, *D. longispina*, *Ceriodaphnia dubia*, *C. affinis* and some others. The most universal test object for sensitivity and adequacy of response to different toxicants can be considered *D. magna*. Biotesting methods using *D. magna* are widely used for the purposes of environmental control, the laboratory culture of *D. magna* is one of the most commonly used in the practice of determining the acute and chronic toxic effects of individual chemicals and water samples with a complex chemical composition. Typically the toxicity is assessed

by the EC50, LC50 end-points and also by a statistically significant change in the parameters of vital activity of daphnids under toxic and control conditions. Mortality of crustaceans is mainly used as a test reaction in acute tests; observations of changes in fertility and quality of offspring are carried out in the study of chronic toxic effects [7].

It is possible to significantly expand the list of recorded parameters by using additional information about the test organism based on its functional characteristics including behavioral responses [8,9] Moreover, analysis of the literature shows that behavioral reactions yielded significant responses at concentrations of toxicants well below reported LC50 values [10].

Behavioral responses most clearly manifested at the organism level, are between the biochemical and ecosystem levels of biomonitoring. However, at the same time behavioral alterations are directly based on biochemical processes, reflect the adaptation of an individual organism as well as the potential effects on the population level. Behavioral responses appear to compare favourably with biochemical and physiological responses in terms of sensitivity and efficiency. Furthermore, in addition to their integrative nature and ecological importance, the assessment of behavioral responses is a non-destructive method that makes possible continuous long-term environmental monitoring [11, 12].

Behavioral responses of aquatic organisms have been used for decades as methods for environmental monitoring but these types of studies have previously received much less attention than others focused on different subject such as developmental or reproductive toxicology [10]. Probably this was due to the problems in recording and quantification of the behavioral characteristics of test organisms. Many difficulties eventually were removed and at present, in addition to visual registration, a quantitative description of behavioral reactions is carried out by various methods: optical, ultrasonic, electrical, magnetic etc.

At the current stage of the development of technology, it is possible to sensitive determination of the presence of toxic substances in water by the behavioral characteristics of test objects, including such small organisms like Daphnia, using digital analysis of video recording [13–16]. To assess the toxicity used such daphnids behavioral characteristics as swimming time and speed, swimming trajectory, hopping frequency, vertical and horizontal distribution, distance travelled, number of turnings and turning angle, resting time, duration of quiescence, sinking rate, gravitaxis, swarming and spinning. Swimming mobility expressed by scalar quantity-speed and its vector quantity-velocity (usually expressed in millimeters per second) is one of the most reliable and widely used parameter of Daphnia behavioral activity [16].

Most studies have evaluated the toxicity of aqueous solutions of pure chemicals by the behavioral alterations of the test organisms. In addition to these studies, it is of interest to estimate the toxicity of other environmental objects (wastes, sewage sludges, soils, sediments etc.) using a digital analysis of the behavioral responses of test organisms exposed to water extracts of solid sample. In this case, there are methodological difficulties associated with sample preparation, such as staining and increasing the turbidity of the water solution. This can restrict the application of computer vision technology since this will make it impossible to recognize objects. The aim of this study was to assess the alterations in the swimming behavior of *D. magna* in elutriates from both uncontaminated natural and artificially chromium-contaminated bottom sediments.

2. Material and methods

Sediment samples for the experiment were collected in 2015 from the Almetyevsk reservoir (54°55'1" N 52°16'24" E). The reservoir is located in the northern part of Almetyevsk (Republic of Tatarstan, Russia). The reservoir exists within the riverbed and the low floodplain of the Steppe Zay River. The reservoir is shallow; the prevailing depth is about 0.9 m. Water area is 1.11 km^2, the maximum length and width is 1.78 and 1.0 km respectively.

Sediments were dried at the temperature of 105 °C and ground, than they were sieved through a 1 mm mesh. To simulate contaminated bottom sediments in the initial non-polluted sediments (“IS”) potassium dichromate ($K_2Cr_2O_7$) was added in an amount of 1.41 grams per 100 grams of dry weight

and mixed thoroughly ("CS"). Chromium was selected because of the high toxicity it represents to different zooplankton organisms. The resulting samples were filled with distilled water (Milli-Q, Millipore) at a ratio of "solid phase : liquid" – "1:10" in beakers. Then they were exposed on the platform shaker for 8 hours; the supernatant was allowed to settle for 12 hours and filtered ("IS-elutriate" and "CS-elutriate"). The sediments and extracts of the ones were analyzed for metals content by emission spectrometer ICPE-9000 (Shimadzu).

