

## Development and Efficiency Assessment of New Methods on Rapid Assessment of Toxicity in Environmental Monitoring

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The transformation of hazardous pollutants in the environment usually results in formation of hard-to-identify compounds that may have a significant impact on ecosystems. They are difficult to identify by conventional methods of quantitative chemical analysis, but their detrimental effects can be detected by biological methods. The goal of this study was to develop novel methodological approaches to the express assessment of the state of the environment as well as toxicity of its components in the process of biological monitoring at hazardous industrial facilities. The article presents the results of comprehensive toxicity assessment of environmental objects using originally developed system of express methods. The objects of research were water fleas (*Daphnia magna* Straus and *Ceriodaphnia affinis* Lilljeborg), infusoria (*Paramecium caudatum*), preparation of lyophilized fluorescent bacteria based on recombinant *Escherichia coli* strain M-17 (Ecolum), algae (*Chlorella vulgaris* Beijer, *Scenedesmus quadricauda* (Turp.) Breb.), and mammalian gonads. The original methods for determining toxicity by changing the motor activity of water fleas at elevated temperatures of the studied environment are described. The description of the following original utility models is presented: “Method of bioassay for water samples and device for its implementation”, “Device for express assessment of natural environment quality” and “Device for comprehensive assessment of natural environment quality”, confirmed by the patents of the Russian Federation. Their use may significantly reduce the cost of ecotoxicological studies in terms of time and money expenditures. The effectiveness of the system of express methods was established during a biological monitoring of a sanitary protection zone of the Balakovo NPP. Toxicity of the samples of bottom sediments, soil, as well as water samples from the cooler pond and the adjacent water area of the Saratov reservoir were identified. The bioassay results were matched by both conventional methods’ results and originally developed system of express methods.

**Keywords:** biomonitoring, biotesting, test objects, express methods, toxicity, hazardous industrial facility.

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## INTRODUCTION

In recent decades, the Russian Federation has established a system of environmental control and monitoring with a characteristic structure that has never been used before. Great importance is currently given to biological laboratories conducting direct analysis of the toxicity of the natural objects using various sensitive plant and animal organisms without adaptive capabilities as bioassay objects (Ashikhmina, 2002; Ashikhmina et al., 2008; Melekhova et al., 2010).

Biological monitoring of abiotic and biotic components of the natural environment is one of the key elements in the system of ensuring environmental safety in the areas exposed to hazardous industrial facilities. The complex of biological studies of such system consists of ecotoxicological monitoring, as well as the monitoring of animal and plant life.

Ecotoxicological monitoring includes: studies of the toxicity of components of the natural environment by bioassay methods on bioassay objects belonging to different systematic groups (cell cultures, microorganisms, lower plants, invertebrates); studies of genotoxicity and mutagenicity of contaminated components of the natural environment (genetic monitoring).

Monitoring of flora and fauna includes: studies of the state of plant communities using bioindication methods; studies of the state of representative species of higher animals from the changes in their hematological, biochemical and immunological parameters.

In addition to the evaluation of the toxic properties of contaminated environmental components, biological monitoring data are used to assess an effectiveness of biodiversity conservation and ecosystem characteristics, along with damage assessment at emergency or extraordinary situations, and for decision-making on limiting the impact of some industrial enterprise on the environment.

The transformation of environmentally hazardous pollutants in the environment leads to the formation of, as a rule, more stable and difficult to identify compounds. Small and ultra-low doses of these substances, safe from the point of view of sanitary and hygienic standards, can have a significant impact on ecosystems. These substances are extremely difficult to identify by standard methods of quantitative chemical analysis, but their effects can be detected by biological methods.

Today, hydrobionts are widely used to assess the quality of environmental components: protozoa (ciliates), crustaceans (daphnia), fish (roach, ruff and others), algae (scenedesmus and chlorella), as well as some groups of higher plants. A special place as test objects is occupied by warm-blooded animals (mice, rats).

