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Methods for Contaminated Water Biotesting

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Abstract: The response of living organisms to pollution is manifested in physiological, morphological and behavioural changes, which makes biomonitoring a very convenient and predictable method. *Daphnia magna*, in particular, is the best indicator in the ratio of the time spent and of course the accuracy of the readings. Increasing the information content of biotesting waters of surface sources of water supply and drinking water on mollusks *Daphnia magna S*. through the use of a test feature of heart rate. The test durations were 48 and 3 hours, respectively. The heart rate measurement results indicate a low level of toxicity (range, 26.2 to 35.4%). The results of the study suggest that the rapid assessment of water quality with the use of Daphnia should rely more on the heart rate measurement. This method proved to be highly effective in estimating the water samples from the Flathet Reservoir, yielding data 73.11% faster than the immobilisation test. The sensitivity level was also 28.33% higher.

Key words: Biotesting, *Daphnia magna* S., immobilisation, toxicity level.

Introduction

The introduction of potentially hazardous chemical agents into drinking water sources has been rapidly increasing over the recent few decades. This tendency made it vital to assess the quality of drinking water via biotesting. The said strategy for water quality analysis provides an integrated assessment of water toxicity (investigates the combined effect of toxic substances on the human body) while considering the synergism and antagonism between various solutes (Arkhipchuk and Malinovskaya, 2000; Braginskii et al., 1983). As regards drinking water quality, there are no clear criteria for health hazards of exposure to toxic substances unless they are present in high concentrations. Bioassays (or biotests), on the other hand, can assess water quality even with low solute concentrations (Arkhipchuk and Goncharuk, 2003; Braginskii et al., 1983; Goncharuk et al., 2016).

Biotesting involves using living organisms (test objects) or their test functions to determine the degree of water toxicity (Dorsey and Tchounwou, 2004). In a broad sense, it is a procedure, which evaluates the adverse effect of environmental factors on an organism and its separate functions or a system of organisms (Goncharuk and Kovalenko, 2012). Unlike the bioindication approach, laboratory bioassays assess the water quality based on what aquatic species

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are present (Goncharuk et al., 2016). It is possible because bioassay organisms exhibit varying responses to metals, organic substances (e.g., polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides, and pharmaceuticals) and biogenic compounds in the water. The test organisms could be both animals and plants. In general, such an approach reduces the number of necessary procedures and simplifies the investigation process.

Biotesting of water quality is carried out in the laboratory, not in the field. Different authors use different criteria to distinguish between assays, but the most popular one is the type of test organism. Bioassays are capable of detecting viruses and bacteria contaminating the water. They also identify toxic, mutagenic or carcinogenic substances and evaluate their effect on organisms (Kovalenko et al., 2016; Krainyukova, 2009).

Single bioassays should be used in conjunction with analytical chemistry procedures. Meantime, if the goal is to estimate the risk to human health, then biotesting should be combined with a standard sanitary inspection (Goncharuk et al., 2005). There is no doubt that the biological approach to water quality analysis is important and effective. This paper discusses the basic principles and approaches to assessing the quality of water with low pollutant concentrations. The present study seeks to find a way how to make the biotesting of water quality with the use of *Daphnia magna* more informative.

Materials and Methods

The test organisms (animals and plants) included *Daphnia magna* and *Ceriodaphnia affinis*, freshwater coelenterate (*Hydra attenuata*), zebrafish (*Brachidanio rerio*) and common wheat (*Triticum vulgare*). They belong to different taxonomic groups and trophic levels. The selected test objects are easy to grow under laboratory conditions, and show high sensitivity to common pollutants, and provide reproducible results. The selection of the test species was in line with three essential criteria, but they may also be subject to additional requirements. The criteria applied for the selection of test organisms were as follows:

(1) being from at least two different trophic levels (this will ensure a specific sensitivity range);
(2) high sensitivity to the types of toxic substances;
(3) existence of cost-effective bioassay kits containing all necessary materials, including the test organisms themselves, to do toxicity testing with species from any region or ecosystem with results within 5 days following preparation.

