Biol. Stud. 2015: 9(3–4); 107–118 • DOI: https://doi.org/10.30970/sbi.0903.446 www.http://publications.lnu.edu.ua/journals/index.php/biology



UDC 594.38: 595.122.2

## CHARACTERISTICS OF DISTRIBUTION OF IONS OF HEAVY METALS IN FRESHWATER MOLLUSKS UNDER THE PARALLEL ACTION OF COPPER IONS AND TREMATODE INVASION

G. Ye. Kyrychuk

Zhytomyr Ivan Franko State University, 40, Velyka Berdychivska St., Zhytomyr 10008, Ukraine e-mail: kyrychuk@zu.edu.ua

We studied the peculiarities of accumulation of copper ions in the freshwater mollusks *Lymnaea stagnalis and Planorbarius purpura* at experimental conditions of chronic effect (0.5, 2, 5 and 10 of maximum-possible concentration for fisheries) in normal conditions and in case of combined action of trematode invasion and specified pollutants. We analyzed the characteristics of distribution of ions of Cu, Pb, Cd and Zn in freshwater mollusks at conditions described. It is found that Trematode infestation leads to changes in the distribution of total content of heavy metal in the body of gastropods. Thus, in the infested *P. purpura*, investigated tissues and organs were placed in the ascending order: hemolymph<shell<hepatopancreas<mantle<leg, in *L. stagnalis* – hemolymph< hepatopancreas< shell<leg<mantle. Using correlation analysis, we calculated Spearman's rank correlation coefficient. It became possible to find out a degree of dependence between the studied ions in different tissues and organs of *P. purpura*. It was established that accumulation of metals is tissue specific, and their level is associated with the affinity of non-specific metals and specific metabolites in cell tissues and organs, metabolic activity and physiological needs for these metals in the mollusks.

**Keywords:** copper ions, heavy metals, freshwater mollusks, accumulation, trematode invasion, *Lymnaea stagnalis*, *Planorbarius purpura* 

#### INTRODUCTION

The well-known law of transformation of hydro- and ecological systems is an increase in their range of quality and quantity of dangerous pollutants that are usually toxic to aquatic organisms [8, 10, 18, 25, 32]. In hydrocenosis, toxicants coming to them from the industrial, agricultural and domestic effluents or appear as a result of breach of hydrochemical balance and circulation of substances, cause a negative effect on aquatic organisms at the level of organisms and at the level of groups [2, 4, 25, 28]. Among the most dangerous pollutants that affect invertebrates, in which mollusks occupy a prominent place, heavy metal (HM) ions are remarkable.

More than 40 chemical elements belong to HM, but of special interest are those metals, which are moving in biogeochemical cycles, are widely used in various fields of

production activities and, because of accumulation in the environment, are dangerous in terms of both their biological activity and toxicity. On one hand, HM relate to the essential metals and are important for the organisms, on the other hand, by increasing of their concentrations in living organisms, they may act as carcinogenic, resulting in disruption of livelihoods of hydrobionts. These HM ions are: zinc, copper, cadmium and plumbum [15].

Copper ion is a part of copper containing proteins and enzymes (over 25): cyto-chrome oxidase, ascorbic acid oxidase, lysine oxidase, ceruloplasmin and other metal ferments [11]. We know that it affects the processes of tissue respiration, is a part of a number of oxidases and haemocyanin, activates gonadotropic enzymes [21], is involved in the processes of tissue respiration, blood formation, spermatogenesis, affects the structure and function of nucleic acids. phenolic metabolism, synthesis of phospholipids, acts as a catalyst in redox reactions [3]. Excessive concentrations of copper are characterized by toxic effects, leading in particular to atrophy of certain organs and tissues and to hemodyscrasia [29].

One of the main factors that determine the environmental dangers of HM is the level of their accumulation in the tissues of organisms that is defined by the speed of inflow, the ability to accumulate and intensity of their withdrawal [13, 21, 24] and redistribution [15]. Adaptive-compensatory response of hydrobionts to actions of pollutants is determined, first of all, by the degree of ion accumulation [7, 13].

According to general theory of stress (stress factor is also the ions of metal) the intensity of metabolism in organisms at the beginning of the stressor activity increases and then decreases. Depending on the intensity and duration of action, it may lead to reverse restore of functions at end of stress factor activity, or to the death of the body [14]. It was noted [19] that the toxicants in mollusks are able to operate at all levels from membranes, enzyme systems of cells, providing power to organisms to disruption of physiological functions, the basic parameters of life (reproduction, development, and modification of the trophic structure and population dynamics of trophic links etc.). Taking into consideration the multilevel and complexity of impact of HM ions on the state of mollusks and their communities, as well as the modification of their actions by other external factors (physical-chemical and hydrological parameters of reservoirs) and internal environment (species, age, sex characteristics and symbiotic interactions, such as the larvae of trematodes etc.) of mollusks, interesting is the system of rating reactions of the organism to these influences to predict their possible adaptation to changing parameters and habitat, use of mollusks as models for the assessment of the influence of hydrocenosis of the HM ions [5, 7, 25].

