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**ASSESSMENT OF THE COMBINED EFFECT OF HEAVY METALS  
AND SURFACTANTS ON CARP FISH ORGANISMS**

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**ОЦІНКА КОМБІНОВАНОГО ВПЛИВУ ВАЖКИХ МЕТАЛІВ І ПОВЕРХНЕВО-  
АКТИВНИХ РЕЧОВИН НА ОРГАНІЗМ КОРОПОВИХ РИБ****ABSTRACT**

Pollution of aquatic ecosystems is one of the current environmental problems that negatively affects aquatic organisms, in particular cyprinid fish. Heavy metals and surfactants are the main anthropogenic toxicants that can cause significant morphological and biochemical changes in the body of fish. Research into their impact is important for assessing the state of water resources and developing measures to reduce environmental risks.

**Purpose of the work:** to assess the impact of heavy metal ions ( $Zn^{2+}$ ,  $Cd^{2+}$ ) and sodium lauryl sulfate on the morphological and biochemical indicators of the carp body. The object of the study is biochemical and morphological transformations in the carp body. The subject of the study is changes in the content of glucose, proteins, lipids and moisture in carp tissues under the influence of toxic substances.

**Methodology.** The study was conducted on two-year-old carp of the scaly and mirror morphotypes (*Cyprinus carpio* L.) weighing 250–350 g. Fish were kept in 200 dm<sup>3</sup> aquariums with settled tap water, in which a stable hydrochemical regime was maintained. Toxic load was created by introducing appropriate concentrations of  $Zn^{2+}$ ,  $Cd^{2+}$  and sodium lauryl sulfate. Morphological changes (damage to the skin, fins, gills) and biochemical parameters (content of glucose, proteins, lipids and moisture in tissues) were analyzed. The results obtained were processed by statistical methods.

**Scientific novelty.** For the first time, the combined effect of heavy metals and surfactants on the morphological and biochemical parameters of the carp organism was studied, which allows using ichthyological parameters as bioindicators of aquatic environment pollution.

**Conclusions.** During the experiment, changes in the appearance of the fish were observed, in particular damage to the fins and skin and scales, increased mucus secretion in carp that were in an aquarium with sodium lauryl sulfate for 14 days. When toxicants of various origins act on the fish body, the glucose content is more sensitive than the total protein content. These indicators can be recommended for monitoring water bodies using fish of the cyprinid family. Under the influence of toxic environmental conditions, a decrease in the lipid content was observed in both white muscles and liver tissues, with the exception of the simultaneous exposure to sodium lauryl sulfate and ions. An increase in the amount of lipids in the liver can lead to exhaustion of the body and indicate tissue degeneration. When studying the effect of toxic substances on the moisture content in various carp tissues, no significant changes were observed. The presence of a multi-stage cell defense system, which has developed during phylogenetic development, determines the complexity of the cause-and-effect relationships between biochemical processes and their focus, primarily, on maintaining the optimal metabolic balance of the cell and the organism as a whole, which is expressed in changes in biochemical indicators.

**Key words:** scaly carp, heavy metals, surfactants, ichthyological indicators, metabolic changes

**АНОТАЦІЯ**

Забруднення водних екосистем є однією з актуальних екологічних проблем, що негативно впливає на гідробіотів, зокрема на корошових риб. Важкі метали та поверхнево-активні речовини є основними антропогенними токсикантами, які можуть спричинити значні морфологічні та біохімічні зміни в організмі риб. Дослідження їхнього впливу є важливим для оцінки стану водних ресурсів та розробки заходів щодо зменшення екологічних ризиків.

**Мета роботи:** оцінити вплив іонів важких металів ( $Zn^{2+}$ ,  $Cd^{2+}$ ) та натрій лаурилсульфату на морфологічні та біохімічні показники організму коропа. Об'єкт дослідження – біохімічні та морфологічні перетворення в

організмі коропа. Предмет дослідження – зміни вмісту глюкози, білків, ліпідів і вологи в тканинах коропа під впливом токсичних речовин.

