# Biotesting of Water Bodies in Metropolitan Areas

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Abstract: Environmental monitoring of natural objects invariably remains a necessary and real reflection of the pollution level under the anthropogenic influence. This work contains a study of surface waters to determine their toxicity using biotesting, which allows to assess the quality of fishery water resources. The rivers of the Moscow city and the Krasnoyarsk city were chosen as the objects of the study. Chlorella vulgaris Beijer served as a test object for assessing the toxicity of waters. The study was conducted using photometry. Based on the results obtained, it is proposed to provide measures to reduce the recreational load and systematic quality control of rivers.

## 1 INTRODUCTION

Water is the most widespread, unique and amazing substance in nature. Due to its special physical, chemical and quantum mechanical properties, it occupies an exceptional position in nature and plays a special role in the existence of organic life. This liquid is a perfect source of life and human life is unthinkable without it. It is the most affordable and cheap substance, that's why its wide application in all spheres.

In order to rationalize the use of biological resources of the rivers and provide people with clean drinking water, systematic quality control of natural waters is necessary. In conditions of intensive production activity, there is a constant increase in the amount of pollutants entering the aquatic environment, including heavy metals, petroleum products, nitrates and other chemical compounds. The identification of this problem is carried out by assessing the level of water bodies pollution and the intensity of their biological self-purification.

The establishment of control over the content of toxicants in the environment by chemical methods presents certain difficulties, in addition, physico-chemical methods of indicating the state of the environment do not provide a direct answer to the question of the possible response of ecosystems to certain pollutants. In this regard, methods of biological analysis of water, soil and air are of great importance, in which algae, due to the stenotopy of many species, their high sensitivity to environmental conditions, play a major role (Onerkhan, Durmekbaeva, Akhmetova, 2019).

## 2 ABOUT THE METHODOLOGY

All methods of biotesting are characterized by their own characteristics. This is the speed of obtaining the result, the availability of the test object, which is determined by the possibility of their cultivation in laboratory conditions, maintaining the necessary conditions of illumination, temperature, complexity of the composition of nutrient media, air purity, and so on. In simple organisms — algae, bacteria and infusoria, the fastest reactions can be recorded to toxic effects.

Such reactions are limited to minutes or several hours. Each method is characterized by its own technical ways of implementation. For all methods, the general rule is to assess the reliability of test cultures (Volchanskaya, Zrazhevskaya, Nikolaenko, 2016).

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In this work, to study the problem of water toxicity, its determination was carried out using a test culture of the green algae Chlorella vulgaris. Chlorella refers to unicellular algae having spherical cells with a thin shell, without mucus. Chlorella has a cup-shaped chromatophore with a pyrenoid, reproduces by autospores, which form 4-8, less often 16 inside the mother cell and are released through a rupture of its shell. This type of chlorella is widespread in the plankton of freshwater reservoirs.

Chlorella algae is the only one of the most well—known species of bacteria that has an extensive distribution in freshwater bodies. Algae are widely used in biotesting due to the available cultivation conditions, high reproducibility and the ability to analyze a wide range of test functions in laboratory culture. One of the main conditions is to obtain the results of sample analysis based on genetically homogeneous organisms. Thus, the differences between the control sample and the experimental one can be explained by a violation of some factor, and not by differences between test organisms (Speedinskaya, 2019).

The method is based on the detection of differences in the optical density of the selected test culture placed in an aqueous medium from various sources.

All laboratory tests were carried out strictly in accordance with the HDPE F T methodology 14.1:2:3:4.10-04. This document establishes a methodology for determining the toxicity of water samples (surface fresh, groundwater, drinking, wastewater) and aqueous extracts from ground, soil, sewage sludge, production and consumption waste by changing the optical density of the test culture of the green protococcal algae chlorella (Chlorella vulgaris Beijer) in laboratory. The optical density of the algae test culture after 22 hours of growth is measured using a photoelectric colorimeter.