It is the studies on these animals that allow us to obtain results that are most correlated with the reaction of the human body to the effects of environmental pollution, since many physiological and biochemical processes that occur in humans and these animals are similar. Methods for assessing environmental genotoxicity are the anaphase-telophase method of accounting for chromosomal aberrations and the nucleolar test, which are successfully used for bioindication and biotesting. The objects of research are plants, of which onions, medicinal dandelion, and seed barley are most convenient. The apical meristem of the roots of bulbs and seedlings is analyzed.

The study of violations of hereditary material at the anaphase-telophase stage consists in the quantitative registration of structural mutations. These approaches allow us to

give a reliable assessment of the impact of hazardous industrial facilities on the environment, which has undergone a wide range of biological changes. The analysis of selected samples using certified methods at bioobjects makes it possible to assess the quality of the environment, which is a natural task of integrated environmental monitoring and the main direction of the transition to environmental regulation.

In the standard formulation of the tasks of control and monitoring the activity of hazardous industrial facilities, they are limited to carrying out analyzes to detect polluting chemicals in the territory of the facility and in the area of its location. This ensures the requirements of environmental quality standards: atmospheric air, soil, natural water, etc. But there are many factors that are difficult to consider in this way. Knowledge on the transformation of various chemicals in the natural environment, their degradation products, accumulation processes, and exposure mechanisms in small doses is limited and is in the process of development. Of great practical importance is the improvement of methodological approaches to the use of biotesting in integrated environmental monitoring, the need for a quick assessment of the state of the environment and the toxicity of its components (Ivanov et al., 2009; Lisovitskaya, Terekhova, 2010; Integrated Environmental Monitoring..., 2014; Lushai, 2018).

The goal of the work was to develop new methodological approaches to a quick assessment of the state of the environment and the toxicity of its components during the biomonitoring of hazardous industrial facilities.

## MATERIAL AND METHODS

The studies were performed in the period from 2007–2018 based on the laboratory of biomonitoring and biotesting of the Federal Budget Institution of the State Research Institute of Industrial Ecology (FBU GosNII ENP, Saratov), scientific and educational center “Industrial Ecology” of the Saratov State Technical University named after Gagarin Yu. A. (SEC IE SSTU), as well as when performing field research on the territory of the sanitary protection zone (SPZ) of the Balakovo NPP in the Saratov Region.

Biotest objects were used as research objects: daphnia (*Daphnia magna* Straus), ceriodaphnia (*Ceriodaphnia affinis* Lilljeborg), ciliates (*Paramecium caudatum*), the preparation of lyophilized luminescent bacteria “Ekolyum” based on the recombinant strain of *Escherichia coli* M-17, algae (*Chlorella vulgaris* Beijer, *Scenedesmus quadricauda* (Turp.) Breb.), mammalian germ cells; environmental objects: natural water, bottom sediments, soil samples.

The studies were carried out using the following generally accepted methods in biomonitoring (Zhmur, 2001 *a, b*; 2007 *a, b*; Guidelines for Determining..., 2002; Grigor'ev, 2004; Method of Determining..., 2004; Grigor'ev, Shashkova, 2006; Chupis et al., 2008 *a*; Eskov et al., 2009; Methodology for Determining..., 2010).

In the process, we have developed and approved the following methods: “Express methodology for assessing the toxicity of water, water extracts from soils, sewage sludge and waste by changing the spontaneous physical activity of daphnia (*Daphnia magna* Straus)”, (Measurement method assesment certificate No. 222.0068/01.00258/2015 (Ural Scientific-Research Institute of Metrology)); “Express methodology for assessing the toxicity of water, water extracts from soils, sewage sludge, waste by daphnia mortal-

