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DISSECTING BIOCHEMICAL MECHANISMS THAT MEDIATE TOLERANCE TO MILITARY CHEMICAL STRESSORS IN DIVERSE MALACOLOGICAL SYSTEMS

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Abstract. The ongoing military conflict in Ukraine has severely contaminated freshwater ecosystems with heavy metal pollutants including lead from ammunition and explosives. This study investigates the physiological and biochemical mechanisms of resistance in the freshwater mollusks. This study examines how freshwater mollusks, specifically *Planorbarius corneus* and *Viviparus viviparus*, resist lead compounds. Lead pollution from military activities poses a significant threat to aquatic life due to its toxicity and bioaccumulation. The research investigated species-specific responses to lead exposure, revealing differences in adaptations. Both mollusk species showed increased levels of carotenoids and proteins when exposed to higher lead concentrations, indicating a compensatory response to oxidative stress. These findings enhance our understanding of adaptive mechanisms against lead toxicity in aquatic environments affected by military pollution.

Keywords: environmental safety, military activity, molluscs, toxicants.

1. Introduction

According to the State Ecological Inspectorate of Ukraine, in the 500 days of of the war on the territory of Ukraine, Russia has caused losses of about 60 billion hryvnias as a result of man-made pollution, water pollution and unauthorized use of water resources.

The problem of heavy metal pollution of water bodies as a result of military operations is real and serious. Heavy metals, such as lead, cadmium, mercury and nickel are toxic to aquatic organisms and humans. It has been established that military operations can lead to the pollution of water bodies with heavy metals in several ways, metals in several ways. First, explosives used in military operations often contain used in military operations often contain heavy metals. During the explosion, these substances can form lead aerosols that can get into the water. Second, fires that occur during military operations can lead to the destruction of buildings and structures containing heavy metals.

This can lead to heavy metal spills into the environment, including water. Thirdly, household waste that is thrown away during military during military operations may contain heavy metals such as paints, batteries, and lead products.

This garbage can end up in water, where heavy metals can dissolve.

Lead (and its compounds) is one of the most poisonous heavy metals. It is found in all components of the environment: in rocks, soils, natural waters, the atmosphere, and living organisms, rocks, soils, natural waters, the atmosphere, and living organisms. Lead can actively disperse into the environment in the course of human economic activity. Lead is a poison that affects all living things, but especially causes changes in the nervous system, blood and blood vessels. It actively effects on protein synthesis, energy balance of the cell

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and its genetic apparatus. All lead compounds act, in general, it seems that the difference in toxicity is explained by by their unequal solubility in body fluids. Numerous observations show that even at high observations, even with a high content of metal ions in the body of animals, some indicators of their functional state can remain unaffected for a long time unaffected.

Lead compounds are not primary constituents in the explosive core of military ordnance. However, lead compounds have historically played several roles in the context of military ammunition and ordnance:

1. Lead compounds, especially lead styphnate and lead azide, have been used in the primers of ammunition. Primers are the small charge that ignites the main propellant in a cartridge or shell. When the firing pin of a firearm strikes the primer, it ignites, and this, in turn, ignites the main propellant that sends the bullet or projectile down the barrel.
2. While the explosive charge does not contain lead, the bullets themselves in many types of ammunition are made of lead or a lead alloy. Lead has been traditionally favored for bullets due to its density, malleability, and relative cheapness.
3. Some military ordnance, like certain types of torpedoes or other equipment, might use lead as counterweights or ballasts because of its high density.
4. Lead-acid batteries have been used extensively in military applications, from vehicles to backup power systems.

However, there has been a shift in recent years due to environmental and health concerns:

- The U.S. military and other organizations around the world have been moving toward “green bullets” or lead-free ammunition. This is primarily due to the environmental impact of lead as well as the health risks to personnel during training exercises.
- Efforts have been made to transition away from lead-based primers in favour of more environmentally friendly alternatives.
- At firing ranges and decommissioned military sites, efforts are often made to manage or remediate lead contamination, whether from bullets, lead-based primers, or other sources.

In summary, while lead compounds are not a primary component in the explosive material of military ordnance, they have historically been associated with various components of ammunition and other military equipment. Modern trends are shifting towards reducing or eliminating lead from these applications due to environmental and health concerns.

