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Article

The procedure for selecting bioassays for different types of pollution

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Annotation. The algorithm is suggested for selecting most appropriate and sensitive bioassay method to a particular type of pollution prevailing in the study area. The algorithm bases on a battery of bioassays, which necessarily includes mortality test for *Daphnia magna* Straus, 1820. Other approaches for comparing sensitivity of methods are selected for particular aim. The algorithm has been tested in model and for in situ samples. According to the proposed algorithm, the mortality tests for *D. magna* and *Ceriodaphnia affinis* Lilljeborg, 1900 are enough informative to assess the pollution by mineral nitrogen compounds. When prevailing contamination are mineral salts of Cu, phosphates, and pyrophosphates, a bioluminescent test with *Escherichia coli* Migula, 1895 has the maximum sensitivity. If the aquatic environment is polluted by Cd, Pb, Zn, oil products, organic herbicides imazetapir and imazamox, chemotactic response reduction test with *Paramecium caudatum* Ehrenberg, 1838 is the most sensitive. The proposed algorithm is general, but it should only be used when the prevailing contaminant is known, and its effects predominate over the effects of other compounds in the sample.

Keywords: bioassay methodology, selection of bioassays, test organism, pollution sensitivity, prevailing pollution, environmental monitoring

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Introduction

Modern features of anthropogenesis include complex and global pollution of the environment, the spread of xenobiotics (substances that are completely alien to living organisms), a combination of the negative effects of physical and chemical factors, etc. Therefore, bioassays of environmental quality, important both for nature and humankind, are becoming more and more relevant every year.

Bioassay is one of the ways to assess the effect a particular component based on the responses of laboratory test organisms. Obtaining reliable conclusion about the toxicity of a sample requires long-term maintenance of test organisms under controlled conditions and compliance with the test protocol. Extrapolation of the results of model tests to the processes occurring in real ecosystems remains a difficult but necessary task for evaluating the impact of a chemical stressor on aquatic and terrestrial ecosystems (Schuijt et al., 2021).

At present, it is possible to select the bioassay methods most suitable for a particular purpose. For example, *Drosophila* traditionally remains a convenient model organism for genetic studies, protocols for testing new drugs cannot avoid mice as the test object, and testing of herbicides is usually carried out primarily on target weed species (Gupta, 2016).

When studying toxicity of natural media (surface waters, bottom sediments, soil, air), it is difficult to use one test organism to obtain reliable results. Therefore, the principle of a battery of bioassays has been developed, which includes several methods of bioanalysis using different organisms. Ideally, a bioassay series should be simple, inexpensive, and multitrophic, have a wide range of responses to toxic substances, and provide fast results (Castillo and Schafer, 2000). For example, L.K. Pandey et al. (2019) propose a bioassay based on six different organisms and their different responses.

For a long time, it was believed that the battery of bioassays was the only correct and environmentally friendly approach to determine the toxicological load on biota. However, this approach has received some criticism later. In particular, they referred to the fact that toxicological studies on animals could not provide the necessary performance in a cost-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 multi-year mode. In this case, after several cycles of observations, it becomes clear which test organism(s) and it (their) test functions are most sensitive to prevailing contamination. However, because of legislation and other bureaucratic aspects, users are forced to apply the entire list of bioassay methods included in the monitoring program. For example, in France, since 1998, a testing strategy has been developed to assess the ecotoxicological properties of waste using a battery of six standardized biological assays. By 2006, multivariate statistical analyzes clearly showed that the number of tests could be reduced to three to obtain the same results. Russian legislation recommends using at least two test organisms of different trophic levels to determine the toxicity of wastes[1] and 2–3 test organisms for monitoring of the state and pollution of land surface waters[2]. There is also a positive practice of increasing the number of bioassays when approving programs for environmental control and monitoring of hazardous and especially hazardous industrial facilities. In this case, the set of bioassays usually includes “basic” and “additional” methods[3].