**Методологія.** Дослідження проводили на дворічних коропах лускатого та дзеркального морфотипів (*Cyprinus carpio* L.) масою 250–350 г. Риб утримували в акваріумах об'ємом 200 дм<sup>3</sup> із відстояною водопровідною водою, в якій підтримувався стабільний гідрохімічний режим. Токсичне навантаження створювали шляхом внесення відповідних концентрацій Zn<sup>2+</sup>, Cd<sup>2+</sup> та натрій лаурилсульфату. Аналізували морфологічні зміни (пошкодження шкіри, плавців, зябер) та біохімічні показники (вміст глюкози, білків, ліпідів і вологи в тканинах). Отримані результати обробляли статистичними методами.

**Наукова новизна.** Вперше досліджено комбінований вплив важких металів та ПАР на морфологічні та біохімічні показники організму коропа, що дозволяє використовувати іхтіологічні параметри як біоіндикатори забруднення водного середовища.

**Висновки.** Під час досліду було зафіксовано зміни у зовнішньому вигляді риб, зокрема пошкодження плавців, шкірних покривів та луски, а також підвищене виділення слизу у коропів, що перебували в акваріумі з натрій лаурилсульфатом протягом 14 діб. Встановлено, що під впливом токсикантів різного походження рівень глюкози в організмі риб є більш чутливим показником порівняно з вмістом загального білка. Отримані результати дозволяють рекомендувати ці показники для моніторингу стану водойм із використанням риб родини коропових. За умов токсичного впливу спостерігалось зниження рівня ліпідів як у білих м'язах, так і в тканинах печінки, за винятком одночасного впливу натрій лаурилсульфату та йонів кадмію. Збільшення концентрації ліпідів у печінці може свідчити про енергетичне виснаження організму та можливі дегенеративні зміни у тканинах. Аналіз впливу токсичних речовин на вміст вологи в різних тканинах коропа не виявив значних змін. Високий рівень захисних механізмів клітини, що сформувалися в ході філогенетичного розвитку, обумовлює складність взаємозв'язків між біохімічними процесами та спрямованість цих змін, перш за все, на підтримку оптимального метаболічного балансу клітини та організму загалом, що проявляється у змінах біохімічних показників.

**Ключові слова:** короп лускатий, важкі метали, поверхнево-активні речовини, іхтіологічні показники, метаболічні зміни

## Formulation of the problem

The rapid pace of urbanization, human activity and the rapid development of science and technology have introduced significant changes into the historically formed relationships between living organisms in natural ecosystems, which are manifested in the disruption of their functioning and the impoverishment of the species composition. Aquatic ecosystems are characterized by significant anthropogenic load, because inland water bodies provide the needs of energy, water transport, industry, agriculture, etc. As a result of intensive exploitation, completely new, often toxic substances enter water bodies or the concentration of already existing ones significantly increases. Therefore, the comprehensive use of water resources leads to complex pollution of water bodies. Among the pollutants, heavy metals and surfactants (surfactants) have recently taken one of the first places. A significant increase in the content of heavy metals in water leads to numerous biochemical, physiological and morphological changes in the body. Since the normal life of fish is determined by the coordinated work of all functional systems and biochemical processes at their core, a deviation from the norm in one of them, caused by a toxicant, can lead to disruption of the life of the whole organism. Surfactants are

able to exert an inactivating or stimulating effect on enzyme systems and disrupt metabolic processes in the body.

In the modern world, pollution with heavy metals and surfactants is often observed simultaneously (Nikolaenko et al., 2023). That is why, to assess the possible combined effect of these toxicants on the fish organism, it is important to conduct studies aimed at establishing patterns, features and levels of accumulation of heavy metals in the organs and tissues of commercially valuable fish species in order to predict the possible impact of toxic substances on the composition and population of ichthyofauna.

**The purpose** of the work: study of the separate and combined effects of toxic concentrations of heavy metal ions (Zn<sup>2+</sup>, Cd<sup>2+</sup>) and surfactant (sodium lauryl sulfate (SLS)) on the content of energy substrates and moisture in fish tissues.

