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# Ecological Monitoring Perspectives from Biotesting of Surface Waters: A Study of Pavlovsk Reservoir (Bashkortostan, Russia)

Dmitry Seifert[1, 2], Yagafar Abdrashitov[2], Elena Bakhonina[1] and Inna Ovsyannikova[1]

*1. Department of Ecology and Environmental Management, Branch of Ufa State Petroleum Technological University, Sterlitamak 453118, Russia.*

*2. Department of Chemistry, Branch of Bashkir State University, Sterlitamak 453103, Russia.*

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**Abstract:** The cost of environmental analysis is becoming astronomically high at the global scale. One of the major trends in the respective research activities is the development of biotesting methods. Such methods, in addition to ecotoxicology, are highly demanded for environmental monitoring and ecological standardization. The development of biotesting in toxicology, however, is limited to the "battery of tests" paradigm, while environmental monitoring and ecological standardization are based on the "uniformity of measurements" paradigm. A "reference bioindicator" is proposed to harmonize these approaches. A reference bioindicator serves for comparison of data obtained by different bioindicators. This method was approved for the state environmental control. Application of reference bioindicators makes analytical procedure substantially cheaper. It requires, however, thorough calibration in relation to specific environmental factors (such as temperature, photoperiod etc.) as well as to specific active agents and their combinations. This problem can be solved with the start-up of calibrating analytical centers and long-term study of the effects of spatiotemporal environmental factors in specific areas and for specific reference objects. This paper demonstrates long-term study results for the surface waters of Pavlovsk reservoir (Bashkortostan, Russia).

**Key words:** Biotesting, phyto-testing, garden cress, eutrophication and toxication of water bodies, environmental monitoring, environmental control.

## 1. Introduction

The cost of the environmental analysis is becoming astronomically high at the global scale. This cost includes expenditures for laboratory equipment purchase and carrying out of analysis. On the other hand, we are facing constant expanding of the list of anthropogenic toxic substances polluting our environment and therefore need less expensive and integral methods for the estimation of environmental conditions [1]. Such methods are supposed to be based on the parameters being the combination of various factors, such as the results of physicochemical analysis, natural conditions and specific ecological indicators considering diversity of species with the domination of specific ones, etc..

One of the major trends in the respective research activities is the development of adequate biotesting. Such methods, in addition to ecotoxicology, are highly demanded for environmental monitoring and ecological standardization. The development of biotesting in toxicology, however, is limited to the "battery of tests" paradigm, while environmental monitoring and ecological standardization are based on the "uniformity of measurements" paradigm [2]. Biotesting in the Russian Federation was mostly developed by the introduction of new experimental methods and not by the analysis of accumulated empirical materials. Such an approach reduces

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**Corresponding author:** Dmitry Seifert, Dr. Sci., main research field: ecotoxicology. E-mail: dseifert@mail.ru.

predicting ability of scientific information and complicates comparison of experimental data from different methods. On the one hand, there is a large number of testing microorganisms. On the other hand, they demonstrate different responses to environmental changes.

Environmental monitoring of surface waters requires spatiotemporal dynamics of parameters under analysis. It is necessary to find the optimal ratio among large number of different analyses important for maintaining high quality of life and their cost-effectiveness. The obtained solution was the development of chemical analysis tests including biological testing methods (biotesting) [3-5]. In addition, transformations occurring in the environment lead, to the synthesis of new substances which can be even more toxic than their precursors. Such substances are, for example, methylmercury, detergents with heavy metals in their structure, pesticides, etc..

The first step to the interpretation of ecotoxicity research results is the development of algorithm for their analysis. The example is the method guidance and recommendations for WET (whole effluent toxicity) Testing developed by the US EPA (Environmental Protection Agency) [6]. This method guidance offers the description of analyzed parameter as a dynamic parameter of toxicity for various test objects and degrees of dilution. Our research was dedicated to the development of a biotesting technique capable of harmonizing with the above US method. The minimal difference of these methods is that the dilution percentage is used in its American variant while we used the dilution ratio.

