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Hematological Indicators of Hydrobionts as a Biomarker of Anthropogenic Pollution of the Aquatic Environment

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Abstract: The problem of preserving the environment, in conditions of intensive development of natural resources, is becoming more and more important every year. In many regions of the world, significant problems with water supply are already noticeable as a result of the quantitative and qualitative depletion of water resources through their irrational use. In the 20th century, hundreds of thousands of tons of organic compounds such as PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls), OCPs (organochlorine pesticides), and inorganic (heavy metals) were produced and partially released into the environment. Water bodies have often been and still are the collectors of many of the listed substances. In civilized countries, the population is seriously concerned about the problem of possible remote and long-term adverse effects of pollutants on the environment in general and aquatic ecosystems in particular. *Objective:* Monitoring the state of aquatic ecosystems and searching for methods to effectively assess the level of pollution and identify its sources. To date, the main assessment of water quality is usually carried out on the basis of chemical-analytical methods. And these methods determine only the presence and amount of chemical elements in the tested water samples, but cannot determine the specifics of the formation of the quality of the tested water samples, due to the very large number of possible combinations of chemical compounds in aqueous solutions, including the behavior of anthropogenic compounds and the natural vulnerability of aquatic ecosystems to the combined effects of their pollution. *Method:* Assessment of the state of organisms living in changing environmental conditions makes it possible to reveal the patterns of responses at different levels of organization (molecular, cellular, organismal), predict the occurrence of diseases, and identify specific biological effects and the etiology of diseases. In this regard, the possibility of using biological markers as tools for assessing water quality is of particular interest. As a *result* of the research, biomarkers have been developed for assessing the quality of the aquatic environment, based on hematological parameters and characteristics of morphological and functional changes in blood cells of aquatic organisms. A change in the blood formula and an increase in the number of cells with nuclear abnormalities when exposed to water samples are shown, for example from the Desna and Dnieper rivers. *Conclusion:* The results obtained can be extrapolated to a certain extent to human health, given the fact that river water is one of the main sources of drinking water for the population of many countries of the World.

Keywords: Aquatic Environment, Biomarker, Biotesting, Hydrobionts, Leukocyte Blood Count, Micronuclei

1. Introduction

The problem of water pollution is becoming more and more acute in most countries of the world, including Ukraine. Changes in environmental factors caused by the growing anthropogenic impact lead to a threatening situation for the survival of living organisms and human health. At the present stage, to determine the degree of impact of anthropogenic pollution, it is necessary and relevant to use cellular biomarkers to assess the quality of natural, including drinking, waters. A polluted aquatic environment has a negative impact on aquatic organisms, leads to an increase in environmental consequences and poses a threat not only to plant and animal organisms, but also to human health [1, 2].

Biotesting is one of the methods of biological control, which involves the targeted use of standard test organisms and methods to determine the degree of toxicity of the aquatic environment, based on measuring the test reaction of an organism, its individual function or system [3, 4]. Biomarkers are understood as any indicators reflecting the interaction of a biological system and a potentially harmful factor, which may be of a chemical, physical or biological nature [5].

Recently, toxicological tests using aquatic organisms have reached significant development. They are used both to study the toxicity of aqueous solutions or natural waters, and to identify carcinogenic, cytotoxic, or genotoxic substances [6].

Therefore, in the complex of measures aimed at preventing negative health effects associated with the factors of the aquatic environment, an important place should be taken by the assessment of water quality, in particular its safety for humans. In this regard, the development of effective methods for assessing both direct and indirect effects of technogenic and other pollutants on living organisms is becoming increasingly important. Anthropogenic changes in aquatic ecosystems cannot but be reflected in the physiological state of aquatic organisms [7].

2. Materials and Methods

2.1. Used Water Samples in Work

To study the impact of anthropogenic pollution, water samples were taken from the Desna River (Oster region) and Dnieper (Hydropark region, Kiev city). In water samples, electrical conductivity and pH were determined, and chemical analysis was performed for the presence of organic carbon and some inorganic substances. Comparison of the obtained indicators with the standards developed for assessing water quality showed that the selected samples of river water were characterized by the absence of significant deviations from the standard indicators, excluding permanganate oxidizability and total organic carbon, the content of which was increased. Control water was prepared in laboratory conditions according to the recommendations of DSTU 4174: 2003 (State Standard of Ukraine).

2.2. Used Test Organisms in Work

The studies were carried out on test organisms - goldfish (*Carassius gibelio*) and clawed frogs (*Xenopus*), grown in laboratory aquariums at the International Academy of Ecology and Medicine. The experiment was repeated twice.