## Materials and methods

The studies were conducted in November 2024 – January 2025 on two-river carp (*Cyprinus carpio* L.) weighing 250-350 g. According to ichthyological observations, no pathogens of parasitic diseases were detected in the fish. Experiments to study the effect of toxicants were carried out in model conditions – aquariums with a volume of 200 dm<sup>3</sup> with

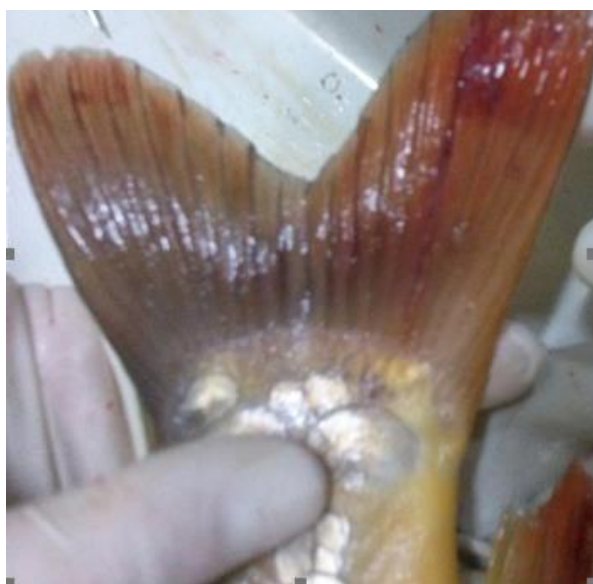
settled tap water, in which the fish were placed at the rate of 1 specimen per 40 dm<sup>3</sup> of water. The acclimation period was 3 days, the exposure to toxicants was 14 days. The fish were not fed. The total number of animals involved in the experiment was 40 fish. In all cases, control was carried out and a constant hydrochemical regime of the water was maintained. The pH value was  $7.30 \pm 0.27$ ; the oxygen content was  $5.6 \pm 0.4$  mg/dm<sup>3</sup>, the temperature was maintained close to natural. Tapeworms were also not recorded. The fish were kept in four variants: control, heavy metal exposure (Zn<sup>2+</sup> or Cd<sup>2+</sup>), surfactant exposure (sodium lauryl sulfate), simultaneous exposure to sodium lauryl sulfate and heavy metal ions (Zn<sup>2+</sup> or Cd<sup>2+</sup>). The concentration of xenobiotics, which corresponded to two maximum permissible concentrations, was created by adding sodium lauryl sulfate, 3CdSO<sub>4</sub> × 8H<sub>2</sub>O and Zn(NO<sub>3</sub>)<sub>2</sub> × 6H<sub>2</sub>O to the aquarium water. After the established time of exposure to xenobiotics (14 days), the animals were decapitated in accordance with the requirements of the International Principles of the Declaration of Helsinki (*World Medical Association, 2001*).

To determine biochemical parameters, tissue homogenate was prepared in 0.25M sucrose in a ratio of 1:10. Glucose content was determined by the glucose oxidase method using the "Reagent" reagent kit. Protein content was determined by the Lowry method (Lowry et al, 1951), which is based on the biuret color reaction. Total lipid content was determined using the "Reagent" reagent kit according to the kit recommendations. Statistical processing of the results was carried out according to general standards using the "Excel" program from the "Microsoft Office-2003" package and the Statistika 6.0 program.

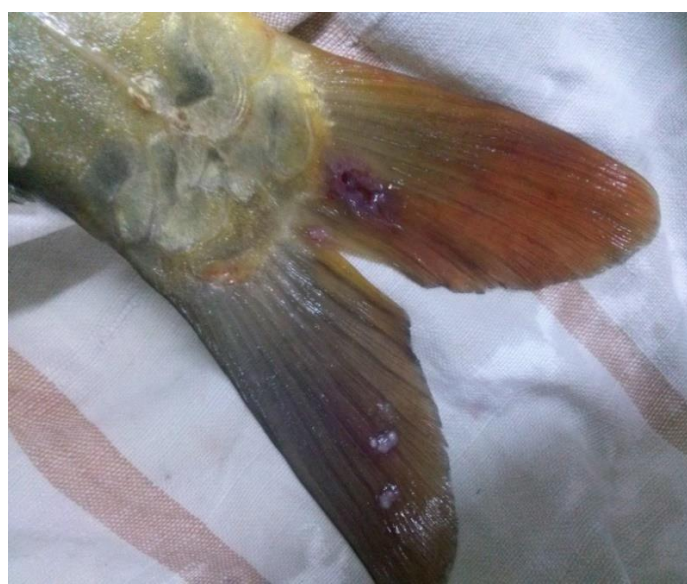
During the experiment, changes in the appearance of the fish were observed, in particular damage to the fins and skin and scales, increased mucus secretion in carp that were in an aquarium with sodium lauryl sulfate for 14 days.

