KnE Life Sciences

BRDEM-2019
International applied research conference
«Biological Resources Development and Environmental Management»
Volume 2020

Knowledge E
enriching | engaging | empowering

Conference Paper

Bioindication of Surface Water of Fish Ponds and Streams for the Dynamics of Phosphatase Activity in Hepatopancreas of Aquatic Organisms

Nikolay Byshov[1], Svetlana Nefedova[1], Alexey Korovushkin[1], Sergey Borychev[2], Ivan Uspenskiy[1], and Elena Vorontsova[2,3]

[1]Ryazan State Agrotechnological University named after P.A. Kostychev, Ryazan, Russian Federation
[2]Federal State Budget Scientific Institution the All-Russian Research Institute of Irrigated Agriculture, Volgograd, Russian Federation
[3]Federal Scientific Center of Agroecology, Complex Meliorations and Protective Afforestation of RAS, Volgograd, Russian Federation

Abstract

Bioindication allows monitoring the ecological state of rivers and ponds. It is the mechanism of the environmental effect on aquatic organisms that the dynamics of interior parameters makes it possible to determine the type of the effect, single emergency or retrospective, even if the environmental damage to surface waters was caused a long time ago. Due to this, the efficiency of taking timely measures to clean toxic water is high. The use of the dynamics of phosphatase activity in hepatopancreas of *Unio pictorum* mollusks as a test reaction is true to present the picture of pollution. In a case of short-term pollution by household or agricultural wastewater, an increase in the activity of phosphatase in the hepatopancreas of mollusks by 1.5 units/g of protein after 12 hours and by 0.8 units/g of protein after 18 hours was noted, etc. As a result of studies, test responses of mollusks allowed graphs to be constructed to determine the toxicity of the environment. They are proposed for preventive work to identify contamination of fish ponds and streams used for watering of farm animals.

Keywords: bioindication, biotesting, mollusk, phosphatase activity, surface water toxicity, copper, zinc, lead.

Corresponding Author:
Elena Vorontsova
fsd_58@mail.ru

Received: 24 December 2019
Accepted: 9 January 2020
Published: 15 January 2020

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the BRDEM-2019 Conference Committee.

1. Introduction

The growth of anthropogenic load on agroecosystems, the deterioration of water quality and the state of water bodies have a significant impact on the performance of a water farm, significantly limit the development of water-intensive sectors of the economy, worsen living conditions and effect of watering of agricultural animals. The expanding scale of irrational water use, taking into account current and future development against

the background of limited water resources, gives rise to a number of environmental problems [1-4]. To predict adaptability to conditions of increasing anthropogenic load, first of all, it is necessary to conduct a comprehensive study of the state of the habitat in conjunction with the physiological aspects of bioindication and biotesting [5-8].

Studies of the adaptation of mollusks for prognostic testing on the basis of biochemical parameters, enzymes in particular, are the actual direction of factorial ecology. Adaptation is essentially a set of biological features of species that provides a specific response to the changing environment. The level of adaptive reactions to the environment determines the molecular mechanisms that ensure the optimal physiological activity of the whole organism.

The interest in indicator organisms with long lifespan and a fairly rapid ``response effect'' to environmental changes is relevant. It is very important that such invertebrates in water bodies under test are numerous and available for study [9-11].

According to the hypothesis, in a case of some regularity between the interior marker indicator and the results of traditional water quality studies for the same period of time, these mollusks can be used to create a method that allows one-time research to determine environmental toxicity.

Hence, the purpose of the research was to identify marker indicators for bioindication of the ecological state of surface waters of ponds and streams used for watering of farm animals, according to the dynamics of phosphatase activity in hepatopancreas of mollusks.

2. Materials and Methods

Mollusks of genus *Unio* were chosen as an experimental object of investigations. Due to the long period of life and the type of food, they were the key to creating a method of bioindication of surface waters used for watering of farm animals. Due to long life expectancy, the use of these mollusks as indicators of the environment allows to obtain data on the toxicity of water in the pond for the required period.

The content of zinc, lead and copper in the mollusk body was determined according to GOST 30178-96, ISO 8288-86. The detection of phosphatase activity in soft tissues of mollusks was carried out in accordance with the method of S.A. Nefedova et al. [10]. Biochemical analysis of phosphatase activity was performed on CFK-2 at a wavelength of 415 nm.

To study the effect of a single release of high concentrations of copper into the water, an experiment was simulated when organisms were divided into 4 groups.

