антирадикальними властивостями щодо 2,2-дифеніл-1-пікрилгідразилу та до 2,2'-азино-біс (3-етилбензотіазолін-6-сульфонової кислоти) у фізіологічно релевантних концентраціях. Синтезовані nZnO з використанням екстрактів *Catharanthus roseus* та *Momordica charantia* характеризуються подібними оптичними, антиоксидантними та антигіперглікемічними характеристиками (Horyn et al., 2019) та можуть бути застосовані у медичній та фармацевтичній галузях для розробки нових лікарських препаратів.

Ключові слова: зелений синтез, нано-цинк оксид, Catharanthus roseus, Momordica charantia, антирадикальні властивості.

Надійшла 14.05.2019.

УДК 597.5:577.12: 57.047 doi: 10.25128/2078-2357.19.2.6

[1]V. Z. KURANT, [1]V. O. KHOMENCHUK, [1]V. Ya. BYYAK, [2]N. G. ZINKOVSKA, [1]V. S. MARKIV

[1]Volodymyr Hnatyuk Ternopil National Pedagogical University
M. Kryvonosa Str., 2, Ternopil, 46027, Ukraine
[2]Taras Shevchenko Regional Humanitarian-Pedagogical Academy of Kremenets, Ukraine
Lyceum lane, 1, Kremenets, 47003
e-mail: khomenchuk@tnpu.edu.ua

INFLUENCE OF HEAVY METALS IONS ON THE CONTENT OF PROTEINS AND NUCLEIC ACIDS IN THE ORGANISM OF FRESHWATER FISH

From the launched research we obtained the aggregate data, that not only confirm and broaden our concept of the important role of protein and nucleic metabolism in the processes of detoxication of heavy metals ions and formation of resistance to them, but also allow making an integral estimation of biochemical reaction of carp organism to chronic intoxication.

Key words: freshwater fish, proteins, nucleic acids, heavy metals.

Contamination of water reservoirs by heavy metals is one of the limiting factors of aquatic ecosystems functioning and their biological productivity. Being part of many organic substances, or engaging them in the interaction, they influence many biochemical processes in aquatic organisms. The ions of metals can form strong connections in the tissues along with various biologically active centres, including the sulphur-containing ligands, that may be enclosed in proteins and amino acids. Their activity is related to the enzymes that contain metal ions in their composition or are actuated by them [6, 10].

One of the basic principles of biochemical adaptation of an organism is to maintain the structural and functional integrity of macromolecules. Much of this is applied to proteins and nucleic acids – biopolymers, that perform an extremely important role in the adaptation of aquatic lives to environmental conditions [10].

Materials and methods

The object of the given research was carp – *Cyprinus carpio* L. For the experiment the 2 year old fish with the mass of 250-300 grams were rummaged from the natural stews of Ternopil region (Zalistsi fish-breeding complex). The experiments were carried out in 200 litre aquariums filled with the precipitated water from the local water supply system under constant gas and temperature operating conditions. During the process the fish were not fed. The effect of Mg, Zn, Cu and Pb ions in two concentrations are complied with 2 and 5 maximum permissible concentrations (MPC) [1]. The period of acclimation was 14 days.

The total content of albumins in tissues was determined by a biuretic method with some modifications [3] while in nucleic acids fractions – by Lowry and co-authors [11]. Nucleic acids were fixed spectrophotometrically by Tsanev R.H. and Markov G.G. [8] in accordance with the authors' recommendations [2]. For the protein fractions of fish blood serum the diagnostic set "Cormay gel protein 100" (Austria) was used. To determine the significant difference the obtained data underwent certain statistic processing [5].

Results and discussion

In our studies significant deviations from the control indices of content as for aggregate proteins and proteins combined with nucleic acids under the influence of higher concentrations of ions of metals were not found. A certain increase in the total number of proteins in the liver may indicate to an active part of this organ in the synthesis of adaptive proteins.

The slightest deviation from the control indexes of the total protein content (table 1) was found in the muscles of carp, which testifies to the fact that along with the increased activity of lysosomal proteases and the rising contents of free amino acids the aggregate protein content remains constant. The latter points rather to deep restructuring of protein metabolism in the body of fish under the influence of heavy metals than to their use in energy processes by means of amino acids oxidation.

Table 1

Effect of heavy metals on the content of total protein in carp tissues

(mg %, M ± m, n = 5)

Group	Manganese	Zinc	Copper	Lead
Liver				
Control	9,84±0,72	9,40±0,42	10,86±0,52	11,22±0,40
2 MPC	10,35±0,73	9,67±0,62	9,94±0,45	11,45±0,33
5 MPC	10,36±0,93	9,03±0,27	12,85±1,26	12,17±0,69
Muscles				
Control	12,58±0,83	12,73±0,38	15,50±0,32	15,08±0,24
2 MPC	13,55±1,49	13,70±0,58	13,81±0,27*	14,84±0,79
5 MPC	13,22±0,39	13,55±0,54	14,19±0,34*	14,92±0,27
Blood				
Control	13,03±0,59	11,21±0,54	13,86±1,29	12,26±0,78
2 MPC	14,58±0,26*	13,94±0,63*	15,56±0,47	12,94±0,64
5 MPC	13,63±1,05	11,09±0,86	17,81±0,73*	12,22±0,81