### Results and discussions

Pus-filled blisters and damage to the caudal fin have been reported (Fig.1, 2).



**Fig. 1. Damage to the caudal fin of fish under experimental conditions**



**Fig. 2. Blisters on the skin of fish under the influence of xenobiotics**

During the study, pure sodium lauryl sulfate was used, which is more aggressive in its properties than impurities in a surfactant containing sodium lauryl sulfate, which are able to slow down the effect of the pure substance on the fish body. According to its chemical properties, sodium lauryl sulfate is able to break down fat molecules, cause irritation, peeling of the epithelium, scales, impaired gas exchange in

the gills, cause hemorrhages in the gills, skin, increase the functional activity of the liver, as a result of a decrease in the immunity of the fish (Musiyenko et al., 2005; Polotnianko & Mekhed, 2024). When removing fish from aquarium water, it was determined by morphological description that all fish in the experimental groups had body damage of various nature.

It is known that the adaptive mechanism of maintaining the glucose level in fish tissues is carried out due to gluconeogenesis, glycogenolysis and intertissue transfer. The level of glycogen in the studied tissues in autumn and winter is low (Grubinko, 2005), under the conditions of the experiment, the penetration of glucose into the carp organism from the environment was excluded, gluconeogenesis

was studied earlier (Grubinko, 2005; Matiushko & Mekhed, 2024), the authors noted the activation of enzymes of this glucose synthesis pathway under the action of toxicants, in particular heavy metals. Therefore, it is interesting to study the change in the content of this metabolite in fish tissues under the combined action of surfactants and heavy metal ions, as presented in Tables 1 and 2.

Table 1

Glucose content in carp tissues under the action of xenobiotics (mg/ml;  $M \pm m$ , n=5)

Organ/Tissue	Control	Zn <sup>2+</sup>	SLS	Zn <sup>2+</sup> + SLS
Muscle	37.9±2.4	30.1±2.4	34.8±3.2	27.8±3.1*
Liver	118.0±6.8	84.2±2.4*	112.6±8.6	72.5±3.5*

Both toxicants, both individually and in combination, cause a decrease in glucose levels in both tissues studied. The maximum changes

were observed in the liver with the simultaneous action of lead and sodium lauryl sulfate.

Table 2

Glucose content in carp tissues under the action of xenobiotics (mg/ml;  $M \pm m$ , n=5)

Organ/Tissue	Control	Cd <sup>2+</sup>	Cd <sup>2+</sup> + SLS
Muscle	44.5±4.6	40.4±3.2	39.2±2.8
Liver	138.4±12.4	122.0±4.8	85.8±6.4

Under the action of cadmium ions, as well as cadmium ions simultaneously with LSN, a decrease in the studied indicator was noted, however, the indicated differences in the glucose level in fish of the control and experimental groups are not probable.

The decrease in the glucose content in the tissues can be explained by metabolic transformations, participation in a number of metabolic systems, which are manifested in an increase in the activity of the corresponding enzymes (Yatsenko et al., 2017). Thus, it can be

concluded that the glucose content in various carp tissues under the action of heavy metal ions and surfactants is sufficiently informative

The results of the study of the content of total protein in the tissues of carp two-year-olds are presented in tables 3 and 4. As the results of the studies showed, the highest protein content corresponded to liver tissues, approximately one third less was found in muscle tissue. Under the influence of extreme factors, these ratios did not change significantly, with the exception of the combined action of toxicants.