Cultivators can work around the clock without requiring the constant presence of a laboratory assistant. The constant temperature is maintained by automatically turning on and off the built-in fan at the command of the device's thermal stabilization unit. The increase in the number of cells is quickly determined by measuring the light transmission of the grown algae suspension using a specialized optical density meter.

Measuring the optical density of algae makes it possible to assess the change in the number of cells in the control and experimental versions, and based on the results and their comparison, it becomes possible to draw conclusions about the toxicity of the samples under consideration. The criterion for the toxicity of aqueous samples is a change in the optical density of the samples under consideration (that is, suppression of growth or vice versa — its stimulation) compared with a control sample prepared on the basis of distilled water.

The equipment used for research is a multi-cuvette algae cultivator.

Biotesting as a method of assessing the toxicity of the aquatic environment is used:

− in the toxicological assessment of industrial, domestic wastewater, agricultural, drainage, polluted natural and other waters in order to identify potential sources of pollution,

− in the control of emergency discharges of highly toxic wastewater,

− to assess the degree of toxicity of wastewater at different stages of formation in the design of local wastewater treatment plants,

− in the control of the toxicity of wastewater supplied to biological treatment plants in order to prevent the penetration of dangerous substances for biocenoses of activated sludge,

− to determine the level of safe dilution of wastewater for aquatic organisms in order to take into account the results of biotesting when adjusting and setting the maximum permissible discharges of substances entering wastewater reservoirs,

− in the environmental assessment of new materials, purification technologies, sewage treatment plant projects and others.

The vital function or toxicity criterion is used in biotesting to characterize the response of the test object to the damaging effect of the environment. Test functions used as bioassay indicators for various objects:

− for infusoria, crustaceans, embryonic stages of mollusks, fish, insects — survival of test organisms;

− for crustaceans, fish, shellfish — fertility, the appearance of abnormal abnormalities in the early embryonic development of the body, the degree of synchronicity of egg crushing;

− for cultures of unicellular algae and infusoria — cell death, change (increase or decrease) in the number of cells in culture, cell division coefficient, average growth rate, daily increase in culture;

− for various plant objects — seed germination energy, primary root length, etc.

## 2.1 The objects of the study

The following rivers were selected as the studied objects for this work:

- The Moskva River;
- Bazaiha river, Krasnoyarsk city;

- Yenisei River, Krasnoyarsk city;
- Kacha River, Krasnoyarsk city.

This choice is due to the high degree of contact the population with the water bodies, for example, the Moscow River flows through the most densely populated Russian city, the Yenisei River divides the Krasnoyarsk into two banks and has close proximity to many industrial enterprises of the city, we are talking about water intakes and emissions.

The Kacha River is a left tributary of the Yenisei River, originates in the northwestern spurs of the Eastern Sayan, in the Yemelyanovsky district, flows through the territory of the Yemelyanovsky district and the city of Krasnoyarsk, flows into the Yenisei in the center of Krasnoyarsk. Kacha is a recreational gem of Krasnoyarsk, as a result, the river is experiencing constant anthropogenic stress. Every year in the spring and summer season, the Kacha River overflows its banks, washing away pollutants of organic and inorganic nature from the soil surface. The negative impact of anthropogenic and man-made factors is manifested at all levels of the ecosystem.

Bazaiha is a river in the Krasnoyarsk Territory, a right tributary of the Yenisei, flows into it within the city of Krasnoyarsk. A large private sector is located in the water protection zone of the Bazaiha River, which has a significant impact on the ecological state of the watercourse.

The main cause of watercourses pollution is unauthorized and polluted discharges of industrial enterprises.

As a result of studying the characteristics of coastal territories, the main sources of problems have been identified. Currently, the condition of urban rivers is assessed as "very dirty" as a result of increased anthropogenic and man-made loads. The key factors of pollution of coastal zones and riverbeds are individual residential buildings closely adjacent to the riverbed, as well as numerous production and storage areas, discharges of industrial enterprises.

