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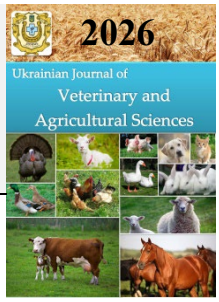

Comparative Assessment of the toxic effects of potassium dichromate, phenol, and lambda-cyhalothrin on the functional activity of carp (*Cyprinus carpio L.*) spermatozoa using biotesting

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Abstract

The results of an experimental study on the toxic effects of compounds of different chemical natures on the functional state of fish germ cells, using the biotesting method, are presented. The study examines the effects of potassium dichromate, phenol, and λ-cyhalothrin on the motility of carp (*Cyprinus carpio L.*) spermatozoa, a sensitive bioindicator of aquatic environmental quality. The work aimed to determine the specific features of the toxic effects of these substances under short-term exposure and to provide a comparative evaluation of their influence on the duration of active sperm motility. The research methodology used freshly obtained carp milt, which was exposed to aqueous solutions of toxicants at specified concentrations. Biotesting was performed by determining the duration of sperm motility after activation in media containing phenol (1, 10, 300 mg/L), potassium dichromate (5, 10, 155 mg/L), and λ-cyhalothrin (37.1 mg/L). Control samples were prepared without the addition of toxic substances. The evaluation was carried out microscopically by recording the time until complete cessation of sperm movement. The obtained results were statistically processed by calculating mean values and assessing the significance of differences. The study established that all tested toxicants under short-term exposure (60 s) cause a decrease in the motility of carp spermatozoa; however, the toxic effect manifests only at concentrations that significantly exceed the maximum permissible levels. It was found that the most pronounced inhibitory effect on the functional activity of spermatozoa is observed with potassium dichromate, which is associated with its ability to disrupt cellular respiration and adenosine triphosphate synthesis rapidly. For phenol and potassium dichromate, no clear "dose–effect" relationship was established within the concentration ranges studied, indicating the initial stage of toxic action development during short-term contact. The effect of λ-cyhalothrin was accompanied not only by a reduction in the duration of motility but also by a disturbance in its character, manifested as chaotic (non-progressive) sperm motility. It is shown that using carp spermatozoa as a test system has limitations due to their short activity, which complicates the assessment of the toxicity of substances with different mechanisms of action. At the same time, this approach may be effective in detecting acute effects of highly concentrated pollutants and could serve as a rapid biotesting method.

Keywords: biotesting, toxicity, carp spermatozoa, motility, potassium dichromate, phenol, λ-cyhalothrin, aquatic environment, hydrobionts, test object, rapid assessment.

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1. Introduction

The main anthropogenic factor negatively affecting the ecological state of water bodies is chemical pollution, which can exert toxic effects on aquatic biocenoses. As a result, there is a decrease in the biological productivity of water bodies, disruption of self-purification processes, and deterioration of water quality (Snizhko, 2001; Abdelkader et al., 2022).

Various approaches are used to monitor the ecological state of aquatic ecosystems, including water biotesting. In most cases, it involves laboratory analysis of water samples from rivers, lakes, and other water bodies. Traditionally, such analyses are performed using physicochemical methods: measurement of temperature, determination of pH, electrical conductivity, dissolved oxygen content, chemical and biochemical oxygen demand, concentrations of heavy metals and biogenic compounds, etc. (Romanenko et al., 1998; Nabyvanets et al., 2007).

The disadvantage of such studies is the difficulty in assessing and predicting the impact of a complex of factors on aquatic organisms. In these cases, synergistic effects (when

the total effect of factors significantly exceeds the sum of individual effects) or antagonism (when one factor weakens or blocks the action of another) are possible (Landis et al., 2017; Newman, 2019).

Thus, to address the complex and multifaceted problem of environmental pollution, biological testing methods have recently gained increasing importance (Gárriz & Miranda, 2020). The essence of biotesting is an analysis that assesses the toxicity of the aquatic environment by observing the reactions of living organisms (test objects) to water samples. This method makes it possible to study the combined (integrated) effect of all substances present in water on representatives of living nature inhabiting it (Nabyvanets et al., 2007; Arystarhova, 2017). An important advantage of bi; otesting is the ability to detect sublethal effects that are not detected by physicochemical methods but can significantly affect the physiological state of hydrobionts (Alavi & Cosson, 2005; Varadarajan et al., 2014; OECD, 2021).

