

Targeted Selecting of Bioassay Methods as an Alternative to the “Battery of Bioassays”

Anna Olkova^{1*}

¹ Vyatka State University, 36 Moskovskaya St, Kirov, 610000, Russian Federation

E-mail: morgan-abend@mail.ru

ABSTRACT

The article describes an algorithm for selecting a targeted bioassay method. It was proposed to consider the most sensitive method to a certain contamination as the targeted method of bioassay. The selection was made from a “battery of bioassays”, which necessarily includes the *D. magna* mortality test. The rest of the methods for comparing sensitivity were selected situationally. The algorithm was tested on model samples. It was found that mortality tests for *D. magna* and *C. affinis* are the most sensitive to contamination with mineral nitrogen compounds. *E. coli* bioluminescence reduction test is preferred when there is contamination with Cu, phosphates and pyrophosphates. It was shown that the test to reduce the chemotactic reaction of *P. caudatum* should be used when the aquatic environment is polluted with Cd, Pb, Zn, oil products, organic herbicides imazethapyr and imazamox. The proposed algorithm is universal, but it should be applied when the priority pollutant is known, the effects of which prevail over the action of other compounds in the sample.

Keywords: bioassay, bioassay methodology, sensitivity of the bioassay method, targeted bioassay, priority pollution, environmental monitoring.

Introduction

Modern features of anthropogenesis include complex environmental pollution, pollution with xenobiotics – substances completely alien to living organisms, a combination of negative effects of physical and chemical factors, globality of pollution by many substances and so on. Therefore, bio-diagnostics of environmental quality every year becomes an increasingly relevant topic that is important from the environmental and social point of view.

Bioassay is one of the methods of biodiagnostics of the quality of the environmental component based on responses of laboratory test organisms. In this case, the accuracy of the conclusion about the toxicity of the sample is based on the long-term keeping test organisms under controlled conditions and adherence to the test protocol. Although extrapolating the measured responses to relevant protection goals remains challenging,

the combination of ecotoxicological experiments and models is key for a more comprehensive assessment of the effects of chemical stressors to aquatic ecosystems (Schuijt et al., 2021).

Currently, there is a possibility of choosing those bioassay methods that are most suitable for a specific research goal. For example, *Drosophila* is traditionally considered a convenient model organism for genetic research, protocols for testing new drugs are not complete without white mice, and it is customary to test herbicides primarily on target species of weeds (Gupta, 2016).

In the field of studying the toxicity of natural components such as surface water, bottom deposits, soil, air it is difficult to identify an obligatory test organism for using. Therefore, the principle of a “battery of bioassays” was formed, which includes several bioassay methods using different organisms. Ideally, the battery of bioassays should be simple, inexpensive, and multitrophic as well as have wide-spectrum response to toxic substances, in addition to yielding fast delivery

results (Castillo, Schafer, 2000). The work (Pandey et al., 2019) proposes multibioassay testing based on six different organisms and different reactions of these organisms.

For a long time it was believed that the “battery of bioassays” is the only correct and environmentally friendly approach to determine the toxicological load on biota. However, later critical works appeared in this area. For example, it has rightly been noted that animal toxicology studies generally fail to provide the required performance in the effective way (Morisseau et al., 2009).

There is also a problem associated with the implementation of environmental monitoring programs, when the “battery of bioassays” is used in a long-term mode. In this case, after several observation cycles it becomes clear which test organisms and their test functions are most sensitive to the prevailing contamination, but for the reasons related to legislation and other bureaucratic aspects, the performers are forced to further use the entire (complete) list of bioassay methods included in the monitoring program. For example, in France a test strategy has been developed to assess the ecotoxicological properties of waste using a battery of six standardized biological analyses since 1998. By 2006, multivariate analyses clearly showed that it was possible to reduce this number of tests without changing the typology of the wastes (Pandard et al., 2006).

Therefore, the introduction of the approach consisting in choosing the most sensitive bioassay will be very effective for obtaining objective data and it will also contribute to saving material and labor costs. This method can be called a targeted bioassay which has a proven high sensitivity to the main pollution in the study area. It should be noted that this approach can only be used if the priority pollutant is known, the effects of which prevail over the effects of other possible toxicants.