The experiment was carried out in three parallels. Group 1 (control, n=75) was under conditions when the concentration of a heavy metal in water was fine, group 2 (n=75) was placed in water with a heavy metal concentration of 1.5 MAC for the first 4 hours, group 3 was kept for 4 hours in water with a heavy metal concentration of 3 MAC and the group 4 had a heavy metal concentration of 10 MAC. After a 4-hour exposure, the mollusks were returned to water with a heavy metal concentration within the normal range, which was typical of their natural habitat in the pond, where invertebrates had been caught for the experiment. To analyze the complex effects of several heavy metals, 4 groups of mollusks were formed. The experiment was carried out in three parallels: group 1 (control, n=75) was under conditions when the concentration of heavy metals in water was normal, group 2 (n=75) was placed in water where the heavy metal content during the first 4 hours was kept within 5 MAC of zinc, 10 MAC of copper and 1.3 MAC of lead. After a 4-hour exposure of the mollusks in the experimental environment, they were returned to clean water, typical of their natural habitat in the pond.

Biometric processing was carried out according to generally accepted methods using the standard Excel software package.

3. Results and Discussion

The surface water of ponds and streams often has concentrations of copper ions dangerous for the environment. The high content of this substance can disrupt the enzyme system of the mollusk liver.

The determination of the effect of copper ions from surface water on the phosphatase activity in *Unio pictorum* hepatopancreas showed that the reaction of mollusks that did not adapt to the high copper content in the medium was the same during the first 12 hours - a sharp increase in the phosphatase concentration of 5.9-8.1 units with previous decrease of 25% during the first 4 hours (Table 1, Figure 1).

The tendency to a slight decrease in the level of phosphatase, followed by a sharp increase, was maintained both in organisms placed in water with low pollution (1 MAC, 3 MAC) and in water with a high content of copper ions (10 MAC). This was due to the primary reaction of the body to enter the stress environment.

The initial reaction to stress is followed by an inhibition reaction. The time of the characteristic inhibition reaction for organisms from different groups differs.

The secondary reaction of inhibition in a group of organisms, placed in an environment with copper ions concentration lower than MAC, exhibits itself after 24 hours by 9% decrease in phosphatase activity, followed by 4.4% increase after 48 hours.

TABLE 1: The dynamics of phosphatase activity of hepatopancreas of *U. pictorum* under the action of short-term contamination with copper ions.

Copper (MAC)	Exposure time					
	before the introduction of copper ions	4 hours	12 hours	18 hours	24 hours	48 hours
	phosphatase activity, units / g of protein					
10 MAC	4.5±0.10	3.5±0.08	8.1±0.20	7.9±0.26	5.4±0.11	4.2±0.13
3 MAC	4.5±0.11	4.0±0.09	6.7±0.13	6.0±0.14	4.9±0.12	4.2±0.09
1 MAC	4.5±0.12	4.2±0.13	5.9±0.20	5.1±0.16	4.1±0.15	4.7±0.07
Norm	4.5±0.09	4.5±0.10	4.5±0.10	4.5±0.10	4.5±0.11	4.5±0.07

The reaction of inhibition in organisms in a case of acute pollution with copper was manifested only after 48 hours, and it was 6.7%.

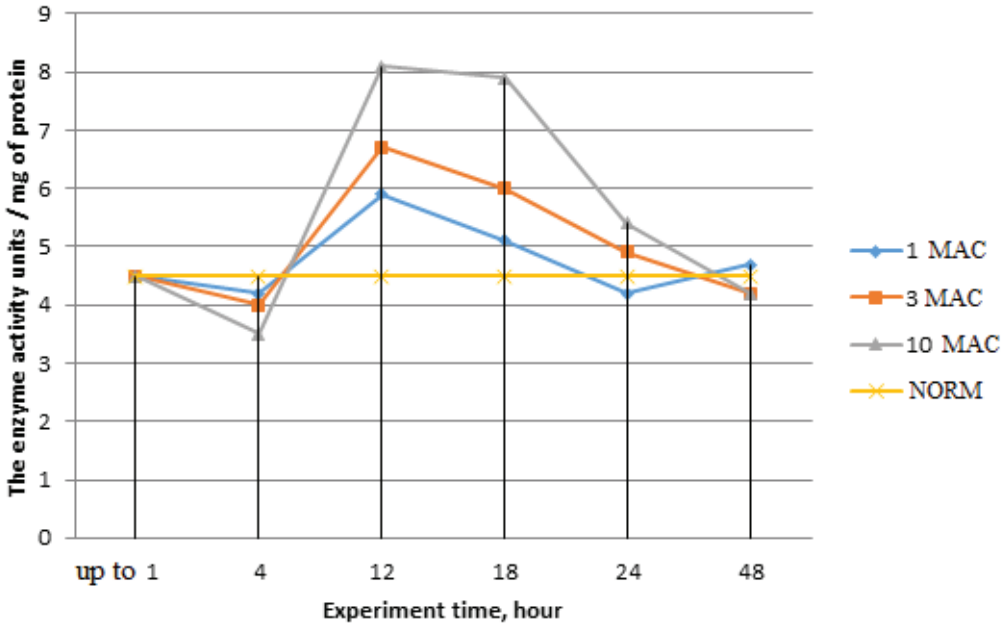

Figure 1: The graph reflecting the dynamics of the phosphatase activity of hepatopancreas of mollusks for bioindication of copper ions in the medium.