One of the main disadvantages of the method is its d; ependence on the species, age, and physiological state of the test organisms, which can cause the same water sample to yield different indicators. Optimal test objects for biotesting are considered to be sensitive, relatively homogeneous (in particular, genetically or physiologically) groups of organisms that reproduce quickly and are easily cultivated under laboratory conditions (Kutluyer et al., 2015; Arystarhova, 2017).

The most suitable test object that meets these parameters is fish milt, particularly its active sperm motility duration (Kollár et al., 2018a, 2018b). The motility of fish spermatozoa is a sensitive indicator of toxic action, as it directly depends on the state of cell membranes, energy metabolism, and ion homeostasis (Cosson et al., 2008; Kutluyer et al., 2015).

The advantages of this test object are that male fish gametes are relatively easy to obtain in sufficient volume. In addition, they are characterized by a relative homogeneity of physiological responses and high sensitivity, making them an effective tool for rapid biotesting of ecotoxicity in aquatic environments. No less important is that such biotesting helps reduce the use of living organisms and thus largely aligns with the principles of bioethics and the ethical foundations of toxicological research (Kutluyer et al., 2015).

It should be noted that organisms' resistance to toxicants is significantly influenced by the nature of the toxic compounds themselves, which in turn affects the results of biotesting. Particularly dangerous are xenobiotics, in particular pesticides, which can exhibit high biological activity even at low concentrations (Singh et al., 2008; Lopes et al., 2020; Yao et al., 2024).

Some toxicants are natural compounds that are constantly present in the aquatic environment. Aquatic organisms have evolved certain protective mechanisms against them over time. Others are synthetic (newly created) substances that hydrobionts have never encountered before and, accordingly, lack effective protective mechanisms (Abdelkader et al., 2022; Mostoviak et al., 2022; Xu et al., 2023).

Our research aimed to determine the negative impact of toxicants of different chemical natures on the motility of fish spermatozoa using the biotesting method.

The objectives of the research were to compare the effects of toxic contaminant compounds (phenol, potassium dichromate) and xenobiotics (λ-cyhalothrin). It should be noted that phenol and potassium dichromate are widely used as model or standard toxicants in toxicological studies and are applied to assess the viability of hydrobionts (Vutukuru, 2005; Lopes et al., 2020; Abdelkader et al., 2022).

2. Materials and methods

The research was conducted at the Bila Tserkva Experimental Hydrobiological Station of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine. The object of the study was freshly obtained milt (sperm) of carp (*Cyprinus carpio L.*). The milt was collected by hand stripping after pituitary injection in 4–5-year-old breeders. Before use, sperm samples were visually assessed and admitted for analysis only in the absence of blood, urine, or water admixtures.

To achieve the set aim, the activity of carp spermatozoa under different toxicant concentrations was studied. The test substances used were generally accepted standard toxicants: phenol (C_6H_5OH), potassium dichromate ($K_2Cr_2O_7$, recalculated to chromium), and the insecticide λ-cyhalothrin ($C_{23}H_{19}ClF_3NO_3$)

Stock (concentrated) solutions were prepared, then diluted to working concentrations: phenol – 1, 10, and 300 mg/L; potassium dichromate (recalculated to chromium) – 5, 10, and 155 mg/L; λ-cyhalothrin was studied at a single concentration – 37.1 mg/L. The control sample was prepared using distilled or settled water without the addition of toxicants.

The determination of toxic effects was performed as follows: 0.1 mL of sperm was added to 5 mL of toxicant solution (or control solution), quickly mixed, and then 0.1 mL of the suspension was placed on a microscope slide. Sperm motility was observed under an AU-12 microscope at ×400 magnification at a temperature of 20–22 °C. The duration of activity (until complete cessation of motility) was recorded with a mechanical stopwatch SPOPR-2a-3-040. Motility assessment was performed immediately after sperm activation. Each concentration was tested in 3–5 biological replicates (sperm from different breeders) and 3 technical replicates. Results were presented as mean values ± standard error (M ± m).

The data obtained were statistically analyzed using Statistica 10 and Microsoft Excel. The significance of differences between groups was assessed using Student's t-test at a significance level of $p < 0.05$.