We have developed the procedure for toxicity testing of drinking, ground and waste waters as well as solutions of chemicals by germinating ability, average length and average dry weight of garden cress (*Lepidium Sativum*) sprouts, federal nature protection documents [7]. This method was approved for the state environmental control [7].

Garden cress (*Lepidium sativum*) is one of the most popular test objects for biotesting of water, bed sediments, soils, natural and anthropogenic substrates and radiation as well as effects of synthesized chemicals and their mixtures [8]. This procedure allows quantitative determination of eutrophication degree (eutrophication index) and the analysis of the interaction of eutrophication and toxication processes [9, 10].

Our procedure has three modifications:

First modification is designed for the evaluation of the most important parameters. It uses growth chambers with controlled temperature and photoperiod;

Second method is based on a room-temperature analysis. The disadvantage of this method is lower accuracy of measurements, while the advantage over the first modification is faster analysis;

Third modification offers analysis in ambient conditions (such as at terraces or in unheated premises) during the vegetation period. This modification does not include determination of the average dry weight (Fig. 1).

This procedure is based on the ecological diagnostics methods and tested for the following objects of natural or anthropogenic origin: natural and waste waters, solutions of various chemicals or pharmaceutical formulations in the environment, tobacco, tobacco ash, etc. [11-15].

The experimental objects studied in natural conditions were the surface waters of the Pavlovsk reservoir headwater. It is possible in principle to track the conditions of surface water in space and time [10, 15].

This procedure proved to be also applicable to the quantitative characterization of eutrophication with the consideration of simultaneous occurrence of eutrophication and toxication processes.

Construction of river dams is considered to be mechanical pollution of the environment according to the present-day definitions. Creation of reservoirs

**Fig. 1 The example of modification for ambient conditions.**

results in lowering or stopping of rivers and massive accumulating of biogenic and organic substances in the backwater zone with large flooded areas. River beds also enrich water with biogenic and organic substances from soil leaching and decomposition of flooded plants. Temperature and light conditions change and increase flooding lowers oxygen concentration in water [16].

## 2. Materials and Methods

Pavlovsk reservoir is formed by the Pavlovsk hydropower station dam at the river Ufa, Republic of Bashkortostan, Russia. Pavlovsk reservoir was flooded in 1959-1961. The total area is 120 km$^2$, the maximum width is 2 km, the volume is 1.41 km$^3$, the length is 150 km, and the average depth is 11.5 m. The reservoir is used for the seasonal control of water flow, water transportation, rafting of timber, water supply; it also contributes to the development of energy industry. The main sources of anthropogenic substances in the reservoir are timber, compounds of agricultural origin (mineral fertilizers, effluents from cattle farms, pesticides and toxic chemicals), municipal and industrial wastes from the regions of Chelyabinsk, Sverdlovsk and the Republic of Bashkortostan.

Anthropogenic influence on water bodies is conditionally subdivided into eutrophication and toxication [17]. The eutrophication effect is usually caused by biogenic elements and organic substances usually considered to be harmless. They stimulate growth of various microorganisms. The result may be the disruption of the ecological balance and secondary pollution by various metabolites. Ecosystems face simultaneous pressure from both toxic and eutrophicating factors. The majority of measurements, however, consider toxic effects only. Eutrophication can be evaluated through changed productivity of water systems, various characteristics of phytoplankton and the change of nitrogen and phosphorus concentrations [18-20]. It is necessary to note that eutrophication parameter is included into regulatory documentation without respective procedures, the methods for quantitative evaluation of the eutrophication level and consideration of the joint effect of eutrophication and toxicity [2].

The total amount of only three biogenic elements dumped into Pavlovsk reservoir over a year is about 17,400 tons (9,200 tons of nitrogen, 2,500 tons of phosphorus and 7,700 tons of potassium) [21]. It creates a problem of removing the "faults" of anthropogenic activities. A solution requires achievement of three interrelated sub-objective to measure the degree of object degradation by various parameters in the process of environmental monitoring; to plan the scale and the duration of activities required for the stabilization of an object; and to carry out all the planned activities at the proper time.

