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ТҮРКІСТАН ОБЛЫСЫНЫҢ СУ АЙДЫНДАРЫНА ДАФНИЯНЫ БИОИНДИКАТОР РЕТІНДЕ ҚОЛДАНУ

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Андатпа

Мақалада индикаторлық жұмыстарға арналған су нысандарының ластану дәрежесі, сондай-ақ дафния мен су биотасының реакциясын көрсететін көрсеткіштер келтірілген. Алғаш рет дафния (*Daphnia magna*) тест-объектілерінің ауыспалы тиімділігі бойынша, Түркістан облысының әртүрлі су қоймаларындағы судың уыттылығын биотестілеуде пайдалану арқылы талдау жұмыстары жүргізілді.

Зерттеу жұмыстарының практикалық маңызы бар, өйткені алынған нәтижелер бойынша дафнияны тест-объект ретінде пайдалану тиімділігі көрсетілген.

Бұл жұмыстың басқа зерттеулерден айырмашылығы, *Daphnia magna* тест-ағзасын зерттеуге комплексті тәсіл арқылы қолдану жүзеге асырылды. *Daphnia magna* индикациялық қасиеттерінің көріністерін зерттеу су қоймасынан олардың қоршаған ортаны қалыптастыру функциясын жүзеге асыруға болатын бірқатар суды анықтауға мүмкіндік берді. Суды биоиндикация әдісімен зерттеу нәтижесінде біз судың жай-күйіне баға бере аламыз; зерделеніп отырған экологиялық проблеманы тұжырымдай аламыз, оның туындау себептерін ұсына және негіздей аламыз; экологиялық жағдайды онтайландыру жөнінде ұсыныстар енгізе аламыз. Бұл жұмыс индикатордың басымдылығын көрсетеді – биотестілеу әдістерін қолдану арқылы жүргізілді. Биотестілеу әдістерінің стандартты әдістерден айырмашылығы судың сапасын, уыттылық дәрежесін бағалау кезінде дафнияның физиологиялық жағдайының және өмір сүру деңгейінің өзгеруіне негізделген.

Түйін сөздер: су қоймасы, дафния, судың ластану дәрежесі, тест-ағзалар, биоиндикация, гидробионт.

ПРИМЕНЕНИЕ ДАФНИИ КАК БИОИНДИКАТОР НА ВОДОЕМОВ ТУРКЕСТАНСКОЙ ОБЛАСТИ

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Аннотация

В статье приведены степень загрязненности водоемов для индикаторных работ, а также показателей, отражающих реакции дафний и биоты воды. Впервые проведен анализ эффективности смены тест-объектов – дафний (*Daphnia magna*), его использования для биотестирования токсичности воды при различных водоемах Туркестанской области.

Исследования имеют практическую значимость, так как применение полученных результатов по эффективности использования в качестве тест-объектов дафний.

В данной работе, в отличие от других исследований, осуществлен комплексный подход к изучению тест-организма *Daphnia magna*. Изучение

проявлений индикационных свойств *Daphnia magna*, позволило определить ряд воды из водохранилище, где возможно осуществление их средообразующей функции. В результате исследования воды методом биоиндикации мы сможем дать оценку состояния воды; сформулировать изучаемую экологическую проблему, выдвигать и обосновывать причины её возникновения; вносить предложения по оптимизации экологической ситуации. В отличие от стандартных методик, где оценка степени токсичности основана на изменении показателя выживаемость дафний, в данной работе показана приоритетность показателя – изменение физиологического состояния дафний при оценке качества воды методами биотестирования.

Ключевые слова: водоем, дафния, степень загрязненности воды, тест-организмы, биоиндикация, гидробионт.

APPLICATION OF DAPHNIA AS A BIOINDICATOR IN RESERVOIRS OF THE TURKESTAN REGION

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Annotation

The article shows the degree of contamination of reservoirs for indicator work, as well as indicators that reflect the reactions of *Daphnia* and water biota. For the first time, an analysis of the effectiveness of changing test objects-*Daphnia* (*Daphnia magna*), its use for biotesting water toxicity in various reservoirs of the Turkestan region.

The research is of practical significance, since the application of the results obtained on the effectiveness of using *Daphnia* as test objects.