The initial data of laboratory tests for all samples are presented in Table 1.

Table 1: Initial data for the experiment.

| Concentration of the water sample, % | Dilution of the sample, times | Biotesting time, hour |
|---|---|---|
| control | - | 22 |
| 1,2 | 81 | |
| 3,7 | 27 | |
| 11 | 9 | |
| 33 | 3 | |
| 100 | 1 | |

### 2.1.1 The Moscow River

The Moskva River is a left tributary of the Oka River (Volga River basin). The length is 473 km (along the old riverbed before straightening 502 km), the basin area is 17.6 thousand square kilometers. It originates on the Smolensk-Moscow upland, flows out of a swamp in the east of the Smolensk region, flows mainly through flat terrain, flows into the Oka near Kolomna. In total, 362 rivers and more than 500 streams flow into Moscow.

It has been established that 83 km of the Moskva River (out of a total of 478 km), which pass in the capital, manage to be heavily polluted by industrial effluents, which affects its unfavorable environmental condition and water quality (Yashin, Vasenev, Gareeva, Chernikov, 2015).

Figure 1 shows a satellite image of the river.

Figure 1: The Moscow River.

The basin of the Moskva River within the city of Moscow is under the influence of an industrial complex, which has a significant impact on the change in the chemical composition of the water of both the Moskva River and its tributaries. Surface runoff from the city is formed by thawed snow and rainwater, as well as irrigation and washing waters. As a rule, the runoff is not cleaned of pollution and directly enters water bodies, carrying with it a large amount of organic, suspended substances, and petroleum products. Most of the pollutants: petroleum products - 63%, suspended solids - 75%, organic substances - 64%, chlorides - 95%, enter the Moskva River with surface runoff in the winter and spring period.

For an objective assessment of the state of water bodies within the city of Moscow and the development of measures for the improvement of reservoirs, a sound system of water quality control and assessment is essential.

Biotesting included the following operations: preparation of control and experimental media,

introduction of test organism cells into them, study of cell growth dynamics, comparative analysis of the data obtained.

The results of biotesting samples of the Moscow River are presented in Table 2.

Table 2: Biotesting of the Moscow River.

| Concentration of the water sample, % | The value of the optical density, D | | The difference D in the tested water, in % of the control | The difference D in the tested water, in fractions to the control |
|---|---|---|---|---|
| | control | experience | | |
| control | - | - | - | - |
| 1,2 | 0,132 | 0,160 | -21 | -0,21 |
| 3,7 | 0,132 | 0,149 | -13 | -0,13 |
| 11 | 0,132 | 0,130 | 2 | 0,02 |
| 33 | 0,132 | 0,055 | 58 | 0,58 |
| 100 | 0,132 | 0,082 | 38 | 0,38 |

Based on experimental data, it can be concluded that toxic effects occur at sample concentrations of 33 and 100 %. At lower concentrations, the growth processes of test objects in the samples are suppressed by 20 %. In the test sample, pollutants had an acute toxic effect on chlorella when diluted by 6.3 times.

Studies have shown a high sensitivity of the test object to contamination with basic pollutants. The use of biotesting technology using chlorella algae to assess the quality of natural waters is advisable.

### 2.1.2 The Yenisei River

The Yenisei River ranks 2nd in length among the rivers of Russia and Eurasia (after the Ob) and 7th among the rivers of the world. The Yenisei basin is characterized by a sharp asymmetry: its right-bank part is 5.6 times more extensive than the left-bank one. The Yenisei is the natural border between Western and Eastern Siberia. The Yenisei River is the most abundant, largest and longest in the Krasnoyarsk Territory.

The total length is 3,487 km, and the total area of the basin is 2,580,000 square km.

The phases of the hydrological regime are smoothed due to the location of the Sayano-Shushenskaya and Krasnoyarsk hydroelectric power plants on the river.