Table 3

The content of total protein in fish tissues under the influence of xenobiotics

(mg/ml;  $M \pm m$ , n=5)

Organ/Tissue	Control	Zn <sup>2+</sup>	SLS	Zn <sup>2+</sup> + SLS
Muscle	8.5±0.7	7.8±0.6	8.3±1.0	7.5±0.8
Liver	13.2±0.4	12.0±0.8	12.5±1.2	13.0±0.7

Both toxicants cause a slight decrease in the level of total protein in both white muscles and liver (Zhelai et al., 2023). Maximum changes in muscle tissue were

observed with simultaneous exposure to lead and sodium lauryl sulfate, in contrast to the liver.

Table 4

The content of total protein in fish tissues under the influence of xenobiotics (mg/ml;  $M \pm m$ ,  $n=5$ )

Organ/Tissue	Control	$Cd^{2+}$	$Cd^{2+}$ + SLS
Muscle	9.6 $\pm$ 0.4	8.8 $\pm$ 0.4	8.5 $\pm$ 0.8
Liver	15.2 $\pm$ 0.4	14.0 $\pm$ 0.8	13.5 $\pm$ 1.7

Thus, in the liver and muscles of carp, the studied toxicants caused a decrease in the content of total protein, however, the indicators did not differ significantly in fish of the control and experimental groups, the observed differences are unlikely.

When studying the effect of toxic substances on the moisture content in various carp tissues (Fig. 3), no significant changes were observed.

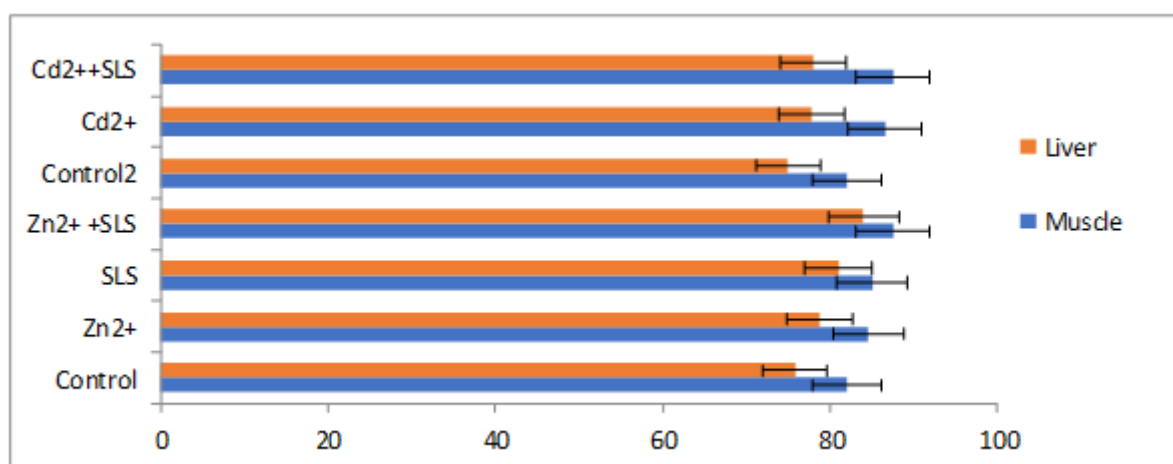


Fig. 3. Moisture content (%) in carp tissues during intoxication with heavy metals and sodium lauryl sulfate ( $M \pm m$ ,  $n=5$ )

The obtained data indicate that fish muscle tissues are least exposed to toxicants, this can be explained by the fact that the main contact organs of fish are the gills and liver.

Analysis of the results obtained during the experiment indicates implausible changes in the lipid content of the liver and white muscles on the fourteenth day under the influence of toxicants (Fig. 4).