The introduction of approach that helps choosing the most sensitive bioassay will be very effective in obtaining objective data and in saving material and labor costs. The desired method can be called a target bioassay, characterized by a proven high sensitivity to the main pollution in the study area. This approach can be applied only if the priority pollutant is known, so its effects prevail over the effects of other possible toxicants.

Despite the widespread pollution of the environment by a great number of substances, as well as the formation and action of their contaminants, there are also common cases for which priority pollutant is known. For example, many agricultural lands are subject to constant exposure to herbicides or insecticides, which makes it necessary to find a targeted bioassay and use it for effective agroecological monitoring. In particular, rapeseed was identified as a suitable plant for atrazine bioassay when testing a series of eight plant species (Ramezanpoor et al., 2021). Several bioassay methods were tested for their evidence-based use for screening the risk associated with soil contamination with zinc (Chapman et al, 2012). High-throughput screening method (HTS) is of particular interest, it comprises nine enzyme-based bioassays and five receptor-based bioassays, which simultaneously detects potentially dangerous chemical compounds and determines the most sensitive biomarker observed in the bioassay (Morisseau et al., 2009).

Comprehensive studies aimed at determining target bioassays for various substances according to a single scheme are still not enough. In this regard, our study aims to develop the algorithm for selecting target bioassay methods for various toxicants and to test them in laboratory and in situ.

Materials and methods

Bioassay methods

We have tested four methods to assess the sensitivity of the battery of bioassays (Table 1).

The presented series includes two classical tests for assessing acute toxicity by mortality of crustaceans (Cladocerans) and two rapid methods for determining acute toxicity by sublethal responses. Two cladoceran species, *Daphnia magna* Straus, 1820 and *Ceriodaphnia affinis* Lilljeborg, 1900, belong to different biological genera and are distinguished by different

[1] Order of the Ministry of Natural Resources and Ecology of the Russian Federation dated December 4, 2014 No. 536 “On Approval of the Criteria for Assigning Waste to Hazard Classes I–V by Degree of Negative Impact on the Environment”.

[2] RD 52.24.309-2016. Organization and conduct of regime observations of the state and pollution of surface waters of land.

[3] RD 52.24.868-2017. Use of methods of biotesting of water and bottom sediments of streams and reservoirs.

sensitivity to the saprobity of water bodies, so their use in a single complex can be considered probatory.

Method using the ciliate *Paramecium caudatum* Ehrenberg, 1838 as a test object is based on its negative chemotaxis to a harmful chemical. Assessing of the chemotactic response of this ciliate has been carried out in the specially designed test equipment "Biotester" (Russia), which belongs to the spectral sensors for concentration measurement. The optical signal passes through the top of the cuvette and enters the receiving device, where it is converted into an electrical signal proportional to the number of ciliates in the measurement zone (Fig. 1). The calculation of the toxicity index is carried out in relation to the control, which is the chemotactic reaction of organisms in pure water.

Bioassay using bacteria *Escherichia coli* Migula, 1895 refers to bioluminescent methods for determining the toxicity level of a sample. A quantitative change in the luminescence of a bacterial suspension in a sample has been measured on a Biotox-10M luminometer (Russia), then the indicator was compared with control data.

The toxicity index (T) for two express methods was calculated as:

$$T = (X_c - X_t)/X_c,$$

where X_c is a quantitative expression of the response of test organisms to the control aqueous medium according to the method used, X_t is a quantitative expression of the response of test organisms to the test medium.

The calculation result is multiplied by 100% for the *P. caudatum* bioassay, for the *E. coli*, this procedure is not provided. Measurement units are set as conventional units (c.u.).

The study was performed in the research laboratory of the Vyatka State University, licensed by the Federal Accreditation Service of Russia.

Substances for modeling tests

The substances used to compare the sensitivity of the four bioassays were conditionally divided into mineral and organic (Table 2). Their frequent presence in anthropogenic emissions and discharges and resulting widespread increase of their pollution level were criteria for their selection as priority pollutants. The range of tested concentrations for all studied toxicants was different; it was determined in preliminary bioassays with *D. magna*. Further doses for other organisms were adjusted if no effect was observed or if it was lethal over the entire concentration range.