Despite the aforementioned pollution of the environment with a complex of substances and the formation and action of their contaminants, the territories characterized by known priority pollution are also widespread. For example, many agricultural lands are constantly exposed to herbicides or insecticides. This creates the need to search for a targeted bioassay and its use for effective agro-ecological monitoring. Thus, from the “battery” of eight plant species, Canola was identified as a suitable plant for the bioassay of atrazine (Ramezanpoor et al., 2021). The work (Chapman et al., 2012) describes the assessment of several bioassay methods for their suitability for screening the risk associated with soil contamination with zinc. An interesting method of high throughput screening (HTS) using nine enzyme-based bioassays and five receptor-based bioassays, which allows simultaneously identifying potentially hazardous chemical compounds and determining the most sensitive biomarker observed in bioassay was described in (Morisseau et al., 2009).

Complex studies aimed at determining targeted bioassays for various substances using a single algorithm are still not enough. In this regard, the purpose of this work was to develop an algorithm for selecting targeted bioassay methods for various toxicants and to test it under laboratory and field conditions.

MATERIAL AND METHODS

Bioassay methods selected for sensitivity comparison

Four methods were included in the “battery of bioassays”, the components of which were assessed for sensitivity (Table 1).

The representation battery contains two classical bioassays for acute toxicity by entomostracans mortality and two methods for determining acute toxicity by sublethal reactions, which are characterized by

Table 1. Characteristics of the assessed bioassays

Test organism	Test function	Exposure	Instrument	Guidance document
<i>Daphnia magna</i> Straus (1820)	Mortality	96 hours	Not needed (visual diagnostics)	Federal Register 1.39.2007.03222 (2007)
<i>Ceriodaphnia affinis</i> Lilljeborg (1900)	Mortality	48 hours	Not needed (visual diagnostics)	Federal Register 1.39.2007.03221 (2007)
<i>Paramecium caudatum</i> Ehrenberg (1838)	Chemotactic reaction	30 minutes	«Biotester» (Russia)	Federal Register 1.39.2015.19242 (2015)
<i>Escherichia coli</i> Migula (1895), strain M17	Bioluminescence	30 minutes	«Biotoks -10M» (Russia)	Environmental Regulatory Document PND F T 14.1:2:3:4.11-04. T.16.1:2:3:3.8-04. (2010)

expressiveness. The selected entomostracans are of different biological genera and have different sensitivities to water saprobity; therefore, their use in a single complex can be considered justified.

The method of using *P. caudatum* is based on the negative chemotaxis of microorganisms in the reverse direction of the hazardous chemical substance. As indicated in the table, determination of chemotactic response reactions of *P. caudatum* is carried out using the “Biotester” instrument, which is a kind of the spectral concentrator. The optical signal passes through the upper part of the cells and proceeds to the receiving device, where the conversion into the electrical signal, which is proportional to the quantity of ciliates in the zone of measurement, takes place (Fig. 1). The calculation of the toxicity index was carried

out in relation to the control, which was the chemotactic reaction of organisms in pure water.

Bioassay with the use of *E. coli* refers to the bioluminescent method to determine the toxicity of the samples. The quantitative change of luminescence of bacterial samples was carried out in the luminometer, and then the indicator was compared with the control data.

The tests were conducted in the scientific-research laboratories of the Vyatka State University, licensed by the Russian Federal Service for Accreditation.

Substances for model experiments

Substances for comparing sensitivities of four bioassays can be divided into mineral and organic (Table 2). The criterion for their selection

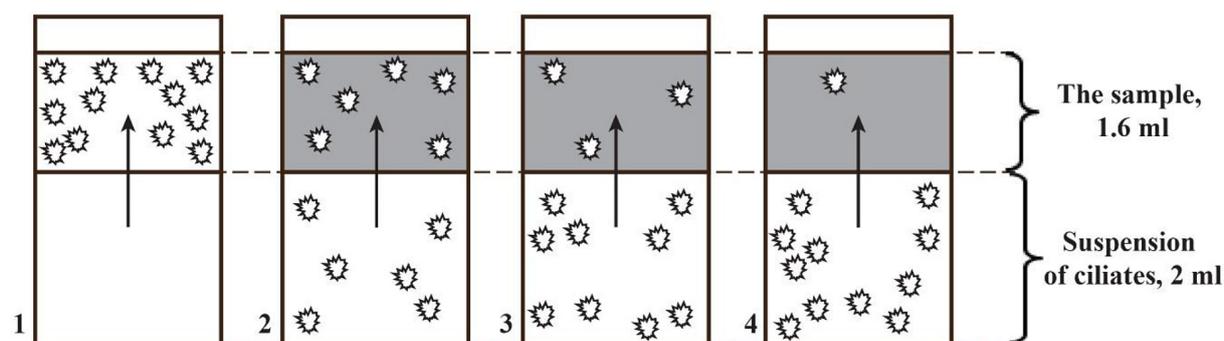


Figure 1. Scheme of bioassay for chemotactic reactions of ciliates:

1 – non-hazardous sample; 2, 3 – moderately toxic sample; 4 – toxic sample

Table 2. The list of the studied substances and their concentrations that were used when determining the targeted bioassay

Substance group	Substance	Toxic element / ion	Range of tested concentrations, mg/l	Compliance with the standard (RF) for fishery reservoirs
Heavy metals (HM)	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Cu^{2+}	0.001-0.1 (Cu^{2+})	1-10 (Cu^{2+})
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	Zn^{2+}	0.01-0.1 (Zn^{2+})	1-10 (Zn^{2+})
	$\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$	Pb^{2+}	0.006-0.06 (Pb^{2+})	1-10 (Pb^{2+})
	$\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$	Cd^{2+}	0.05-0.05 (Cd^{2+})	1-10 (Cd^{2+})
Mineral forms of nitrogen	NaNO_3	NO_3^-	200-4000	5-100
	NaNO_2	NO_2^-	0.4-8.0	5-100
	NH_4Cl	NH_4^+	0.5-50	1-100
	$\text{NaNO}_3 + \text{NH}_4\text{Cl}$	$\text{NO}_3^- + \text{NH}_4^+$	40-400 (NO_3^-) + 0.5-5.0 (NH_4^+)	1-10
	$\text{NaNO}_2 + \text{NH}_4\text{Cl}$	$\text{NO}_2^- + \text{NH}_4^+$	0,08-0,8 (NO_2^-) + 0,5-5,0 (NH_4^+)	1-10
Mineral forms of phosphorus	$\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$	PO_4^{3-}	20-400	100-2000
	$\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$	$\text{P}_2\text{O}_7^{4-}$	13-550	3.7-157
Herbicides	Imazethapyr	$\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_3$	0.01-3.0	1-300
	Imazamox	$\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_4$	0.01-3.0	1-300
Oil products	Petrol	Hydrocarbons C_8H_{15}	10-100	-

Note: * – the addition was made into the soil, the calculation was 1 kg for the air-dry mass of the soil; «-» – the standard is not found.

Table 3. Results of analyses of natural water for experiments

N	Indicator	Units of measurement	Result of measurement	Acceptable level
1	pH	PH units	7.4±0.2	6.5-8.5
2	Nitrates	mg/l	10.2±2.0	Not more than 20
3	Nitrites	mg/l	Less than 0.016	Not more than 0.5
4	Ammonium ions	mg/l	Less than 0.05	Not more than 0.1
5	Phosphates	mg/l	Less than 0.05	Not more than 3.5
6	Total water hardness	mg/eq-l	2.43±0.36	Not more than 7.0
7	Oil products (in total)	mg/l	Less than 0.005	Not more than 0.05
8	Organic carbon	mg/l	Less than 1.2	Not more than 10

is frequent presence of anthropogenic objects in emissions and, as a consequence, an increase in the degree of contamination of certain substances. The range of tested concentrations for all studied toxicants was different. It was identified in preliminary tests on *D. magna*. Further doses for other organisms could be adjusted if no exposure was observed or it was lethal over the entire concentration range.

The substances were introduced into natural drinking-quality water; its source was an artesian well. The water without additives served as a control in all bioassays. The content of basic cations and anions and some pollutants was preliminarily determined in the water. The content of all natural compounds was within acceptable levels, the content of pesticides and their degradation products, as well as HMs, was less than the detection limit of the methods used. Some physicochemical characteristics of this water are presented in Table 3.

Statistical analysis and reliability of results

The tests were carried out under the same laboratory conditions, using the same water to simulate contamination.

The experiments were carried out in three-four replicates. The initial data obtained were processed by standard mathematical methods. The presence of significant differences between the results of bioassays was determined by the Student's test, $p < 0.05$.

RESULTS

Algorithm of selecting the most sensitive bioassay method

The proposed algorithm is based on ranging the sensitivity of several bioassay methods to a priority toxicant, which creates the main factor

of environmental toxicity in the area of environmental monitoring. The main toxicity factor is understood as the presence in the test medium of a substance, the effects of which prevail over the action of other substances in the sample. Preliminary testing of bioassay methods for sensitivity to priority contamination will allow further monitoring work to use the targeted bioassay method, not the whole “battery of bioassays”.