To minimise the risks of sample contamination by the sample collector and to ensure sample integrity, basic precautions must be taken to obtain a representative sample. Samples can become contaminated through careless sampling techniques. So we use precautions to avoid contamination, which are as follows:

1. always use contaminant-free containers and where possible, prepare containers and testing equipment at sampling sites;
2. keep an accurate record of each sample collected using the correct form;
3. always use devices or instruments that have been calibrated at the required frequency (thermometer and pH-meter);
4. reagents that are used for analysis must be kept in a clean, dry, well-ventilated and dark location until use;
5. always take measurements with reagents in a suitable location. Avoid leaving reagents in prolonged sun exposure;
6. seal reagent bottles correctly after use;
7. never place wet fingers on reagent bottles; this may lead to inaccurate results;
8. verify product expiry dates. Once the date indicated is past, you cannot be certain that the results are reliable;
9. discard expired products, in accordance with regulations in effect.

The rapid testing process for determining toxicity in aquatic systems seems to be not burdened with complexities. However, an evaluation of water quality at a low pollution level requires appropriate technology. Not all living organisms can be helpful in this situation. The morphological and physiological differences between the test organisms define their application in biotesting, and no single test object is ideally suited for all toxicity methodologies. A common belief is that no solution exists, but analysing the individual characteristics of multiple organisms and comparing their sensitivity to various toxicants could help overcome that problem.

Toxicity testing took place in the U.S. State of Montana on water samples from freshwater lakes and reservoirs on the South Fork of the Flathead River, including the state's largest Flathead Reservoir. The samples were collected into 100-mL beakers in April

2017 and divided into several groups: samples from the South Fork of the Flathead River (group 1), samples from the Flathead Reservoir (group 2), and those from clear water reservoirs with a capacity of 5.000 and 20.000 m^3 (groups 3 and 4, respectively). Tap water (settled for 24 hours) was taken as a control sample.

The aim was to compare immobility (I) and heart rate (HR) endpoints used in water biotesting. The aquatic invertebrate species, *Daphnia magna* (aged 24h), has been the focus of the study. The specimens were exposed to immobilisation and heart rate (HR) measurement procedures. The number of active and immobilised organisms were recorded after 48 hours of exposure (n = 10). For this, each sample was taken by a pipette and placed on a glass slide with a drop of the test medium. The heart rate of *Daphnia* was counted twice, for 30 seconds and 1 min, under a microscope after 3 hours of exposure (n = 15), and the arithmetic mean was determined. The tests were carried out in triplicate at 20 ± 2°C. The data were compared with the control sample. The toxicity index (%) was calculated for each test sample as per SSsanRN 2.2.4-171-10. The formula is given below:

$$T = \frac{Ik - Io}{Ik} 100 \quad (1)$$

where T is the toxicity index, %; Ik is the level of response in the control group; Io is the level of response in the test group.

Since pollutants have the power to both slow down and speed up the heart rate of test organisms, it is crucial to determine the difference in response level between the test and control samples. The biotest using heart rate as an endpoint is more sensitive compared to the immobilisation test. In addition, it detects contamination in the water much faster than conventional tests. In a short-term biotesting of surviving Daphnia, they are taken into account after 1, 6, 12, 24 and 48 hours. Individuals are considered alive if they move freely in the water column or float from the bottom of the vessel no later than 15 seconds after it is wiggled.

Results

The water toxicity scale derived from the study is consistent with SSanRN 2.2.4-171-10 and more convenient than the standard. It implies that the acceptable level of water toxicity should be less than 50%, regardless of the test objects used in the analysis. The scale encompasses four levels of toxicity: permissible (1-25%), low (26-50%), moderate (51-75%), and high (76-100%) (Table 1).

Another toxicity test exposes organisms to a fluorogenic substrate (galactose). When chemical compounds are present in the water, the sugar decomposition process is disturbed, which inhibits the ability of organisms to emit light. This test takes 75 minutes. As test objects for acute toxicity (high mortality) and chronic toxicity (decreased reproduction) tests, Daphnia are also suitable for this type of biotesting. Table 2 shows other test organisms that were used in water quality analysis.