In this work, in contrast to other studies, a comprehensive approach to the study of the test organism *Daphnia magna* is carried out. The study of the manifestations of the indicative properties of *Daphnia magna* allowed us to determine the number of water from the reservoir, where it is possible to implement their environmental function. As a result of water research using the bioindication method, we will be able to assess the state of water; formulate the environmental problem under study, put forward and justify the reasons for its occurrence; and make suggestions for optimizing the environmental situation. In contrast to standard methods, where the assessment of the degree of toxicity is based on changes in the survival rate of *Daphnia*, this paper shows the priority of the indicator – changes in the physiological state of *Daphnia* when assessing water quality by biotesting methods.

Key words: reservoir, *Daphnia*, degree of water pollution, test-organisms, bioindication, hydrobiont.

Introduction

Water is the only natural resource that has no substitute. As the society develops, the need for it increases all the time. They will inevitably continue to grow wherever terrestrial life forms exist. The problem of water pollution is one of the most urgent. Human activity irrevocably changes the natural regime of water bodies with waste and discharges. Various pollutants that get into the environment can undergo various changes in it, while increasing their toxic effect. Water toxicity – the property of water to cause pathological changes or death of organisms due to the presence of toxic substances in it. Water quality is a characteristic of the composition and properties of water that determines its suitability for specific types of water use [1].

Test organisms are usually selected for those species that, being sensitive to pollution, play a significant role in the ecosystem, being an important link in the trophic network and forming the quality of the habitat. *Daphnia magna*-organisms that are widely used separately for the following properties: indicator-in biotesting, feed value - in fish farming, filtration method of nutrition - in water purification processes [2].

Recently, environmental pollution has become a very urgent problem. The study of *Daphnia* is extremely important for ecological research, since they are a key species for many freshwater ecosystems and can serve as a kind of indicator of the state of the environment [3].

The scale of anthropogenic activity has reached a level where the existing system of environmental monitoring should be supplemented with research using new indicators of bioindication and bioassay, which will allow identifying the main factors of ecosystem stability in critical conditions. Biotesting and bioindication are mandatory elements of a modern water quality control system [4].

The traditional approach to environmental monitoring of the water environment is to use chemical analysis methods to assess the quantitative content of toxic substances. However, chemical analysis does not take into account their integral Toxicological effect on biological objects [5].

Currently, it has been shown that *Daphnia magna* individuals can be cultivated more successfully than others on purely bacterial food and use dissolved organic substances as food. Filtration method of feeding *Daphnia* allows to reduce the degree of trophic reservoirs and increase the transparency of water. The research uses the filtration capacity of *Daphnia* to lighten water in ponds with livestock runoff flowing into reservoirs on the earth's surface [6].

Water quality assessment of reservoirs can be carried out using biological methods that characterize the state of the aquatic ecosystem by the plant and animal population of the reservoir. Bioassay methods are usually carried out in special laboratories and require special conditions. However, their advantage is that they allow you to determine specifically the quality of the pollutant, as well as its quantity and degree of toxicity to living organisms. Bioassay methods can determine the toxicity of water. The criterion of acute toxicity is the death of 50% or more organisms in the analyzed water compared to the control within 24, 48 or 96 hours [7].

Bioindication is a method for determining the quality of the habitat of organisms based on the species composition and indicators of quantitative development of bioindicator species and the structure of their communities.

Bioindicators are organisms whose number or development features serve as indicators of natural processes or anthropogenic changes in their habitat. Their indicator significance is determined by the ecological tolerance of the biological system. Within the zone, a tolerant organism is able to maintain its homeostasis. Any factor that goes beyond the "comfort zone" for a given organism is stressful. In this case, the body reacts with a response of varying intensity and activity, the manifestation of which depends on the type of test object. It is the response that determines the methods of bioindication. We choose *Daphnia* as the most sensitive organisms to pollutants. Crustaceans are in constant motion. Toxic substances can react with a sharp increase in motor response, chaotic movement in space, rapid rotation in one place, slowing down rowing movements, and immobilization [8].

Daphnia belong to the Cladocera crustaceans. They can be found in a wide variety of reservoirs: puddles, ponds, ditches, lakes. *Daphnia* feed on the smallest organisms that live in fresh water: algae, infusoria, etc. *Daphnia* allows you to determine the toxicity of both

wastewater and natural water. Because of its anatomical features, *Daphnia* constantly hovers in the water column and only dead individuals gradually sink to the bottom. The crustacean moves in the water with the help of thick bristles, which they wave like a fan. Vertical movements are usually caused by the search for food, changes in temperature and light. *Daphnia* are endowed with a fairly strong chitinous shell, which is periodically replaced with a new one. Due to the metamorphosis of the chitinous cover, *Daphnia* change their weight. The crustacean has an elongated head with a thin nose and one faceted eye. The organ of vision allows you to detect accumulations of algae or bacteria. Once in the area where food objects are found, *Daphnia* uses its abdominal legs to draw water and bacteria into a kind of filter made of furry legs. And finally, after passing the abdominal formations, food enters the mouth. By passing large volumes of water through their bodies, they are able to accumulate significant amounts of toxic substances, thereby contributing to the natural self-purification of water. The rate of accumulation of pollutants in this group of organisms is very high. *Daphnia* are sensitive even to small concentrations of some salts, for example, adding copper salts at a concentration of 0.01 mg / l causes a slowdown in the movements of crustaceans, they either sink to the bottom or freeze at the surface film of water [9].