Note. * – significant difference compared to the control, $P < 0,05$

The change of the content of proteins in the structure of nucleoprotein complexes is probably related to the functional characteristics of these complexes. It is a well known fact, that some proteins can act as the repressors of genome. Therefore, their number in the tissue may be an indicator of the size of the protein blockade of nucleic acids molecules. In our studies we could not find any statistically significant deviations from the control indices of protein content in the fractions of RNA and DNA (tables 2, 3). It is possible that under these experimental conditions the body of fish does not undergo any significant functional changes at the genetic level and its adaptation passes on the level of phenotype through modification of the quantitative and qualitative composition of molecules.

Changing of the chemical structure of water environment inevitably leads to the changes in protein composition of fish blood. The obtained data proves the alteration of the total protein concentration and the ratio of protein fractions in the serum of carp, its body exposed to higher concentrations of heavy metal ions. Thus, the total protein content in the blood serum of fish increases when affected by manganese, zinc, lead, and especially copper. Deviations of this index from the control indices increase along with the rise of metal concentration in water.

Table 2

Effect of heavy metals on the content of RNA in carp tissues (mg% P, M ± m, n = 5)

Group	Manganese	Zinc	Copper	Lead
Liver				
Control	64,23±1,56	47,33±4,18	43,76±2,99	45,72±1,69
2 MPC	69,84±1,56*	50,49±4,05	40,67±1,09	53,01± 4,80
5 MPC	67,04±3,72	59,95±2,99*	52,45±1,86*	73,49±6,19*
Muscles				
Control	14,44±0,48	13,67±1,68	6,73±0,52	13,04±0,36
2 MPC	13,88±0,81	15,01±1,12	5,76±0,14	12,48±0,81
5 MPC	14,55±1,05	12,34±0,57	6,17±0,46	15,00±0,52*
Blood				
Control	27,49±2,20	17,18±2,09	23,28±1,69	26,65±0,99
2 MPC	28,40±2,01	18,51±1,56	28,89±1,30*	21,32±1,90*
5 MPC	23,49±2,88	12,27±1,20	28,61±1,05*	22,16±0,69*

Note. * – significant difference compared to the control, P < 0,05

Table 3

Effect of heavy metals on the content of DNA in carp tissues (mg% P, M ± m, n = 5)

Group	Manganese	Zinc	Copper	Lead
Liver				
Control	23,20±3,14	21,00±1,29	15,00±1,73	20,50±0,96
2 MPC	26,40±2,31	22,00±1,67	13,20±1,02	23,00±1,73
5 MPC	22,80±1,03	21,60±1,17	22,40±1,17*	29,50±1,26*
Muscles				
Control	9,25±0,75	8,00±0,32	3,40±0,51	6,40±0,24
2 MPC	6,60±0,87*	7,80±0,20	3,00±0,32	6,00±0,00*
5 MPC	6,00±0,45*	8,40±0,40	2,60±0,24	7,40±0,24*
Blood				
Control.	51,60±3,06	51,20±3,38	36,00±4,97	31,60±0,98
2 MPC	52,00±3,74	56,00±1,41	37,00±4,36	32,00±2,53
5 MPC	56,67±3,71	45,20±5,98	44,00±2,28	42,50±1,71*

Note. * – significant difference compared to the control, P < 0,05

An increase of the total protein content in the serum of carp due to heavy metals, in our opinion, should be considered primarily as a result of the synthesis of the acute phase of proteins, growth of the level of blood haemolyse in the experimental fish, augmentation of the number of transporting proteins which bind and transfer the ions of metals, and also of blood coagulation. Besides, a higher content of proteins in the blood serum of experimental fish may be caused by the enhanced dissolution of proteins in tissues resulting from the increase of activity of proteolytic enzymes under intoxication.

Determination of the fractional composition of carp blood serum exposed to the influence of heavy metals has an important diagnostic value. Thus, under both of the studied metals concentrations the content of albumin in blood serum of fish increases (figure 1). The only exception is lead at 2 MPC of metal in water. This protein plays an important role in maintaining the osmotic pressure in the blood and in transporting of a number of substances, including amino acids and inorganic ions [9]. Therefore, there becomes clear the increase of quantity of albumin which under the intoxication and active proteolysis of tissue proteins and transports of free amino acids. The largest growth of albumin concentration in the serum of fish is observed under the influence of copper ions. This phenomenon is

consistent with the data that albumin carries the fast-exchange fractions of copper, while the slow-exchange fractions of this metal are transported by α_2–globulins [9]. The ability of albumin to bind calcium ions and magnesium is also well known [4]. It is possible, that according to the similar principle of this protein binds other divalent ions, there by reducing their toxicity to the body. On the other hand, the ions of the investigated metals may exhibit a stimulating effect on the biosynthesis of albumin.