ity (*Daphnia magna* Straus) with increasing temperature of the cultivation environment”, (Measurement method assesment certificate No. 222.0069/01.00258/2015 (Ural Scientific-Research Institute of Metrology)); “Express methodology for assessing the toxicity of water, water extracts from soils, sewage sludge, waste by the mortality of ceriodaphnia afferentis (*Ceriodaphnia affinis* Lilljeborg) with increasing temperature of the cultivation environment”, (Measurement method assesment certificate No. 222.0070/01.00258/2015 (Ural Scientific-Research Institute of Metrology)); Methodology for determining the toxicity of drinking, natural, wastewater, bottom sediments, water extracts from soils, sewage sludge, waste using a nucleolus test on seedlings and plant bulbs of seeds and bulbs of plants (Measurement method assesment certificate No. 224.01.17.066/2015 (Ural Scientific-Research Institute of Metrology)); Methodology for determining the toxicity of drinking, natural, wastewater, bottom sediments, water extracts from soils, sewage sludge, waste by changing the level of chromosomal aberrations in the apical meristem of seeds and bulbs of plants (Measurement method assesment certificate No. 224.01.03.067/2015 (Ural Scientific-Research Institute of Metrology)).

Statistical processing of the experimental results was carried out using the Statistica for Windows 6.0 software package according to well-known methods taking into account Student's criteria.

## RESULTS AND DISCUSSION

At the preliminary stage of work, we analyzed literature data on ecotoxicological monitoring and biotesting methods used in practice, used to assess the quality of the environment. The experience of applying biotesting methods in monitoring hazardous industrial facilities, their advantages, disadvantages and prospects of application, which made it possible to justify the need to develop and study a system of express methods for biotesting environmental objects, are examined. Common methods in practice are:

- a method for assessing the toxicity of aquatic environments, based on the survival of *Daphnia* crustaceans as a result of their exposure in the test solution (Pavlenko, Denisova, 1986);
- a method for biotesting sewage, surface, groundwater and water extracts from the death of crustaceans *Daphnia magna* Straus;
- a method of biotesting water samples and aqueous extracts by assessing the toxicity of the test solution based on the behavioral reaction (phototaxis) of *Daphnia magna* Straus.

Of the known methods for assessing the toxicity of aquatic environments based on survival and studying the behavioral response of crustaceans *Daphnia magna* Straus (Larchenko, 2005; Jun et al., 2006; Zhmur, 2007 *a, b*; Methodology for Determining..., 2010), the disadvantages are low sensitivity and duration of determination.

There is also an element of ambiguity in the results of the determination, caused by the superposition of two factors at once on the behavior of daphnia: illumination, which, in general, can affect both positive and negative motor activity of invertebrates and toxicity, which, in turn, can both increase, and lower the physical activity of daphnia.

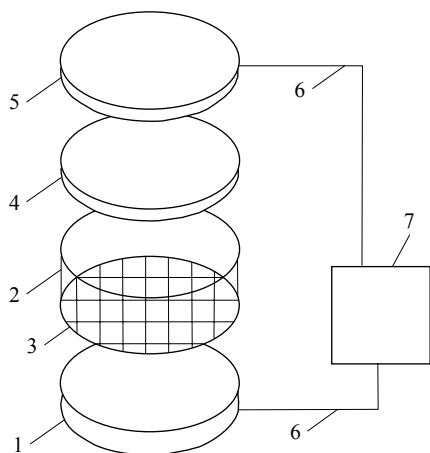
The disadvantages include the features of the used device for recording phototaxis in *Daphnia magna*. This is the complexity of manufacturing: the presence of parts that

require fitting (cover), the ambiguity of the design – the size of the outlet is not strictly regulated, which requires preliminary measurements of the dimensions of the daphnia and, as a consequence, the need to make the device directly during the biotesting process; the location of the hole on the rigidly fixed side wall of the working chamber, and not on a removable lid, which makes it necessary to have a large number of chambers with different hole sizes or each time re-manufacture the chamber to the size of the currently existing daphnia.

Based on the analysis data, the priorities of our own research and the main methodological approaches to accomplish the tasks were determined. The need to reduce time costs, the value of the choice of test objects depending on the object of pollution is revealed.