It is known that in chronic administration of toxicants in doses and concentrations close to threshold, adaptive capacities of organisms may be implemented more effectively [26]. In our experiments, we used concentrations of copper ions, corresponding to 0.5, 2.0, 5.0, 10.0 MPC. The first of these, based on common patterns of ecological and toxicological action of HM on different organisms, was accepted as the threshold and as one that will call prognostic adaptive changes, while the others regarded as being capable of causing irreversible pathological changes.

Taking into consideration the above information, we tried to find out the peculiarities of distribution of ions of copper, cadmium, zinc and plumbum in the body typical representatives of hydrocenosis – gastropods at joint action of trematode infestation and chronic exposure to low concentrations of ions of copper.

#### **MATERIALS AND METHODS**

To determine the level of accumulation of HM ions (Cu, Zn, Cd, Pb) we used 56 specimens of uniform in size *Lymnaea stagnalis* (Linnaeus, 1758) (height of shells  $-39.768\pm0.131$  mm, weight of mollusk  $-3.893\pm0.094$  g). 106 specimens of uniform in size *Planorbarius purpura* (Müller, 1774) (diameter of shells  $-23.413\pm0.831$  mm, weight of mollusk  $-3.324\pm0.164$  g), which were collected manually in 2005-2009 in the Middle Dnieper basin (Teteriv river, Zhytomyr).

The toxicological experiments were performed using the method of V. A. Alekseieva [1]. Acclimatization of mollusks to laboratory conditions took 14 days [12]. As toxicant we used salt CuCl<sub>2</sub>·2H<sub>2</sub>O of analytical reagent grade type at concentrations corresponding 0.5; 2; 5 and 10 of maximum-possible concentration (MPC) for Fisheries (0.001 mg/dm³). The calculation of concentrations was conducted for cation. Exposure lasted 14 days. Replacement of toxic environment was performed daily to remove metabolites of animals and to maintain a constant concentration of toxicants. All experiments were accompanied by control.

Trematode infestation was found in temporary histological preparations, made from mollusk hepatopancreas tissue, and studied under a microscope MBI-3 (10×20). The species belonging of trematodes was determined [31] solely on living material. The experiments involved only those infected individuals who were infested by larvae and parthenita of *Echinoparyphium aconiatum Dietz*.

During the experiment, each specimen was weighed on the electronic scales of WPS 1200/C type with the accuracy up to a 0.01 g. Dimensional characteristics were determined using calipers. To determine the level of HM we used shell, hemolymph, hepatopancreas, mantle and leg. To determine the HM, the material was prepared according to Kjeldahl method [22]. The organ or tissue were removed completely and fixed with 96% ethanol and evaporated in 6–12 hours at a temperature of 105 °C [22]. The sample then was burned in nitric acid for 12–24 hours until complete bleaching of mixture. Quantitative HM ion content was determined using atomic adsorption spectrophotometer C-115M with flame analyzer (standard C9B 5346). Totally, we executed 3240 tests. Metal concentration was expressed as mg/kg of wet weight of animals under conditions natural humidity. Statistical processing of the material was made by the conventional method [16].

#### **RESULTS AND DISCUSSION**

Determination of total content of the studied ions (Cd, Cu, Pb, Zn) made it possible to analyze the role of different organs and tissues of mollusks in the accumulation of HM under the experimental conditions. provided by chronic activity of copper ions. Thus, uninfected individuals in the control group *P. purpura*, hemolymph is characterized by the lowest rates of HM content (3.57% of the total content of HM), the highest rate belongs to hepatopancreas – 32.21 %. According to total content of HM ions, investigated tissues and organs were included in a series (in ascending order): hemolymph<shell< mantle< leg<hepatopancreas. In uninfected *L. stagnalis* the share of hemolymph accounted for only 1.83 % of the total HM, and the series is as follows: hemolymph<shell<hepatopancreas<leg<mantle.

Trematode infestation leads to changes in the distribution of total content of HM in the body of gastropods. Thus, in infested *P. purpura.* investigated tissues and organs were placed in ascending order in following way: hemolymph<shell<hepatopancreas<

mantle < leg, in *L. stagnalis* – hemolymph < hepatopancreas < shell < leg < mantle. The additional physiological stress in the form of trematode infestation leads to destruction of acini of hepatopancreas [33], which, in turn, causes differences in contents of the HM in the organisms of infested animals. Long-term chronic (14 days) stress on the organisms of mollusks by ions of copper (2.0 MPC) led to a redistribution of HM content in the body of gastropods. According to total content of the studied ions (Cd, Cu, Pb, Zn) in intact *P. rurpura*, the following series are built: hemolymph < shell < leg < mantle < hepatopancreas, and in *L.stagnalis* we observed the following tendency: hemolymph < shell < hepatopancreas < mantle < leg. Another sequence was recorded in the infested individuals of *P. purpura*: shell < hemolymph < leg < hepatopancreas < mantle, and in *L. stagnalis* – hemolymph < shell<mantle</p>

