



---

<https://doi.org/10.15407/scine22.02.084>

TYAGNIJ, L. M. (<https://orcid.org/0000-0002-4954-002X>),  
and STEPOVA, O. V. (<https://orcid.org/0000-0002-6346-5484>)

National University "Poltava Polytechnic" named after Yuriy Kondratyuk,  
24, Vitaliy Hrytsayenko Ave., Poltava, 36011, Ukraine,  
+380 53 256 1604, [eco.poltntu@gmail.com](mailto:eco.poltntu@gmail.com)

## BIOTESTING OF SURFACE WATERS OF THE VORSKLA RIVER USING THREE TEST ORGANISMS AS INDICATORS

---

**Introduction.** *In accordance with the Water Framework Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy, standards for monitoring, surveying, sampling, and laboratory analysis of water bodies have been developed.*

**Problem Statement.** *In Ukraine, a significant problem has remained the insufficient development of protection technologies and control methods capable of predicting the ecological state of water bodies based on toxicological parameters. Conventional approaches have not fully ensured reliable assessment of the integrated toxic effects of pollutants. The application of biotesting has enabled determination of wastewater impacts using biological responses, thereby providing conclusions regarding the toxicological state of aquatic ecosystems.*

**Purpose.** *This study has aimed to assess the quality of surface waters using biotesting methods and to determine the impact of wastewater on aquatic biocenoses involved in self-purification processes, based on toxicological parameters.*

**Materials and Methods.** *The Bioindication Water Test method has been applied, involving staged laboratory tests using a laboratory culture of *Daphnia* and natural populations of the rotifer *Rotaria rotatoria* as test organisms collected from the studied water bodies. Laboratory, microscopic, biotesting, and statistical methods have been employed.*

**Results.** *Biotesting on crustaceans has demonstrated that water from the Vorskla River has exerted an acute toxic effect on *Daphnia* and *Rotaria rotatoria*. A 50% decrease in the survival rate has been recorded over 96 hours of exposure, indicating that water quality has not met established standards for*

Citation: Tyagnij, L. M., and Stepova, O. V. (2026). Biotesting of Surface Waters of the Vorskla River Using Three Test Organisms as Indicators. *Sci. innov.*, 22(2), 84–98. <https://doi.org/10.15407/scine22.02.084>

© Publisher PH "Akademperiodyka" of the NAS of Ukraine, 2026. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

toxicological indicators. Toxicological analysis of surface waters has shown that the highest toxicity has been observed in samples collected from the sewage discharge zone near the Poltava stormwater collector.

**Conclusions.** Biotesting as an integral method for assessing aquatic toxicity has been shown to be a necessary complement to physicochemical analysis. It has provided direct evidence of organismal responses to pollution and has enabled effective control of wastewater discharges that contribute to ecosystem degradation. The implementation of biotesting has supported compliance with the requirements of Water Framework Directive 2000/60/EC aimed at improving the ecological status of water bodies in Ukraine.

**Keywords:** biotesting, wastewater, *Rotaria rotatoria*, *Daphnia magma*, *Daphnia moina*.

The selection of the research topic has been influenced by a combination of factors affecting water bodies in Ukraine. One of the most significant factors is the discharge of large quantities of phosphates into river surface waters with wastewater, which has a detrimental effect on the viability of rare aquatic biota species [1]. This phenomenon is mainly associated with industrial development, as many production processes involve the use of various synthetic compounds and chemical raw materials. Each year, increasing consumer demand for specific products in urban areas and surrounding regions has led to growth in industrial output [2–3].

In every region of Ukraine, *Rules for Wastewater Discharge* into municipal sewerage systems of cities and settlements are developed and approved by Regional Councils or local self-government authorities [4–7]. According to these regulations, enterprises are required to comply with established standards, including the installation of local wastewater treatment facilities. However, as production volumes increase, the need for additional financial investment in infrastructure reconstruction arises. Consequently, some industrial operators illegally connect to stormwater drainage systems and discharge untreated industrial effluents.

During the second half of the twentieth century, anthropogenic pollution processes affected the majority of lakes in Central and Southern Europe, the United States, several lakes in Northern Europe and neighboring countries of the former USSR, Asia — particularly China, India, and Japan — as well as lakes in Central and South America, Africa, and Australia. Numerous cases of lake degradation have been documented in reviews add-

ressing the development of eutrophication research. With the expansion of eutrophicated water bodies during this period, the number of experimental studies devoted to these processes increased substantially [1, 2, 8–13].

Over the past two decades, water resources management has become one of the key environmental challenges facing the countries of Eastern Europe, the Caucasus, and Central Asia (EECCA) [11–14]. Following the dissolution of the Soviet Union, each state was required to establish its own water management framework. Water quality in EECCA countries has been influenced by fluctuating economic conditions and changing climate patterns. The recovery of industry and agriculture after the sharp decline of the 1990s has resulted in renewed growth of water pollution in the region, originating from a different spectrum of sources.

The anticipated — and already evident — effects of climate change, including rising water temperatures and increased frequency of floods and droughts, have intensified contamination of waters by substances released from bottom sediments, nutrients, dissolved organic carbon, pathogenic organisms, pesticides, and salts, thereby contributing to thermal and chemical pollution. These challenges are likely to be further complicated by issues related to transboundary waters.

To support structural transformations aimed at implementing a “green” economic model and its diversification, as well as to facilitate adaptation to climate change, it is necessary to establish a flexible system for water quality regulation. In this context, the development and application of the *Bioindication Water Test* method in operational

assessments of water bodies have been considered appropriate for evaluating the effects of toxic water based on toxicological parameters, through which the ecological status of aquatic ecosystems can be characterized.

Eutrophication of water bodies has been influenced by numerous factors [14], which are conventionally divided into two groups: abiotic and biotic. Abiotic factors include light availability, transparency, temperature, hydrodynamic regime, salinity and mineral composition, acidity, and concentrations of nutrients. Biotic factors encompass phylogenetic, zoogenic, and anthropogenic components, with anthropogenic influence having acquired decisive importance in recent decades [15].

The systemic interdependence of all processes occurring within a water body as an integral natural object has been emphasized since the late nineteenth and early twentieth centuries, particularly in the classical works of the Swiss limnologist F. Forel [9–12, 16, 17]. In the 1920s, A. Thienemann (Germany) and E. Naumann (Sweden) demonstrated the possibility of subdividing lakes and other water bodies according to biolimnological types (oligotrophic, mesotrophic, eutrophic, dystrophic, and others) [11, 12, 14, 15]. The problem of typology and classification of natural waters has continued to be developed to the present day.