Table 1. Characteristics of the assessed bioassays.

Test organism	Test function	Test duration	Equipment	Guidance
Daphnia magna Straus, 1820	Mortality	96 hours	Not required (visual diagnostics)	FR 1.39.2007.03222[4]
Ceriodaphnia affinis Lilljeborg, 1900	Mortality	48 hours	Not required (visual diagnostics)	FR 1.39.2007.03221[5]
Paramecium caudatum Ehrenberg, 1838	Chemotactic reaction	30 min	Biotester	FR 1.39.2015.19242[6]
Escherichia coli Migula, 1895, strain M17	Bioluminescence	30 min	Biotox-10M	PND F T 14.1:2:3:4.11-04[7]

[4] FR 1.39.2007.03222. Biological methods of control. Methodology for determining the toxicity of water and water extracts from soils, sewage sludge, waste by mortality and changes in the fertility of *Daphnia*.

[5] FR 1.39.2007.03221. Biological methods of control. Methodology for determining the toxicity of water and water extracts from soils, sewage sludge, waste by mortality and changes in the fertility of *Ceriodaphnia*.

[6] FR 1.39.2015.19242. Methodology for determining the toxicity of samples of natural, drinking, domestic drinking, domestic wastewater, treated wastewater, wastewater, melted water, process water by an express method using a device of the Biotester series.

[7] PND F T 14.1:2:3:4.11-04. A method for determining the integral toxicity of surface, including sea, ground, drinking, waste water, aqueous extracts of soils, waste, sewage sludge by changing bacterial bioluminescence with the Ecolum test system.

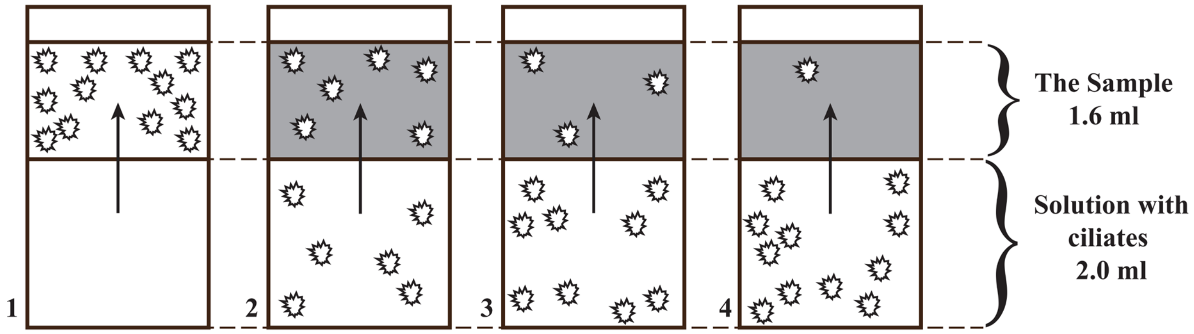

Fig. 1. Scheme of biotesting by chemotactic reaction of ciliates: 1 – harmless sample; 2, 3 – moderately toxic sample; 4 – toxic test.

Substances were introduced into artesian water of drinking quality. Water without additives served as control in all bioassays; the concentrations of the main cations and anions, as well as some pollutants, were determined prior to the start of the experiment. The content of all natural compounds was within acceptable ranges[8]; the content of pesticides and their degradation products, as well as HMs, did not reach the detection limit of the methods used. Some physical and chemical characteristics of water are presented in Table 3.

Statistical analysis and reliability of results

The bioanalyses were carried out under controlled laboratory conditions in three or four replicates, using the same water to simulate contamination. The obtained initial data were processed by standard mathematical methods. Significancy of differences was determined by Student's t-test, $p < 0.05$.

Results

Algorithm for choosing the most sensitive bioassay method

The proposed algorithm is based on ranking the sensitivity of several bioassay methods to the priority toxicant, which is a main factor of environmental toxicity in the study area belonging to natural or transformed ecosystems. The main factor of toxicity refers to the substance effects, which prevails over the effects of other substances in the sample. Preliminary testing of bioassay methods for sensitivity to priority contamination will allow further monitoring work to use the target bioassay method, but not the entire battery of bioassays.