The investigation results showed that primary stress reactions, even when mild contamination, appear strong enough to use the phosphatase reaction in hepatopancreas of mollusks as a test reaction, especially to find the source of contamination quickly. It should be noted that the duration of stress reactions when acute pollution is much longer. The phosphatase level in the test group of organisms that were in an

environment with a 10-fold excess of MAC of copper ions between the 12th and 18th hours of the experiment decreased only by 2.5%. At the same time, the organisms from the test group kept in the medium with a 3-fold excess of MAC of copper ions showed a decrease in the activity of phosphatases by 10%.

The practice shows that surface water pollution from industrial enterprises is complex. Different components of effluent streams may act as antagonists to each other. In other cases, a synergistic effect may occur, which should affect the enzymatic activity in organs of mollusks. The use of mollusks during bioindication gives a technique with high sensitivity, integrity and versatility.

In the next experiment, the stress response of mollusks to a single complex environmental pollution with heavy metals was analyzed. The concentration of zinc in the experiment was 5 MAC (sample 1), that of copper was 10 MAC (sample 2) and that of lead was 1.3 MAC (sample 3) (Table 2, Figure 2).

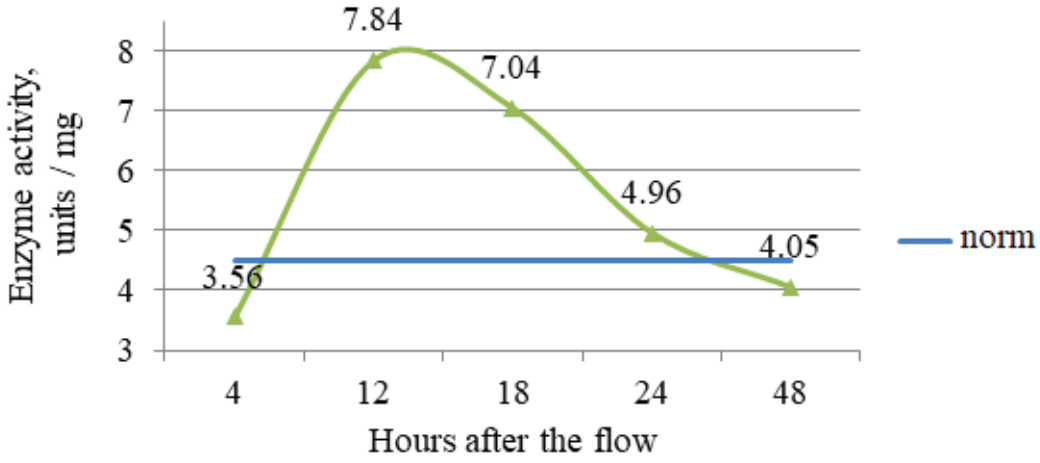

Figure 2: The graph reflecting the dynamics of phosphatase activity for a single complex pollution of the environment with heavy metals.

The dynamics of mollusks' stress reaction manifests itself in the same way when complex pollution of the environment with several heavy metals and when water is polluted with one of heavy metals.

In this case, the phosphatase activity reaches the maximum during the first 12 hours, then a gradual decrease in this parameter occurs and after 48 hours of the experiment the enzymatic activity of hepatopancreas of mollusks is restored to its normal value. This should be taken into account when conducting bioindication of surface water with a single exposure to heavy metals in order to promptly find the source of contamination. Bioindication studies of the dynamics of enzymatic activity in *Unio pictorum* hepatopancreas allow for an operational search for a source of unauthorized exposure

to a water body. It causes a short-term change in hydro chemical parameters of water, while bottom sediments do not have time to accumulate pollutants and remain clean.

Table 2: The activity of mollusk phosphatase in experimental environments.

Sample	Hours after exposure				
	4	12	18	24	48
Sample 1	3.9±0.09**	7.26±0.07***	5.7±0.09***	4.72±0.06*	4.26±0.05*
Sample 2	3.34±0.05***	8.13±0.09***	7.98±0.09***	5.38±0.06*	4.24±0.03**
Sample 3	3.56±0.19**	7.84±0.21***	7.04±0.33***	4.96±0.29	4.06±0.27
Norm	4.5±0.07	4.5±0.05	4.5±0.07	4.5±0.06	4.5±0.06

Note: πa * - ≤ 0.05; ** - ≤ 0.01; *** - ≤ 0.001.

In such situations, it is effective to use the dynamics of phosphatase activity in hepatopancreas of mollusks, caused by the stressing of hydrobionts as a result of a sharp short-term change in the environment as a test reaction.