Long-term biotesting in the field conditions faces certain limitations such as weather and photoperiod differences. The purpose of the research was the estimation of the surface water conditions at the headwater of Pavlovsk reservoir (Fig. 2). The scale of ecological effects was studied at the "SOLUNI" research and industrial test park in 2011-2013. Four reference cross-sections were chosen for taking water samples. The samples were taken in June 2011, every month of the vegetation period in 2012, in June 2013 and August 2013.

The field modification of the procedure does not include the measurement of the average dry weight of sprouts.

It was demonstrated that the concentration of biogens reduces over a vegetation period in water reservoirs located in the studied natural zone [22].

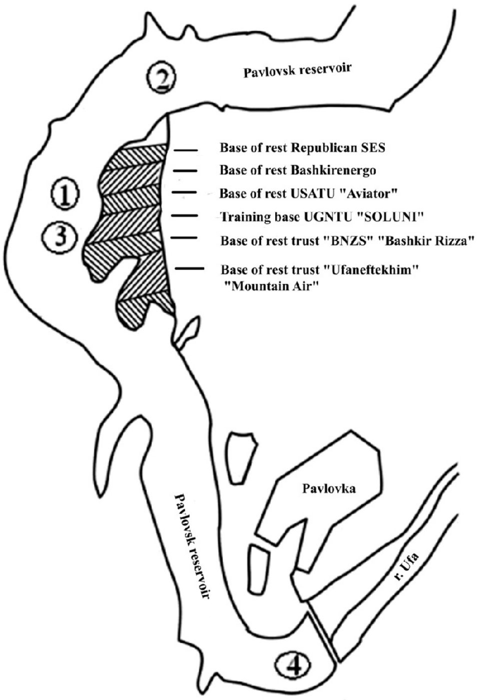


**Fig. 2 Reference cross-sections at the headwater of Pavlovsk reservoir: 1-"Aviator" biological treatment plant discharge site; 2-the recreation cottage area; 3-"SOLUNI" biological treatment plant discharge site; 4-in quiet reach.**