Discussion

Living organisms can exhibit varying responses to toxicants. The difference in their response depends on the nature and level of water pollution. According to available data (Goncharuk et al., 2017; Kotova et al., 1989; Kovalenko et al., 2016), more subtle responses (e.g., inhibition of growth) indicate lower concentrations of toxic substances, while lethal responses represent a measure of high toxicity. Sensitivity is not the only

Table 1: Efficiency of toxicity tests using different endpoints

Test group	*Efficiency of toxicity tests using different endpoints*			
	I (T%)	*Toxicity level*	*HR (T%)*	*Toxicity level*
Group 1 (South Fork of the Flathead River)	Absent	Moderate	35.4	Low
Group 2 (Flathead Reservoir)	Absent	Moderate	27.6	Low
Group 3 (Clear WaterReservoir, 5 000 m^3)	Absent	Moderate	24.1	Moderate
Group 4 (Clear WaterReservoir, 20 000 m^3)	Absent	Moderate	26.2	Low
Exposure duration (hours)	48	—	3	—
Preparation and data analysis, hours	0.5	—	0.75	—
Number of test objects	10	—	15	—

Note: I — immobility; HR — heart rate; T — toxicity index.

Table 2: Comparison of bioassay kits used to assess surface water quality

Test organism	*Test*	*Realisation time*	*Result*	*Standard*
Brachionuscalyciflorus	AcuterotoxkitF	24 hours	Death rate percent	ASTME1440-91
Brachionuscalyciflorus	Short-chronicRotoxkitF	48 hours	Inhibition of growth	AFNOR T90-377, ISO20666
Brachionusplicatilis	RotoxkitM	24-48hours	Death rate	ASTME1440-91
Larvae of the salt water genus Artemia	ArtoxkitM	24-48hours	Death rate	ASTME1440-91
Daphniamagna, Daphniapulex	DaphtoxkitF	48 hours	Immobilization, death rate percent	OECDGuideline202, ISO6341
Ceriodaphniadubia	CeriodaphtoxkitF	24 hours	Death rate percent	OECDGuideline202, ISO6341
Thamnocephalusplatuyurus	ThamnotoxkitF	24-48hours	Death rate percent	ISO14380
Thamnocephalusplatuyurus	RapidtoxkitF	30-60 minutes	Reduction or no food	ISO14380
Tetrahymenathermophila	ProtoxkitF	24 hours	Inhibition of growth	OECDGuideline202
Selenastrumcapricornutum	AlgaltoxkitF	72 hours	Inhibition of growth	ISO8692,OECD Guideline201

crucial test characteristic. The resulting time and ease of observation are also important.

The adequacy of testing is closely related to how the test is organised and its integral toxicity estimation ability. Therefore, it is vital to choose a proper criterion for evaluation, which may vary depending on the case. Some authors propose to choose the measurement endpoint in accordance with desirable requirements (Goncharuk et al., 2017; Krainykova, 1991). However, the following factors should also be considered: efficiency, versatility, measurability, ease of interpretation and calculation, comparability, and biological value. Studies show that aquatic organisms respond to lower concentrations of deadly toxicants compared to warm-blooded animals (Braginskii et al., 1983; Goncharuk et al., 2016). The difference is one or two orders of magnitude. Thus, the death rate can be used to establish acute and chronic toxicity. An earlier response to unfavourable factors can be obtained with tests examining biochemical and physiological parameters. For example, changes in blood characteristics are recorded long before the test object dies from toxic effects (Gandziura and Grubinko, 2008; Goncharuk et al., 2012, 2016). In general, toxicity response is influenced by a whole complex of external factors. The primary one is the sample composition. Usually, scientists deal with complex pollution cases.

The value of biotesting is largely determined by the ability of the test to estimate integral toxicity (Arkhipchuk and Malinovskaya, 2000; Goncharuk et al., 2017; Krainykova, 1991). However, the combined action of multiple toxic substances on living organisms needs more research. It is generally accepted that the combined effect of several substances is not additive. Some toxic substances can exert smaller toxic effects in the presence of other substances, while others tend to become more toxic (Scott and Sloman, 2004).

Given the above complexities of water toxicity testing, biotests appear to belong to a category of tests that give an accurate idea of the combined effect of various chemicals on the living systems. When conducting biological tests, researchers often face the need to adjust the threshold of detection response (Braginskii et al., 1983; Kovalenko et al., 2016; Scott and Sloman, 2004). For this purpose, the so-called functional loads can be used. They serve to force changes in the state of the test system (Goncharuk et al., 2017; Kotova et al., 1989). When put under stress, the test organism is forced to compensate through the change in metabolism and distribution of internal resources. In this way, the organism loses the resources necessary for counteracting the toxic effects and becomes more sensitive.