The mortality bioassay for *D. magna* is proposed to be used as a basic, that is, the first and obligatory element of the “battery of bioassays”, since:

- it is one of the most widely used bioassay methods in the world, its variations are described in international and national protocols for the determination of the toxicity of samples (EPS 1/RM/11, 1996; EPA 821/R-02/012, 2001; Federal Register 1.39.2007.03222, 2007; ISO 6341: 2012, 2018);
- reactions of *D. magna* can be used to determine both subacute and acute and chronic effects (if necessary);
- *D. magna* possesses a successful combination of biological features that are important for the bioassay process: clonal reproduction, the presence of most organs and their systems characteristic of highly organized organisms, a relatively short life cycle, etc.

The algorithm for selecting a targeted bioassay in the case of a known priority contamination is as follows:

1. First of all, it is necessary to establish the non-lethal and lethal doses of the test substance for the basic test organism *D. magna* according to the international (ISO 6341: 2012, 2018) or national protocol for determining mortality (Federal Register 1.39.2007.03222, 2007). Determination of the moderately lethal concentration of the toxicant is not necessary, if this is not the task of the study. This approach significantly reduces the amount of work.

The guidelines for the selection of test concentrations can be:

- national regulations on the harmful effects of substances, if they have been developed;
- the results of published scientific works, if the toxicant was previously subject to study;
- effective doses of homologous substances (for organic compounds) or substances closest in genesis (for inorganic), if the toxicant is not studied.

The planned doses for testing should be introduced into natural water typical for the ecological monitoring area. Distilled water is not recommended. This will make it possible to obtain the data that are adequate to the natural chemical background of natural waters in the studied area.

2. At the second stage, it is necessary to select other bioassay methods available for further use in the process of environmental monitoring. The criteria for the formation of the initial “battery of bioassays” are:

- use of test organisms of different trophic groups, which is recommended by the Directive in the field of water policy (Directive 2000/60 / EC..., 2000);
- inclusion of express methods in the “battery of bioassays”;
- use of bioassays to determine chronic effects (if necessary).

3. Lethal and non-lethal doses of the priority toxicant established at the first stage for *D. magna* should be tested using the rest of the selected bioassays. If necessary, the effects of additional doses of substances are assessed:

- if the test organisms exhibit low or excessively high sensitivity to the tested concentrations of the toxicant;
- if it is necessary to differentiate the sensitivity of bioassays with the help of which similar results were obtained.

The control environment used for the simulation remains the same, it is the natural water initially selected.

4. Establishment of additional effects of the priority toxicant, for example, chronic and delayed effects, mutagenic effects, and others. This procedure is performed if it is a part of the research objectives or the priority pollutant can potentially have a specific effect.

5. Comparison of the results obtained and their distribution in order of increasing sensitivity to the priority pollutant.

The study scheme with an indication of the used test organisms is shown in Figure 2.

Thus, the experimental parts of the study and the analytical work were carried out. Further, the series of sensitivity of bioassays were built and the information was generalized.

Sensitivity analysis of various bioassay methods to mineral and organic toxicants

The intermediate results of bioassays for each sample are not presented here, since the tasks of the presented work included the description of the algorithm for the targeted selection of bioassay