The behavioral experiments were performed with *D. magna* Straus, cultured in accordance with the Russian standard procedure [10]. For experiments we used parthenogenetically reproducing laboratory clones of Daphnia cultivated at the Department of Applied Ecology at the Kazan Federal University (Kazan, Russia). *D. magna* are cultivated at constant conditions of temperature (20±2°C), under 2000–2500 lux light intensity and 12/12 h light/dark photoperiod in a laboratory incubator.
Daphnids were fed daily with 1–2 mL *Chlorella vulgaris* suspension cultured in Tamiya medium. The daphnids used in this experiment originate from one single female to eliminate variation among clones. *D. magna* individuals were selected according to size (3.23±0.03 mm). Size measurements carried out using an eyepiece micrometer.

Measuring of swimming activity of the daphnids were determined and analyzed in real time by the hardware-software system "TrackTox". The scheme of experiment is presented in figure 1. Three daphnids were transferred from permanent culture into transparent polycarbonate test chamber (100 mm × 45 mm × 10 mm) with 25 ml of culture medium (1) and subsequently was placed into "TrackTox" (2) under static conditions (20±2 °C), without any water exchange during the experiment. The transfer of daphnids was carried out rapidly and carefully by micropipette in order to minimizing the stress to the test organisms. The next 30 min measuring of swimming activity (with rate 3 times s^{-1}) was implemented by "TrackTox" (3). In order to observe the behavioral response of the *D. magna* to elutriate from non-polluted and contaminated sediments daphnids consecutively were transferred from test chamber with culture medium ("CM") into test chambers with "IS-elutriate" (4) and "CS-elutriate" (5). In each case swimming activity was determined within 60 min by "TrackTox". During the experiment data are visualized on display (6). At the end of the experiment the data have been stored and exported to a statistical software package for further processing. Thus the total exposure time for each group of three was 150 (30+60+60) minutes. The acute toxicity of the same samples was additionally assessed (within 48 hours).

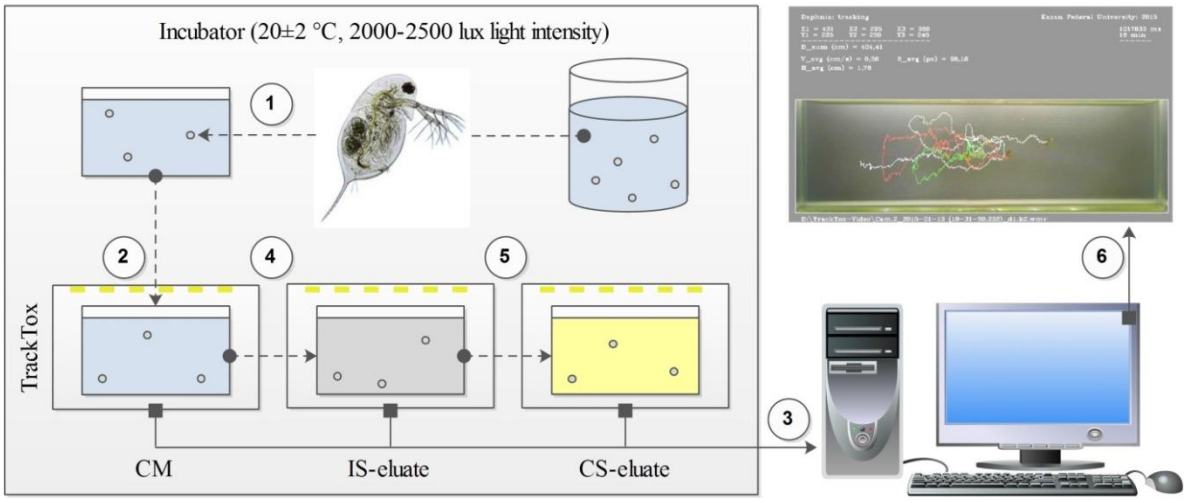

Figure 1. Scheme of Daphnia swimming activity measuring (dashed lines – movement of objects, solid lines – receiving the information). Explanations of experiment stages are given in the text above.

Normal distribution of data (Kolmogorov-Smirnov test) was previously verified. Differences were considered significant at values of $P < 0.05$. The full range of variation, arithmetic mean, standard

deviation (SD), standard error of the mean (SE), median, and interquartile range were calculated. The swimming speeds were averaged for time intervals (every minute) as well as for the consolidated group of thirty daphnids (number of experiments is N=10). Statistical analysis was performed using STATISTICA 8.0 (StatSoft).