The functional characteristics of aquatic organisms can be elucidated only through experimental studies of metabolism, growth, nutrition, and chemical composition. Foundational contributions to the development of this research area have been made by N. S. Gaevskaya, V. S. Ivlev, and S. Skadovsky [12, 13].

In 2022, the President of Ukraine, Volodymyr Zelenskyy, requested Ukraine's immediate accession to the European Union under a "new special procedure." The first step toward Ukraine's European integration consists in compliance with and implementation of specific water policy requirements, primarily those of the Water Framework Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy [4].

In June 2022, Ukraine has obtained candidate status for EU membership; however, fulfillment of a number of conditions and adherence to adopted and implemented legislation and regulations in the environmental protection sector remain necessary. In accordance with the Association Agreement, as well as Decision No. 2455/2001/EC, Directive 2009/31/EC, and Directive 91/271/EEC on urban wastewater treatment, as amended by Directive 98/15/EC and Regulations (EC) No. 1882/2003 and No. 1137/2008, the Ukraine–EU framework for water policy requires the development and implementation of methods for controlling water quality and managing water resources of rivers, lakes, and other water bodies [5, 6].

Pursuant to the Presidential *OSCE Support Program for Ukraine*, which identifies the need to adopt 643 European and international standards (EN ISO) in the field of environmental protection, interregional and territorial bodies of the State Environmental Inspectorate have been mandated, under martial law, to monitor surface waters through sampling in order to document environmental conditions across Ukraine. All monitoring activities must be conducted in accordance with international and European standards.

In November 2022, Ukraine has obtained the status of an affiliated member of CEN-CENELEC, effective from 1 January 2023. This status has been granted to national standardization bodies of countries officially recognized as EU candidates or potential candidates and provides access to European standards (EN, EN ISO). Consequently, existing discrepancies between national standards and EU standards shall be eliminated.

Annex V, Section 1.2.6 of Directive 2000/60/EC [4] specifies ecological quality standards aimed at limiting the input of hazardous chemical substances into surface waters based on a "basic set of taxa." In research practice, river water sampled from the studied water body has been used to determine the effects of pollutants on zoobenthos, employing selected taxa as test organisms for biotesting. The development of a biotesting method based on the above-mentioned standards has cont-

ributed to the preservation of endangered aquatic hydrobiont species. In addition, cyanobacterial toxins present in water have posed a significant threat to human health.

In biological monitoring practice, biotesting, bioindication, and bioaccumulation approaches have been widely applied. Numerous studies have been devoted to bioindication methods, which have demonstrated substantial advantages over purely chemical and physical techniques [1–3, 14–18].

Prior to the initiation of experimental investigations, the fundamental principle of practical laboratory biotesting of surface waters implemented in the European Union in accordance with Directive 2000/60/EC [4] has been adopted. This principle involves the simultaneous application of three to four methods using test organisms representing different trophic groups, specifically organisms from aquatic ecosystems such as macrophytes, phytobenthos, aquatic invertebrates, algae, and higher plants. Primary producers form the basis of most food chains in water bodies. An example of a key trophic link is crustaceans, which represent some of the main filter feeders and sedimentators in freshwater ecosystems.

Before the start of experimental studies, standards included in Directive 2000/60/EC have been analyzed to identify the most commonly used test organisms for assessing hazards posed by pollutants. These standards recommend the use of standardized test organism sets, including [4, 19–24]:

- ◆ Algae: *Scenedesmus subspicatus*, *Scenedesmus quadricauda*, *Selenastrum capricornutum*, *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*;
- ◆ Crustaceans: *Daphnia magna*, *Ceriodaphnia dubia*, *Ceriodaphnia affinis*, *Hyalella azteca*;
- ◆ Fish: *Danio rerio*, *Oncorhynchus mykiss*, *Cyprinus carpio*;
- ◆ Macrophytes: *Lemna minor*.

Monitoring of surface waters is an integral and essential component of water quality control in aquatic ecosystems. Following the adoption of the Water Framework Directive (WFD) by the European Union in 2000, EU member states have progressively developed and implemented its provisions.

This process has been reflected in the development of bioindication methods for water bodies as one of the foundations of surface water monitoring [4].

Directive 2000/60/EC [4] incorporates established standards and regulatory requirements [19], including:

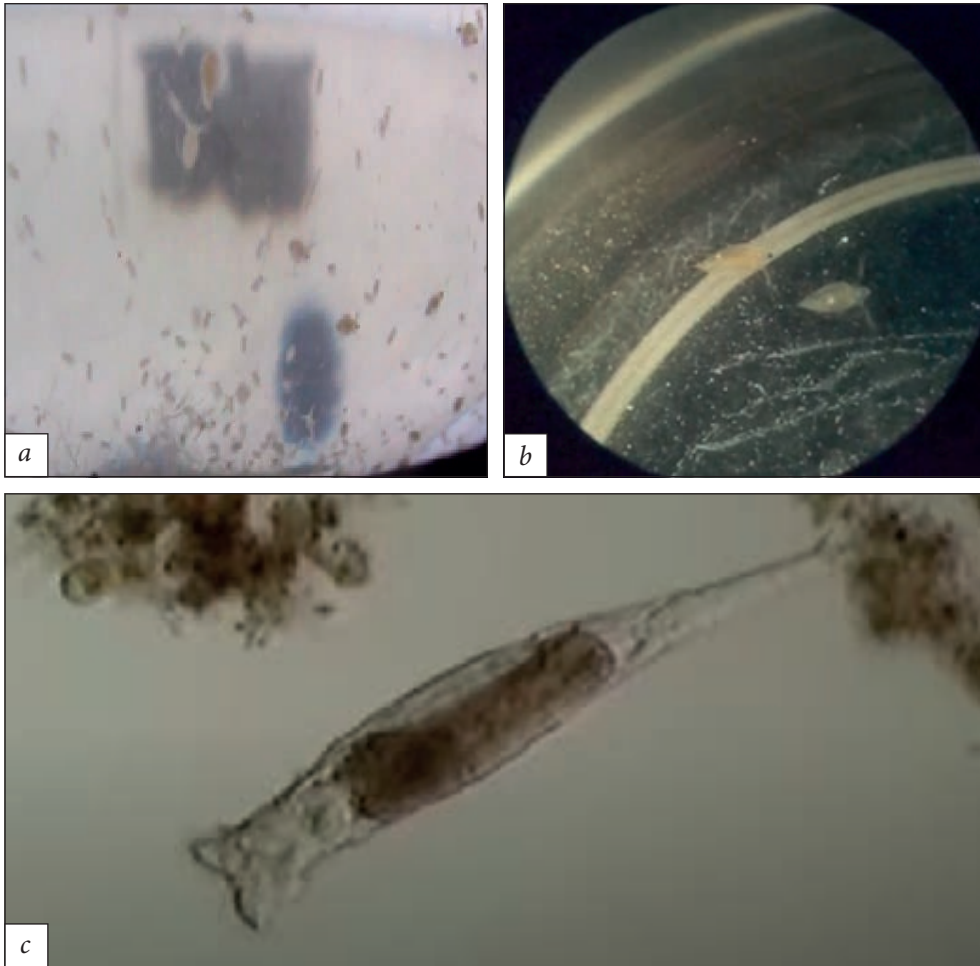
- ◆ EN ISO 5667-3:2024 (ISO 5667-3:2012) Water quality — Sampling — Part 3: Preservation and handling of samples.  
Standards for benthic invertebrates [20, 21]:
- ◆ EN ISO 10870:2012 Water quality — Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters;
- ◆ EN 16150:2012 Water quality — Guidance on proportional sampling of benthic macroinvertebrates from multiple habitats in wadeable rivers.