However, the level of accumulation of metals by certain mollusks differs significantly (Fig. 1). Thus, in the uninfected individuals of *P. purpura* and *L. stagnalis*, the largest number of Cu found in the mantle; Cd – in hemolymph. As for the other two investigated ions, hepatopancreas and foot were characterized by maximum content of Zn ions (*L. stagnalis*) (*P. purpura*) ions of Pb were found in shell (*P. purpura*) and hepatopancreas (*L. stagnalis*).

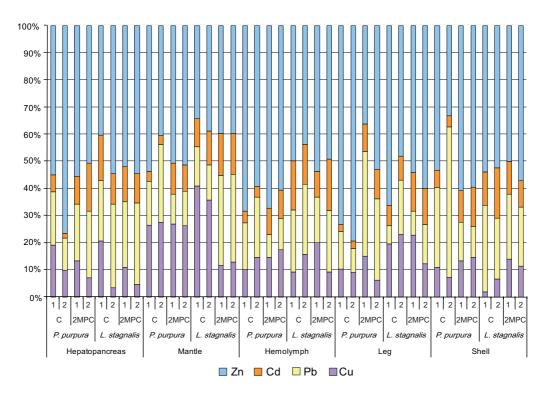


Fig. 1. The content (%) of heavy metals in the body of freshwater mollusks under combined action of trematode infestations and low concentrations (2 MPC) ions of copper (14 days): 1 – infested mollusks, 2 – uninfected mollusks; C – control (MPC – maximum-possible concentration)

Рис. 1. Вміст (%) важких металів в організмі прісноводних молюсків за сумісної дії трематодної інвазії та низьких концентрацій (2 ГДК) іонів купруму (14 діб): 1 – інвазовані молюски, 2 – неінвазовані молюски; С – контроль, 2 МРС – 2 ГДК (ГДК – гранично допустима концентрація)

In the infested individuals of P. purpura and L. stagnalis monotype prevalence of individual ions content in the studied tissues and organs was observed. So, marker body for Cu was the mantle; Cd – hepatopancreas, Zn - leg, Pb - shell.

Chronic (14 days) stay in toxic environment leads to migration of investigated HM ions, except copper ions and redistribution and the maximum of their location in the body of infested and intact P. purpura: Zn – hemolymph, Pb – leg and Cd – hepatopancreas (uninfected) and shell (infested animals). In contrast to the control group of individuals for uninfected L.stagnalis, the influence of the highest rates of Pb content is found in mantle and Zn – leg. In the infested parts, the same differences were marked for ions of Cu – leg and Cd – mantle (Fig. 1).

To clarify the interaction between HM ions during the actions of different concentrations of copper ions (0.5-10.0 MPC) in the body of infested and intact P. purpura using correlation analysis, we calculated Spearman's rank correlation coefficient (Table). It became possible to find out a degree of dependence between the studied ions in different tissues and organs of P. purpura. It was established, that under influence of Cu ions with concentration of 0.5 MPC in uninfected animals, we observed a significant relationship (P<0.05) only for mantle between pairs Pb/Cd and Cu/Zn, trematode infestation is inhibitory factor which leads to an increase in correlation hemolymph communication between pairs of Pb/Cd. Zn/Cd and Cu/Zn, in hepatopancreas Cu/Cd, in leq - Pb/Cd, in shell - Pb/ Cd and Cu/Zn. This suggests different HM regulation of metabolism in the body of mollusks. Raising of toxic effects (2.0 MPC) leads to growth (6 pairs) number of ion pairs in uninfected animals, which are characterized by correlative relationship (Table). The further growth of stress load (5.0 MPC) leads to a possible connection between metals in uninfected individuals in hemolymph – Pb/Zn, in hepatopancreas – Pb/Cd, Zn/Cd and Pb/ Zn, in mantle – Zn/Cd, in foot – Cu/Zn. In infested individuals the number of such pairs is growing: in the hemolymph and in hepatopancreas – Zn/Cd, Cu/Pb, in hepatopancreas, mantle – Cu/Zn, in hepatopancreas, shell – Cu/Cd, in mantle – Cu/Pb, in shell – Pb/Zn.

Further elevation of the presence of copper ions in the environment (10.0 MPC) increases the number of ion pairs with correlation connection recorded for uninfected mollusks (9 pairs) and a sharp decrease in infested individuals (5 pairs) (Table).