The release of lead compounds into water bodies as a result of military operations is a concern because of the potential impacts on environmental health and ecosystems, particularly aquatic organisms. Here's an overview of this issue:

Sources of Lead from Military Operations:

- Historically, bullets and shot for firearms have been made primarily of lead. When these bullets or pellets end up in water bodies, they can leach lead over time.
- Military bases often have firing ranges where substantial amounts of ammunition are discharged. If these ranges are near or within watersheds, lead can make its way into water bodies.
- Beyond ammunition, some military equipment and materials may also contain lead, though these are less commonly direct sources of aquatic contamination.

Toxicity of Lead to Aquatic Organisms:

1. Like many heavy metals, lead can be bioaccumulated. This means that organisms at the base of the food chain, like plankton or small invertebrates, might ingest lead. Predators that eat these smaller organisms can then accumulate higher concentrations of lead in their bodies, which poses a risk to any organisms that feed on them.
2. At high enough concentrations, lead can be directly toxic to aquatic organisms. This can lead to physiological disruptions, reproductive issues, or even death.
3. Even at lower levels, chronic exposure to lead can affect the growth, behaviour, reproduction, and overall health of aquatic species.
4. Some species are more sensitive to lead contamination than others, and exposure can lead to significant declines in their populations.
5. Once lead enters the food chain, it poses a threat not only to aquatic organisms but also to larger predators, including birds and mammals that feed on aquatic life. For example, waterfowl may ingest lead pellets directly, leading to lead poisoning.
6. If lead-contaminated water is used as a source of drinking water or if aquatic organisms with accumulated lead are consumed by humans, there are potential health risks. Chronic exposure to even low levels of lead can have harmful effects on human health, particularly in children.

Analysis of recent research and publications. In the work “Ecological safety of of Ukraine”, M. Khylko warns that it is dangerous to eat fish caught in the waters of the Black and Azov Seas is dangerous to consume because of possible poisoning with fatal poisoning.

In their joint work, Garasym A., Kelm N. draw attention to Russia's seizure of water supply facilities, which leads to shallowing of rivers and reduction of flora and fauna in aquatic ecosystems. Also, the temporary supply of water to Crimea of water to Crimea may also result in an aggravation of water shortages both on the mainland mainland and on the peninsula. Basically, an increase in water resources to Crimea from the South of Ukraine resources to Crimea from the South of Ukraine is necessary because of the greater availability of water resources in the southern regions with water resources, as well as the water of the southern are the largest consumers. Another problem is the significant impact of climate change on of climate change on a drier climate in Crimea. Scientists say that starting in 2041 local surface runoff may stop in dry years in Kherson, Odesa, Mykolaiv, Dnipro, and Zaporizhzhia regions. For example, in Zaporizhzhia region, the “climate runoff” could decrease by 10 times, in Dnipropetrovska oblast – by 6 times, in Mykolaivska oblast – by 3.6 times, and in Crimea – by half.

Khylko M. in his work emphasizes: as a result of emergencies situations in operational water supply and sewerage facilities during the conduct of hostilities and due to the cessation of the functioning of enterprises in the the occupied territories, surface and groundwater is being polluted of the region. In particular, intense pollution occurs due to the failure of industrial and municipal industrial and municipal wastewater treatment plants, emergency discharges polluted waters due to power outages, as well as pollution by the combustion products of ammunition and because of fuel and lubricant spills materials.

The intensity of metal intake into the body depends on the form of the metal compound and the presence of complexing agents. Most of the metals accumulates in the kidneys, liver, bone tissue, spleen, and some glands of animals. They are usually bound to lipoprotein membrane formations.

Most often, irreversible binding of sulfhydryl groups occurs of enzymes. Changes in enzymatic activity lead to disorders of transport, respiration, and protein synthesis (Jorge, 2007).

The ability of aquatic organisms to accumulate metals has been used to indicate water pollution. The following organisms can serve as indicator organisms’ aquatic organisms that have been in the study area for a long time.

Metals enter the body of aquatic organisms mainly with food. This was revealed in the absorption of zinc, cobalt, and iron by the mollusk Mytilus edulis. Aquatic organisms directly through body surface, gills, scales, and urine (De Lisi, 2013) excrete excess metals.