D. magna mortality bioassay is proposed to be used as the basic one and the very first and mandatory element of the battery of bioassays, because:

– this is one of the most widely used methods of bioassay worldwide, its variations are described in international and national protocols for determining the toxicity of samples[9, 10, 11, 12];

– according to the responses of *D. magna,* it is possible to determine subacute, acute, and chronic effects (if necessary);

– *D. magna* has 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 target bioassay in the case of a known priority contamination comprises five stages.

1. Searching for non-lethal and lethal doses of the test substance for *D. magna* according to the international[12] or national[11] protocols for determining its mortality. Determining the mean lethal concentration of the toxicant is not necessary, if this is not the task of the study. The latter significantly reduces the amount of work.

Guidelines for selecting test concentrations are:

– following national standards for the harmful effects of substances, if they are developed;

– referring to the results of published scientific papers, if the toxicant has been previously studied;

[8] SanPiN 2.1.4.1074-01. Drinking water. Hygienic requirements for water quality of centralized drinking water supply systems. Quality control.

[9] EPS 1/RM/11. Biological test method: Acute lethality test using *Daphnia* spp.

[10] EPA 821/R-02/012. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms.

[11] FR 1.39.2007.03222. Biological methods of control. Methodology for determining the toxicity of water and water extracts from soils, sewage sludge, waste by mortality and changes in the fertility of *Daphnia*.

[12] ISO 6341:2012. Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test.

Table 2. The list of studied substances and their concentrations used for setting target bioassays. Estimated concentrations and compliance with MPC are given for the toxic ion (except for herbicides and oil products).

Substance group	Substance	Toxic ion / Substance	Range of tested concentrations, mg/L	Compliance with MPC (if any)
Heavy metals (HM)	$CuSO_4 \cdot 5H_2O$	Cu^{2+}	0.001–0.1	1–10 (Cu^{2+})
	$ZnSO_4 \cdot 7H_2O$	Zn^{2+}	0.01–0.1	1–10 (Zn^{2+})
	$Pb(CH_3COO)_2 \cdot 3H_2O$	Pb^{2+}	0.006–0.06	1–10 (Pb^{2+})
	$Cd(CH_3COO)_2 \cdot 2H_2O$	Cd^{2+}	0.05–0.5	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
	$NaNO_3$ + NH_4Cl	NO_3^- + NH_4^+	40–400 (NO_3^-); 0.5–5.0 (NH_4^+)	1–10
	$NaNO_2$ + NH_4Cl	NO_2^- + NH_4^+	0.08–0.8 (NO_2^-); 5–5.0 (NH_4^+)	1–10
Mineral forms of phosphorus	$Na_3PO_4 \cdot 12H_2O$	PO_4^{3-}	20–400	100–2000
	$Na_4P_2O_7 \cdot 10H_2O$	$P_2O_7^{4-}$	13–550	3.7–157
Herbicides	Imazetapir	$C_{15}H_{19}N_3O_3$	0.01–3.0	1–300
	Imazamox	$C_{15}H_{19}N_3O_4$	0.01–3.0	1–300
Oil products	Petrol	C_8H_{15}	10–100	–

Table 3. Results of analyzes of natural water for pollution modeling.

No.	Parameter	Units	Measurement result	Permissible level
1	pH	pH units	7.4 ± 0.2	6.5–8.5
2	Nitrates	mg/L	10.2 ± 2.0	No more than 20
3	Nitrites	mg/L	Less than 0.016	No more than 0.5
4	Ammonia	mg/L	Less than 0.05	No more than 0.1
5	Phosphates	mg/L	Less than 0.05	No more than 3.5
6	General water hardness	mg-eq/L	2.43 ± 0.36	No more than 7.0
7	Oil products (total)	mg/L	Less than 0.005	No more than 0.05
8	Organic carbon	mg/L	Less than 1.2	No more than 10

– using effective doses of homologous substances (for organic compounds) or substances closest in genesis (for inorganic), if the toxicant has not been studied.