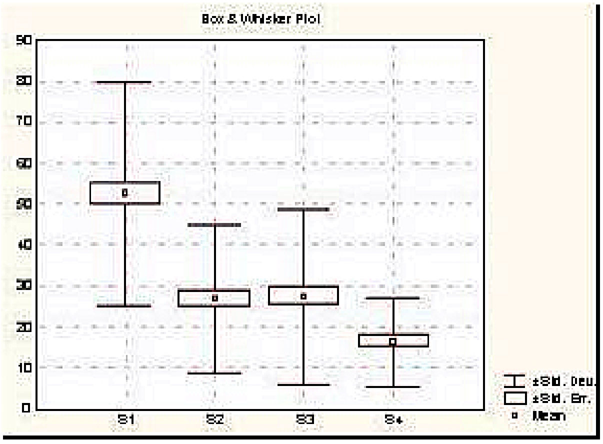


**Fig. 3 The water eutrophication index values (mm) at the reference cross-sections in June 2011.**

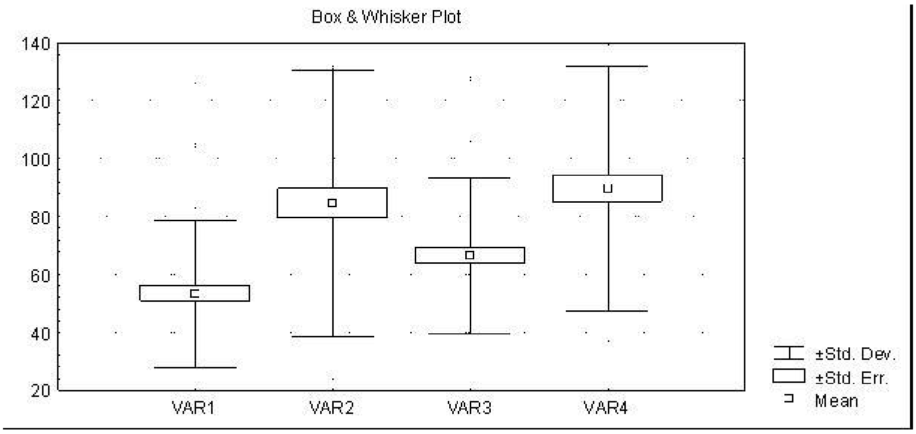
## 3. Results

The following eutrophication index values were obtained in 2011 (Fig. 3). The research results reveal both yearly dynamics of the water eutrophication index and its changes during the vegetation period. In June 2011, the highest index values were observed at "Aviator" biological treatment plant effluent discharge site (cross-section 1), while the minimal were obtained in the area in front of the cross-section (cross-section 4), other values demonstrated no differences. In June 2012, index values were highest at the cross-section 2 and in front of the cross-section (cross-section 4). Cross-section 2 revealed the opposite correlation between the degree of effluent dilution and the average length of sprouts ($r$ = -0.80) indicating sufficiently strong water eutrophication.

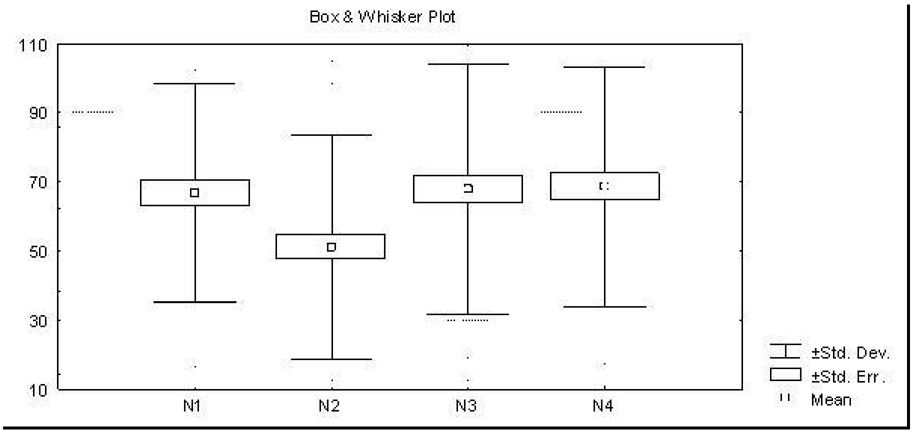
This correlation was not reliable in the other periods (correlation factors were in the range between -0.15 and 0.02). Similar correlation was revealed earlier for the surface waters of the "Belaya" river in the Sterlitamak city area [8]. At the same time, the "Aviator" biological treatment plant effluent discharge site demonstrated no changes in the index values after June 2011 due to the "negative" effect of effluents. Similar reliable but less negative effect of "SOLUNI" biological treatment plant was observed at the cross-section 3.

In 2012, there were short spring and early summer temperatures. These values were obtained again in June, July and August 2012 (Fig. 4).