At present, more than 60 biotesting methods have been applied worldwide, incorporating various characteristics of zoobenthos [12–14, 18].

In 2013, M. M. Sydorovych published the scientific article “Use of biometric indicators of the ALLIUM TEST for determining the quality of urban drinking water,” which describes a biotesting method based on assessing the responses of living microorganisms to the toxicological effects of waters of different origin, including wastewater from the city of Kherson [8]. The author conducted a series of studies using seeds of *Allium cepa* L. as test organisms.

A biotest represents a set of techniques for obtaining information on the toxicity of water and bottom sediments that affect the vital activity of invertebrates, based on the registration of responses of test organisms [6, 7, 11, 12].

It has been established that a toxicant represents a factor that promotes entropy and acts as a destructive force for living systems. Within an ecological system, counteracting processes have developed that aim to eliminate the entropic factor, thereby forming a specific property of ecosystems — buffering capacity [12]. An ecosystem is capable of absorbing and transforming toxicants within certain limits. Only when this resistance potential has been exhausted does the actual toxic effect begin to manifest itself.



**Fig. 1.** Synchronized cultures used in biotesting: *a* — *Daphnia magna*; *b* — *Daphnia pulex*; *c* — *Rotaria rotatoria*

During the study, particular attention has been paid to the selection of sampling locations at monitoring cross-sections (stations). A mandatory criterion has been their placement directly within ecologically sensitive zones, specifically at points of local stormwater discharge and wastewater outfalls in the city of Poltava.

In the summer of 2023, experiments have been conducted by observing the responses of living organisms. The following test organisms have been used in the biotests: *Daphnia magna* (*a*); *Daphnia pulex* (*b*) (note: corrected to commonly used species); and *Rotaria rotatoria* (*c*) (Fig. 1). Synchronized cultures have been applied, in particular third-

generation organisms obtained through acyclic parthenogenesis under controlled cultivation conditions [22—24].

To obtain a vegetative culture of the rotifer *Rotaria rotatoria* from the Vorskla River, water together with bottom sediment was collected from the shoreline in the vicinity of the village of Vakulentsi, Poltava Region. After transportation, the material was transferred to a crystallizing dish, where the water was allowed to stand for 24 h to enable suspended particles to settle. Without feeding, the culture was maintained in a ventilated room at a water temperature of 19—22 °C.

Following the appearance of rotifers after 14 days, the culture was fed with small fragments of dry baker's yeast (approximately 1 mm<sup>2</sup>) and supplemented with a suspension of the unicellular alga *Chlorella vulgaris* until the medium acquired a light-green coloration. After one month, the obtained juveniles were transferred to a new crystallizing dish to achieve the required sensitivity corresponding to LC<sub>50</sub><sup>24</sup>.

The rotifer *Rotaria rotatoria* is capable of sensing physicochemical changes in the water column and is therefore widely used in laboratories for toxicological studies. When resting eggs obtained from research institutions or laboratory cultures established in-house are available, continuous cultivation for several years is possible [18, 22].

During testing, specific culture sensitivity requirements have been met, namely an LC<sub>50</sub><sup>24</sup> value within the concentration range of 0.9—2.0 mg/l<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> when exposed to potassium dichromate solution.

Subsequently, the total volume of surface water prepared for biotesting of one sample was 0.6 l, given triple water replacement; in the case of short-term tests, 2.0 l was prepared.

*Daphnia* spp. and *Rotaria rotatoria* were collected from the crystallizing dish using a pipette and carefully transferred into conical flasks containing water (Reference 1, Reference 2, and Reference 3). Each flask was filled with 0.2 l test water from labeled samples, and 10 individuals of one-day-old juveniles were introduced.

Water collected from a conditionally clean (background) site of the water body was used as Reference 1. Simultaneously, Reference 2 and Reference 3 were established using settled and paper-filtered river surface water [18].

For each sample, three replicates were established (a total of 30 individuals per water sample). Excess liquid was then removed using a pipette, and the test water was immediately and carefully added to avoid injuring *Daphnia* spp. and the rotifer *Rotaria rotatoria*.

After three days from the start of the experiment, water in the crystallizing dishes used for culturing *Daphnia* and *Rotaria rotatoria* was replaced with

freshly collected water or with water from the same sample stored under refrigerated conditions in accordance with storage requirements. To perform water replacement, test organisms were captured and transferred to another crystallizing dish.

During the first two days, organisms were not fed; beginning on the third day, feeding was conducted daily. The amount of food introduced into each crystallizing dish during biotesting was kept constant. When water replacement was performed, *Daphnia* were fed 3 h prior to the procedure.

Observations of test organisms were carried out continuously during the first hour, then hourly for the next 6—8 h, and subsequently twice daily. Survival rate and fecundity were recorded. In addition, behavioral responses of crustaceans were noted, including changes in body coloration and other indicators of physiological activity. Mortality was determined by immobility: individuals lying on the bottom of the flask and exhibiting no swimming movements after gentle shaking of the flask for 15 s (even if antennae movements were observed) were considered dead. Dead individuals were counted visually without removing them from the vessel.