Planned doses for study should be injected into natural water typical of the environmental area under monitoring (the use of distilled water is not recommended). This will make it possible to obtain data adequate to the natural chemical background of the natural waters of the study area.

2. Selection of 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:

– using test organisms of different trophic groups in accordance with the European Union Water Framework Directive[13];

– including express methods in the battery of bioassays;

– using bioassays to determine chronic effects (if necessary).

3. Testing of lethal and non-lethal doses of the priority toxicant, established during the first stage, on *D. magna* with 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;

– it is necessary to distinguish between the sensitivity of bioassays with close results obtained.

The control medium used for the simulation is the same (natural water), which has been used initially during stage 1.

4. Assessing additional effects of the priority toxicant: for example, chronic and delayed effects, mutagenic effects, etc. This procedure is performed only if it is included in the objectives of the study or the priority pollutant can potentially have a specific effect.

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

The scheme of our studies, indicating the test organisms used, is presented at Fig. 2. According to the above algorithm, the experimental blocks of the study and the analytical block were performed. Next, series of bioassay sensitivity were built and the information was summarized.

Analysis of sensitivity of various bioassay methods to mineral and organic toxicants

In this paper, we do not present intermediate results of bioanalysis of each sample, since our task is to describe the algorithm for the targeted selection of bioassays and to generalize analytically many series of experiments. Most of the results of individual experiments have been published, including a comparison of the sensitivity of bioassays to mineral forms of phosphorus (Kondakova et al., 2014), oil products (Olkova et al., 2017), mineral forms of nitrogen (Olkova and Makhanova, 2018), herbicides (Olkova and Berezin, 2018), and heavy metals (Olkova, 2020).

The results of the experimental and analytical blocks of this study are presented in Table 4, which clearly indicates the methods non-sensitive to certain contamination and bioassays that signal on contamination at the lowest doses. The sensitivity of bioassays is ranked into four categories according to the number of compared methods. The degree of the organism response to the tested doses of toxicants and the time of its manifestation are main criteria. As the battery of bioassays increases, the number of sensitivity levels will also increase.

The results summarized in Table 4 show that bioassay methods can be both maximally sensitive and insensitive to the impact, depending on the pollutant origin. This also applies to exposure to the toxicants, which have similar mechanisms of toxic action. The *E. coli* bioluminescence bioassay is characterized by minimal sensitivity to salts of Cd, Pb, and Zn, but at the same time it has the highest sensitivity to Cu salts compared to other methods.

Regard must be paid to variability of susceptibility to tested toxicants in taxonomically related species *D. magna* and *C. affinis*. According to the results summarized in this work, *C. affinis* mortality bioassay is often preferred over that with *D. magna.* At the same time, test for *D. magna* was more sensitive than that for *C. affinis* when exposed to Cd and Zn.

The obtained data emphasize the need to implement the battery of bioassays approach at the stage of preliminary tests and the possibility to apply them further on in the target bioassay method.

Information presented in Table 4 may serve as a basis for planning the environmental studies in the areas exposed to these substances.

Approbation of the target selection of bioassays on real samples

It is known that modern environmental pollution is characterized by the combined action of a number of toxicants, however, each industrial enterprise has its own specific impact, i.e., a chemical trace marker in the environment (Blais et al., 2015). We tested the "operability" of target bioassays in areas characterized by precisely such relationships ("enterprise (research area) – chemical trace").

Here we describe a case of establishing the battery of bioassays and its further use as the most sensitive target bioassay by the example of urbanozems

[13] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

sampled near the Electrozinc enterprise (Vladikavkaz, Russia).

According to atomic absorption spectroscopy, high level of heavy metal pollution (Table 5) is a main feature of urbanozems near the Electrozinc metallurgical enterprise (Fokina et al., 2016).

The maximum excess of the maximum permissible concentration (MPC) for HMs in soil was observed for zinc and lead by 92 and 37.5 times, respectively, in different areas. In half of the studied samples, the content of copper and iron was found to be higher than MPCs.