The use of the dynamics of phosphatase activity in *Unio pictorum* hepatopancreas reliably reflects the picture of short emergency pollution of a pond. At the same time, the marker parameter of the test reaction of the dynamics of enzymatic activity during short-term contamination of surface water is an increase in phosphatase activity in hepatopancreas of mollusks by 1.5 units/g of protein after 12 hours and by 0.8 units/g of protein after 18 hours.

4. Conclusion

The use of mollusks *Unio pictorum* as test objects for bioindication of surface water, namely the dynamics of their phosphatase activity in the liver, showed a high indicator ability of this species of invertebrates. The developed method makes it possible to identify one-time short-term pollution and long-term exposure to toxic substances from surface water used when watering farm animals. This is relevant for the rapid search for a source of pollution.

Mollusks are universal hydrobionts for bioindication, since their response to exposure to pollutants is of the same type, not specific both to complex environmental pollution by a wide spectrum of toxicants, and to a certain pollutant. The mechanism of influence of environmental conditions on the organism of mollusks is such that by changing their interior parameters one can not only accurately determine the type of impact (chronic or one-time) and that of exposure - even if environmental damage to the water body was caused some time ago, but also to predict the succession changes of this water body. Having studied the mechanism of pollutants accumulation in the body of mollusks,

identified patterns of their impact on biochemical parameters of the object under study and determined the correlation links between these data, graphs for bioindication of the surface water used to water farm animals were made.

The introduction of the developed methodology to identify the dynamics of phosphatase activity in hepatopancreas of mollusks to search for single sources of pollution has proven itself. It showed not only a high degree of reliability, but also excellent work in conditions of high background copper content. So, it allowed to use this technique in order to search for sources of pollution in regions with different physiographic conditions, manifested in high salinity of surface water.

Acknowledgement

The authors would like to thank their colleagues for their contribution and support of the research. They are also thankful to all the reviewers who gave their valuable inputs to the manuscript and helped in completing the paper.

Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Aleksandrova, M. A. (2017). Analysis of environmental problems of the Barents Sea and their influence on the condition of the sanitary and nestles stock of the main wealth of Barents Sea north-waterstone of the arctic cod. *Modern Science Success,* vol. 2, issue 12, pp. 153-156.

[2] Baranova, T. V. (2017). Evaluation of the environment at betula pendula cytogenetic parameters. *Modern Science Success,* vol. 1, issue 9, pp. 97-104.

[3] Vikhman, A. A. and Generalova L. P. (2017). *Guidelines for the quantitative analysis of humoral factors of resistance in organs and tissues of fish*, Moscow, VNIIPRH.

[4] Vikhman, A. A., Sitnova, O. V., Generalova, L. P., Shart, L. A. and Borschev, V. N. (2015). *On the methods of studying the variability of the immunophysiological reactivity of fish. The development of aqua- culture in inland water bodies*, Moscow, Moscow University Press, pp. 58-59.

[5] Gabysheva, N. S., Protopopova, A. V., Sorokopudov, V. N. (2017). Gene pool of ribes nigrum l. on stability to illnesses and wreckers in the con- ditions of Yakutia. *Modern*

Science Success, vol. 1, issue 9, pp. 174-180.

[6] Golovina, N. A., Romanova, N. N. and Golovin, P. P. (2017). Ecologo- faunistic analysis of parasites of fish of the Belgorod and Staroos- kolsky reservoirs in *Scientific statements of Belgorod State University.*

[7] Golovina, N.A., Romanova, N.N. (2013). On the occurrence of Pseud- amphistomum truncatum (Rudolphi, 1919) in carp fish in water bodies of central Russia. In the book: Parasitology in the changing world in *Proceedings of the 5th Congress of the Parasitological Society of the Russian Academy of Science* (Novosibirsk, September 23-26, 2013), Novosibirsk.

[8] Dorovskikh, G.N. (2001). *Biodiversity of the European North. Abstracts of international scientific conferences*, Petrazovodsk, pp. 57-58.

[9] Korovushkin, A. A., Nefedova, S. A. (2016). The current state and prospects of commercial aquaculture development, In coll. of the national conference *Innovative development of the modern agro- industrial complex of Russia*, FSBEI HE RSATU, December 12, 2016, pp. 360-362.

[10] Nefedova, S. A., Korovushkin, A. A., Shashurina, E. A. (2015). Bio-testing techniques with the use of aquaculture representatives as test objects, *Agrarian Russia*, Moscow, Folium, no. 4, pp. 35-39.

[11] Nefedova, S. A., Korovushkin, A. A., Minin, D. G., Zutova, L. B. and Ipatov, I. A. (2014). On the issue of selecting test-sensitive hydrobionts for water bio-testing in laboratory, industrial and natural conditions, *Theoretical and Applied Ecology*, no. 3.