The analysis of accumulation of copper ions in the body of *P. purpura* has shown that during the chronic action (14 days) it is stored in the mantle, hepatopancreas and less in leg, and even less in the shell and the hemolymph (Fig. 2). The accumulation of copper ions in the mantle was highest in animals infested with 0.5 (2 times) and 2.0 MPC (1.5 times), and at 5.0 and 10.0 MPC ions of copper decrease its content in an aqueous medium at 2-3 times in the mantle. This accumulation was higher in infested animals (0.5 and 2.0 MPC) and its output (5.0 and 10.0 MPC) - in uninfected individuals. Active accumulation of copper at concentrations close to the MPC, is explained by active absorption of copper as a nutrient. As a result of nutrition of organ, the mechanisms to prevent the penetration of copper ions into the cells probably turn on, and accelerate the withdrawal of its surplus [9]. In hepatopancreas and foot, the level of accumulation of copper with increasing of MPC has a close trend. At MPC values 0.5 and 5.0 in both we bodies observed increase of copper ions in 1.7-3.9 times against the control, and at the concentrations of 2.0 and 10.0 MPC hepatopancreas decreased in 1.5-3.3 times in infested individuals and 10.0 MPC 16 times in uninfected individuals compared with individuals of the control group. For the leg, downward trend in cumulative properties of the investigated ion was found. In hepatopancreas and leg, we detected concentration differences in accumulation, connected with the change of mechanisms for linking ions.

Spearman's rank correlation coefficient in calculation of connection strength between metals in infested and uninfected *P. purpura* during chronic effects of various concentrations of copper ions

Коефіцієнти кореляції рангів Спірмена при розрахунках сили зв'язку між металами в інвазованих та неінвазованих *Р. ригрига* за хронічної дії різних концентрацій іонів купруму

	Uninfected mollusks			Infested mollusks						
	Cd <sup>2+</sup>	Zn <sup>2+</sup>	Pb <sup>2+</sup>	Cd <sup>2+</sup>	Zn <sup>2+</sup>	Pb <sup>2+</sup>				
0.5 MPC(maximum-possible concentration)										
Hemolymph										
Cu <sup>2+</sup>	0.0971	0.1088	-0.0677	0.4000	0.8000*	-0.2000				
Pb <sup>2+</sup>	0.0735	0.4059	-	-0.8000*	-0.4000	-				
Zn <sup>2+</sup>	0.3324	-	-	0.8000*	-	-				
Hepatopancreas										
Cu <sup>2+</sup>	-0.1077	0.3802	-0.6231	0.9000*	0.3000	-0.1000				
Pb <sup>2+</sup>	0.2363	-0.1308	-	0.2000	0.6000	-				
Zn <sup>2+</sup>	-0.3319	-	-	0.4000	-	-				
Mantle										
Cu <sup>2+</sup>	0.1648	0.5088*	0.3495	0.7000	0.1000	0.2000				
Pb <sup>2+</sup>	0.5209*	0.3923	-	0.3000	-0.5000	-				
Zn <sup>2+</sup>	0.1725	_	-	0.4000	-	-				
Leg										
Cu <sup>2+</sup>	0.1677	-0.0412	0.3382	0.4000	-0.1000	-0.2000				
Pb <sup>2+</sup>	0.0265	0.0206	-	0.8000*	0.2000	-				
Zn <sup>2+</sup>	-0.0559	_	-	-0.4000	-	_				
Shell										
Cu <sup>2+</sup>	-0.2571	-0.2440	-0.0945	0.1000	-0.9000*	0.0000				
Pb <sup>2+</sup>	-0.0462	0.1385	-	0.9000*	-0.1000	-				
Zn <sup>2+</sup>	0.1868	-	_	-0.2000	-	_				
			2.0 MPC							
			Hemolymph							
Cu <sup>2+</sup>	-0.2919	-0.2430	-0.0367	0.0857	-0.0286	0.0285				
Pb <sup>2+</sup>	0.1748	-0.2238	-	0.8286*	0.8286*	-				
Zn <sup>2+</sup>	-0.0839	_	_	0.6571	-	-				
		Н	epatopancrea	ıs						
Cu <sup>2+</sup>	0.2857	0.1429	-0.0476	0.0000	-0.8000*	0.8000*				
Pb <sup>2+</sup>	0.4286	0.5952*	-	0.4000	-0.4000	-				
Zn <sup>2+</sup>	0.7143**	_	-	0.6000	-	-				
			Mantle							
Cu <sup>2+</sup>	0.3714	0.3494	-0.0319	0.5179	0.6250	0.1518				
Pb <sup>2+</sup>	-0.2517	0.5374*	-	0.2589	-0.0446					
Zn <sup>2+</sup>	0.3275	-	-	0.3571						
			Leg							
Cu <sup>2+</sup>	0.3000	0.4545	0.0545	0.2571	0.7714 <sup>*</sup>	0.3714				
Pb <sup>2+</sup>	0.3454	0.5818*		-0.0857	-0.1429	-				
Zn <sup>2+</sup>	0.1455			0.3714	-	_				
Shell										
Cu <sup>2+</sup>	-0.4455	0.1181	0.5000*	0.5714	0.0357	-0.0357				
	5100	0101	0.0000	0.07 1 1	0.0001	0.0001				