When offspring appeared, juveniles were removed from the flask, counted, and transferred to a crystallizing dish. Data on the number of surviving *Daphnia* and the number of produced offspring for each day of observation were recorded in Tables 1 and 2.

During short-term biotesting [18]:

1. For the test organism *Daphnia moina* after 96 h of exposure, the following results have been obtained:

- ◆ In Reference 1 (K1; settled conditionally clean river water from the Vorskla River), all 30 individuals survived (three replicates, 10 individuals each);
- ◆ In Reference 2 (K2; river water sampled upstream of the urban impact zone), all 24 individuals survived;
- ◆ In Reference 3 (K3; water from the Vorskla River collected at the pollution source), 15 out of 30 *Daphnia* survived, corresponding to 50% of the initial number.

**Table 1. Template for Recording Results of Surface River Water Biotesting**

**Sample No. Vorskla River (Poltava city limits)**

**(water body, site, point)**

**Sampling date: 07.08.2023; 11.08.2023; 21.08.2023**

Registration date and biotesting start time	<i>Daphnia</i> survived, ind.									Offspring produced, ind.								
	Reference K1			Reference K2			Reference K3			Reference K1			Reference K2			Reference K3		
	Replicate																	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
<i>Daphnia magna</i> Cladocera																		
07.08.23 10:00	10	10	10	7	8	8	5	6	4	5	6	4	0	0	0	0	0	0
11.08.23 11:00	10	10	10	8	9	8	6	5	4	8	7	8	0	0	0	0	0	0
21.08.23 12:00	10	10	10	8	7	7	4	5	5	5	5	7	0	0	0	0	0	0
<i>Daphnia moina</i> Macrocopa																		
07.08.23 10.30	10	10	10	8	9	8	4	5	5	4	5	5	0	0	0	0	0	0
11.08.23 11:30	10	10	10	9	8	8	5	6	5	5	6	5	0	0	0	0	0	0
21.08.23 12:30	10	10	10	8	8	9	5	5	4	8	7	8	0	0	0	0	0	0

**Table 2. Results of Short-Term Bioassay of Surface River Water**

Date of sampling	Series	Survival rate, % of the initial number	Student criterion, <i>tst</i>	Reduction in survival rate, % of reference			Water toxicity assessment
				K1 (sample 1)	K2 (sample 2)	K3 (sample 3)	
<i>Daphnia magna</i> Cladocera							
07.08.2023	1—1	10; 10; 10	2.23	100		100	No effect
11.08.2023	1—2	10; 10; 10					
21.08.2023	1—3	10; 10; 10					
07.08.2023	2—1	7; 8; 8	2.37	100	47		Acute toxic effect
11.08.2023	2—2	8; 9; 7	2.31				
21.08.2023	2—3	8; 8; 7	2.37				
07.08.2023	3—1	5; 6; 4	2.57	100		20	Acute toxic effect
11.08.2023	3—2	6; 5; 4	2.57				
21.08.2023	3—3	4; 5; 5	2.78				
<i>Daphnia moina</i> Macrocopa							
07.08.2023	1—1	10; 10; 10	2.23	100		100	No effect
11.08.2023	1—2	10; 10; 10					
21.08.2023	1—3	10; 10; 10					
07.08.2023	2—1	8; 9; 8	2.31	100	50		Acute toxic effect
11.08.2023	2—2	9; 8; 8	2.31				
21.08.2023	2—3	8; 8; 9	2.31				
07.08.2023	3—1	4; 5; 5	2.78	100		29	Acute toxic effect
11.08.2023	3—2	5; 6; 5	2.57				
21.08.2023	3—3	5; 5; 4	2.78				

2. For the test organism *Daphnia magna* (Cladocera) (Tables 1 and 2), the following has been recorded:

- ◆ In Reference 1 (K1; settled conditionally clean river water from the Vorskla River), all 30 individuals survived (three replicates, 10 individuals each);
- ◆ In Reference 2 (K2; river water sampled upstream of the urban impact zone), all 25 individuals survived;
- ◆ In Reference 3 (K3; water from the Vorskla River collected at the pollution source), 16 out of 30 *Daphnia* survived, corresponding to 55% of the initial number.

The significance of differences has been determined using Student's *t*-test [11]. For Reference 1 (K1) and the tested water samples (K2 and K3), the arithmetic mean of toxicity indicators ( $\bar{X}$ ), the standard deviation ( $\sigma$ ), the standard error of the mean ( $S$ ), and the *t* statistic were calculated. The following formulas (1) have been applied:

$$\bar{X} = \sum X_i / n, \tag{1}$$

where  $\bar{X}$  is the arithmetic mean;  $n$  is the number of replicates;  $X_i$  is the  $i$ -th recorded indicator of water toxicity;

$$\sigma = \sqrt{\sum (X_i - \bar{X})^2 / (n - 1)}, \tag{2}$$

$$S = \sigma / \sqrt{n}, \tag{3}$$

$$t = \frac{(X_{ref} - X_{test})}{\sqrt{S_{ref}^2 + S_{test}^2}}, \tag{4}$$

where  $X_{ref}$  and  $X_{test}$  are the arithmetic mean values of the toxicity indicators calculated for K1 (reference) and for K2 and K3 (Vorskla River test water samples);  $S_{ref}$  and  $S_{test}$  are the standard error of the arithmetic mean of toxicity indicators for K1 (reference) and for K2 and K3 (tested water samples).

During the calculations, values of the *t* statistic (significance criterion) have been obtained and compared with the critical (standard) values of Student's *t* ( $t_{st}$ ) at a significance level of 0.05 and degrees of freedom:

$$k = n_{ref} + n_{test}.$$

The calculations have followed the recommendations in [25]. Specifically, if the calculated value  $t \geq t_{st}$  the difference between the sample and mean toxicity indicators is considered statistically significant. In this case, there are grounds to conclude that the tested water exerts a toxic effect on the test organism.

Critical values of Student's *t* ( $t_{st}$ ) at a significance level of 0.05 are presented in Table 3.

Survival rates of *Daphnia* in the tested water (References 2, Reference 3) and in Reference 1 are calculated using Eq. (5):

$$X_{\sum_i} = (\sum X_i / \sum N) 100, \tag{5}$$

where  $X_{\sum_i}$  is the survival rate of *Daphnia* in the experiment for the  $f$ -th replicate, expressed as a percentage of the initial number;  $X_i$  is the number of surviving *Daphnia* in each replicate, and  $N$  is the total number of *Daphnia* introduced in the experiment (initial population).