Cations Hg^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+} inhibit the activity of adenylate cyclase in the smooth muscle tissue and hepatopancreas of freshwater bivalves (*Anodonta cygnea*) and gastropods (*Coretus corneus*, *Viviparus contectus*) molluscs in the following order: Hg^{2+}; Cu^{2+}; Pb^{2+}; Zn^{2+}; Cd^{2+} (Harbar, 2021).

The toxicity of substances for aquatic organisms is affected by the presence of other compounds, forms of the substance under study, water hardness, light and temperature conditions, oxygen concentration, pH, flow velocity, lighting, the presence of complexing agents, synergism, and the condition of biological objects. Toxicity can be determined by the ability of metals to concentrate (Gandziura, 2023).

Metals in the body of animals affect many vital organs, tissues, and structures, including the gills organs, tissues, and structures, including the gills. These toxicants change the function of blood; hearts of aquatic animals, damage the gills, and disrupt biochemical processes. All this is reflected in the overall functional state of aquatic animals and their respiration (Uvayeva, 2023).

Chemically active cell groups are sensitive to the action of metals, especially heavy ones, which are associated with the membrane, and as a result, the permeability of the membranes is impaired.

The high sensitivity of the respiratory function to toxic substances is due to by the fact that the respiratory surface of the gills of aquatic organisms is in direct contact with pollutants and is the first to be affected by them. With increase in gill ventilation in response to mechanical impact, the amount of of suspended particles that come into contact with the gills increases. All this causes damage to the of the epithelium and the entire gas exchange surface (Alam, 2023).

In the course of research, it was found that in case of intoxication with many chemicals in animals, the direct relationship between the activity of the between the activity of the gill ventilation apparatus and the activity of the heart (fish, daphnia, molluscs), as well as between the intensity of total gas exchange and the activity of cytochrome oxidase in in the gills of fish. The same disorders are observed in animals exposed to temperature, hypoxia, and hypercapnia (Shahbaa, 2020).

According to the results of our own field research of post-military water of the village of Moshchun, Bucha district, Kyiv region in the aquatic environment and bottom sediments of fishery and recreational water bodies were found all classes of heavy metals, including lead compounds, a highly toxic a highly toxic heavy metal. For all items, there are significant exceedances of the MPCs fish. (Maksymenko, 2022).

The aim of the study is to establish physiological and biochemical mechanisms of resistance of *Planorbarius corneus* L. and *Viviparus viviparus* L. to the effects of chemical war stressors (in our case, lead compounds). Thus, because the of the analysis, one of the main chemical stressors of war, which violates enantiostasis and homeostasis of hydroecosystems is lead and its compounds.

2. Materials and Methods

Before starting the study, animals were acclimatized to laboratory conditions for 14 days at temperature of 18–20 ºC. pH = 7.2–7.4. In the laboratory, the mollusks were placed in glass 6 liter glass containers with 10 specimens each. To prevent the influence of their own exometabolites on the experimental mollusks, the water in the aquariums was changed daily with fresh water of the same quality every day. Only non-invasive individuals were selected for the study in order to avoid the influence of the biotic factor on the studied parameters. The animals were placed in Pb^{2+} solutions at concentrations of 1/2CL50, 1/4CL50, 1/8CL50. As a control was used settled (24 hours) tap water.

The toxicological experiment consisted of an orientation (aimed at determination of the CL50 value for individuals exposed to different concentrations of of selected pesticides and heavy metals) and the main experiment (3 concentrations (1/8CL50, 1/4CL50, 1/2CL50) were selected and used in the main experiment). The solutions were prepared in water dechlorinated by settling (1 day).

The used solutions were replaced with fresh ones after 24 hours. In the toxicological experiment, the exposure was 7 days. The experimental data obtained were processed using the “Excel” program package.

The content of total carotenoids was determined by the method of V.M. Karnaukhov (and modern modifications of the method) (Uvayeva, 2023). Chromatographic method for the separation of carotenoids. The method of thin-layer chromatography (TLC) involves the application of tissue extracts onto a silica gel plate with subsequent separation in a particular or other solvent system. Spectra of the visible region. This method is one of the of the criteria in the identification of carotenoids and is used for quantitative determination of the identified carotenoids. In the study, the spectra of the spectrophotometer SF-2000 were used in the study to take spectra of the visible region. The obtained spectra were compared with those described in the works of other authors (Jorge, 2007). After identification, the content of carotenoids in the sample was determined. To do this, according to the data absorption spectra, the optical density of the pigment at 450 nm was noted. After determining the content of each carotenoid, their percentage ratio was calculated.