To date, a large number of molecular, cellular and tissue biomarkers have been developed [5]. Biochemical and pathophysiological disorders can be detected in various aquatic organisms, however, indicators of the physiological state of fish are more often used in diagnostics of the consequences of water pollution [8, 9]. For a number of reasons, fish are recognized as the most convenient and representative objects for biomonitoring pollution of aquatic ecosystems. Fish are recommended to be used for screening substances potentially hazardous to humans that cause deformities and cancer, as well as genotoxic substances that get into drinking water [5, 9]. Fish inhabit all water bodies, have a long life cycle and occupy the upper level in the trophic system of water bodies. Compared to invertebrates, fish are easier to identify by species, sex, age, and other biological and ecological characteristics [8]. There is another reason for studying the effects of pollution on fish. Many species are fished and used for human consumption.

Xenopus Spur frogs, this type of test organism is widespread in developmental biology. The ease of handling amphibians and their embryos has made them an important subject in embryology, developmental biology, and aquatic toxicology [9, 10]. This animal is used because of its powerful combination of experimental pliability and close evolutionary bond with humans, at least when compared to many model organisms. *Xenopus* has long been an important tool for in vivo research in the molecular, cellular and developmental biology of vertebrates. However, the wide breadth of *Xenopus* research stems from the fact that additional cell-free extracts made from *Xenopus* are a leading in vitro system for studying fundamental aspects of cellular and molecular biology. Thus, *Xenopus* is the only vertebrate model of the system that allows high-throughput in vivo analysis of gene function and high-throughput biochemistry. They are also widely popular among aquarium animals for studying the toxicology of the aquatic environment, as well as fish, which are relatively undemanding to the conditions of pets [10-13].

2.3. Working Process

To determine the effect of the toxicity of aquatic samples, 30 specimens of each test organism of *Carassius gibelio* fish weighing 25-30 grams and *Xenopus* clawed frogs weighing 60-65 grams were used.

The test organisms were divided into three groups of 10 individuals. Each group was placed in aquariums with control water (1), water samples from the Desna (2) and Dnieper (3) rivers. After 96 hours of exposure, blood samples were taken from each individual from the vein. Blood was taken from fish from the tail vein, from frogs - from the vein of the hind leg. Cytological preparations were prepared according to the standard procedure [14] and analyzed under a light microscope at a total magnification of x1000. 3000 cells were viewed on each preparation. Statistical data processing was performed using Student's t-test, $p < 0.05$ was considered statistically significant [15].

3. Research Results

A study was carried out of the effect of the selected water samples on hematological parameters and indicators of cytogenetic stability of erythrocytes in aquatic organisms. Under the influence of the investigated water samples, a tendency towards a decrease in the percentage of lymphocytes in both test organisms was observed (Table 1). Also, water samples caused a significant increase in the percentage of segmented neutrophils almost twofold, a significant (p<0.05) increase in the number of eosinophils was observed.

Table 1. *Leukocyte formula (%) of fish (Carassius gibelio) and clawed frogs (Xenopus), when exposed to the investigated water samples.*

Corpuscular elements of blood	Control		Desna (Oster)		Dnipro (Hydropark)	
	Fish n = 10	Frog n = 10	Fish n = 10	Frog n = 10	Fish n = 10	Frog n = 10
Rod neutrophils	1,39 ± 0,18	1,45 ± 0,19	1,45 ± 0,19	1,75 ± 0,08	2,54 ± 0,19	2,77 ± 0,12
Segmented neutrophils	2,51 ± 0,19	2,11 ± 0,12	2,66 ± 0,22 *	2,16 ± 0,19 *	3,83 ± 0,22 *	2,98 ± 0,29 *
Eosinophils	0,26 ± 0,08	0,28 ± 0,04	0,76 ± 0,13 *	1,46 ± 0,53 *	4,80 ± 0,14 *	5,77 ± 0,18 *
Basophils	4,52 ± 0,33	4,37 ± 0,36	6,95 ± 0,43 *	5,15 ± 0,63	7,75 ± 0,42 *	8,05 ± 0,61 *
Monocytes	5,33 ± 0,32	5,48 ± 0,42	6,09 ± 0,41	7,02 ± 0,12 *	7,48 ± 0,42 *	7,83 ± 0,45 *
Lymphocytes	85,91 ± 0,57	84,80 ± 0,64	81,69 ± 0,70	82,19 ± 0,54	73,60 ± 0,70 *	72,60 ± 0,66 *

Note: * - p <0.05 compared with the control group.