Given that *Daphnia* live and actively reproduce in clean reservoirs, their absence in the water is quite significant. These small crustaceans are very sensitive to chemical and radioactive contamination and can therefore serve as indicator organisms for water purity. Even urban wastewater diluted by 10 times is toxic to *Daphnia* in the place of wastewater discharge, the mortality rate of these crustaceans reaches 94-100%. Small *Daphnia* species (*Daphnia pulex*, *D. longispina*) are less sensitive to contamination than large *Daphnia magna*, but they also do not occur in wastewater, where the degree of purification is close to the minimum [10].

Advantages of *Daphnia* as an object of biological research:

1. Transparency of the body of *Daphnia* allows you to observe changes in the physiological parameters of the object during the experiment, such as growth, reproduction, heart rate, etc.

2. The Short life cycle makes it possible to use *Daphnia* in fertility tests, as well as rapid growth and reproduction make it possible to conduct experiments quickly.

3. *Daphnia* are very sensitive to changes in the environment, so they are often used as indicators of pollutants in the aquatic environment. Also, in response to various environmental factors, they change shape and color, grow mustaches, spikes, and so on.

4. Under good conditions, *Daphnia* reproduce by parthenogenesis. In this type of reproduction, the offspring is a clone of the parent, which is a necessary condition for conducting experiments based on comparing the reaction of genetically identical animals to various factors. Under unfavorable conditions, only males are born, usually this indicator is used to find out the survival and well-being of the species in these conditions.

5. *Daphnia* Fecundity is a sensitive criterion for radiation studies.

6. *Daphnia* is an accessible test object, which is important for many tests [11].

Daphnia is a standard test object that is used mainly in determining toxic substances in water. Methods of bioassay using *Daphnia* are mainly based on recording their mortality from the action of certain toxic substances on them (Fig. 1).

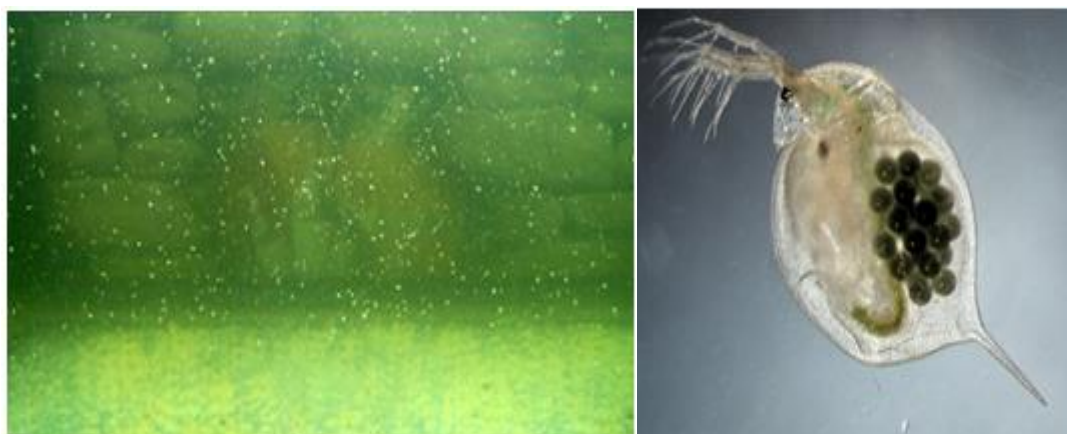


Figure 1 – Appearance of the *Daphnia magna* test organism

At an early stage of water bioassay, changes in the behavioral activity of *Daphnia* (heart rate, motor activity, reproduction intensity) are also an indicator of the presence of toxicants in the studied environment. *Daphnia* are sensitive to even small concentrations of toxic substances that cause their movements to slow down [12].

The purpose of this work is to assess the quality of reservoirs in Turkestan by the survival and fertility of *Daphnia*, which can be defined as water quality.