The content of carotenoid pigments was determined in hemolymph, hepatopancreas, mantle, and foot. The content of total protein was determined by the Lowry method. The calibration graph was constructed in the range of concentrations from 0.025 to 0.250 mg of the standard protein sample by measuring the optical density of the solutions at 750 nm.

3. Results and Discussion

The creation of a compensatory of a compensatory reaction by the mollusk organism based on the variability of the amount of of carotenoid pigments and protein levels in the hemolymph.

Species specificity of carotenoid pigments and total protein content in the hemolymph of the studied freshwater mollusks under standard conditions of the aquatic environment.

According to the data obtained on the content of carotenoid pigments and and total protein in the hemolymph of mollusks (*Planorbarius corneus, Viviparus viviparus*) revealed species specificity. Thus, the content of total carotenoids under under standard conditions (t = 18 °C, pH = 7.2-7.4) in *Planorbarius corneus* is 0.3202 ± 0.0217 mg/100g of tissue, while in the foregill mollusk *Viviparus viviparus* is twice as high (Table 1).

The content of carotenoid pigments (β-carotene, xanthophylls) was determined in hemolymph, hepatopancreas, mantle, and foot (Figs. 1, 2).

Table 1

Content of total carotenoids (mg/100g) in the studied mollusks

Mollusc species	Carotenoid content
Planorbarius corneus	0.3202±0.0217
Viviparus viviparus	0.6942±0.0476

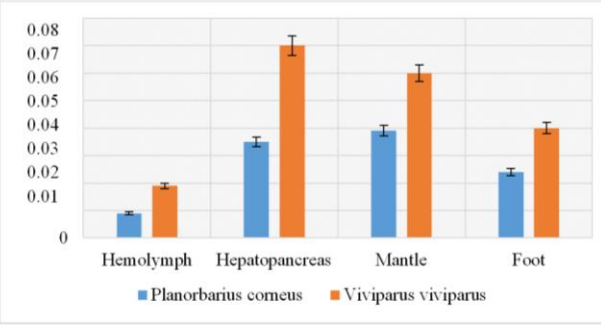

Fig. 1. β-Carotene content in Planorbarius corneus and Viviparus viviparus

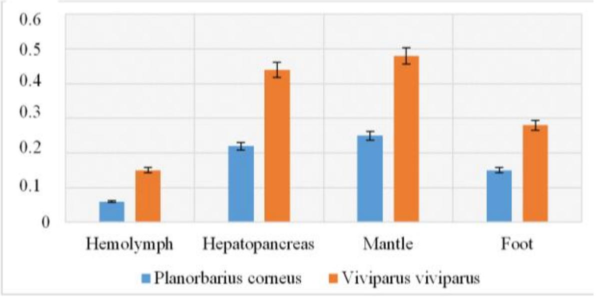

Fig. 2. Xanthophyll content in Planorbarius corneus and Viviparus viviparus

The increased content of *β*-carotene and xanthophyll's was observed in the mollusk *Viviparus viviparus*. At the same time, their distribution is the same for the two mollusk species is characterized by an increased amount in the hepatopancreas and mantle.

The content of total protein in the hemolymph of molluscs is presented in Table 2.

The data obtained suggests that the species studied react differently to extreme environmental changes to extreme changes in environmental factors.

Planorbarius corneus.

The semi-lethal concentration (CL_{50}) of lead ions is – 0.13 mol/L^{-1}. When studying the effect of lead on the content of carotenoids in the tissues of in mollusc tissues, the results are presented in Table 3.

The content of carotenoids in the tissues of coils was significantly higher than in control animals at toxicant concentrations of $1/2CL_{50}$ and $1/8CL_{50}$. The results of lead exposure on the content of β-carotene and xanthophylls are shown in Figs. 3, 4.

In molluscs, the content of carotenoids in lead salt solutions in almost all concentrations increased, which confirms Karnaukhov's assumption about the role of carotenoids in overcoming environmental pollution. Thus, in biochemical sphere adequately responds to the effects of heavy metal salt in lungfish metal salt.