Based on the data presented in Tables 4 and 5, it is hypothesized that the results of the *P. caudatum* chemotaxis bioassays are the most informative, and *E. coli* bioluminescence bioassay can be excluded as insensitive to the main toxicity factor.

During the experimental verification of the hypothesis, aquatic extracts from urbanozem samples were tested using all four tested bioassay methods (Table 6).

The samples turned out to be toxic only for ciliates; high toxicity indices were obtained in the bioassay on changes in their chemotaxis. Mortality rates of *D. magna* and *C. affinis* did not differ significantly from control. In bioassay using *E. coli,* stimulation of bioluminescence of the bacterial preparation was observed. Therefore, our hypothesis and the data on different sensitivity levels of bioassays are correct.

Similarly, when testing real samples, the rest of the sensitivity battery of bioassays were confirmed. Therefore, if there is a need to study the level of toxicity repeatedly, the scientific approach of choosing target bioassay method, which the most sensitive to priority pollution, is approved.

Discussion

Undoubtedly, any test environment is a multicomponent system, where excluding contamination of many natural substances and compounds of anthropogenic origin is impossible (Altenburger et al., 2018). However, we hypothesize here that it is possible to single out a priority pollutant for many areas experiencing anthropogenic load and it is possible to choose a method most sensitive to this pollution within a battery of bioassays.

The use of a battery of bioassays is a well-known bioassay approach (Wieczerzak et al., 2016). The “battery” is often chosen to identify the risks associated with a wide range of chemical pollutants and their transformation products, allowing at the same time to identify the groups of compounds that may cause specific effects (De Baat et al., 2019). This study supports the idea of establishing targeted bioassays.