Pb <sup>2+</sup>	-0.6364**	-0.1546	-	0.4286	0.8571 <sup>*</sup>	-					
Zn <sup>2+</sup>	0.0000	-	-	0.3929	-	-					
5.0 MPC											
Hemolymph											
Cu <sup>2+</sup>	-0.1177	0.0824	0.2265	0.0000	0.1000	-0.8000*					
Pb <sup>2+</sup>	-0.2882	-0.7265**	-	-0.4000	0.1000	-					
Zn <sup>2+</sup>	0.4206	-	-	0.9000*	-	-					
	Hepatopancreas										
Cu <sup>2+</sup>	0.4882	0.4294	0.2654	0.8000*	0.8000*	-0.8000*					
Pb <sup>2+</sup>	0.6272*	0.5801*	_	-0.4000	-0.4000	_					
Zn <sup>2+</sup>	0.7206**	_	_	0.9000*	_	_					
			Mantle								
Cu <sup>2+</sup>	0.3535	0.0217	-0.0093	0.2000	0.8000*	-0.8000*					
Pb <sup>2+</sup>	0.2977	0.3127	-	-0.4000	-0.4000	0.0000					
Zn <sup>2+</sup>	0.6331*	-	-	0.4000	0.1000						
Leg											
Cu <sup>2+</sup>	0.0999	0.5931*	-0.4001	-0.2000	-0.1000	-0.6000					
Pb <sup>2+</sup>	0.1642	0.0545	-	-0.2000	0.6000	0.0000					
Zn <sup>2+</sup>	0.1042	-	-	0.2000	-	-					
Z11	0.3911	-	Shell	0.2000	-	-					
Cu <sup>2+</sup>	0.3642	0.2652	0.1806	0.8000*	0.2000	0.4000					
Pb <sup>2+</sup>	-0.1971	0.2032		0.0000	-0.8000°	0.4000					
Zn <sup>2+</sup>		0.0402	-		-0.6000	-					
Z11-	0.1661	-	- 40.0 MDO	0.4000	-	-					
			10.0 MPC								
2+	0.0000	0.5504*	Hemolymph	0.0400	0.0400*	0.7574					
Cu <sup>2+</sup>	0.0839	0.5594*	0.4685	0.0429	0.8429*	0.7571					
Pb <sup>2+</sup>	0.4615	0.7273**	-	-0.6000	0.6571	-					
Zn <sup>2+</sup>	0.4196	-	-	-0.0857	-	-					
- 0			lepatopancrea								
Cu <sup>2+</sup>	0.3427	0.1538	0.2308	0.3143	0.2000	0.1429					
Pb <sup>2+</sup>	0.0559	0.3706	-	-0.5429	-0.0857	-					
Zn <sup>2+</sup>	0.4685	-	-	0.0857	-	-					
Mantle											
Cu <sup>2+</sup>	0.0070	0.7745**	0.0559	-0.5000	0.8750	-0.9320*					
Pb <sup>2+</sup>	0.8951**	0.2849	-	0.5000	-0.6250	-					
Zn <sup>2+</sup>	0.2255	-	-	0.1250	-	-					
			Leg								
Cu <sup>2+</sup>	0.3352	0.3857	0.5769*	0.4857	0.4857	0.1571					
Pb <sup>2+</sup>	0.5703*	0.4274	-	0.3000	0.3000	-					
Zn <sup>2+</sup>	0.5385*	-	-	0.2571	-	-					
Shell											
Cu <sup>2+</sup>	0.5912*	0.4500	0.2941	0.6000	0.8000*	0.4000					
Pb <sup>2+</sup>	0.3941	0.4676	-	0.4000	0.8000*	-					
Zn <sup>2+</sup>	0.7353**	-	-	0.8000*	-	-					

Comment: P < 0.05; P < 0.01 Примітка: P < 0.05; P < 0.01

In the shell, the accumulation of copper ions was detected only at 0.5 MPC, and at the increasing of MPC index the intensity of this process decreased (in 1.3–2.8 times against control). The content of copper ions in the hemolymph was lower compared with its content in hepatopancreas and leg, but had the same dynamics of concentration dependence of the accumulation.

Thus, the mantle reveals a specific behavior towards accumulation of copper depending on the concentration, that allows to emphasize the vital role of this organ in the accumulation of the physiologically essential ions and detoxification of others [15].

As for the differences in accumulation of copper ions by the uninfected and infested animals, both in control and at the chronic effect of high concentrations of ions in investigated metal, a trend to its greater accumulation by uninfected animals, especially in hepatopancreas, hemolymph and leg was observed.