The reduction in *Daphnia* survival rate in the tested water samples (Reference 2 and Reference 3) relative to Reference 1 is calculated using Eq. (6):

$$A = \left[ (\bar{X}_k - \bar{X}_0) / \bar{X}_k \right] 100, \tag{6}$$

where  $A$  is a reduction in *Daphnia* survival rate as compared with Reference, %;  $\bar{X}_k$  is the arithmetic mean number of *Daphnia* surviving in the Reference, and  $\bar{X}_0$  is the arithmetic mean number of *Daphnia* surviving in the tested samples (Reference 2 or Reference 3).

Table 3. Critical Values of Student's *t* ( $t_{st}$ ) at a Significance Level of 0.05

Number of degrees of freedom	1	2	3	4	5	6	7	8	9	10
$t_{st}$	12.71	4.30	3.18	2.78	2.57	2.45	2.37	2.31	2.26	2.23

Fecundity of *Daphnia* in the tested water and in the reference is calculated using Eq. (7):

$$X_i = \sum M_i / \sum V_i, \quad (7)$$

where  $M_i$  is the number of juveniles produced by each brooding female, units;  $V_i$  is the number of initial females that produced offspring and survived at the time of fecundity assessment

Pathological deviations (e.g., aborted eggs, still-born offspring) were included in the calculation of fecundity (potential reproductive output) according to Eq. (7).

**Table 4. Statistical Analysis of Biotesting Data for Collected Water Samples**

Series	$X_i$ (arithmetic mean)	$X$	$(X_i - \bar{X})$	$(X_i - \bar{X})^2$	$\sigma$	$S$
<i>Daphnia moina</i> Cladocera						
K1	10	10	0	0	—	—
	10		0	0		
	10		0	0		
K2	23	7.67	15.33	30.66	46.67	15.55
	25	8.33	16.67	33.34		
	22	7.33	14.67	29.34		
				$\Sigma 93.34$		
K3	15	5.0	10.00	20.00	20.00	6.66
	15	5.0	10.00	20.00		
	14	4.67	9.33	18.66		
				$\Sigma 40.00$		
<i>Daphnia magna</i> Macrocopa						
K1	10	10	0	0	—	—
	10		0	0		
	10		0	0		
K2	25	8.33	16.67	33.34	50.04	16.68
	25	8.33	16.67	33.34		
	25	8.33	16.67	33.34		
				$\Sigma 100.08$		
K3	14	4.67	9.33	18.66	29.32	9.77
	16	5.34	10.66	21.32		
	14	4.67	9.33	18.66		
				$\Sigma 58.64$		

To verify the results for the tested water samples, statistical analysis of the *Daphnia* data has been performed. For convenience, the data have been organized in Table 4.

Let us calculate the *f*-criterion for *Daphnia moina* Cladocera:

$$t = \frac{(8.33 - 4.67)}{(8.33^2 + 7.67^2 + 5.0^2 + 4.67^2)} = 0.071.$$

The number of degrees of freedom is equal to:

$$5 + 5 - 0.017 = 10.07.$$

For this number of degrees of freedom:

$$t_{st} = 2.23.$$

Let us calculate the *f*-criterion for *Daphnia magna* Macrocopa:

$$t = \frac{(5.34 - 4.67)}{(8.33^2 + 5.34^2 + 4.67^2)} = 0.018.$$

The number of degrees of freedom is equal to:

$$5 + 5 - 0.018 = 9.98.$$

For this number of degrees of freedom:

$$t_{st} = 2.26.$$

Thus, the condition of statistical significance  $t_\alpha > t_{st}$  has been satisfied; therefore, survival rate in the experiment is significantly lower than in the Reference.

Mortality of *Daphnia moina* (Cladocera) has been high relative to the reference: 100% compared with K1, 73% compared with K2, and 50% compared with K3, exceeding the accepted significance threshold. Mortality of *Daphnia magna* (Cladocera) has also been high: 100% compared with K1, 77% as compared with K2, and 50% compared with K3, similarly exceeding the significance criterion [18, 25].

Thus, based on the results of short-term biotesting of Vorskla River water, the data have been compiled in Table 5. In Reference 3, survival rate is significantly lower than in Reference 1. These results have indicated that water from sample No. 3 exerts a clear “acute toxic effect” on *Daphnia*. The experiment was terminated, as the toxic effect of the water had already been established during the short-term biotest [18].

Based on the results of biotesting of natural water using the selected test organisms and the data presented in Table 6, the toxicity of the tested water has been assessed. The obtained indicators show a 20% reduction in the survival rate of *Rotaria rotatoria* and a 70% deviation in fecundity.

During the study, the third test organism, the rotifer *Rotaria rotatoria* from the Vorskla River, was placed into nine flat-bottom flasks arranged in three series: three series with Reference 1 (K1), three series with Reference 2 (K2; water collected upstream of the impact area), and three series with Reference 3 (K3; water collected downstream of the impact area). Using a capillary pipette under a microscope, ten age-synchronized *Rotaria rotatoria* individuals were transferred into each flask.

Water carried together with the rotifers was removed using filter paper, after which Vorskla River water was added to the flasks. The flasks were pla-

ced in trays containing water to maintain a temperature of 22–23 °C and to prevent evaporation.

After 1, 3, 5, 24, 48, 72, and 96 h, the numbers of surviving *Rotaria rotatoria* individuals and newly produced juveniles were recorded. Juveniles were removed immediately after counting. Observational data were recorded in accordance with the format of Table 4. The number of juveniles at each subsequent observation time was calculated as the sum of individuals produced during the previous observation period (and removed) and those produced by the time of the current observation.

At the end of the 24 h exposure, the total number of juveniles in Reference 1 was 18 individuals, which were removed. After 48 h, the cumulative number of juveniles was 18 + 12 = 30 individuals (18 individuals produced during the first 24 h and removed, and 12 individuals produced between the first and second observations).