3. Results and discussion

A chemical analysis both of initial non-polluted sediments and water elutriates of them showed a low level of metals contamination (table 1). The chromium content in natural bottom sediments was 23±4.6 mg kg^{-1}, in artificially contaminated sediments – 9100±1820 mg kg^{-1}. The concentrations of chromium in "IS-elutriate" and "CS-elutriate" were 90±4.5 μg l^{-1} and 2300±115 μg l^{-1} respectively. In the latter case, difficulties were encountered with the recognition of daphnids by "TrackTox" (due to staining of the solution), so that the sample was further diluted to a concentration of 115±5.7 μg l^{-1} ("CS-elutriate$_d$").

Table 1. The concentration of metals in bottom sediments (mg kg^{-1}) and in eluents of them (μg l^{-1}).

	Cr	Al	Cd	Cu	Fe	Mn	Ni	Pb
IS	23±4.6	2025±526.5	<0.05	15±3	5000±1400	950±285	21±7.35	6.5±1.63
CS	9100±1820	1850±481.0	<0.05	18±3.6	4875±1365	900±270	19±6.65	4.9±1.23
IS-elutriate	24.4±1.22	52±3.12	0.3±0.03	6.5±0.39	81±4.05	20±1.4	3.9±0.23	9±0.72
CS-elutriate	2300±115.0	23±1.38	0.1±0.01	11±0.66	75±3.75	63±4.41	5.5±0.33	11±0.88

The summary results of the *D. magna* behavior alteration experiments in the following sequence: "CM" → "IS-elutriate" → "CS-elutriate$_d$" are shown in figure 2.

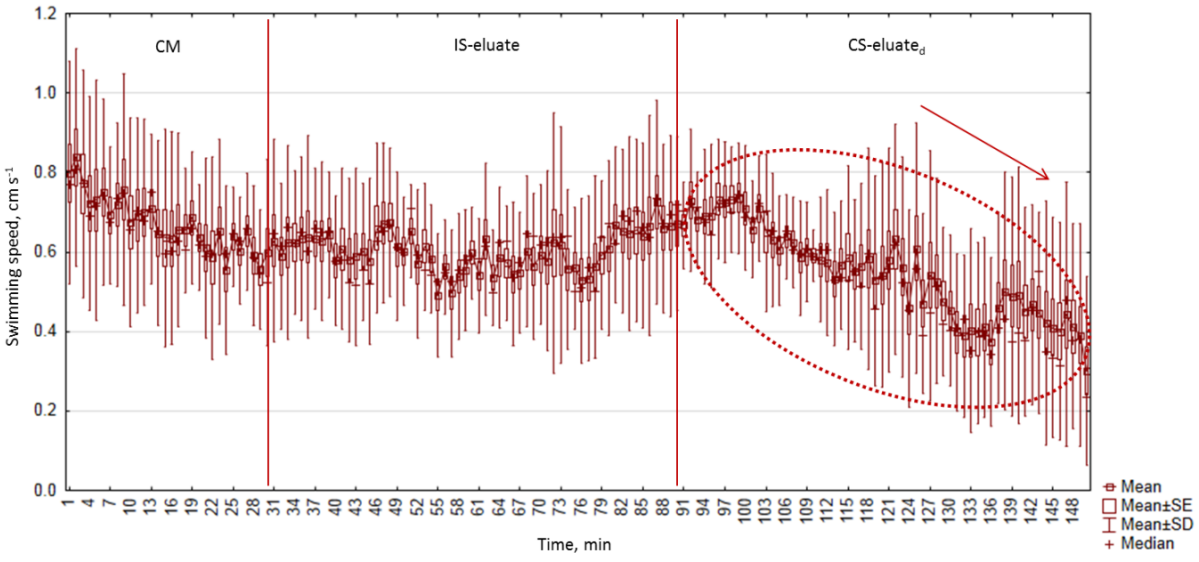

Figure 2. Summary results of the swimming activity of Daphnia in a series of experiments.

The average swimming speed of daphnids in culture medium was 0.67±0.01 cm s^{-1}, after transfer to the "IS-elutriate" it practically did not change and was 0.60±0.01 cm s^{-1}, after transfer to the "CS-elutriate$_d$" it it further decreased to 0.55±0.01 cm s^{-1} (table 2). More detailed information about swimming activity of Daphnia can give a per-minute analysis of the dynamics – in this case, the inhibiting effect of chromium can be traced particularly clearly.

Table 2. Parameters of Daphnia swimming activity (cm s^{-1}) in different mediums.