We have developed a “Method for bio-testing water samples and a device for its implementation” and received a certificate for the invention No. 2409813. The developed method allows to significantly increase the sensitivity of the method and the reliability of the results while reducing the analysis time.

The essence of the proposed method is that two groups of *Daphnia* are simultaneously kept in the test and control water samples, and then one by one is transferred to a measuring device, which is a flat glass vessel with a bottom marking, filled with a neutral aqueous medium. In the process of observation, the number of intersections of the marking lines by daphnia that have passed exposure in the tested water sample is compared with the same number of intersections of daphnia that have passed exposure in the settled tap water. The method and device allows you to quickly and with high sensitivity to conduct biotesting of water samples in laboratory and field conditions.



**Fig. 1.** A device for biotesting water samples in laboratory and field conditions: a photo-detector 1, a working vessel 2 with a marking of the bottom of the working vessel 3, a transparent cover 4, a light source 5, connecting wires 6, a power and control unit 7

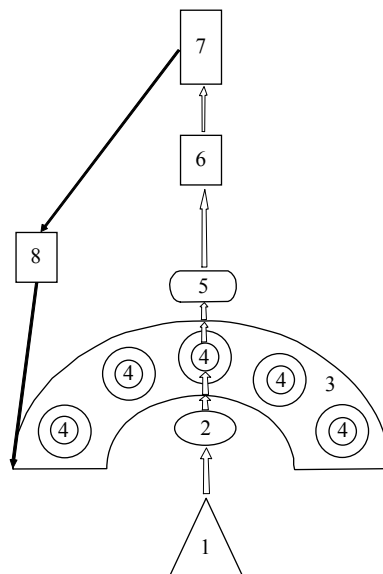
The task of increasing the sensitivity of the method and the reliability of the results obtained is achieved through a comparative assessment of the approximate research activity and the dynamics of the decrease in the activity of daphnia, previously aged in the test and control solutions, respectively. To reduce the time of biotesting and increase the sensitivity of the method, before assessing the activity of daphnia, they are kept in the test and control solutions for 1 hour, plus or minus 5 minutes in a thermostat with a temperature of 35°C plus or minus 0.5°C. As a control solution can be used neutral aqueous medium (cultivation or standing tap water). To fully realize the capabilities of the proposed biotesting method, a device was developed that is described in the same patent, which includes a vessel with a lid filled with a neutral aqueous medium, which is a lightproof cubic chamber with an outlet (Fig. 1).

The objective of the invention is to reduce the preparation time and analysis time, simplifying the design of the device and test procedures. The problem is solved thanks to the device, which is a flat vessel (working vessel) with a transparent base (bottom) and with a transparent lid (a glass Petri dish is possible), on the lower surface of which a marking is applied in the form of a grid with a square mesh.

In an embodiment of the device, the marking is applied to the transparent cover of the working vessel. The linear dimensions of the grid grid cells are set to exceed two lengths of a typical *Daphnia*, but not more than four lengths (from 2 mm to 2.5 mm), i.e. from 5 mm to 10 mm and a line thickness exceeding one quarter, but not more than one second, of the length of a typical daphnia, i.e. from 0.5 mm to 1.2 mm.

Cell sizes depend on the chosen test option. In the automated (main) version, in the diagram under number 3, the cell size is preferably selected as the minimum of the specified, and the thickness of the lines is maximum. The given dimensions are determined by the features of the behavior of daphnia and are optimal (preferred). With significant size deviations from the above, the test results become irreproducible and unreliable. The working vessel is placed between the light source and the photodetector connected to the pulse recorder. The pulse recorder is combined with an electrical power and control unit. Each intersection of the daphnia of the marking line forms a light pulse captured by the photodetector and detected by the pulse recorder. The time interval between pulses corresponds to the unit distance traveled by daphnia (the distance between the marking lines). The number of recorded pulses per unit time corresponds to the speed of movement of daphnia. The proposed method also allows simple visual registration of the movement of *Daphnia*. In this case, for the convenience of work and the elimination of errors, the side of the square is taken maximum (from 8 mm to 10 mm), and the thickness of the lines is minimal (from 0.5 to 0.7 mm). The toxic substances of the tested water sample affect the physical activity of daphnia, which leads to its significant change (decrease or increase). The degree of change is estimated by the difference in the number of line intersections (square borders) per unit time for the test and control samples. At the same time, the rate of change in the speed of movement of daphnia in time is assessed (dynamics of a decrease in activity).