3. Results and discussion

The effects of phenol, potassium dichromate, and λ-cyhalothrin on the activity of carp (*Cyprinus carpio L.*) spermatozoa revealed somewhat unexpected toxic effects.

It is known that the maximum permissible concentration (MPC) of phenol for natural water bodies of fishery designation is 0.001 mg/L. In our study, only at a concentration of 1 mg/L phenol was a slight decrease in sperm motility observed, by 6.6 % relative to the control (Fig. 1). At higher concentrations (10 and 300 mg/L), sperm motility decreased by 17.3 % relative to the control. At the same time, the degree of activity suppression in the groups with 10 mg/L and 300 mg/L was practically the same.

Thus, the negative effect of phenol on carp sperm motility was observed only at concentrations that significantly exceeded the established MPC. In addition, a 30-fold in-

crease in phenol concentration (from 10 to 300 mg/L) did not significantly enhance the toxic effect. This indicates that the toxic action of phenol on spermatozoa in the studied ranges is only beginning to manifest and is likely to have a threshold character.

Obviously, the relatively weak effect of phenol on carp sperm activity is attributable to the short exposure duration. The contact time of spermatozoa with the toxic environment was insufficient for full interaction between the biological object and the toxicant.

The literature indicates that the negative effect of phenol on hydrobionts usually manifests after several hours of exposure (Hatef et al., 2013; Landis et al., 2017). In our study, the exposure duration of spermatozoa in phenol solutions was significantly shorter, owing to the physiological characteristics of fish spermatozoa.

A mature spermatozoon is a cell with minimal cytoplasmic volume. After leaving the body, it remains in an immotile state with a low metabolic rate. Upon contact with water, spermatozoa are activated, accompanied by a sharp increase in oxygen consumption and ATP utilization. Spermatozoa begin to move actively at a speed of 164–330 µm/s. However, their motility is short-lived due to the cell's limited energy reserves. The duration of active motility of fish spermatozoa, as a rule, does not exceed 1–3 min (Cosson et al., 2008; Zietara et al., 2009; Cosson, 2010).

Thus, our study showed that phenol at a concentration of 1 mg/L caused a slight decrease in carp sperm activity (by 6.6 %), while in the concentration range of 10–300 mg/L, an almost identical decrease in motility was observed, averaging 17.3 %. This indicates that only the initial stage of the negative effect of phenol was observed at the studied concentrations.

More pronounced toxic effects could not be registered due to the extremely short physiological activity of fish spermatozoa.

The negative effect of potassium dichromate on carp sperm activity was similar to that of phenol. As seen in Fig. 2, the decrease in sperm motility in the experimental groups was practically the same.

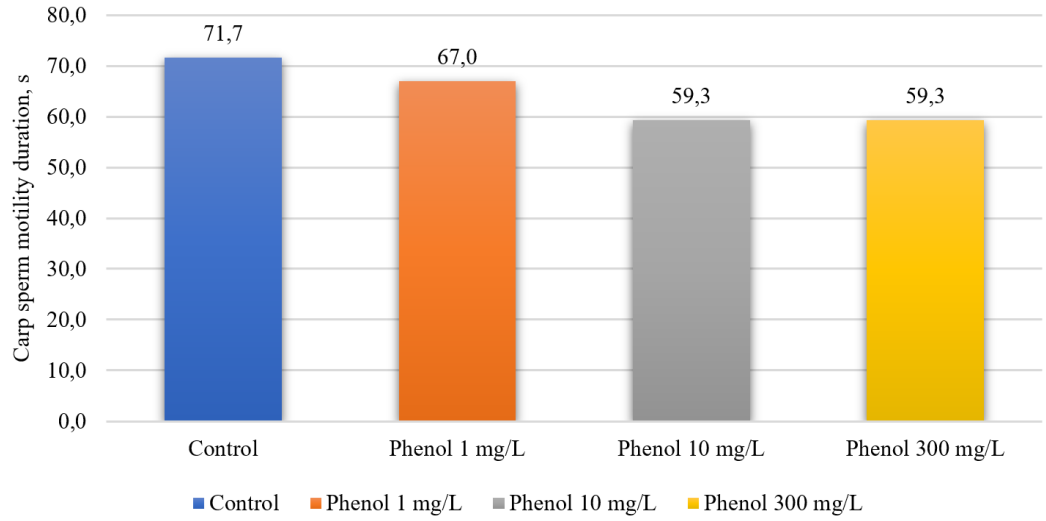

Fig. 1. Duration of active motility of carp (*Cyprinus carpio L.*) spermatozoa under the action of different phenol concentrations in water, s (n = 5)