Analysis of special literature shows an existence of two stages of biological accumulation of studied metal ions [17]. At the first stage, there is quite rapid absorption of HM ions as a result of various processes and phenomena – from surface adsorption at the interface of the body and the environment to the ion exchange in chemical interaction of metal ions on the surface of the substrate and their systems. At the increasing of saturation of the system with ions, their accumulation from the environment is slowing and other factors begin to dominate, that leads to the inclusion of metabolism in the body, excretion rate and other processes causing a dynamic equilibrium between the inflow of HM ions and their withdrawal.

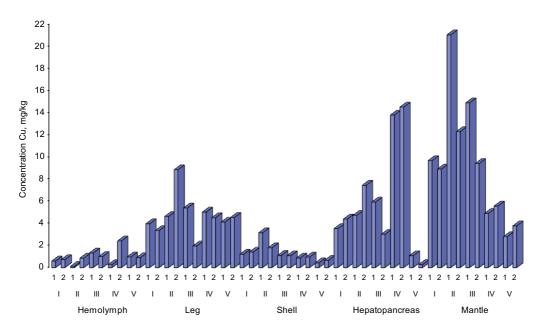


Fig. 2. The content of copper ions in the body of *P. purpura* during trematode infestations and chronic effect of copper ions: 1 – infested mollusks 2 – uninfected mollusks; I – control. II – 0.5 MPC. III – 2.0 MPC. IV – 5.0 MPC. V – 10.0 MPC (MPC – maximum-possible concentration)

Рис. 2. Вміст купруму в організмі *Р. ригрига* за трематодної інвазії та хронічної дії іонів купруму: 1 – інвазовані молюски 2 – неінвазовані молюски; І – контроль. ІІ – 0,5 ГДК. ІІІ – 2,0 ГДК. IV – 5,0 ГДК. V – 10,0 ГДК (ГДК – гранично допустима концентрація)

There are two mechanisms of resistance of cells to the action of heavy metals: extracellular – prevention of penetration of metal ions in the body; intracellular - metabolic adjustment aimed at binding, detoxification and excretion of the metal [23]. Excessive concentration of metal ions in the body can carry toxic effects at the cellular, tissue and organ levels, so the amount of inflow and animal excretion by the body is regulated carefully [20].

Another regulatory factor is the mutual influence of metals on the processes of absorption in each other. The processes of copper absorption are competitive related with zinc and cadmium, and the amount of absorbed metal is determined by its shape and composition of the diet [5]. Therefore, in the uninfected and in infested mollusks antagonism in the accumulation of zinc and other ions of investigated metals was found. An important role in the homeostasis of heavy metals play metallothioneins – low molecular proteins with high content of cysteine (over 30 % of the total content of amino acids) that do not outline hydrophobic amino acids and have a high affinity for divalent HM ions because of sulfhydryl groups. Metallothioneins are involved in both maintaining constant concentration of free ions of physiologically important metals in normal conditions (copper, zinc) [27] and in detoxification of toxins, such as plumbum, mercury, cadmium.

#### CONCLUSIONS

Intracellular detoxification systems are implemented in three main ways: limiting inflow of metal ions into the cell, growth of toxicant export from cells and binding to the metal in a form that does not carry toxic effects (chelates, soluble compounds) [6]. Individual organs play a leading role in accumulation and redistribution of metal ions in the body of mollusk. For cadmium, zinc and copper at chronic exposure, mantle shows the highest affinity to them. Plumbum is mainly accumulated in the shell. The role of hepatopancreas during the actions of all studied metals, lies in the initial association with binding of specific and nonspecific proteins and their subsequent redistribution in other organs and tissues. The role of hemolymph is in transfer and participation in the redistribution of ions between the organs and tissues, and, therefore, its relative metal content compared to other studied organs and tissues is the smallest. The accumulation of metal ions is largely governed by so important biotic factor as trematode invasion. As a whole, the mantle invasion promotes grabbing of metals, especially nutrients. In other tissues and organs, especially hepatopancreas and leg, the presence of trematode infestations generally reduces their accumulation capacity.

<sup>1.</sup> *Alekseev V.A.* Basic principles of comparative toxicological. **Hydrobiological Journal**, 1981; 7(3): 92–100. (In Russian).

<sup>2.</sup> Arsan O.M. The state and the outlook for development of aquatic ecotoxicology. **Hydrobiological Journal**, 2007; 43(6): 50–64. (In Russian).

<sup>3.</sup> *Arsan V.O.* Influence of the ionic form of copper (II) in the aquatic environment on the contents of metabolites of glycolysis and tricarbonic acid cycle in carp tissues. **Hydrobiological Journal**, 2003; 39(2): 109–115. (In Russian).

<sup>4.</sup> *Braginskiy L.P. Linnik P.N.* On the method of toxicological experiments with hydrobionts using heavy metals. **Hydrobiological Journal**, 2003; 39(1): 92–104. (In Russian).

<sup>5.</sup> Davies N.T., Campbell J.K. The effect of cadmium on intestinal copper absorption and binding in the rat. Life Science, 1977; 20: 955–960.