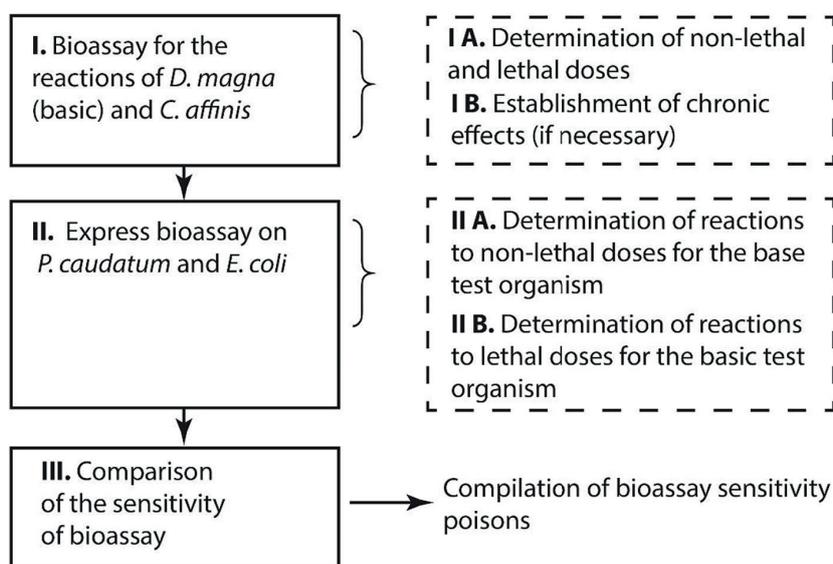


Figure 2. Block diagram of the algorithm for selecting the targeted bioassay

Table 4. Comparison of the sensitivity of bioassays to mineral and organic toxicants

Toxicant			Bioassays			
			Mortality of <i>D. magna</i> , %	Mortality of <i>C. affinis</i> , %	Bioluminescence of <i>E. coli</i>	Chemotaxis of <i>P. caudatum</i>
Minerals	Cu	Heavy metals	+	++	++++	+++
	Cd		+++	++	+	++++
	Pb		++	+++	+	++++
	Zn		+++	++	+	++++
	NO ₃ ⁻	Mineral forms of nitrogen	+++	++++	+	++
	NO ₂ ⁻		+++	++++	+	++
	NH ₄ ⁺		+++	++++	++	+
	NO ₃ ⁻ + NH ₄ ⁺		+++	++++	+	++
	NO ₂ ⁻ + NH ₄ ⁺	+++	++++	++	+	
(P _x O _y) ^{z-}	Phosphates and pyrophosphates	+	++	++++	+++	
Organic matter	Imazethapyr	Herbicides	+	++	+++	++++
	Imazamox		+	++	+++	++++
	Oil products		+	++	+++	++++

Note: the sensitivity level of bioassays is shown by signs and colors: “+” – minimum, “++” – medium, “+++” – “high”, “++++” – maximum sensitivity.

and the analytical generalization of many series of experiments. Most of the results of individual experiments have been published. A comparison of the sensitivity of bioassays to mineral forms of nitrogen is reflected in the article (Olkova, Machanova, 2018), to mineral forms of phosphorus – in the article (Kondakova et al., 2014), to petroleum products – in the article (Olkova et al., 2017), to herbicides in the article (Olkova, Berezin, 2018), to heavy metals in (Olkova, 2020).

The result of the experimental and analytical parts of these works was information-analytical table 4, which clearly shows the methods that are not sensitive to a certain contamination, and bioassays that signal contamination at the lowest doses. In Table 4, the sensitivity of bioassays is ranked into 4 categories according to the number of compared methods. With an increase in the “battery of bioassays” the number of sensitivity levels will also increase.

The results summarized in Table 4 show that depending on the nature of the contamination, the bioassay methods can be both the most sensitive and not sensitive to the impact. This also refers to the effects of toxicants belonging to a group of substances with a similar mechanism of toxic action. Thus, the bioassay of *E. coli* bioluminescence was characterized by the minimum sensitivity to Cd, Pb, Zn, but at the same time it showed the maximum sensitivity to Cu in comparison with other methods.

It is also interesting that systematically close species of *D. magna* and *C. affinis* differ in their sensitivity to the tested toxicants. According to the results summarized in this work, the mortality bioassay of *C. affinis* is more often preferable than the use of *D. magna*. However, the bioassay of mortality *D. magna* reflects better the exposure to Cd and Zn than the method using of *C. affinis*.

These data once again emphasize the need to implement the principle of “battery of bioassays” at the stage of preliminary tests and the possibility of further transition to the use of a targeted bioassay method.

The data presented in Table 4 can serve as a reference material when planning environmental studies in areas exposed to the action of these substances.

DISCUSSION

Of course, any tested environment is a multicomponent system, in which it is impossible to exclude the contamination of many natural substances and compounds of anthropogenic origin (Altenburger et al., 2018). At the same time, many industrial enterprises have their own specific pollutants, which are their markers of the chemical trace in the environment (Blays, Rosen, Small, 2015). The hypothesis of this work was that for many territories experiencing anthropogenic

pressure it is possible to single out, firstly, a priority pollutant and, secondly, among the methods that make up the “battery of bioassays” it is possible to determine the analysis characterized by a high sensitivity to this pollution.