It was shown that under the influence of the studied samples, in comparison with the control, the level of basophils and monocytes increased, where these indicators almost doubled, and the number of eosinophils increased more than twenty times.

Thus, the study of the leukocyte blood count of fish (*Carassius gibelio*) and clawed frogs (*Xenopus*) reveals the presence of impurities of an undetermined nature in river water, which negatively affect living organisms. In the results [16] obtained allow us to assert that the sample from the Desna River is relatively less contaminated than the samples taken from the Dnieper River. The results obtained directly indicate the possibility of using the leukocyte formula of fish to determine the degree of pollution of aquatic biotopes.

The results of micronucleus analysis are presented in Table 2. The results obtained indicate the influence of anthropogenic pollution of river water on the frequency of appearance of blood cells with a violation of the genetic apparatus. All investigated water samples significantly (p <0.01) increased the proportion of erythrocytes with micronuclei and double nuclei in the blood of experimental aquatic organisms.

Table 2. *Frequencies of nuclear disturbances (‰) in erythrocytes of fish (Carassius gibelio) and clawed frogs (Xenopus) when exposed to the investigated water samples.*

Indicators		The investigated water samples		
		Control	Desna (Oster)	Dnipro (Hydropark)
Fish n = 10 erythrocytes (3000 cells)	MN, ‰	0	2,99 ± 0,61 *	3,75 ± 0,67 *
	2N, ‰	0	1,67 ± 0,50 *	3 ± 0,61 *
Frog n = 10 erythrocytes (3000 cells)	MN, ‰	0	2,67 ± 0,55 *	5 ± 0,77 *
	2N, ‰	0,33 ± 0,20	1,99 ± 0,40*	2,75 ± 0,58*

Note: MN - cells with a micronucleus, 2N - cells with two nuclei, * - p <0.05 compared with the control group.

After 96 hours of incubation in the blood of hydrobionts that were in river water samples, the number of erythrocytes with micronuclei and double nuclei increased significantly (p <0.05). Among these disorders, cells with micronuclei were more common.

It should be noted that the most pronounced was the genotoxic effect in water samples from the Dnieper River, which were obtained from the treatment facilities of the city of Kiev in the Hydropark area.

Anthropogenic pollution of natural waters leads to a weakening of the antioxidant system of aquatic organisms, which is accompanied by an increase in the frequency of genetic disorders in the blood cells of fish and frogs. It should be noted that it is erythrocytes that are the most sensitive target to the action of reactive oxygen species. It is known that heavy metal ions can catalyze the formation of reactive oxygen species [17, 18], and, on the other hand, play the role of inhibitors of individual enzymes of the antioxidant system [19]. In this context, the determination of the number of cells with micronuclei and double nuclei makes it possible to evaluate the integral effect of a fairly wide range of polluting factors of the aquatic environment on the state of marine and freshwater fish. From a methodological point of view, the combination of hematological and cytological methods for studying aquatic organisms makes it possible to obtain information on the mechanism of the toxic action of factors of anthropogenic pollution of the aquatic environment. Taking into account the growing amount of pollutants in the river waters adjacent to regions with a high level of industrialization, these vital signs of aquatic organisms can be used for continuous environmental monitoring of natural waters, as well as for assessing the potential toxicological risk of chemicals present in the water for human health [20].

Thus, the studies carried out to determine the influence of anthropogenic pollution of river waters made it possible to reveal changes in hematological and cytogenetic parameters of aquatic organisms, which can be proposed for bioindication.

4. Conclusions and Recommendations

1) The results of identifying changes in the genetic apparatus of aquatic organisms under the influence of fresh water pollutants can be extrapolated to a certain extent on human health, given the fact that river water is one of the main sources of drinking water for the population of Ukraine and other countries of the World.
2) Relatively simple and fast methods of cytological analysis of the blood of aquatic organisms make it possible to assess the toxicological risk of the presence of anthropogenic pollutants in fresh water.
3) The results of the study can be used in carrying out work on assessing the impact and calculating damage to aquatic ecosystems, both at the stage of designing economic activities, and during its implementation; in environmental expertise of the consequences of emissions of pollutants into water bodies, in the development of programs for monitoring the state of the environment.
4) The objectivity and reliability of biomarkers makes it possible to use the results of this work for assessing and predicting the state of the fish population, as well as for complex ecotoxicological characterization of water bodies.
5) The advantage of such an analysis for the aquatic environment is that both tests - the detection of genetic disorders and changes in the leukocyte formula - can be carried out on the same preparation. The proposed method can be considered relatively humane in that the production of peripheral blood preparations does not require the slaughter of experimental animals.

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