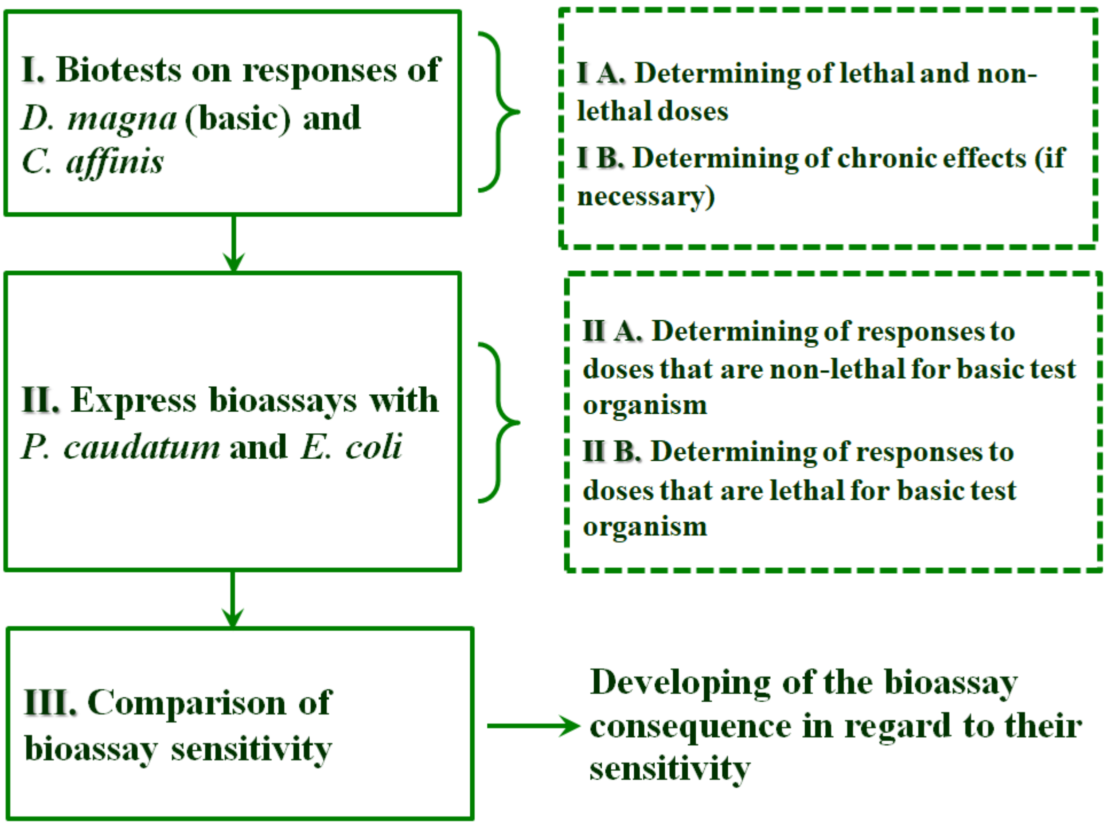

Fig. 2. Block diagram of the algorithm for selecting the target bioassay.

"Effect – directed analysis" (EDA) approach aimed at fractionating the sample and identifying the specific effects of each fraction is also becoming popular (Brennan et al., 2020). EDA approach and the battery of bioassays are now being contrasted, calling classical non-targeted bioassay methods (Oberleitner et al., 2020). However, we have shown that it is possible to move from a battery of bioassays to the use of one of the most sensitive bioassay methods, which will be the target method of laboratory biological diagnostics aimed at long-term monitoring. The importance of such developments is reported by S.J.P. van den Berg et al. (2021): they note that risk assessment can benefit most from describing sensitivity in terms of environmentally relevant and robust effects.

The choice of a target bioassay cannot be based solely on theory or data from 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 the resistance of organisms will increase as they become more complex. However, the discussed results indicate the opposite: for example, *C. affinis* and *D. magna* have maximum and high sensitivity to water pollution by mineral forms of nitrogen, and unicellular ciliates *P. caudatum* and bacteria *E. coli* are characterized by moderate and minimal responses, respectively. Such an inversion of the general toxicological pattern is not uncommon. The rat liver *in vitro* has greater detoxifying potential of organophosphate and N-methylcarbamate pesticides than human liver cell samples do (Animal models in toxicology, 2016). Crustaceans *D. magna* are more sensitive to ionic liquids based on 1-alkyl-3-methylimidazolium nitrate than the green algae *Chlorella vulgaris* Beijer in experiments assessing the acute toxicity of this compound (Zhang et al., 2017). It should be noted that in the case of diagnosing pollution with nitrates, nitrites, and ammonium ions, the significance of small crustaceans increases even more if one takes into account that their mortality has been estimated in these experiments, but sublethal responses were considered for unicellular organisms.

In the present study, we suggest choosing a target bioassay according to an algorithm based on determining lethal and non-lethal concentrations of a toxicant for the basic test species *D. magna*. Fur-

Table 4. Comparison of sensitivity of bioassays to mineral and organic toxicants. The sensitivity level of bioassays is indicated by signs and colors: "+" – minimum (white), "++" – medium (red), "+++" – high (yellow), "++++" – maximum sensitivity (green).

Pollutant			Bioassay / assessed response			
			Mortality of *D. magna*	Mortality of *C. affinis*	Bioluminescence of *E. coli*	Chemotaxis of *P. caudatum*
Mineral	Cu	Heavy metals	+	++	++++	+++
	Cd		+++	++	+	++++
	Pb		++	+++	+	++++
	Zn		+++	++	+	++++
	NO_3^-	Mineral forms of nitrogen	+++	++++	+	++
	NO_2^-		+++	++++	+	++
	NH_4^+		+++	++++	++	+
	$NO_3^- + NH_4^+$		+++	++++	+	++
	$NO_2^- + NH_4^+$		+++	++++	++	+
	$(P_xO_y)^{z-}$	Pyro-phosphates and phosphates	+	++	++++	+++
Organic	Imazetapir	Herbicides	+	++	+++	++++
	Imazamox		+	++	+++	++++
	Petroleum products (gasoline)		+	++	+++	++++

Table 5. Characteristics of urbanozems sampled in Vladikavkaz (Fokina et al., 2016). "–" – no standard exists. Bold font indicates values that exceed Russian standards for the content of HMs in soil[14, 15].