The use of a “battery of bioassays” is a well-known approach in bioassaying (Wieczerek, Namiesnik and Kudlak, 2016). The battery of bioassays is often chosen so that it can identify the risks associated with a wide range of chemical pollutants and their transformation products, while simultaneously it allows targeted identification of groups of compounds that may cause specific effects (De Baat et al., 2019). This study supports the idea of the need to determine the targeted bioassay.

“Effect-directed analysis” (EDA) aimed at fractionating a sample and identifying the specific effects of each fraction is also gaining popularity (Brennan et al., 2020). Currently, the approaches of EDA and the “battery of bioassays” are contrasted, classical bioassay methods are called “non-targeted” (Oberleitner et al., 2020). However, in the presented work it is shown that it is possible to make the transition from a “battery of bioassays” to the use of one of the most sensitive bioassay methods. This method of bioassaying will be the targeted method of laboratory biodiagnostics aimed at monitoring the situation for a long time. The importance of such developments is reported in (van den Berg et al., 2021): risk assessment can benefit most from modeling approaches when sensitivity is described based on ecologically relevant and robust effects.

Selecting the targeted bioassay cannot be based solely on theory or data for closely related species. For example, according to the principles of general toxicology (Gupta, 2016) and aquatic toxicology (Nikinmaa, 2014), it could be assumed that unicellular organisms will always be more sensitive than multicellular organisms, and further the resistance of organisms will increase as organisms become more complex. However, the discussed results show that more highly organized species may be more sensitive than unicellular organisms. Thus, *C. affinis* and *D. magna* exhibited maximum and high sensitivity to water pollution by mineral forms of nitrogen, and unicellular *P. caudatum* and *E. coli* showed medium and minimum responses in a comparative relation. Such an inversion of the general toxicological pattern is not uncommon. In the study of the detoxification of organophosphate and N-methylcarbamate

pesticides it was shown that rat liver (in vitro) has a greater detoxification potential than the samples of human liver cells (Animal models in toxicology, 2016). The crustaceans *D. magna* were found to be more sensitive than the green alga *Chlorella vulgaris* in an experiment to assess the acute toxicity of ionic liquids based on 1-alkyl-3-methylimidazolium nitrate (Zhang et al., 2017). It should be noted that in the case of diagnostics of pollution with nitrates, nitrites and ammonium ions, the value of crustaceans increases even more if we take into account that mortality was assessed for them, and pre-lethal reactions were taken into account in unicellular organisms.

In the presented work, the choice of the targeted bioassay is proposed to be carried out according to the algorithm based on the determination of toxicant concentrations, which are lethal and non-lethal for the basic test organism *D. magna*. Further, the “battery of bioassays” should be increased in accordance with the capabilities and objectives of the study. On the basis of the results of preliminary laboratory tests, a series was found that reflects an increase in the sensitivity of bioassays to a priority pollutant. The stage of confirming the high sensitivity of the targeted bioassay in comparison with other methods when testing real samples is also obligatory.

The proposed approach to selecting a targeted bioassay has an important limitation: the study area should be characterized by the presence of a priority pollutant, the effects of which are much greater than the effect of other toxicants on living organisms.

CONCLUSIONS

Thus, as a result of a series of experiments, the effectiveness of the targeted selection of bioassays was substantiated using a universal algorithm to determine the most sensitive and preferred methods for bioassay of natural and anthropogenic environments contaminated with mineral and organic toxicants. The algorithm is aimed at a clear sequence of actions to determine the sensitivity of bioassays to the toxic effects of pollutants.

The algorithm was tested on model and natural environments contaminated with mineral compounds of nitrogen and phosphorus, salts of heavy metals (Cu, Zn, Pb, Cd), oil products (gasoline), herbicides imazethapyr and imazamox. The series

of sensitivity of bioassays based on the reactions of *D. magna*, *C. affinis*, *P. caudatum* and *E. coli* were experimentally determined. As a result, it was found that the mortality tests for *D. magna* and *C. affinis* are most sensitive to contamination with mineral nitrogen compounds. It was shown that the *E. coli* bioluminescence reduction test is preferable when contamination with Cu mineral salts, phosphates and pyrophosphates occurs. If the aquatic environment is polluted with mineral salts of Cd, Pb, Zn, oil products, organic herbicides imazethapyr and imazamox, a test to reduce the chemotactic reaction of *P. caudatum* should be used.

For the convenience of using the algorithm for selecting bioassays the table which can serve as a reference material when planning environmental studies in the areas exposed to the action of tested substances was compiled.