At a lead concentration of $1/2CL_{50}$, metabolic stimulation was detected, which led to a significant increase in β-carotene and xanthophylls in the foot by 2.7–2.9 times and in the hepatopancreas by 3.6–3.8 times compared to the control.

When studying the effect of lead on the content of total hemolymph protein of mollusks, the results presented in Table 4 were obtained.

Table 2

Total protein content (%) in hemolymph of P.corneus and V.viviparus

Type of mollusk	min-max	M±m
Planorbarius corneus	4.3-8.2	5.96±0.43
Viviparus viviparus	4.0-10.2	7.93±0.04

Table 3

Carotenoid content in mollusc tissues exposed to different concentrations of lead solution

Characteristics	Toxicant concentration			
	Control (pure water)	1/8 CL_{50}	1/4 CL_{50}	1/2 CL_{50}
Carotenoid content, mg/100g	0.3202±0,0217	0.6071±0,0602*	0.3703±0.0511	0.7253±0.0412*

* P<0.05.

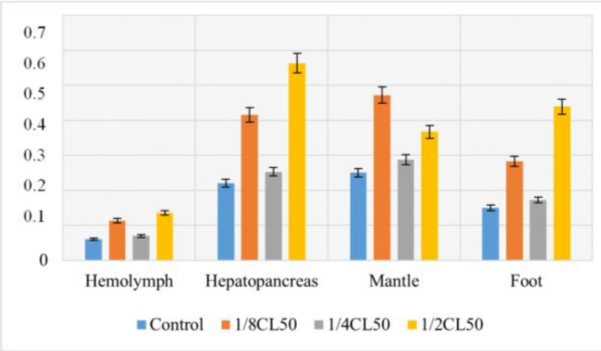

Fig. 3. The content of β-carotene in molluscs exposed to different concentrations of different concentrations of lead solution

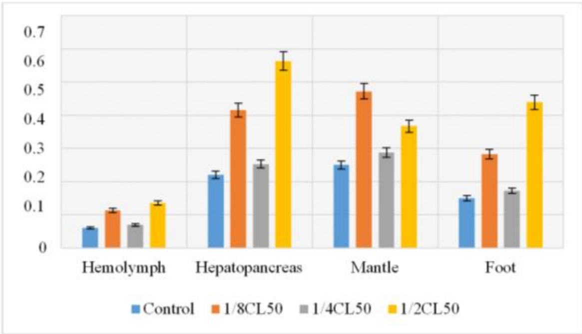

Fig. 4. Xanthophyll content in molluscs exposed to different concentrations of lead different concentrations of lead solution

Table 4

The content of total protein in the hemolymph of mollusks exposed to different concentrations of under the influence of different concentrations of lead solution

Controlling		1/8 CL_{50}		1/4 CL_{50}		1/2 CL_{50}	
Min-max	M±m	Min-max	M±m	Min-max	M±m	Min-max	M±m
4.3–7.1	5.21±0.43	5.6–8.7	7.89±0.17	5.0–6.5	5.42±0.11	4.7–6,9	6.0±0.16

Table 5

The content of carotenoids in the tissues of mollusks exposed to different concentrations of lead solution

Characteristics	Toxicant concentration			
	Control (pure water)	1/8 CL_{50}	1/4 CL_{50}	1/2 CL_{50}
Carotenoid content, mg/100g	0.6942±0.0476	0.9611±0.0241*	1.1474±0.0475*	2.9828±0.1394*

* $P<0.05$.

Table 6

The content of total protein in the hemolymph of mollusks exposed to different concentrations of under the influence of different concentrations of lead solution

Controlling		1/8 CL_{50}		1/4 CL_{50}		1/2 CL_{50}	
Min-max	M±m	Min-max	M±m	Min-max	M±m	Min-max	M±m
4.0–10.2	7.93±0.04	4.9–9.5	7.02±0.18	5.0–10.9	8,42±0.14	6.2–11.7	10.10±0.07

Under the influence of lead, the content of total protein in the hemolymph statistically increases statistically. This means that mollusks are in the second stage of the pathological process, namely the stage of process, namely the stimulation stage. The greatest increase was found at 1.5 times the concentration of 1/8CL_{50}.

Viviparus viviparus.

