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EVALUATION OF TOXICITY OF WATERS FROM THE SURFACE SOURCES OF WATER SUPPLY AND DRINKING WATER OF ZHYTOMYR CITY BY THE METHOD OF SHORT-TERM CHRONIC BIOTESTING

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*Обґрунтовано підвищення інформативності тестування вод поверхневих джерел водопостачання та питної води завдяки використанню гладенької шпоркової жаби (*Xenopus laevis* Daudin, 1802). Як метод досліджень використовували біотестування за тест-реакцією іммобілізації озерної жаби (*Rana ridibunda* Pallas, 1771) і *X. laevis* з 8-добовою експозицією особин. Встановлено, що під час біотестування за відсутності іммобілізації *R. ridibunda* не виявлено токсичності дослідних вод, а за іммобілізації *X. laevis* визначено низький рівень водної токсичності у межах 25–50%. Оцінювання хронічної токсичності вод поверхневих джерел водопостачання та питної води доцільно проводити методом біотестування на *X. laevis*, які виявились більш чутливими до низьких рівнів забруднень, у середньому на 28,2% — у гострому досліді тривалістю 4 доби і на 41,3% — у хронічному досліді тривалістю 8 діб, ніж традиційні тест-об'єкти *R. ridibunda*.*

Ключові слова: біотестування, води джерел водопостачання, питна вода, іммобілізація, *R. ridibunda*, *X. laevis*, токсичність.

The assessment of water quality in surface water sources and drinking water using physical and chemical analysis does not provide complete information on the complex impact of pollutants on aquatic ecosystems. Therefore, the use of biological methods to determine the toxicity of these waters is particularly relevant [1, 2]. Moreover, the toxicity of drinking water surface waters should be evaluated not only for humans, but also for aquatic organisms [3–8], especially for those which are actively involved in the processes of water quality formation.

Due to the urgent need for monitoring the state of the water from existing methods of biomonitoring, it is expedient to use the

biological testing as the obligatory method, which allows, due to high sensitivity, to detect quickly the effects of pollution of surface water sources and drinking water, and at the same time it is much simpler and cheaper in execution, has a time constraint even when determining the chronic toxicity of water and does not require highly qualified labor as physical and chemical methods of research [9–11].

In literature during the last decade much attention has been paid to the feasibility of using sets of several highly sensitive organisms related to different levels of biological organization, through which it is possible to assess the state of the aquatic environment [9–11]. Obligatory representatives of such test kits should be vertebrate animals [10,

11], including frogs. However, not all species of frog behave equally in the same type of water pollution. In addition, some species are able to react more actively to organic compounds, others – on heavy metals, synthetic surfactants or nanomaterials, etc. [2, 11]. To identify the creatures that can provide more accurate information on the level of total water toxicity, a prerequisite is to conduct a comparative analysis of a few or more species of frogs, the most sensitive of which are the prospect of being used in biotesting of low contaminated waters.

Taking into account the above mentioned features and the existing literary data on the significant increase in the effectiveness of bioassay for the use of highly sensitive organisms [10, 11], we consider that it is necessary to compare two types of unified frogs of marsh (*Rana ridibunda* Pallas, 1771) and african clawed (*Xenopus laevis* Daudin, 1802), which are used in international and national standards for determining the toxicity of water [10, 11]. For the first time, for assessing the toxic effects of water using frogs, the proposed scale of toxicity levels tested on daphnia [4], which is fully consistent with SSanRN (State sanitary rules and norms) 2.2.4-171-10, has been applied.

The goal of the research is to increase the informativeness of the method of testing water from the surface sources of water supply and drinking water using african clawed frog.

MATERIALS AND METHODS OF RESEARCH

In order to realize the research objective, a comparative analysis of the biotesting of water toxicity was carried out, a sample of which was selected in prepared dishes from the Denyshivske reservoir and Vidsichne water intake of Teteriv river and from the clean water reservoirs (CWR) of CE «ZhytomyrVodokanal» in April 2016 [4]. After sampling the water was poured into aquariums (10 dm³) and formed control and test groups according to the following scheme:

- Control group – a test of a settled (24 hours) tap water;

- Test group D-1: water samples – from the Denyshivske reservoir;
- Test group D-2: water samples – from the Vidsichne water intake;
- Test group D-3: water samples – with CWR 5000 m³;
- Test group D-4: water samples – with CWR 20 000 m³.

Test-objects: marsh frog (*R. ridibunda*) and african clawed frog (*X. laevis*).

Retention: in aquariums, 10 dm³ of water, the concentration of oxygen in which is not less than 4 mg/dm³, the temperature +20°C±2.5°C, and the density of planting – individual/3 dm³; in conditions of natural light and replacement of water of appropriate quality every 2 days.

Feeding: animal feed (mosquito larvae, sludge worm).

Test Reaction: immobilization, including mortality of individuals.

Exposure: acute lethal toxicity – for 4 days and short-term chronic toxicity (short-term chronic toxicity) – within 8 days.

Biotesting: daily calculation of active and immobilized (including dead) individuals from the 1st to the 8th day.

Experiments were carried out in 3-time repeat, using standard techniques and own developments [4, 5, 10, 11].