The proposed bioassay strategy, including a preliminary targeted selection of bioassays, is intended to be implemented immediately before large-scale and/or long-term environmental studies, including when performing the biodiagnostic part of environmental monitoring programs. The procedure allows choosing the most sensitive bioassay methods for the most common and hazardous pollutants in the study area and focusing on them when interpreting the results.

REFERENCES

1. Altenburger R., Scholze M., Busch W., Escher B., Jakobs G., Krauss M., Krueger J., Neil P., Ait-Aissa S., Almeida A.C., Seiler T.B., Brion F., Hilscherova K., Hollert H., Novak J., Schlichting R., Serra H., Shao Y., Tyndall A., Tolefsen K.E., Umbuzeiro G., Williams T.D., Kortenkamp A. 2018. Mixture effects in samples of multiple contaminants – An interlaboratory study with manifold bioassays. *Environment International*, 114, 95–106. DOI: 10.1016/j.envint.2018.02.013
2. Animal models in toxicology. Ed. S.C. Gad. Boca Raton, FL: CRC Press, 2016, 1152.
3. Blais J.M., Rosen M.R., Smol J.P. 2015. Environmental contaminants: using natural archives to track sources and long-term trends of pollution. *Book Series Developments in Paleoenvironmental Research*. Netherlands: Springer, 18, 509. DOI: 10.1007/978-94-017-9541-8
4. Brennan J.C., Gale R.W., Alvarez D.A., Berninger J.P., Leet J.K., Li Y., Wagner T., Tillitt D.E. 2020. Factors Affecting Sampling Strategies for Design of an Effects-Directed Analysis for Endocrine-Active Chemicals. *Environmental toxicology and chemistry*, 39(7), 1309–1324. DOI: 10.1002/etc.473
5. Castillo G., Schafer L. 2000. Evaluation of a bioassay battery for water toxicity testing: A Chilean experience. *Environmental toxicology*, 15(4), 331–337. DOI:10.1002/1522-7278(2000)15:4<331::AID-TOX9>3.0.CO;2-E
6. Chapman E.E.V., Hemer S.H., Dave G., Murimboh J.D. 2012. Utility of bioassays (lettuce, red clover, red fescue, Microtox, MetSTICK, Hyalella, bait lamina) in ecological risk screening of acid metal (Zn) contaminated soil. *Ecotoxicology and environmental safety*, 80, 161–171. DOI: 10.1016/j.ecoenv.2012.02.025
7. De Baat M.L., Kraak M.H.S., Van der Oost R., De Voogt P., Verdonschot P.F.M. 2019. Effect-based nationwide surface water quality assessment to identify ecotoxicological risks. *Water research*, 159, 434–443. DOI: 10.1016/j.watres.2019.05.040
8. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy – EU Water Framework Directive (as amended on October 20, 2014). 2000. [Internet resource] <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32000L0060> (Accessed: 6.12.2021).
9. Environmental Regulatory Document PND F T 14.1:2:3:4.11-04. T.16.1:2:3:3.8-04. 2010. Method for determining the integrated toxicity of surface waters, including marine, ground, drinking, waste waters, water extracts from soils, waste, sewage sludge by changes in bacterial bioluminescence using the Ecolum test-system. Moscow: Nera-S, 30. (in Russian).
10. EPA 821/R-02/012. 2001. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5th ed. US Environmental Protection Agency (USEPA). Washington DC, USA.
11. EPS 1/RM/11. 1996. Biological test method: Acute lethality test using *Daphnia* spp. Environment Canada.
12. Federal Register 1.39.2007.03221. 2007. Methodology for determining the toxicity of water and water extracts from soils, sewage sludge, and waste by mortality and changes in fertility of *Ceriodaphnias*. Moscow: Akvaros, 54. (in Russian)
13. Federal Register 1.39.2007.03222. 2007. Biological control methods. Method for determining the toxicity of water and water extracts from soils, sewage sludge, waste by mortality and changes in fertility of daphnia. [Internet resource] <https://meganorm.ru/Index2/1/4293842/4293842234.htm> (Accessed: 6.12.2021). (in Russian)
14. Federal Register 1.39.2015.19242. (2015). Environmental Regulatory Document PND F T 16.2:2.2-98. Methodology for determining the toxicity of samples of natural, drinking, domestic and drinking, household