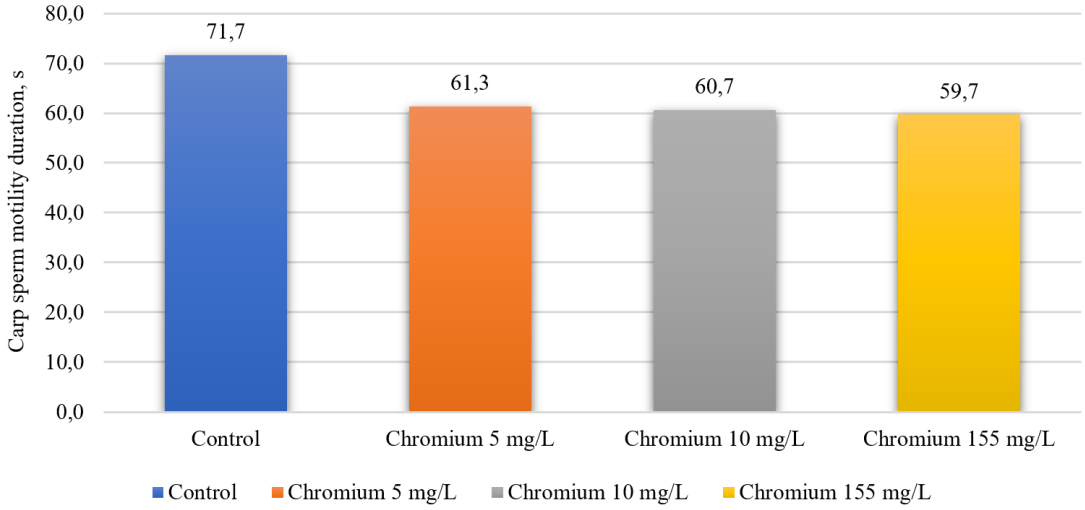

Fig. 2. Duration of active motility of carp (*Cyprinus carpio L.*) spermatozoa under the action of different potassium dichromate concentrations in water, s (n = 5)

Thus, at concentrations of 5 mg/L, 10 mg/L, and 155 mg/L, sperm activity decreased by 14.5 %, 15.4 %, and 16.8 %, respectively, compared to the control.

Therefore, as in the case of phenol, the toxic action of potassium dichromate on carp spermatozoa manifests only at the initial stage. At this stage, there is still no clear differentiation of the negative effect by concentration gradient. It should be noted that the concentrations of potassium dichromate used exceed the maximum permissible concentration (MPC) of 0.05 mg/L by tens and hundreds of times.

Comparison of the toxic effects of the two reference toxicants showed that potassium dichromate is more toxic to carp spermatozoa than phenol. Thus, at concentrations exceeding the respective MPCs by 100 times (1 mg/L phenol and 5 mg/L potassium dichromate), the decrease in sperm motility in the potassium dichromate solution was 38.2% stronger than in the phenol solution. This difference is probably due to the differing specificities of action of these toxicants on cellular biochemical processes.

It is known that the toxic effect of phenol on fish at the biochemical level manifests through disruption of energy metabolism. The main effects include inhibition of enzymatic activity, protein denaturation, and disruption of transamination processes (Mudra, 2014).

The toxic effect of potassium dichromate is due to its inhibition of the respiratory chain. The compound inhibits key enzymes, particularly succinate dehydrogenase, leading to the cessation of oxidative phosphorylation, the loss of membrane potential, and a significant decrease in ATP synthesis (Cosson, 2010).

Thus, when comparing the toxic effects of the studied substances, it can be concluded that both toxicants significantly reduce the viability of carp spermatozoa. Still, the mechanisms of their toxicity differ substantially. Potassium dichromate acts as a chemical oxidizing agent that directly disrupts energy metabolism, particularly ATP synthesis. In contrast, phenol is a structural poison that triggers a chain reaction of lipid peroxidation, ultimately leading to the destruction of sperm membranes.