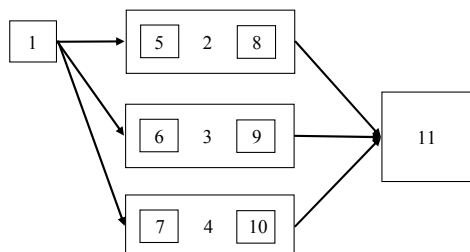
We have also developed devices that facilitate the conduct of rapid biotesting methods. The first is the "Device for the rapid assessment of the quality of natural environments", which provides the possibility of analyzing microscopic video images of localized changes in shoe ciliates concentration and changes in the motility of mammalian germ cells in the range from 2  $\mu\text{m}$  to 400  $\mu\text{m}$  (Fig. 2).



**Fig. 2.** Diagram of a device for rapid assessment of the quality of natural environments

The device is interconnected to form an optoelectronic path, a light source, filters, a carriage with a drive having at least three slots for accommodating working vessels with control and test solutions in them and made in the form of an optical-mechanical unit, combined with a sample preparation unit, which is a dry thermostat with the ability to maintain a temperature in the range of  $30 \div 50^\circ \text{C}$ , a photodetector with a lens, a portable personal computer having a monitor and input devices for text and coordinate information, and a drive and temperature control unit for the carriage.

The second “Device for a comprehensive assessment of the quality of natural environments” was proposed to register the concentration of ciliates, measure the intensity of the bioluminescence of bacteria, and record the time dependence of the mobility of a suspension of mammalian germ cells. The device contains a sample preparation unit, measuring cuvettes, optical-mechanical and electronic units that record the time dependence of the mobility of a suspension of mammalian sex cells, characterized in that a pulse photometer unit is used to record the concentration of ciliates, and a lumen meter unit that measures the intensity of bioluminescence bacteria, and all three work units are connected to one electronic unit (Fig. 3).



**Fig. 3.** Device for a comprehensive assessment of the quality of natural environments

reservoir water area, water extraction of soil samples from a zone of possible influence of a nuclear power plant (Chupis et al., 2008 *b*). Ecotoxicological analysis of the samples was carried out on several test objects belonging to different systematic groups of living organisms (bacteria, ciliates, algae, lower crustaceans) using the methods certified and developed by us.

The acute toxic effect of the samples was determined by assessing the mortality of daphnia and ceriodaphnia, by recording differences in the optical density of the chlorella algae and by changing the fluorescence of algae, by determining the chemotactic reaction of the ciliates, and by changing the intensity of bacterial bioluminescence. In total, since 2007, about 576 samples were analyzed (of which 360 soil samples, 108 water samples and 108 samples of bottom sediments), about 2880 analyzes were carried out using certified biotesting methods (Lushai et al., 2011). 1256 samples were analyzed according to generally accepted methods and using the bioassay method developed by us. In 98.1% of cases, the results coincided, indicating the effectiveness of the proposed method.

Ecotoxicological studies in general have shown that the state of aquatic and terrestrial ecosystems is normal. The Balakovo NPP does not have a significant negative impact on the water and land ecosystems of the area of its location.