- waste, treated sewage, waste, thawed, technological water by the express method using the Biotester device. St. Petersburg: SPEKTR-M, 21 p.
15. Gupta PK. 2016. Fundamentals of Toxicology: Essential Concepts and Applications. London, England: Academic press LTD-Elsevier science, 398.
 16. ISO 6341:2012. 2018. Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test. <https://www.iso.org/standard/54614.html>
 17. Kondakova L.V., Domracheva L.I., Ogorodnikova S.Y., Olkova A.S., Kudryashov N.A., Ashikhmina T.Y. 2014. Bioindication and bioassay reactions of organisms to the action of methylphosphonates and sodium pyrophosphate. Theoretical and Applied Ecology, 4, 63–69.
 18. Morisseau C., Merzlikin O., Lin A., He G.C., Feng W., Padilla I., Denison M.S., Pessah I.N., Hammock B.D. 2009. Toxicology in the Fast Lane: Application of High-Throughput Bioassays to Detect Modulation of Key Enzymes and Receptors. Environmental health perspectives, 117(12), 1867–1872. DOI: 10.1289/ehp.0900834
 19. Nikinmaa M. 2014. An introduction to aquatic toxicology. London: Academic press, 252.
 20. Oberleitner D., Stutz L., Schulz W.G., Bergmann A., Achten C. 2020. Seasonal performance assessment of four riverbank filtration sites by combined non-target and effect-directed analysis. Chemosphere, 261, 127706. DOI: 10.1016/j.chemosphere.2020.127706
 21. Olkova A.S., Zimonina N.M., Lyalina E.I., Bobretsova V.R. 2017. Diagnostics of local pollution of urbanozem in the areas of petrol stations. Theoretical and Applied Ecology, 1, 56–62.
 22. Olkova A.S. 2020. Development of a strategy for bioassay of aquatic environments taking into account the multifactority of response reactions of test organisms. Dissertation of Doctor of Biological Sciences, Vladimir, 358.
 23. Olkova A.S., Berezin G.I. 2019. Study on the sensitivity of certified bioassays to water pollution with modern herbicides: model experiments. Water and ecology: problems and solutions, 24, 2(78), 111–119. DOI: 10.23968/2305-3488.2019.24.2.111-119
 24. Olkova A.S., Machanova E.V. 2018. Selection of bioassay for ecological research of water, polluted by mineral nitrogen forms. Water and ecology: problems and solutions, 4(76), 70–81. DOI: 10.23968/2305-3488.2018.23.4.70-81
 25. Pandard P., Devillers J., Charissou A.M., Poulsen V., Jourdain M.J., Ferard J.F., Grand C., Bispo A. 2006. Selecting a battery of bioassays for ecotoxicological characterization of wastes. Science of the total environment, 363(1–3), 114–125. DOI: 10.1016/j.scitotenv.2005.12.016
 26. Pandey L.K., Lavoie I., Morin S., Depuydt S., Lyu J., Lee H., Jung J., Yeom D.H., Han T., Park J. 2019. Towards a multi-bioassay-based index for toxicity assessment of fluvial waters. Environmental monitoring and assessment, 191(2), 112. DOI: 10.1007/s10661-019-7234-5
 27. Ramezanpoor M., Salehian H., Babanezhad E., Rezvani M. 2021. The Leaching of Atrazine and Plant Species Sensitivity to Atrazine using Bioassays and Chemical Analyses. Soil & Sediment contamination. DOI:10.1080/15320383.2021.1963667
 28. Schuijt L.M., Peng F.J., van den Berg S.J.P., Dingemans M.M.L., Van den Brink P.J. 2021. (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. Science of the total environment, 795, 148776. DOI: 10.1016/j.scitotenv.2021.148776
 29. van den Berg S.J.P., Maltby L., Sinclair T., Liang R.Y., van den Brink P.J. 2021. Cross-species extrapolation of chemical sensitivity. Science of the total environment, 753, 141800. DOI: 10.1016/j.scitotenv.2020.141800
 30. Wiczerzak M., Namiesnik J., Kudlak B. 2016. Bioassays as one of the Green Chemistry tools for assessing environmental quality: A review. Environment international. 94, 341–361. DOI: 10.1016/j.envint.2016.05.017
 31. Zhang C., Zhang S., Zhu L.S., Wang J.H., Wang J., Zhou T. 2017. The acute toxic effects of 1-alkyl-3-methylimidazolium nitrate ionic liquids on *Chlorella vulgaris* and *Daphnia magna*. Environmental pollution, 229, 887–895. DOI: 10.1016/j.envpol.2017.07.055