Objects and methods of research

Materials and methods of research the necessary information is provided on the research work that was carried out at the laboratory of the research Institute of Ecology.

For biotesting of water taken from reservoirs the following locations: Orangay, Kentau, Karnak, Zhuynek.

Determination of mortality and fecundity of *Daphnia*, according to the "Guidelines for determining the toxicity of water, sediments, pollutants and drilling fluids by bioassay" (2002) [13], "Methods for determining the toxicity of water and water extracts from soils, sewage sludge, waste by mortality and changes in fecundity of *Daphnia*" (2007) [14].

The bioassay was performed at a temperature of $20 \pm 2^\circ\text{C}$. For the analysis, one-day specimens of *Daphnia magna* culture were used, which were planted in the test water, and the control was the cultivation water. Accounting for mortality and changes in their fertility was determined for a period of up to 96 hours.

For cultivation of *Daphnia*, glass vessels with a capacity of 250 ml are used. The initial density of *Daphnia* is from 10 individuals per 100 ml. In the room where the *Daphnia* culture is located, there should be no harmful gases and vapors. Experiments are performed in three repetitions.

This study was conducted in the fall of 2020. samples were taken from reservoirs in Turkestan. Biotests were performed by the aquatic invertebrate branchial crustaceans *Daphnia Magna*, which can determine both water quality and toxicity.

Daphnia is not fed during the experiment. In short-term experiments, the main indicator of environmental toxicity is the survival rate of crustaceans.

The time of death of crustaceans is marked by the onset of immobility (immobilization): *Daphnia* lie at the bottom of the glass, swimming movements are absent and do not resume with a light touch of a jet of water or swaying the vessels. After the end of the experiment, the survival rate of *Daphnia* was calculated. The account of the surviving *Daphnia* were repeated at 1, 6, 24, 48, 72 and 96 hours (Fig. 2).



Figure 2-the course of the experiment, the degree of toxicity of wastewater using the Daphnia Magna test object

Equipment-aquarium, magnifying glass, funnels, thermometer, pipettes, glass tubes, Petri dish, microscope, glass containers for water sampling, glass tubes with a diameter of 5-7 mm for Daphnia sampling.

The results of the study and their discussion

After the end of the experiment, Daphnia is counted. Calculate the percentage of surviving individuals. A water sample is assessed as toxic if more than 50% of Daphnia die in it during the 24-hour experiment compared to the control. When determining the water pollution zone, the behavior of Daphnia was taken into account (table 1).

Table 1-Bioindication using Daphnia

Pollution zone	Indicative changes in Daphnia
1st – heavy pollution (close to the source of pollution)	Partial destruction of specimens specimens are kept in natural coat, the part loses its activity, there are cases of "whirligig". There is precipitation on the antennas, clogged filtration devices. Dying individuals have a pink diffuse color.
2nd – average pollution	Increased activity is replaced by depression, Daphnia periodically lie on the bottom, individuals have an empty intestine, a cloudy yellow color, heartbeats are weakened, there are no fat drops.
3rd – low pollution (remote from the source)	The presence of increased activity in some individuals, in others-periods of activity are replaced by a normal state; the intestines are poorly filled.

The results of monitoring the behavior of individuals and counting dead Daphnia in experimental and control samples are shown in table 2.

Table 2 - Assessment of water quality in the cities of Turkestan and Kentau

Selection point	Time from the start of the bioassay	Number of survivors		The mortality rate of Daphnia in the experience, % of control
		Control	Experience	
Pond village Zhuynek	1 hours	10	10	0 %
	6 hours	10	10	0 %
	24 hours	10	7	30 %
	48 hours	10	7	30 %
	72 hours	10	7	30 %
	96 hours	10	6	40 %
Reservoir village of Karnak	1 hours	10	9	10 %
	6 hours	10	7	30 %
	24 hours	10	4	60 %
	48 hours	10	3	70 %
	72 hours	10	3	70 %
	96 hours	10	3	70 %
Reservoir of Kentau city	1 hours	10	10	0 %
	6 hours	10	10	0 %
	24 hours	10	9	10 %
	48 hours	10	9	10 %
	72 hours	10	8	20 %
	96 hours	10	8	20 %
Orangay village reservoir	1 hours	10	10	0 %
	6 hours	10	10	0 %
	24 hours	10	9	10 %
	48 hours	10	8	20 %
	72 hours	10	8	20 %
	96 hours	10	8	20 %

We found the arithmetic mean number of Daphnia survivors in the control and experiment. To calculate the percentage of Daphnia death in the experiment in relation to the control, the formula was used:

$$X_1 - X_2 / X_2,$$

where X_1 is the arithmetic mean number of Daphnia survivors in the control; X_2 is the arithmetic mean number of Daphnia survivors in the experiment.