Anthropogenic factors of the formation of the qualitative composition of the Yenisei River in the area of the city can be attributed the presence of industrial facilities such as the Krasnoyarsk housing and communal complex, the Krasnoyarsk Metallurgical Plant, the Krasnoyarsk Aluminum Plant, the Krasnoyarsk Reinforced concrete plant (Badmaeva, Sokolova, 2017).

Figure 2 shows a satellite image of the river.

Figure 2: The Yenisei River.

On the territory of the city of Krasnoyarsk, the main pollutants of the Yenisei River are industrial and production areas, motor transport, boiler houses, cultural and household facilities.

Pollution of the river in the area of the Krasnoyarsk occurs due to:

- untreated and poorly treated wastewater;
- flushing of pollutants from fields, lands and roads by rain and snow precipitation;
- soil erosion;
- wind transfer of pollution from the environment into the water;
- smoke pollution;
- unauthorized drains;
- contaminated underwater sources;
- mechanical contamination of water.

The study of samples from the Yenisei River at the test facility was carried out in a similar way to the samples of the Moskva River.

The criterion of toxicity of the studied waters was also a significant difference in the number of chlorella

cells observed in the upper zone of the cuvette in a sample containing no toxic substances (control), compared with the same indicator in the test sample (experiment).

The results of biotesting samples of the Yenisei River are presented in Table 3.

Table 3: Biotesting of the Yenisei River.

| Concentration of the water sample, % | The value of the optical density, D | | The difference D in the tested water, in % of the control | The difference D in the tested water, in fractions to the control |
|---|---|---|---|---|
| | control | experience | | |
| control | - | - | - | - |
| 1,2 | 0,132 | 0,132 | 0 | 0,00 |
| 3,7 | 0,132 | 0,132 | 0 | 0,00 |
| 11 | 0,132 | 0,112 | 15 | 0,15 |
| 33 | 0,132 | 0,118 | 11 | 0,11 |
| 100 | 0,132 | 0,073 | 45 | 0,45 |

Based on experimental data, it can be concluded that toxic effects occur at sample concentrations of 100 %, that is, in an undiluted sample. At lower concentrations, the growth processes of test objects in the samples are suppressed by 20 %. In the test sample, pollutants had an acute toxic effect on chlorella when diluted by 2.2 times.

Studies have shown a high sensitivity of the test object to contamination with basic pollutants. The use of biotesting technology using chlorella algae to assess the quality of natural waters is advisable.

### 2.1.3 The Bazaiha River

The object of the study is The Bazaiha River, which belongs to the basin of The Yenisei River, flowing into it near the city of Krasnoyarsk. It originates from the uninhabited settlement of Sukhaya Bazaiha. The length is 128 km, the catchment area is 1000 square km.

A large private sector is located in the water protection zone of The Bazaiha River, which has a significant impact on the ecological condition of the watercourse (Shpet, Spitsyna, 2021).

Figure 3 shows a satellite image of the river.

Figure 3: The Bazaiha River.

The results of biotesting samples of The Bazaiha River are presented in Table 4.

Table 4: Biotesting of The Bazaiha River.

| Concentration of the water sample, % | The value of the optical density, D | | The difference D in the tested water, in % of the control | The difference D in the tested water, in fractions to the control |
|---|---|---|---|---|
| | control | experience | | |
| control | - | - | - | - |
| 1,2 | 0,132 | 0,135 | -2 | -0,02 |
| 3,7 | 0,132 | 0,129 | 2 | 0,02 |
| 11 | 0,132 | 0,133 | -1 | -0,01 |
| 33 | 0,132 | 0,113 | 14 | 0,14 |

| | | | | |
|---|---|---|---|---|
| 100 | 0,132 | 0,043 | 67 | 0,67 |

Based on experimental data, it can be concluded that toxic effects occur at sample concentrations of 100 %, that is, in an undiluted sample. At lower concentrations, the growth processes of test objects in the samples are suppressed by 20 %. In the test sample, pollutants had an acute toxic effect on chlorella when diluted by 2.6 times.