The proposed toxicity grading scale for water allows for differentiating non-toxic (1-25%), low-toxicity, and

even very toxic (76-100%) samples that can lead to the death of people. Immobilisation data obtained in the study suggest that all samples exhibit toxicity at low doses. However, drinking water stored in Flathead Reservoir and water from surface sources obviously cannot have the same toxic effects. Therefore, the criterion choice shifted toward changes in Daphnia's heart rate. The responses of Daphnia were then compared.

The rate of immobilisation is considered a standard toxicity test. Despite its simplicity (this test involves counting the number of active and immobile daphnids), the heart rate of the organism has more advantages as a toxicity measure. In particular, immobilisation-based testing is associated with a long exposure period (48 hours), within which the state of animals is registered at a time interval of every 6 hours. Such procedure limitations make it difficult to quickly obtain information. With heart beat frequency as a criterion, it is not the case, as the heart rate can be measured after 3 hours of exposure. Even though the measurement of Daphnia heart rate is somewhat more complicated and requires more time to examine each individual (0.5 min per test object), it is fully compensated for by the substantially shorter duration of the investigation and higher sensitivity.

The literature shows that Daphnia heart rate can be successfully used as an evaluation criterion to detect water contamination with pesticides (Goncharuk et al., 2005; Scott and Sloman, 2004). The present study process is also suitable for determining water toxicity. The respiratory function in lower crustaceans is closely related to cardiac contraction. Thus, a substantial impact on the respiratory system will cause an alteration in the heart rate. The response to pollutants can also change over time. Hence, the cardiac activity of Daphnia should be different depending on whether the harmful substances are present or not in the water. If this difference is more than 50%, the level of water pollution should be considered significant.

As almost all toxicants are biologically active substances, their presence in water is accompanied by oxidation and, consequently, a gradual decrease in dissolved oxygen. In these circumstances, lower crustaceans are forced to activate physiological mechanisms to compensate and their heart rate increases as a result (Krainyukova, 2009; Scott and Sloman, 2004). Therefore, the respiratory and cardiovascular systems can be considered one of the most sensitive components of the body in Daphnia — they are the first to respond to pollutants in the water. When it comes to rapid toxicity testing, measuring the heartbeat rate will give more informative results than the generally accepted technique concerning lethality (immobilisation test). This criterion is of particular importance when the testing is done on drinking water supplies that undergo specific treatment (such as chlorination).

Water samples from reservoirs on the South Fork of the Flathead River and the Flathead Reservoir, tested using the heart rate of Daphnia as a toxicity endpoint, were found to exhibit low toxicity. Those from the Clear Water Reservoir (the smaller one) were non-toxic, with a toxicity index of 24.1%. Overall, the toxicity values of samples obtained with Daphnia heart rate as an experimental endpoint were 24.1 to 35.4% higher than those obtained using immobilisation.

Conclusions

More and more chemical and biological wastes are introduced into surface waters. Therefore, it is vital to monitor the water quality. Over the past years, biological methods for water quality assessment have been developed. Such methods are increasingly incorporated into technological solutions, i.e., the use of biosensors or biologically active deposits in water purification. The advantage of biological methods is that results show how a living organism would react to pollution. The response of living organisms to pollution is manifested through physiological, morphological and behavioural changes. Research based on the use of biological methods is often less expensive and time-consuming because some of the sample preparation steps can be skipped. Another advantage of such methods is that the analysis can be conducted both in a laboratory and in the natural habitat of the organisms used for testing. Bioassays can also be used to supplement standard analyses. Individual tests vary in sensitivity to various compounds; therefore, it is recommended to use multiple tests to obtain more reliable results.

Based on the results from this study, it can be concluded that the heart rate of Daphnia has a more suitable toxicity endpoint for assessing potential water quality hazards than Daphnia mortality. Due to higher sensitivity to low toxicity dose, relying on this measure for toxicity testing was 28.33% more informative and took 73.11% less investigation time.

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Conflict of interests

The authors declare that they have no conflict of interests.

Data Availability

Data will be available on request.

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