June



July



August

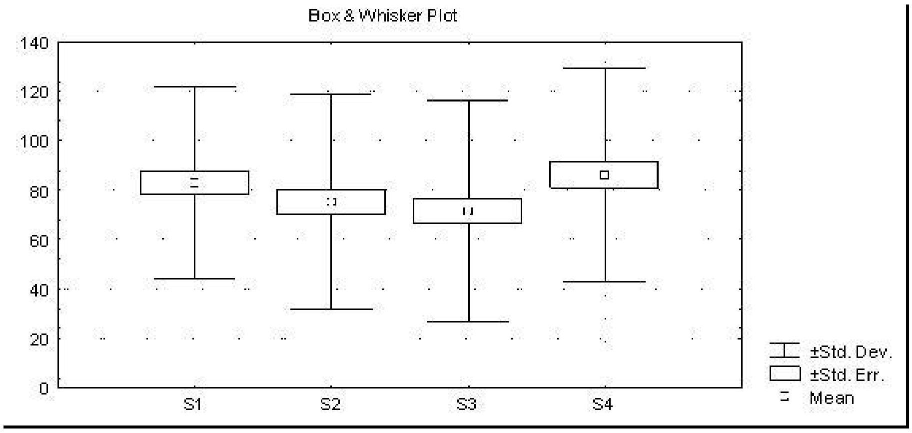


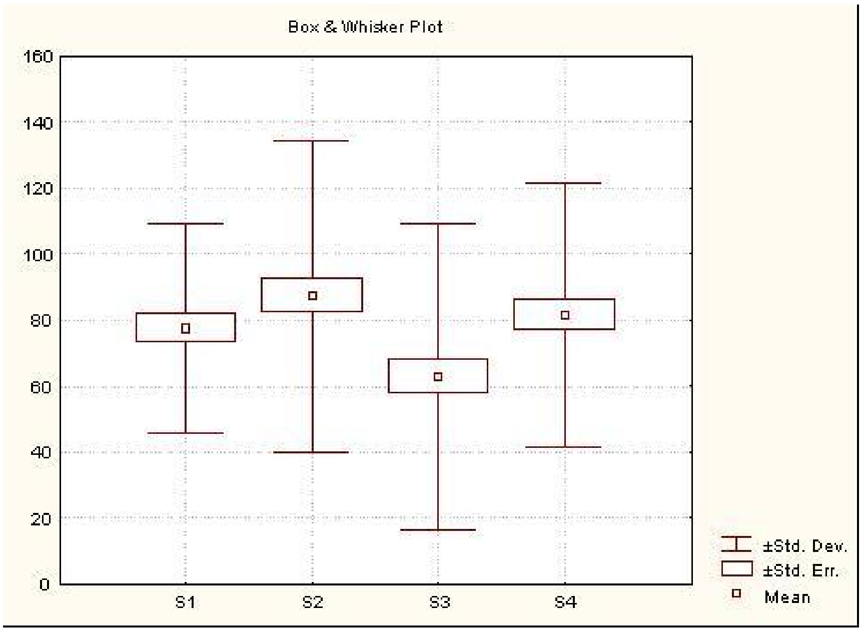
**Fig. 4 The water eutrophication index values (mm) at various sample collection sites in 2012.**

In July 2012, the lowest eutrophication index value was obtained for the cross-section in front of the recreation cottage area (Fig. 4). These values, however, were in total lower than in June 2012 and corresponded to the highest index values in June 2011.

In August 2012, the index values were reliably different for the cross-sections 3 and 4 (Fig. 4). The highest values corresponded to the ones in June 2012.

In June 2013, the index values were reliably different for the cross-sections 1 and 3 as well as for the cross-sections 3 and 4. The reliable occurrence of a toxic effect was observed for the cross-sections 1 (*r* = 0.64), while reliable eutrophication effect was detected for the cross-sections 1, 2 and 3 by the average length of sprouts (*r* = -0.68, *r* = -0.56, *r* = -0.73, respectively) (Fig. 5).