Swimming speed	Mean	Min	Max	Std.Dev.	Std.Err.	Q25	Median	Q75
Values in culture medium								
CM_p (whole period)	0.67	0.12	1.65	0.23	0.01	0.52	0.65	0.78
CM_{fm} (first minute)	0.80	0.44	1.43	0.28	0.07	0.55	0.77	1.04
CM_{lm} (last minute)	0.60	0.37	1.29	0.23	0.06	0.43	0.52	0.73
Values in IS-elutriate medium								
IS_p (whole period)	0.60	0.09	1.53	0.20	0.01	0.46	0.61	0.72
IS_{fm} (first minute)	0.63	0.13	1.03	0.25	0.07	0.42	0.65	0.86
IS_{lm} (last minute)	0.67	0.26	1.10	0.22	0.06	0.54	0.72	0.78
Values in CS-elutriate$_d$ medium								
CS_p (whole period)	0.55	0.02	1.38	0.24	0.01	0.37	0.58	0.71
CS_{fm} (first minute)	0.67	0.50	0.86	0.11	0.03	0.57	0.70	0.73
CS_{lm} (last minute)	0.30	0.04	0.81	0.24	0.06	0.10	0.24	0.50

An additional estimate is the toxicity index widely used in practice:

$$T,\% = 100 \times (V_{bc} - V_{ac}) / X_{bc} \quad (1)$$

V_{bc} – is the swimming speed in the baseline, V_{ac} – is the swimming speed in the analyzed conditions. It can be noted that the toxic effect in the series with chromium, despite a pronounced decrease in speed by the end of the experiment, is not revealed by this approach. The toxicity index for the average speed in this case is only 8.3%.

Probably this is a feature of data aggregation, when there is a significant variation in values of speed during the experiment. To eliminate the influence of this factor, it is possible to use for the calculation of the toxicity index not the integral value of the swimming speed for the entire observation period, but only the boundary values that characterize the final state of Daphnia (at the last minute), both in the control and in the experimental conditions (table 3).

Table 3. Variants of the toxicity indexes calculated by the different values of swimming speed.

Toxicity index	By mean values	By median values
$(CM_p - IS_p) / CM_p$	10.4%	6.2%
$(IS_p - CS_p) / IS_p$	8.3%	4.9%
$(CM_p - IS_{lm}) / CM_p$	0.0%	–10.8%
$(IS_p - CSl_m) / IS_p$	50.0%	60.7%
$(IS_{fm} - CS_{lm}) / IS_{fm}$	52.4%	63.1%
$(CM_{lm} - IS_{lm}) / CM_{lm}$	–11.7%	–38.5%
$(IS_{lm} - CS_{lm}) / IS_{lm}$	55.2%	66.7%

The toxicity index calculated by this way was 55 and 67% for the mean and median speed, respectively. Since the data distribution in the samples differs from the normal one, it is possible to recommend the use of a toxicity index calculated just by the median. The values obtained correspond more accurately to the actual picture of the swimming activity of Daphnia observed in the experiment.

Daphnia swimming behavior is complex, multiparametric and it may be characterized by several parameters reflecting changes induced by various compounds on sensitive (i.e. nervous and endocrine) systems [16]. A number of results indicate that various substances may alter swimming speed. In particular, our earlier studies showed that *D. magna* change their behavior under the exposure of potassium dichromate, zinc sulfate, esfenvalerate pesticide, microcystin-LR [17–19]. In the case of the exposure of metal salts, there was a statistically significant decrease, and in the case of a pesticide and cyanotoxin, an increase in the swimming speed of Daphnia with a short exposure time of 10–30 minutes.

Some authors also suggested that the swimming speed may be also altered by some metals. In particular, daphnids exposed to sublethal cadmium [20,21] or copper stress [22] tends to manifest inhibited swimming activity when compared to the unexposed, similarly-sized control organisms. The mechanisms by which metals decrease Daphnia speed have not been completely clarified, however it could be hypothesized that oxidative stress known to be induced by these substances in other animal species may also be responsible for the toxic effects on the behavior of crustaceans [16].

4. Conclusion

The conducted study showed the possibility of using a biotesting method based on computer vision to assess the quality of contaminated bottom sediments. In the elutriate from chromium contaminated bottom sediments, the Daphnia swimming speed decrease from 0.61 cm s^{-1} (median speed over the period) to 0.50 cm s^{-1} (median speed at the last minute of the experiment). The relocation of daphnids from the culture medium to the extract from the non-polluted sediments does not essential changes the swimming activity.

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