Table 5. Results of Short-Term Biotesting of Vorskla River Water, Poltava

Number of sample	Water Body / Site / Sampling Point / Sampling Date	Series	Survival rate, % of Initial Number	Water Toxicity Assessment
<i>Daphnia moina Macrocopa</i>				
1	The Vorskla River 7.08.23/11.08.2023/21.08.2023 shore	Reference K1	100	No toxic effect
2	The Vorskla River 7.08.23 /11.08.2023/21.08.2023 Shore, local beach	Reference K2	47	Acute toxic effect
3	The Vorskla River 7.08.23 /11.08.2023/21.08.2023 Shore, waste water discharge site	Reference K3	20	Acute toxic effect
<i>Daphnia magna Cladocera</i>				
1	The Vorskla River 7.08.23 /11.08.2023/21.08.2023 shore	Reference K1	100	No toxic effect
2	The Vorskla River 7.08.23 /11.08.2023/21.08.2023 Shore, local beach	Reference K2	50	Acute toxic effect
3	The Vorskla River 7.08.23 /11.08.2023/21.08.2023 Shore. Waste water discharge site	Reference K3	29	Acute toxic effect

*Rotaria rotatoria* were fed from the second day onward by adding one drop of a suspension of the microalga *Chlorella vulgaris* to each flask.

The procedure for processing observational data and evaluating biotesting results is demonstrated using calculations for the 96 h exposure period.

Let us calculate the survival rate in three References by Eq. (8):

$$x = (24/30) 100 = 80\%. \quad (8)$$

Survival rates in the reference series have been compared. In both references (K1 and K2), no mortality of *Rotaria rotatoria* has been observed, and the survival rate is 100%.

The decrease in the survival rate in series K3 (Vorskla River water collected at the pollution source) relative to references K1 and K2 is calculated using Eq. (9):

$$A = [(30 - 24)/30] 100 = 20\%. \quad (9)$$

The data are entered into Table 7.

The decrease in survival rate of *Rotaria rotatoria* in the K3 series is less than 25%; therefore, ac-

ording to this parameter, the tested water does not exhibit a toxic effect. To obtain a final assessment of the nature of toxicity, the fecundity rate was calculated. For this purpose, the total number of offspring produced across replicates during the exposure period was determined.

For Reference K2, the number of offspring over 96 h of exposure was  $14 + 16 + 16 = 46$  individuals. For Reference K1, this value was 51 individuals, while for K3 it was 30 individuals.

Fecundity in series K3 and in References K1 and K2 is calculated using Eq. (10). For Reference K2, fecundity equals:

$$E = 46/30 = 1.5. \quad (10)$$

For Reference K1,  $E$  equals 1.7, and for K3,  $E$  equals 1.0. These data are presented according to the format of Table 4.

The deviation of the fecundity rate between the two references is calculated using the corresponding equation. For Reference K1, the deviation equals:

$$n = [(46 - 51)/51] 100 = -11.2\%. \quad (11)$$

Table 6. Data of the Vorskla River Surface Water Biotesting Using *Rotaria rotatoria*

Number of sample	Series	Replicate No.	Survivors (No.)				Offspring (No.)			
			Exposure time, h*							
			24	48	72	96	24	48	72	96
1	Reference K1	1	10	10	10	10	2	5	11	15
		2	10	10	10	10	22	8	13	19
		3	10	10	10	10	1	8	12	17
		Total	30	30	30	30	6	21	36	51
2	Reference K2	1	10	10	10	10	4	8	12	16
		2	10	10	10	10	2	6	8	14
		3	10	10	10	10	0	4	10	16
		Total	30	30	30	30	6	18	30	46
3	Reference K3	1	10	10	10	8	1	7	9	8
		2	10	10	8	9	3	6	9	11
		3	10	10	9	7	2	8	11	11
		Total	30	30	27	24	6	21	29	30

Note: The number of juveniles at each subsequent observation time is determined by summing the individuals born during the previous observation period (transferred) and those born by the time of the current observation.

Table 7. Data of the Express Biotest Using *Rotaria rotatoria*

Number of sample	Series	Calculation number	Number of <i>Chlorella vulgaris</i> Cells per Square of a Goryaev or Fuchs-Rosenthal Chamber		Number of Rotifers in the Crystallizer	Start of the test 10:00	End of the test 12:00
			At the beginning	At the end			
1	Reference 1	13:00	160	115	10	10	10
2	Reference 2	13:00	115	106	10	8	12
3	Reference 3	13:00	184	135	10	7	9
1	Reference 1	15:00	165	164	10	10	10
2	Reference 2	15:00	187	137	10	10	8
3	Reference 3	15:00	174	169	10	10	9
1	Reference 1	14:00	124	122	10	10	10
2	Reference 2	14:00	132	96	10	10	8
3	Reference 3	14:00	98	75	10	10	4

Table 8. Results of Natural Water Biotesting on *Rotaria rotatoria*

Number of sample	Water Body, Sampling Site, Point, Sampling Date	Series	Exposure time	Survival rate, % of Initial number	Reduction in Survival rate, % vs. Reference	Fecundity	Fecundity Deviation, % vs. Reference	Toxicity Assessment
1	The Vorskla River 7.08.23; 11.08.2023; 21.08.2023 Shore	Reference 1	72	100	—	1.7	—	—
2	The Vorskla River 7.08.23; 11.08.2023; 21.08.2023 Shore, local beach	Reference 2	72	100	0	1.5	-11.2	No chronic toxic effect
3	The Vorskla River 7.08.23; 11.08.2023; 21.08.2023 Shore, waste water discharge site	Reference 3	72	80	20	1.0	-70	Chronic toxic effect

Table 9. Conclusion on the Toxicological Status of the Water Ecosystems

Number of sample	Water body	Toxicologic parameters based on the biotesting data
1	The Vorskla River 7.08.23; 11.08.2023; 21.08.2023 shore	Class I — Surface water does not exert toxic effects on biota
2	—	Class II — Surface water exerts subchronic toxic effects on biota
3	The Vorskla River 07.08.23; 11.08.2023; 21.08.2023 Shore, waste water discharge site	Class III — Surface water exerts acute toxic effects on biota

Thus, based on the combined deviations of survival and fecundity rates over a 96 h exposure period, and according to the criteria presented in Tables 5, 6, the water from the background site does not exhibit chronic toxic effects.

Next, the deviation of the fecundity index of the experimental series relative to the reference is calculated using the corresponding formula:

$$n = \left[ \frac{(30 - 51)}{30} \right] 100 = -70\%. \quad (12)$$

The results are presented in Table 8.

Based on the results of biotesting using crustaceans, the surface water from the Vorskla River exerts an acute toxic effect on *Daphnia* (a 29% decrease in the survival rate over 96 h of observation is recorded in Table 9), indicating non-compliance of water quality with toxicological parameters and classification into Class III.