June



August

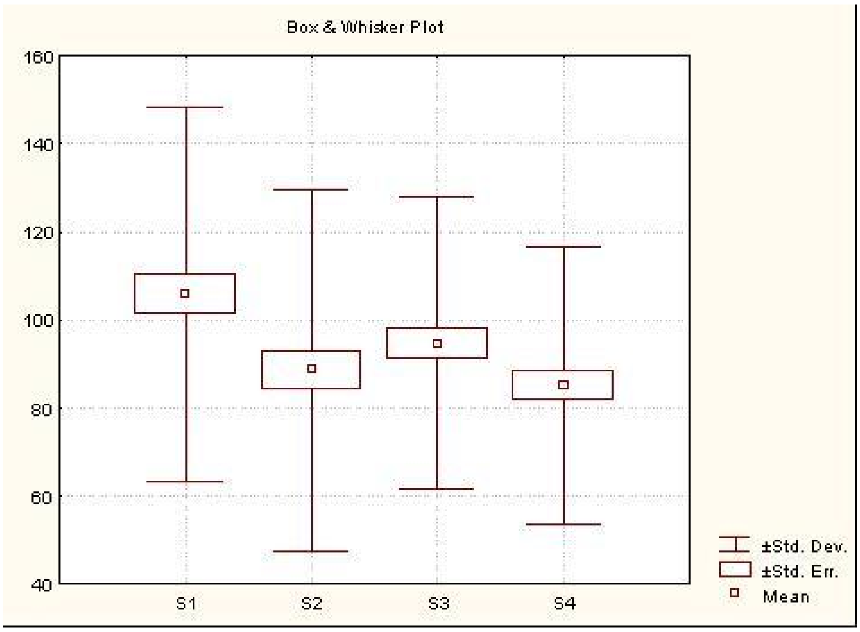


**Fig. 5 The water eutrophication index values (mm) at various sample collection sites in 2013.**

**Table 1 The matrix of correlation coefficients between eutrophication index values at the studied period (2011-2013).**

| Reference cross-sections | Cross-section 1 | Cross-section 2 | Cross-section 3 | Cross-section 4 |
|---|---|---|---|---|
| Cross-section 1 | 1.00 | 0.58 | 0.80 | 0.51 |
| Cross-section 2 | 0.58 | 1.00 | 0.79 | 0.92 |
| Cross-section 3 | 0.80 | 0.79 | 1.00 | 0.85 |
| Cross-section 4 | 0.51 | 0.92 | 0.85 | 1.00 |

The reliable correlation coefficients are marked with bold type.

A reliable influence of toxicity on germinability ($r = 0.68$) was revealed in August 2013, while cross-section 3 demonstrated a long-term eutrophication effect ($r = -0.54$). Eutrophication effects were also revealed in the cross-sections 3 and 4 ($r = -0.62$ and $r = -0.93$ respectively) from the analysis of the average length of sprouts. The influence of toxicity on germinability was detected earlier only for the waste waters meant for biological treatment plants (before and after treatment).

Reliable differences of eutrophication index values were obtained for the cross-sections 1, 2 and 4 as well as for the cross-sections 3 and 4 in August 2013.

## 4. Discussion

It is very important to provide ecologically adequate interpretation of results. The lowest values of eutrophication index for the entire period of studies were observed in 2011. Such differences are probably caused by weather factors. These observations are confirmed by the simultaneous changes of eutrophication index values at various study periods (Table 1).

Due to different correlations between biotesting parameters and the dilution rate of affecting samples, it is possible to suppose that the index value is the resulting combination of the eutrophication level and the degree of water toxicity. In 2011, all the collected samples demonstrated neither suppression nor stimulation of the growth of seeds. In 2012, the eutrophication effect was observed at the reference cross-section only in July (cross-section 2). In 2013, the same effect was revealed at the reference cross-section in June. The stimulating effect was observed for the cross-sections 1 and 3 in June and for the cross-sections 1 and 4 in August. Opposite germinability behavior was revealed in that year for the first time: the toxic effect for the cross-section 1 and the stimulating effect for the cross-sections 3.

The cross-section 2 is located upstream to the local discharge sources and therefore, is considered as the reference site demonstrating integral conditions of the surface waters at the reservoir region under study. The values of eutrophication index at the cross-section 2 change in a way similar to the behavior of the cross-section 4 (Table 1).

The “Aviator” base effluent discharge site is the most toxic of the local sources. “SOLUNI” biological treatment plant effluent site also contributes to the resulting negative outcome in certain periods. The most stable behavior of the studied area of Pavlovsk reservoir was observed in 2011, with the most unstable one in 2013. This research needs to be continued to draw a solid conclusion on increased eutrophication level at the studied area of Pavlovsk reservoir. Further research activities will involve the analysis of collected data and the contribution of weather factors and will result in the development of a model predicting eutrophication levels and toxication of water levels. Such a model will be easily applicable to the characterization of other water reservoirs in the Republic of Bashkortostan, Russia.

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