The obtained data were compared with the control. For each experimental water sample, the toxicity index (%) for the SSanRN 2.2.4-171-10 [5] was calculated using the following formula:

$$T = \frac{I_k - I_0}{I_k} 100,$$

where: T – index of toxicity, %; I_k – the value of the test-reaction of individuals in the control; I₀ – the value of the test-reaction of individuals in the experiment.

The water toxicity index should not exceed 50% regardless the test objects used in the studies.

RESULTS AND DISCUSSION

One of the most important issues in the context of biotesting of water toxicity is the choice of test objects that must be standardi-

zed organisms, with which standard experiments have been developed, and which should be adequately responsive to certain levels of pollution of the tested medium. Among the organisms that test biotesting of the aqueous medium the most commonly used are infusoria, daphnia, ceriodaphnia, guppies, common onions, salad dressings, etc. [5–8]. For a long time, these test objects were used to determine the toxicity of natural and sewage, and the development of SSanRN 2.2.4-171-10 contributed to their use in water testing of surface water sources and drinking water [5].

However, for biotesting the quality of these waters, which are much cleaner than other species, traditional organisms were not sufficiently informative. In this regard, the search for unified organisms, by means of which higher efficiency compared with generally accepted test objects, low levels of water pollution can be found.

As test organisms, we tested for sensitivity to toxic components of water 2 species of marsh and african clawed frogs for the test-reaction of immobilization of individu-

als, the advantage of which, in comparison with many other frogs reactions, is the simplicity of its determination for the calculation of the number of active and immobilized individuals.

A comparative analysis of the environmental effectiveness of biotesting on marsh frog and african clawed frog of water samples taken in the reservoirs of the Teteriv river and the clean water reservoirs (CWR) at the Communal Enterprise «ZhytomyrVodokanal» is presented in the table:

The obtained data indicate that the test organism proposed for conducting the assessment of the water *X. laevis* by the reaction of immobilization of individuals is more sensitive to the determination of water toxicity than *R. ridibunda*. According to the biotesting data, the scale of the water toxicity levels proposed by us is used, which can be used both for the determination of its acute and chronic toxic effects [4]: 1–25% – acceptable level of toxicity; 26–50% – low toxicity; 51–75% – average toxicity; 76–100% – high toxicity.

Comparative analysis of biotesting with *R. ridibunda* and *X. laevis* of waters from the reservoirs of the Teteriv river and the CWR of Communal enterprise «ZhytomyrVodokanal» (n = 30)

Test groups and figures	Toxicity after immobilization of test objects (T, %):			
	<i>R. ridibunda</i>	toxicity level	<i>X. laevis</i>	toxicity level
<i>Acute toxicity</i>				
D-1 (Denyshivske Reservoir)	absent	ATL	35.7	LTL
D-2 (Vidsichne water intake)	absent	ATL	27.1	LTL
D-3 (CWR 5 000)	absent	ATL	25.1	ATL
D-4 (CWR 20 000)	absent	ATL	24.9	ATL
Exposure duration, days	4		4	
<i>Chronic toxicity</i>				
D-1 (Denyshivske Reservoir)	absent	ATL	49.6	LTL
D-2 (Vidsichne water intake)	absent	ATL	42.5	LTL
D-3 (CWR 5 000)	absent	ATL	38.2	LTL
D-4 (CWR 20 000)	absent	ATL	34.9	LTL
Exposure duration, days	8	–	8	–

Note: I_M – immobilization of frogs; T – index of water toxicity (in relation to control); levels of toxicity of water: ATL – admissible, LTL – low.

African clawed frog is a model for investigation in medical and ecological biotechnology. This species of frog should be sensitive enough to the chronic toxic effects of water components of surface water sources and drinking water. Therefore, it is important to compare the sensitivity to the chronic toxicity of water given by *X. laevis* with such a widespread aquatic toxicology as a species of frog like *R. ridibunda*.

In the experiment, in the test-reaction of immobilization of *R. ridibunda*, only the permissible level of toxicity was detected in all investigated water species. However, it is obvious that water from surface water sources and drinking water can not have the same toxicity. This fact has been proved by us with the use of a more sensitive species of *X. laevis*. Thus, for the determination of acute and chronic effects of water, not only the permissible levels of their toxicity (ATL) were revealed, which in fact indicate a lack of harmful effects on organisms, but also low levels (LTL) within the toxicity index of 25–50%. The highest value of the toxicity index was detected determining the chronic effect of water in the reservoir of Denyshivske district of Teteriv, somewhat lower – in the water

intake of the Attic. The determination of the acute effects of water in these groups also revealed higher levels of toxicity compared with the levels of toxicity of drinking water. Although the 50% limit on water hazard was not exceeded, biotesting with african clawed frog was on 26.3% average (in the acute study) and 36.6% (in the chronic study) more effective than testing on marsh frog.

Consequently, under the same conditions of biotesting with *R. ridibunda* and *X. laevis*, the latter showed a significantly higher sensitivity to the toxic components of water sources of water supply and drinking water in the conditions of the Communal enterprise «ZhytomyrVodokanal».

CONCLUSIONS

The evaluation of chronic toxicity of water from surface sources of water supply and drinking water is proposed to be carried out by the method of biotesting with *X. laevis*, which turned out to be more sensitive to low levels of contamination on average 28.2% in an acute study of duration 4 days and 41.3% in the short-term chronic study duration of 8 days than the traditional test-object of *R. ridibunda*.

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