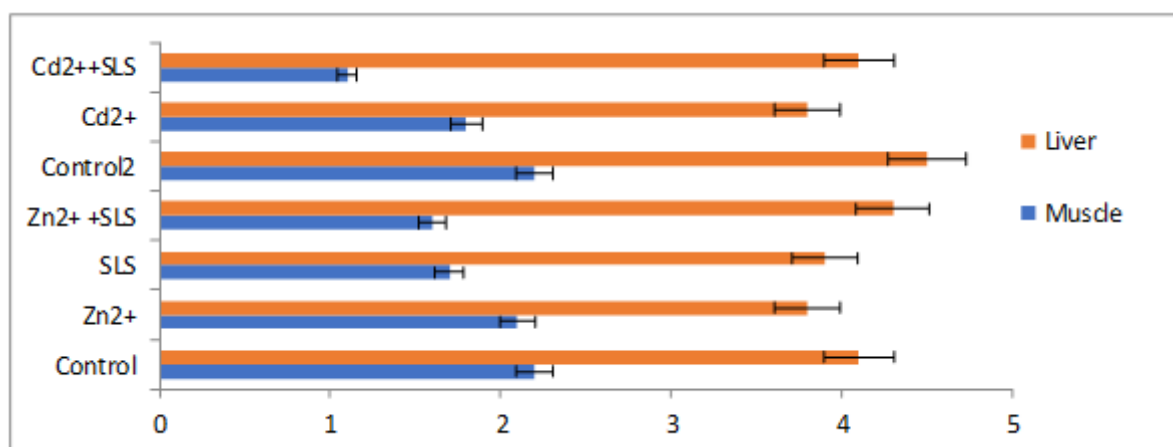


Fig. 4. Content of total lipids (g%) in carp tissues during intoxication with heavy metals and sodium lauryl sulfate ( $M \pm m$ ,  $n=5$ )

According to the results presented in Fig. 4, a decrease in lipid content was observed both in white muscles and in liver tissues under the influence of toxic environmental conditions. The exception is the indicated indicator under the simultaneous influence of sodium lauryl sulfate and lead ions. The increase in the amount of lipids in the liver can be explained by the fact that the fish organism, being on endogenous nutrition, does not use lipids as a nutritional substrate, but on the contrary, synthesizes and accumulates them at the expense of other substrates – glucose, glycogen, depleting itself energetically. At the same time, fat accumulation is observed in the liver. This may be a consequence of blocking their exit from the liver as part of lipoproteins, which can lead to fatty degeneration of the liver.

### **Conclusions**

During the experiment, changes in the appearance of the fish were observed, in particular damage to the fins and skin and scales, increased mucus secretion in carp that were in an aquarium with sodium lauryl

sulfate for 14 days. When toxicants of various origins act on the fish body, the glucose content is more sensitive than the total protein content. These indicators can be recommended for monitoring water bodies using fish of the cyprinid family. Under the influence of toxic environmental conditions, a decrease in the lipid content was observed in both white muscles and liver tissues, with the exception of the simultaneous exposure to sodium lauryl sulfate and lead ions. An increase in the amount of lipids in the liver can lead to exhaustion of the body and indicate tissue degeneration. When studying the effect of toxic substances on the moisture content in various carp tissues, no significant changes were observed. The presence of a multi-stage cell defense system, which has developed during phylogenetic development, determines the complexity of the cause-and-effect relationships between biochemical processes and their focus, primarily, on maintaining the optimal metabolic balance of the cell and the organism as a whole, which is expressed in changes in biochemical indicators.

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### **Заява про доступність даних / Data Availability Statement**

Набір даних доступний за запитом до авторів / Dataset available on request from the authors.

### **Заява інституційної ревізійної ради / Institutional Review Board Statement**

Експериментальні процедури були схвалені Комісією з біоетики Національного університету «Чернігівський колегіум» імені Т.Г. Шевченка (№ протоколу: 5, 3 жовтня 2024 р., Чернігів, Україна) / The experimental procedures were approved by the Bioethics Committee of T.H. Shevchenko National University «Chernihiv Colehium» (Protocol Number: 5, 3 October 2024, Chernihiv, Ukraine).

### **Заява про інформовану згоду / Informed Consent Statement**

Не застосовується / Not applicable.

### **Конфлікт інтересів / Conflicts of Interest**

Автори заявляють про відсутність конфлікту інтересів / The authors declare no conflicts of interest.

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