Sample no.	Water pH	pH_{KCl}	Content of organic matter, %	Gross content of HM, mg/kg				
				Fe	Cu	Ni	Zn	Pb
1	7.02	5.93	2.7 ± 0.5	**31000 ± 170**	14.5 ± 0.7	28.57 ± 0.24	59 ± 4	**35.51 ± 0.10**
2	6.55	5.53	7.3 ± 0.7	**31800 ± 1000**	44.4 ± 0.6	30.1 ± 0.8	**1165 ± 12**	**325.5 ± 11**
3	7.31	6.46	11.5 ± 1.1	**29660 ± 320**	**62.5 ± 0.7**	31.1 ± 1.5	**1468 ± 18**	**405 ± 7**
4	7.60	6.93	11.0 ± 1.1	17080 ± 210	**100.5 ± 0.8**	28.4 ± 0.9	**1985 ± 21**	**1240 ± 110**
5	6.60	6.06	5.0 ± 0.7	**35070 ± 360**	**383.8 ± 1.8**	31.6 ± 0.4	**3750 ± 90**	**2760 ± 230**
MPC/APC	–	–	–	25000	55	85	100	30

Table 6. Toxicity of samples of urbanozems in Vladikavkaz. "*" – stimulation of bioluminescence; bold type indicates the values corresponding to the conclusion "the sample is toxic"; the results of biotesting for mortality of *D. magna* and *C. affinis* are acceptable according to the metrological recommendations of the methods used. C.u. – conditional units.

Sample no.	Bioluminescence of *E. coli*, T (c.u.)	Change in chemotaxis of *P. caudatum,* T (c.u.)	Mortality of *D. magna,* %	Mortality of *C. affinis,* %
1	(−39.9 ± 3.0)*	**0.45 ± 0.09**	3.3	10
2	(−41 ± 9)*	**0.43 ± 0.09**	0	0
3	(−39.9 ± 3.0)*	**0.51 ± 0.01**	0	0
4	(−63 ± 5)*	0.32 ± 0.05	0	0
5	(−37.6 ± 1.1)*	**0.40 ± 0.05**	0	0

ther, the battery of bioassays should increase in accordance with the possibilities and objectives of the study. Based on the results of preliminary laboratory tests, a series is established that reflects the increase in the sensitivity of bioassays to a priority pollutant. The stage of confirming the high sensitivity of the target bioassay in comparison with other methods when testing real samples is also mandatory.

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

Conclusions

According to a series of experiments, the effectiveness of the targeted selection of bioassays using a universal algorithm for determining the most sensitive and preferred methods for bioassay of natural and anthropogenic environments contaminated with mineral and organic toxicants has been substantiated.

The algorithm has been tested on model and natural media contaminated with mineral compounds of nitrogen and phosphorus, salts of heavy metals (Cu, Zn, Pb, and Cd), oil products (petroleum), herbicides imazethapyr and imazamox. Sensitivity

[14] GN 2.1.7.2041-06. Maximum permissible concentrations (MPC) of chemicals in soil.

[15] GN 2.1.7.2042-06. Approximate permissible concentrations (APC) of chemicals in soil.

battery of bioassays based on responses of *D. magna, C. affinis, P. caudatum,* and *E. coli* were experimentally determined. The mortality tests for *D. magna* and *C. affinis* are the most sensitive to contamination with mineral nitrogen compounds. The *E. coli* bioluminescence reduction test is preferable when assessing Cu mineral salts, phosphates, and pyrophosphates. The chemotactic response reduction test with *P. caudatum* should be used for pollution of the aquatic environment with mineral salts of Cd, Pb, and Zn, oil products, organic herbicides imazetapyr and imazamox.

For the convenience of using the algorithm for selecting bioassays, we compiled the table that may serve as a reference material when planning environmental studies in areas exposed to the tested substances.

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 bioasay in environmental monitoring programs. The proposed procedure allows choosing the most sensitive and reliable bioassay methods to the most common and dangerous pollutants in the study area.

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