Since sperm motility directly depends on ATP levels in the cell (Perchec et al., 1995; Dietrich et al., 2011), significant disruption of energy metabolism by potassium dichromate causes a faster and more pronounced suppression of their activity than phenol. Therefore, the difference in the toxic effects of these two substances is associated with their distinct abilities to influence the energy processes that enable the active movement of fish spermatozoa (Paravani et al., 2019).

No less interesting results were obtained under the action of λ-cyhalothrin on the duration of active motility of carp spermatozoa. This potent insecticide, a synthetic pyrethroid, acts by grossly disrupting ion channel function in cell membranes (Abdelkader et al., 2022; Xu et al., 2023; Sharma et al., 2024).

The results of our studies confirmed that a 60 s exposure duration is insufficient for full-scale biotesting of carp spermatozoa. A significant decrease in sperm motility was observed only at a concentration of 37.1 mg/L, by 8.0% relative to the control (Fig. 3).

The concentration of λ-cyhalothrin (37.1 mg/L) used in the study exceeds the maximum permissible concentration for household and drinking water bodies, which is 0.0001 mg/L, by many orders of magnitude.

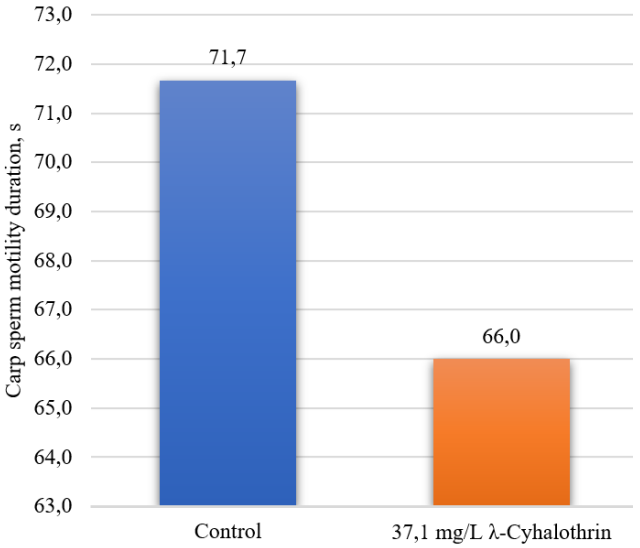

Fig. 3. Duration of active motility of carp (*Cyprinus carpio L.*) spermatozoa under the action of different λ-cyhalothrin concentrations in water, s (n = 5)

Unlike phenol and potassium dichromate, the action of λ-cyhalothrin, in addition to reducing the duration of active motility, was also accompanied by a disturbance in the character of sperm motility. About 50 % of the spermatozoa moved chaotically (non-progressively). When assessed on the 5-point Persov scale, sperm motility corresponded to 3 points ("satisfactory").

Such motility disturbance is obviously a direct consequence of ionic and energy disorders in the cell. For fish spermatozoa, this means biological death, as they lose the ability to fertilize eggs.

Thus, the results obtained confirm our assumption that the negative effect of the studied toxicants (phenol, potassium dichromate, and λ-cyhalothrin) under short-term exposure (60 s) is observed only at concentrations that significantly exceed the current maximum permissible concentrations.

4. Conclusions

It was established that under short-term exposure (60 s), the studied toxicants (phenol, potassium dichromate, and λ-cyhalothrin) reduce the motility of carp spermatozoa; however, the toxic effect is observed only at concentrations that significantly exceed the maximum permissible levels.

It was found that potassium dichromate exerts the most pronounced inhibitory effect on the functional activity of carp spermatozoa during short-term exposure, likely due to its ability to disrupt cellular respiration and ATP synthesis rapidly.

The absence of a clear "dose–effect" relationship for phenol and potassium dichromate across the studied concentrations was observed, indicating the initial stage of toxic action development under short exposure times.

It was established that carp spermatozoa, as a test object, have limitations in biotesting the aquatic environment, particularly for assessing the toxicity of substances with different mechanisms of action. Still, they can be used to detect acute effects of highly concentrated toxicants.

The prospect for further research is to increase the informativeness of biotesting using fish spermatozoa, in par-

ticular by optimizing the exposure duration, expanding the range of studied concentrations, and involving additional indicators of the functional state of cells, as well as a comparative study of the sensitivity of spermatozoa of different fish species to the action of toxicants of different chemical nature.

Conflict of interest

The authors declare no conflict of interest.

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