The presented developments were carried out on the basis of the biomonitoring and biotesting laboratory of the Federal State Institution GosNIIENP and were confirmed by patents of the Russian Federation (Ivanov et al., 2009; 2012 *a, b*). During biological monitoring of the region where the Balakovo NPP is located, biotesting methods evaluated the toxicity of water samples, water extraction of bottom sediments from a cooling pond and reser-

## CONCLUSION

Thus, the emphasis in our own experimental studies was on improving methodological approaches to the ecotoxicological part of biomonitoring. We have developed methods for rapid assessment of the quality of natural environments and the negative impact of industrial facilities on the environment by changing the level of chromosomal aberrations in the apical meristem of seeds and bulbs of the nucleolus test on seedlings and bulbs of plants of seeds and bulbs of plants, as well as a method for determining toxicity by changing motor activity of daphnia and ceriodaphnia at elevated temperature of the investigated environment.

A “Method for biotesting water samples and a device for its implementation”, “A device for rapid assessment of the quality of natural environments” and “A device for a comprehensive assessment of the quality of natural environments” have been developed, confirmed by patents for utility models and the invention. The developed and proposed methodological approaches for biomonitoring and biotesting methods (certified in Rosstandart) can significantly reduce the economic and time costs of ecotoxicological studies.

The effectiveness of their use is confirmed by a set of studies conducted on the territory of the Balakovo NPP in determining the toxicity of water samples from the cooler pond and the adjacent water area of the Saratov reservoir, samples of bottom sediments and soils within the sanitary protection zone. It should be noted 100% coincidence of the results of biotesting according to generally accepted methods and using the system of express methods developed by us, including the author's method and devices for biotesting.

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## РАЗРАБОТКА И ИССЛЕДОВАНИЕ ЭФФЕКТИВНОСТИ НОВЫХ МЕТОДОВ БЫСТРОЙ ОЦЕНКИ ТОКСИЧНОСТИ КОМПОНЕНТОВ ОКРУЖАЮЩЕЙ СРЕДЫ ПРИ БИОМОНИТОРИНГЕ

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Трансформация экологически опасных загрязнителей в окружающей среде приводит, как правило, к образованию трудно идентифицируемых соединений, которые могут оказывать существенное влияние на экосистемы. Их сложно идентифицировать стандартными методами количественного химического анализа, однако эффекты можно обнаружить биологическими методами. Цель работы заключалась в разработке новых методических подходов к быстрой оценке состояния окружающей среды и токсичности ее компонентов в процессе биомониторинга опасных промышленных объектов. Представлены результаты оценки комплексной токсичности объектов окружающей среды с использованием разработанной системы экспресс-методов. Объектами исследования были дафнии (*Daphnia magna* Straus), цериодафнии (*Ceriodaphnia affinis* Lilljeborg), инфузории (*Paramecium caudatum*), препарат лиофилизированных люминесцентных бактерий «Эколюм» на основе рекомбинантного штамма *Escherichia coli* M-17, водоросли (*Chlorella vulgaris* Beijer, *Scenedesmus quadricauda* (Turp.) Breb.), половые клетки млекопитающих. Описаны разработанные методики определения токсичности по изменению двигательной активности дафний и цериодафний при повышенной температуре исследуемой среды. Представлено описание разработанных полезных моделей: «Способ биотестирования проб воды и устройство для его осуществления», «Устройство для экспресс-оценки качества природных сред» и «Устройство для комплексной оценки качества природных сред», подтвержденные патентами РФ. Их использование позволяет существенно сократить экономические и временные затраты на экотоксикологические исследования. Эффективность системы экспресс-методов подтверждена при биомониторинге санитарно-защитной зоны Балаковской АЭС. Определяли токсичность проб донных отложений, почвы, а также проб воды из пруда-охладителя и прилегающей акватории Саратовского водохранилища. Показано совпадение результатов биотестиро-

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вания по общепринятым методикам и с использованием разработанной системы экспресс-методов.

**Ключевые слова:** биомониторинг, биотестирование, тест-объекты, экспресс-методы, токсичность, опасный промышленный объект.

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