The semi-lethal concentration of lead ions is – 0.13 mol/L^{-1}. When studying the effect of lead on the content of carotenoids in the tissues of in mollusc tissues, the results are presented in Table 5.

The results of lead exposure on the content of β-carotene and xanthophylls are shown in Figs. 5, 6.

The content of carotenoid pigments in the tissues and organs of live births significantly increased at all concentrations of lead ions.

There was a significant increase in xanthophylls (2.3 times) in the hepatopancreas and foot at lead concentrations of 1/2CL_{50}. This indicates the development of a compensatory reaction of the body to the action of a toxic environment, and activation of free radical oxidation processes.

In the study of the effect of lead on the content of total protein in the hemolymph in the hemolymph of mollusks, the results presented in Table 6 were obtained.

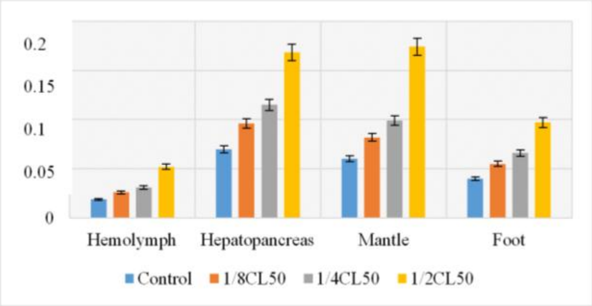

Fig. 5. The content of β-carotene in molluscs exposed to different concentrations of lead solution

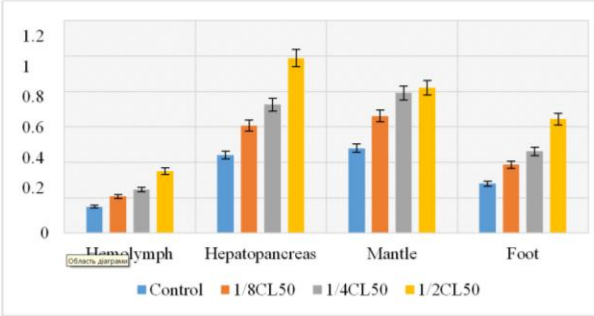

Fig. 6. The content of xanthophylls in the organisms of mollusks exposed to different concentrations of under the influence of different concentrations of lead solution

Table 6

The content of total protein in the hemolymph of mollusks exposed to different concentrations of under the influence of different concentrations of lead solution

Controlling		1/8 CL_{50}		1/4 CL_{50}		1/2 CL_{50}	
Min-max	M±m	Min-max	M±m	Min-max	M±m	Min-max	M±m
4.0–10.2	7.93±0.04	4.9–9.5	7.02±0.18	5.0–10.9	8,42±0.14	6.2–11.7	10.10±0.07

After exposure of animals to a solution of 1/8CL 50, the metabolic rate slightly decreases slightly. The mollusks acclimatize to the conditions of this environment. At concentrations of 1/4CL_{50} and 1/2CL_{50}, an increase in total protein in the hemolymph, which indicates an increase in metabolism as a protective reaction of the organism.

4. Conclusion

1. As a result of the analysis, one of the main chemical stressors of of war, which disrupts the enantiostasis and homeostasis of hydroecosystems, is lead and its and its compounds.

2. To determine the adaptive mechanisms of *Planorbarius corneus* L. and *Viviparus viviparus* L. to the toxic effects of chemical warfare stressors, the compensatory reaction of the the compensatory reaction of the mollusk organism based on the variability of the amount of carotenoid pigments and protein levels in the hemolymph.

3. According to the data obtained for the studied mollusks for all studied indicators under standard conditions of the aquatic environment (without the influence of toxicants), species specificity was found. Thus, the maximum content of of carotenoids (β-carotene and xanthophylls) under standard conditions was noted in **Planorbarius corneus* is more than 2 times lower. Indicators of total protein in hemolymph of control animals are also species-specific. *V. viviparus*, this indicator is 1.3 times higher, compared to *P. corneus*. The data obtained suggest that the studied species respond in different ways to extreme changes in environmental factors environmental factors.

4. The content of carotenoid pigments and hemolymph proteins in two species of the studied mollusks increased significantly with increasing concentration of of lead chloride solution. The degree of resistance to lead compounds: *Viviparus viviparus=Planorbarius corneus*.

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