30% of Daphnia died on the sample taken from the reservoir of the village of Zhuynek, which indicates that the water in these samples is slightly toxic. Zone of pollution – 2nd (Increased activity is replaced by depression, Daphnia periodically lie on the bottom).

As a result, we received the following reaction of the test object to the tested water in Karnak village: - 1 hour after the start of testing, 1 individual died;- after 6 hours, the number of dead individuals was-3; after 24 hours, 6 individuals died (total from the beginning of testing), which was 60 % of the individuals placed in the tested water. In connection with the death of 50% of individuals a day later, testing was stopped. The death of individuals in this or more numbers during any testing period indicates an acute toxic effect of the tested water on the test object. In relation to the control, 70% died during

testing, i.e. the water sample is assessed as toxic. Contamination zone - 1 (the specimens are kept in natural coat, the part loses its activity, there are cases of "whirligig beetles").

In the sample taken from the Kentau reservoir and the orangay village reservoir, 20% of *Daphnia* died, which indicates that there is no toxicity in these water samples. Zone of contamination - 3rd (the Presence of increased activity in some individuals, the rest - periods of activity are replaced by a normal state).

The results of *Daphne* mortality in experiments on reservoirs in Turkestan and Kentau are shown in figure 3.

When biotesting reservoirs using *Daphnia magna* as test objects, differences were found in their stress responses to water changes.

When analyzing the mortality of *Daphnia*, the following trend was found: the mortality rate in *Daphnia magna* increases by 24 %.

The results of the research showed that in all 4 sampling points there was a difference in the state of *Daphnia* from the control.

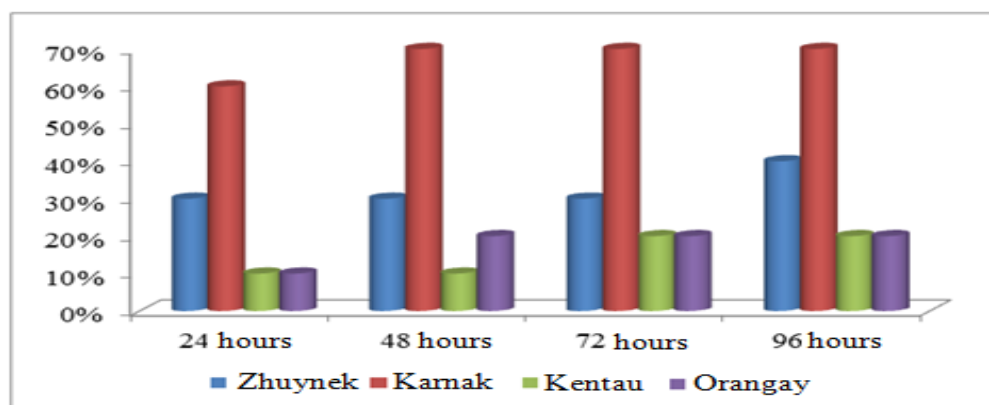


Figure 3 – *Daphnia* Mortality in experiments on reservoirs in the cities of Turkestan and Kentau

Conclusion

Our work may become part of a larger project on the use of aquatic crustaceans in research using bioindication. Recently, environmental pollution has become a very urgent problem. The situation is getting worse and worse every day. But instead of carrying out a series of chemical reactions at each waste release, it is easier to check the degree of water contamination on crustaceans found in almost all reservoirs. A change in the behavior of *Daphnia* allows you to quickly establish the fact of water contamination.

The bioindication method is based on determining changes in the survival rate of *Daphnia* when exposed to toxic substances contained in the tested water compared to the control.

The bioindication study allows us to take a deeper look at natural processes, provides a basis for explaining the cycle of substances in water, and only in the aggregate of all methods of study: organoleptic and chemical studies, bioindication - you can get reliable information about the ecological state of reservoirs.

As a result of water research using the bioindication method, we will be able to assess water, formulate the environmental problem under study, and make suggestions for optimizing the environmental situation.

Each water ecosystem is interesting and unique in its own way. Environmental assessment of a reservoir involves long-term monitoring, which allows you to get a number

of observations that are necessary for statistical processing of information. This work requires considerable time and effort. We have defined our approach to the study of water bodies in the area. This will allow, without having expensive equipment, to conduct an Express assessment of the state of the nearest reservoirs, using only your own hands, with effort and observation.

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