Ershov Y.A., Pletneva T.V. Mechanisms of toxic action of inorganic compounds. Moscow: Medicine, 1989. 272 p. (In Russian).

- 7. Handzyura V.P., Hrubinko V.V. Harmfulness concept in ecology. Ternopil: Edition TNPU after name V. Hnatyuk, 2008. 144 p. (In Ukrainian).
- 8. *Hilenko O.F.* Some universal regularities action of chemical agents for aquatic organisms: Abstract of dissertation for the degree of Doctor of Biological Sciences: specialty 03.00.16 "Hydrobiology". Moscow: Moscow State University, 1990. 36 p. (In Russian).
- 9. *Hilmy A.M., Domiati N.A., El. Daabees A.J.* et al. The toxicity to *Clarias lozera* of copper and zinc applied jointly. **Comparative Biochemistry and Physiology**, 1987; 2: 309–314.
- 10. *Izrael Yu.A*. **Ecology and control of the natural environment**. L.: Hydrometeoizdat, 1979. 375 p. (In Russian).
- Kharchenko T. A., Timchenko V., Kovalchuk A.A. et al. Hydroecology of Ukrainian section of the Danube and adjacent bodies of water. Kyiv: Science. Dumka, 1993; 327. (In Russian).
- 12. Khlebovich V.V. Acclimation animal organisms. L.: Science, 1981. 135 p. (In Russian).
- 13. *Khomenchuk V.O.* **Biochemical features of penetration and distribution of some heavy metals in the body scaly carp**: Abstract dissertation for the degree of candidate of biological sciences 03.00.04 specialty "Biochemistry". Lviv, 2003, 19 p. (In Ukrainian).
- 14. *Kirillov O.I.* From theory orthobiosis Mechnikov Selye stress concentration. Bulletin FEB RAS, 1996; 1: 29–34. (In Russian).
- 15. Kyrychuk G.Ye. Accumulation of cadmium and zinc in organism of planorbarius purpura on background of trematods invasion. Scientific notes Ternopil Pedagogical University after name V. Hnatyuk. Series Biology, 2010; 4(45): 54–61. (In Ukrainian).
- 16. Lakin B.F. Biometrics. Moscow: Higher School, 1973; 343. (In Russian).
- Levina E.N. General Toxicology metals. L: Medgiz (Leningrad Branch), 1972. 183 p. (In Russian).
- 18. Linnik P.N. Coexisting forms of heavy metals in natural waters and comparative assessment of their toxicity to aquatic organisms. Linnik K., Dep. VINITI. 1986; No 7633-V86: 39. (In Russian).
- 19. Lukyanova O.N. Molecular biomarkers (assessment of marine invertebrates in chronic contamination of the environment). Vladivostok: Publishing House DVGAEU, 2001; 191. (In Russian).
- Milne D.B. Assessment of copper nutritional status. Clinical Chemistry, 1994. 40: 1479– 1484
- 21. *Moore Dzh., Ramamurti S.* **Heavy metals in natural waters**. Moscow: World, 1987. 288 p. (In Russian).
- 22. *Nikanorov A.M., Zhulidov A.V., Pokarzhevsky A.D.* Biomonitoring of heavy metals in freshwater ecosystems. L.: Hydrometeoizdat, 1985. 143 p. (In Russian).
- 23. *Ochiai E.I.* Toxicity of heavy metals and biological defense: principles and application in bioinorganic chemistry. **Journal Chemical Education**, 1995; 72(60): 479–484.
- 24. *Patin S.A., Morozov N.N.* Trace elements in marine organisms and ecosystems. M: Light and food industries, 1981; 153. (In Russian).
- 25. Romanenko V.D. Basics of Hydroecology. Kiev: Genesa, 2004. 664 p. (In Russian).
- 26. Sidorin G.I., Lukovnikova L.V., Frolova A.D. Adaptation as a basis for protecting the body from the harmful effects of chemicals. **Russian Chemical Journal**, 2004; 48(2): 44–50. (In Russian).
- 27. Stolyar O.B. Metallothioneins role in the detoxification of copper ions. zinc. manganese and lead in the body of freshwater fish and shellfish: Abstract of dissertation for the Degree of Doctor of Biological Sciences: 03.00.04 specialty "Biochemistry". Lviv, 2004. 30 p. (In Ukrainian).
- 28. Stroganov N.S. Influence of pollutants on hydrobionts ecosystems and water bodies. Materials of two Soviet-American Symposium. L.: Science, 1979. 53–76 p. (In Russian).