Studies have shown a high sensitivity of the test object to contamination with basic pollutants. The use of biotesting technology using chlorella algae to assess the quality of natural waters is advisable.

### 2.1.4 The Kacha River

The Kacha River originates near the village of Kacha in the Yemelyanovsky district of the Krasnoyarsk Territory, in the spurs of the Eastern Sayan. It flows into The Yenisei River in Krasnoyarsk. The length of the river is 103 kilometers. The original name of The Kacha River is Izyr-Su.

The Kacha River is a left tributary of the river. The Yenisei River flows through the territory of the Kozulsky and Yemelyanovsky districts. The length of The Kacha River is 120 km, the mouth is located within the city of Krasnoyarsk. The catchment area is 1,270 square kilometers. The average flow rate of the river is 0.4 – 0.5 m / sec, the depth is from 0.1 to 2 m, the average width of the riverbed is 10 – 15 m, in high water it can be 35 – 40 m. The nature of the soil is heterogeneous, rocky soil prevails in the upper reaches, pebble-sandy, silty soils in the lower reaches (Kosarikova, Turovets, Nizovtseva, 2020).

The rivers of the Krasnoyarsk Territory have repeatedly been included in the list of the dirtiest rivers in the territory of the Russian Federation, including The Kacha River.

The Kacha River is a typical mountain river with a rather narrow valley covered with taiga. In the forest-steppe zone, it has a well-developed valley with some contours of terraces. The surface of the floodplain is flat, in places excessively moistened, covered with shrubs and meadow grasses.

Over the past five years, the level in the river has decreased, and there are clear signs of pollution. Common pollutants in the river are phenols, petroleum products, metal compounds: common iron, aluminum, copper, manganese, zinc and chemical oxygen consumption. The river flows through several settlements, which grow every year, thereby increasing the load on The Kacha River system (Kalekulin, Gudaeva, 2022).

Figure 4 shows a satellite image of the river.

Figure 4: The Kacha River.

The results of biotesting samples of The Kacha River are presented in Table 5.

Table 5: Biotesting of Kacha River.

| Concentration of the water sample, % | The value of the optical density, D | | The difference D in the tested water, in % of the control | The difference D in the tested water, in fractions to the control |
|---|---|---|---|---|
| | control | experience | | |
| control | - | - | - | - |
| 1,2 | 0,126 | 0,109 | 13 | 0,13 |
| 3,7 | 0,126 | 0,112 | 11 | 0,11 |
| 11 | 0,126 | 0,111 | 12 | 0,12 |
| 33 | 0,126 | 0,097 | 23 | 0,23 |
| 100 | 0,126 | 0,084 | 33 | 0,33 |

Based on experimental data, it can be concluded that toxic effects occur at sample concentrations of 33 and 100 %. At lower concentrations, the growth processes of test objects in the samples are suppressed by 20 %. In the test sample, pollutants had an acute toxic effect on chlorella when diluted by 4.0 times.

Studies have shown a high sensitivity of the test object to contamination with basic pollutants. The use

of biotesting technology using chlorella algae to assess the quality of natural waters is advisable.

## 3 CONCLUSION

Chlorella algae is a good biological object to reduce pollution of reservoirs, since they use pollutants as a food source.

With a high reproduction of Chlorella in the reservoir, the number of zooplankton increases, as a result of which the natural nutrition in the reservoir increases, which is a favorable factor for fish reproduction. But, despite all the advantages of this method of reducing the toxicity of waters, Chlorella tends to multiply rapidly, so there is a need to monitor and control the population of algae.

Optical measurement of cell density made it possible to observe the stimulation of their growth. This fact tells us about the increased toxicity of samples taken from different water bodies in Krasnoyarsk and Moscow. The sample taken from The Moscow River has the greatest toxicity.