Using calculations at an exposure time of 96 h and based on the decrease in the survival rate (20% < 25%) and deviation in fecundity of the rotifer *Rotaria rotatoria* (70% > 25%), the toxicity of the studied water was assessed. In this case, water from Reference 3 (K3) exhibits an acute toxic effect on *Rotaria rotatoria*.

According to the experimental results and the developed classification presented in Table 9, the tested river water is classified as Class I by toxicological parameters and does not comply with established water quality standards.

Using the developed *Bioindication Water Test* method, the effect of toxic water has been determined based on toxicological parameters of tested water samples collected from a water body, employing laboratory cultures of *Daphnia* and the rotifer *Rotaria rotatoria* obtained from the same water body as an indicator organism, since it is capable of detecting adverse changes in water quality.

Our method involves the simultaneous use of three different bioindicators within a short period of time. This approach is justified by the fact that crustaceans differ in their sensitivity to toxic substances present in wastewater. The calculation of parameters is based on Student's distribution hypotheses. The main limitation of the method is that biotesting using natural populations is technically more labor-intensive; however, it provides prospects for predicting the impact of toxic pollution on the biota of a specific aquatic ecosystem.

During environmental monitoring conducted by national regulatory authorities, when surveying water bodies in accordance with the international regulatory documents and European standards referenced in our study, it is necessary to collect samples not only for physicochemical analysis but also to assess the effects of pollutants under laboratory conditions by observing the responses of living organisms. Upon completion of biological testing, a report should be prepared indicating deficiencies and violations that lead to ecosystem degradation and mortality, and enforcement notices should be issued to responsible parties.

**Conflict of Interest.** The authors O. V. Stepova and L. M. Tyagnij declare that they have no conflicts of interest related to this study, including financial, personal, authorship, or other relationships that could influence the research or the results presented in this article.

**Funding.** This research is funded solely by the authors.

**Acknowledgements.** The authors are grateful to the administration of Yuri Kondratyuk Poltava Polytechnic National University (Poltava, Ukraine) for providing the opportunity to conduct this study.

## REFERENCES

1. Arystarkhova, E. O. (2017). Rapid Assessment of Potential Water Hazard Using Biotesting on *Daphnia Magna* S. *Bulletin of Agricultural Science*, 95(2), 50–54. <https://doi.org/10.31073/agroviznyk201702-09> (in Ukrainian).
2. Barabash, O. V. (2019). Assessment of the Toxicity Level of Surface Waters in Kyiv. *Ecological Safety*, 2(28), 31–37. <https://doi.org/10.30929/2073-5057.2019.2.31-37> (in Ukrainian).
3. Stytsiuk, L. M. (2013). Using Bioindication and Biotesting Methods to Assess the State of Aquatic Ecosystems. *Bulletin National University of Water and Environmental Engineering*, 2(62), 175–184 (in Ukrainian).

4. *Water Framework Directive 2000/60/EC. Key terms and their definitions.* (2006). Kyiv.
5. *Directive of the European Parliament and of the Council 2009/31/EC as amended by Decision No. 2455/2001/EC.* URL: <https://eur-lex.europa.eu/eli/dir/2009/31/oj/eng> (Last accessed: 20.11.2025).
6. *Directive 91/271/EC of the European Parliament and of the Council concerning urban waste water treatment, as amended by Directive 98/15/EC and Regulation (EC) No 1882/2003 and Regulation (EC) No. 1137/2008.* URL: <https://eur-lex.europa.eu/eli/dir/1991/271/oj/eng> (Last accessed: 20.11.2025).
7. DSTU 7525:2014 *Drinking water. Requirements and methods of quality control.* Order of 23.10.2014 No. 1257. Developer: Institute of Colloidal Chemistry and Water Chemistry named after A. V. Dumansky of the National Academy of Sciences of Ukraine (ICCHW of the National Academy of Sciences of Ukraine). URL: [https://www.ksv.biz.ua/publ/dstu/dstu\\_7525\\_2014/3-1-0-2467](https://www.ksv.biz.ua/publ/dstu/dstu_7525_2014/3-1-0-2467) (Last accessed: 20.11.2025).
8. Skok, S. V. (2015). Assessment of Drinking Water Quality in Kherson Using Biotesting Method. *Agroecological journal*, 2, 26—30.
9. Loboda, N. S., Pylypiuk, V. V. (2013). Analysis of Changes in Water Quality of the Psel and Vorskla Rivers Over Time. *Proceedings of the II International Conference “Youth in Addressing Environmental and Socio-Economic Issues of Today” (10—15 Juny, 2013, Odesa)*, 109—111. Odessa.
10. Sydorovych, M. M. (2013). Use of biometric indexes of *Allium* test for determination of quality of drinking-water of city. *Naukovi Chasopys Dragomanov Ukrainian State University. Series 20. Biology*, 5, 182—192. URL: <https://ekhsuir.kspu.edu/handle/123456789/364> (Last accessed: 20.11.2025).
11. Didukh, Ya. P. (2012). *Basics of Bioindication: monograph.* Kyiv [in Ukrainian].
12. Dudnik, S. V., Yevtushenko, M. Yu. (2013). *Aquatic toxicology: Basic theoretical provisions and their practical application: monograph.* Kyiv [in Ukrainian].
13. Klymenko, M. O., Pryshchepa, A. M., Klymenko, O. M., Stetsyuk, L. M. (2014). *Assessment of the state of aquatic ecosystems by biotesting indicators.* Rivne [in Ukrainian].
14. Skok, S., Breus, D. (2021). Bioindication Assessment of Drinking Water Toxicity in Large Cities of Ukraine. *Indian Journal of Ecology*, 48(6), 1692—1697. URL: <https://dspace.ksaeu.kherson.ua/handle/123456789/7938?show=full> (Last accessed: 20.11.2025).
15. Remeshevska, I., Trokhymenko, G., Gurets, N., Stepova, O., Trus, I., Akhmedova, V. (2021). Study of the Ways and Methods of Searching WaterLeaks in Water Supply Networks of the Settlements of Ukraine. *Ecological Engineering & Environmental Technology*, 22(4), 14—21. <https://doi.org/10.15421/jchemtech.v31i4.287024>
16. Naumann, E. (1933). *Daphnia magna* Straus als Versuchstiere. *Kgl. Fysiog. Saliskap, Lund for hunde*, 2, 1—49.
17. Osmalenyy, M. S., Holovkov, A. M., Naniieva, A. V., Vergolias, M. R. (2015). Comprehensive evaluation of toxicity of water samples using plant and animal test organisms. *Factors in experimental evolution of organisms*, 16, 74—77. URL: <https://nasplib.isoftware.kiev.ua/items/04bf98bd-8fc8-4ea8-b92a-7fdd826b1487> (Last accessed: 20.10.2025).
18. Stepova, O. V., Tyagniy, L. M. (2024). Comprehensive assessment of the bioecological system of the Vorskla River based on diagnostics of bioindicators. *Abstracts of the 76th scientific conference of professors, teachers, researchers, postgraduates and students of the university (14—23 May, 2024, Poltava)*, 1, 332—333 [in Ukrainian].
19. EN ISO 5667-3:2022. Water quality. Sampling. Part 3. Storage and processing of water samples (EN ISO 5667-3:2018, IDT; ISO 5667-3:2018, IDT) URL: <https://standards.globalspec.com/std/10381531/iso-5667-3#:~:text=Water%20quality%20-%20Sampling%20-%20Part%203%3A%20Preservation,all%20water%20samples%20including%20those%20for%20biological%20analyses> (Last accessed: 20.10.2025).
20. EN ISO 10870:2012. Water quality. Recommendations for the selection of methods and devices for sampling benthic macroinvertebrates in freshwaters. URL: <https://www.en-standard.eu/iso-10870-2012-water-quality-guidelines-for-the-selection-of-sampling-methods-and-devices-for-benthic-macroinvertebrates-in-fresh-waters/#:~:text=This%20International%20Standard%20specifies%20criteria%20for%20the%20selection,in%20fresh%20waters%20%28rivers%2C%20canals%2C%20lakes%2C%20and%20reservoirs%29> (Last accessed: 20.10.2025).
21. EN 16150:2012. Water quality — Guidance on proportional sampling of benthic macroinvertebrates from multiple habitats in rivers passing through a watercourse. ISO 16150:2012. URL: <https://www.nen.nl/en/nen-en-16150-2012->