- 29. Subbaiah M., Balaven Kata, Akhilender Naidu K. et al. Heavy metal toxicity to some freshwater organisms. **Geobios**, 1983; 10(3): 128–129.
- 30. The EU Water Framework Directive 2000/60/EC. Basic terms and their definitions. Kyiv, 2006. 240 p. (In Ukrainian).
- 31. *Zdun V.I.* **Trematode larvae in freshwater shellfish Ukraine**. Kyiv: Publishers USSR, 1961. 141 p. (In Ukrainian).
- 32. Zhulidov A.V., Yemets V.M., Shevtsov A. Biomonitoring Pollution of rivers by heavy metals in reserves based on the accumulation of metals in the body of large aquatic invertebrates. **Report USSR Academy of Sciences**, 1980; 252(4): 1018–1020. (In Russian).
- Zhytova O.P., Khomych V.T. The emission of trematode cercariae as an index of the infection level and pathohistological changes in mollusc hepatopancreas. Science magazine National Pedagogical University M.P. Drahomanov. Series 20: Biology, 2011; 3: 109–115. (In Ukrainian).

### ОСОБЛИВОСТІ РОЗПОДІЛУ ІОНІВ ВАЖКИХ МЕТАЛІВ У ПРІСНОВОДНИХ МОЛЮСКІВ ЗА СУМІСНОЇ ДІЇ ІОНІВ КУПРУМУ І ТРЕМАТОДНОЇ ІНВАЗІЇ

Г. Є. Киричук

Житомирський державний університет імені Івана Франка вул. Велика Бердичівська, 40, Житомир 10008, Україна e-mail: kyrychuk@zu.edu.ua

Досліджено особливості накопичення іонів купруму у прісноводних молюсків Lymnaea stagnalis і Planorbarius purpura за експериментальних умов хронічної дії (0,5; 2; 5 і 10 гранично допустима концентрація (рибогосподарська)) у нормі та за сумісної дії трематодної інвазії і зазначеного полютанта. Проаналізовано особливості розподілу іонів Cu, Pb, Cd і Zn в організмі молюсків за згаданих умов. З'ясовано, що трематодна інвазія призводить до зміни розподілу сумарного вмісту важких металів в організмі черевоногих молюсків. Так, у інвазованих Р. purpura досліджені тканини й органи розмістилися в порядку зростання: гемолімфа < черепашка < гепатопанкреас < мантія < нога, а у L. stagnalis – гемолімфа < гепатопанкреас < черепашка < нога < мантія. За допомогою кореляційного аналізу розраховано ранговий коефіцієнт кореляції Спірмена. Це дало змогу виявити ступінь залежності між дослідженими іонами в різних тканинах і органах Р. purpura. Встановлено, що акумуляція металів є тканиноспецифічною, а рівень їх накопичення пов'язаний зі спорідненістю металів до неспецифічних і специфічних метаболітів, метаболічною активністю й фізіологічною потребою організму молюсків у цих метапах.

**Ключові слова:** іони купруму, важкі метали, прісноводні молюски, накопичення, трематодна інвазія, *Lymnaea stagnalis, Planorbarius purpura.* 

# ОСОБЕННОСТИ РАСПРЕДЕЛЕНИЯ ИОНОВ ТЯЖЕЛЫХ МЕТАЛЛОВ ПРЕСНОВОДНЫХ МОЛЛЮСКОВ ПРИ СОВМЕСТНОМ ВОЗДЕЙСТВИИ ИОНОВ МЕДИ И ТРЕМАТОДНОЙ ИНВАЗИИ

Г. Е. Киричук

Житомирский государственный университет имени Ивана Франко ул. Большая Бердичевская, 40, Житомир 10008, Украина e-mail: kyrychuk@zu.edu.ua

Исследованы особенности накопления ионов меди в пресноводных моллюсках Lymnaea stagnalis и Planorbarius purpura в экспериментальных условиях при хроническом воздействии (0,5; 2; 5 и 10 предельно допустимая концентрация (рыбохозяйственная)) на фоне трематодной инвазии. Проанализированы особенности распределения Cu, Pb, Cd и Zn в организме моллюсков при указанных условиях. Выяснено, что трематодная инвазия приводит к изменению распределения суммарного содержания тяжелых металлов в организме брюхоногих моллюсков. Так, у инвазированных Р. purpura исследованные ткани и органы расположены в порядке возрастания их содержания: гемолимфа < раковина<гепатопанкреас < мантия < нога, а у L. stagnalis – гемолимфа < гепатопанкреас < раковина < нога < мантия. С помощью корреляционного анализа рассчитан ранговый коэффициент корреляции Спирмена. Это позволило выяснить степень зависимости между исследованными ионами в разных тканях и органах Р. purpura. Установлено, что аккумуляция металлов тканеспецифична, а уровень их накопления определяется сродством металлов к неспецифическим и специфическим метаболитам, метаболической активностью и физиологической потребностью организма моллюсков в этих металлах.

**Ключевые слова:** ионы меди, тяжелые металлы, пресноводные моллюски, накопление, трематодная инвазия, *Lymnaea stagnalis*, *Planorbarius purpura*.

Одержано: 21.10.2015