The fact that a water body is located in an urban area increases the anthropogenic load on the river system. For this reason, the toxicity of the waters of The Moskva River in Moscow and The Kacha River in Krasnoyarsk is similar.

In turn, the high level of chemical pollution of The Yenisei River is known, but given that The Yenisei River occupies the first place in Russia in terms of runoff, it is obvious that the degree of dilution and the ability to self-purify contribute to reducing the overall toxicity of water samples.

The Bazaiha River, despite the fact that it flows to a greater extent outside the territory of the urban agglomeration, organic runoff from agricultural and forest lands affects the increase in water toxicity.

The significance of the study lies in the fact that the possibility of an adequate assessment of the toxicity of analyzed water samples using test organisms has been established.

At the same time, the high sensitivity of the test object, as the weakest link in the ecological system, allows predicting the final result of the toxicity assessment in vivo with high accuracy and reliability.

Thus, the use of biotesting technologies using chlorella algae to assess the quality of natural reservoirs is advisable.

The implementation of the proposed unified method of water toxicity can serve as the basis for a systematic analysis of environmental factors for the development of information and analytical models of population health, providing an assessment of risk factors.

## REFERENCES


Onerkhan, G., Durmekbaeva, S. N., Akhmetova, N. P., 2019. Biotesting of Lake Kopa pollution using Chlorella sp-3K cells. *Bulletin of Science and Education*. №18 (72).

Volchanskaya, A. A., Zrazhevskaya, M. S., Nikolaenko, S. N. 2016. Assessment of water toxicity in various regions of the Southern Federal District of the Russian Federation. Young scientist. № 17 (121). P. 315-318.

Speedinskaya, A. A., 2019. Determination of the toxicity of Kurgan water using chlorella algae. Young scientist. № 5 (243). P. 100-101.

The method of determining the toxicity of samples of surface fresh, groundwater, drinking, wastewater, water extracts from soil, sewage sludge and waste by changing the optical density of chlorella algae culture (Chlorella vulgaris Beijer) HDPE 14.1:2:3:4.10-04 16.1:2.3:3.7-04. Moscow, 2004. 26 p

Yashin, I. M., Vasenev, I. I., Gareeva, I. V., Chernikov, V. A., 2015. Ecological monitoring of the waters of the Moskva River in the metropolitan metropolis. Izvestiya TSKHA. №5.

Badmaeva, S.E., Sokolova, Yu.A., 2017. Monitoring of the hydrochemical state of the Yenisei River in Krasnoyarsk. *Bulletin of the KrasGAU.* №8.

Shpet, S.D., Spitsyna, T.P., 2021. Dynamics of the surface water pollution index of the Bazaiha river of the Krasnoyarsk agglomeration for 1999-2020. *Actual problems of aviation and cosmonautics*..

Kosarikova, D. D., Turovets, V. E., Nizovtseva, P. V., 2020. Problems of small rivers of Siberia the ecological state of the Kacha River water in the city of Krasnoyarsk. Actual problems of aviation and cosmonautics : A collection of materials of the VI International Scientific and Practical Conference dedicated to Cosmonautics Day. In 3 volumes, Krasnoyarsk, April 13-17, 2020 / Under the general editorship of Yu.Yu. Loginov. Volume 2. Krasnoyarsk: Federal State Budgetary Educational Institution of Higher Education "Siberian State University of Science and Technology named after Academician M.F. Reshetnev", P. 491-493.

Kalekulin, V. B., Gudaeva, E. A., 2022. The state and quality of the Kacha river of the Krasnoyarsk Territory. Reshetnev readings : materials of the XXVI International Scientific and Practical Conference dedicated to the memory of the General Designer of rocket and Space Systems, Academician M. F. Reshetnev, Krasnoyarsk, Volume Part 1. – Krasnoyarsk: Federal State Budgetary Educational Institution of Higher Education "Siberian State University of Science and Technology named after Academician M.F. Reshetnev", P. 772-775.