- en-171108#:~:text=This%20European%20Standard%20gives%20guidance%20on%20procedures%20for,of%20benthic%20macro-invertebrates%20in%20wadeable%20rivers%20and%20streams (Last accessed: 20.10.2025).
22. DSTU 4173: 2003. Water quality. Determination of acute lethal toxicity on *Daphnia magna* Straus and *Ceriodaphnia affinis* Lilljeborg (*Cladocera, Crustacea*). ISO 6341: 1998, MOD. URL: [https://online.budstandart.com/ua/catalog/doc-page.html?id\\_doc=72858](https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=72858) (Last accessed: 20.10.2025).
23. KND 211.1.4.046-95. Biotesting and determination of acute lethal toxicity levels of return waters discharged into water bodies. Methodology. Approved by order of the Ministry of Environmental Protection of Ukraine dated 30.05.95 No. 47.
24. KND 211.1.4.056-97. Methodology for determining chronic toxicity of water on the crustacean *Ceriodaphnia affinis* Lilljeborg. Approved by order of the Ministry of Environmental Protection of Ukraine dated 21.05.97 No. 68.
25. Student. The probable error of a mean. (1906). *Biometrika*, 6(1), 1—25. URL: <https://academic.oup.com/biomet/article-abstract/6/1/1/225634> (Last accessed: 20.10.2025).

Received 26.06.2025

Revised 17.09.2025

Accepted 29.10.2025

Л.М. Тягній (<https://orcid.org/0000-0002-4954-002X>),

О.В. Степова (<https://orcid.org/0000-0002-6346-5484>)

Національний університет «Полтавська політехніка імені Юрія Кондратюка»,  
просп. Віталія Грицаєнка, 24, Полтава, 36011, Україна,  
+380 53 256 1604, [eco.poltntu@gmail.com](mailto:eco.poltntu@gmail.com)

#### БІОТЕСТУВАННЯ ЗАБРУДНЕНИХ ПОВЕРХНЕВИХ ВОД РІЧКИ ВОРСКЛИ З ВИКОРИСТАННЯМ ТРЬОХ ІНДИКАТОРІВ «ТЕСТ-ОБ'ЄКТІВ»

**Вступ.** Відповідно Водно рамкової Директиви 2000/60/ЄС Європейського Парламенту і Ради «Про встановлення рамок діяльності Співтовариства в галузі водної політики» розроблено стандарти для моніторингу, обстеження, відбору проб та проведення лабораторно аналізу водних об'єктів.

**Проблематика.** Проблемою в Україні є відсутність технологій захисту та методів контролю, які б передбачали стан водного об'єкта за токсикологічними параметрами на основі «токсикології». За допомогою біотестування можливо визначити вплив стічних вод за цими параметрами, відповідно до висновку про токсикологічний стан водних екосистем.

**Мета.** Дослідження поверхневих вод водойм методом біотестування та визначення впливу стічних вод на біоценози, які беруть участь у процесах самоочищення водних об'єктів, за токсикологічними параметрами.

**Матеріали й методи.** Використано метод «*Bioindication Water Test*», заснований на проведенні окремих постановочних тестів з використанням лабораторної культури *Daphnia*, та на природних популяціях коловороток *Rotaria rotatoria* як «тест-об'єктах» з досліджуваних водойм. Застосовано: лабораторні, мікроскопічні, біотестування, статистичні методи.

**Результати.** За результатами біотестування на рачках вода з річки Ворскла справила «гостру токсичну дію» на *Daphnia* та *Rotaria rotatoria* (zareєстровано 50% зниження виживання за 96 год спостережень), що вказує на невідповідність якості води за токсикологічними показниками встановленим нормам. За результатами токсикологічного аналізу поверхневих вод найбільш забрудненими є води взяті з місця скиду стоків у районі дощового колектору м. Полтави.

**Висновки.** Біотестування як інтегральний метод оцінки токсичності водного середовища є обов'язковим доповненням до фізико-хімічного аналізу. Він дає змогу практичним шляхом проводити спостереження за реакцією живих організмів у водоймі, контролюючи скиди стічних вод, що призводять до руйнування унікальних екосистем, при цьому виконавши умови Водно Рамкової Директиви 2000/60/ЄС щодо покращення стану водойм в Україні.

**Ключові слова:** біотестування, стічні води, *Rotaria rotatoria*, *Daphnia magna*, *Daphnia moina*.