Because of intoxication the carp blood serum globulins undergo certain changes. These proteins are involved in the transportation of lipids, hormones, vitamins, metal ions; form important complexes of blood coagulation, while γ-globulins fraction contains antibodies of the immune system. It is logical to assume that the change of globulins in the content of blood serum leads to the violation of performance of the described functions by them.

Higher concentrations of ions of the investigated metals in particular caused a slight increase in the content of α_1-globulin at 2 MPC of metals in water, while the α_2-fraction responded in the same way to 5 MPC of the investigated metals. Zinc was considered to be an exception, for the action of which the reduction of the content of fractions α_1– and α_2–globulins was observed in both cases. Taking into account that zinc inhibits the activity of certain proteases, the decrease of α_1-globulins containing antitrypsin and antichimotrypsin might be a response of the carp serum protein system to the increased level of zinc in water. One should also admit the growth of the content of α_2-globulins under the effect of copper ions at the concentration of 5 MPC, which is consistent with the data [4] that exactly this fraction contains ceruleoplasmin – an acute phase protein which actively transports the ions of copper.

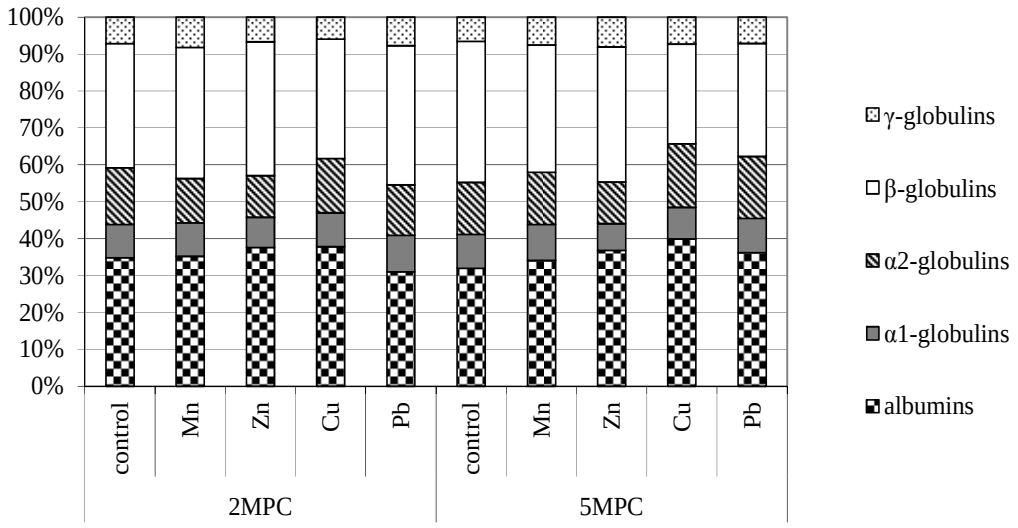

Fig. 1. Relative content of carp blood serum proteins in control groups and under intoxication (% of total proteins)

Somewhat different dynamics was detected as for the content of *β*-globulins of carp blood serum under the influence of ions of the investigated metals. At the level of 2 MPC of metals in water we have noticed the growth in the total proteins and their fraction under the effect of ions of manganese, zinc and lead, and only copper ions were reducing this index. At 5 MPC of metals in water all of the investigated chemical elements caused the reduction of *β*-globulins content in the blood serum of fish. Thus more significant deviations from the control group were observed due to the influence of copper ions and lead.

One of the main functions of *β*-globulins is the transportation of iron ions [4], which is part of haemoglobin structure, and thus participates in the processes of oxidation. The decrease of the amount of this metal in the blood leads to a decrease of oxidation processes in the whole organism, which we observe in the conditions of intoxication when anaerobic ways of energy formation is dominated over the aerobic.

Because under the action of ions of the investigated metals the content of *γ*-globulins, especially at the concentration of 5 MPC, grows in the carp blood serum. With this fraction of proteins, that contains antibodies, are mainly associated the protective properties of the body and therefore it is clear why their number increases under intoxication.

A very important diagnostic value has the determination of fish blood serum lipoproteins content — complexes of proteins and lipids, whose role in the process of adaptation of the body of fish to the environmental conditions is rather significant. Our studies revealed *α*- and *β*-lipoprotein fractions: the α-fraction accounted for 72-78% of the proteins, and *β* – for only 22-28% (Table 2). The dynamics of changes in these fractions under the influence of ions of metals investigated was also different. While the number of *α*-lipoproteins in the blood serum of experimental fish at both studied concentrations of metals in water was increasing, the amount of *β*-lipoprotein, in contrast, declined. The only exception was the indicator of the impact of lead ions at 2 MPC of metal in water.

The reduction of the content of *β*-lipoproteins, that are a low-density lipoprotein fraction, is probably happening due to the fact, that these protein-lipid complexes are absorbed by tissues and undergo disintegration in lysosomes [7]. Therefore, the fortified catabolism of *β*-lipoproteins and the decrease of their content may be the result of the increased activity of lysosomal enzymes in the studied tissues of fish under intoxication.

Table 4

Dynamics of the content of lipoproteins in the carp blood serum under the influence of heavy metal ions (%, M ± m, n = 5)

Group	*α*- lipoproteins		*β*- lipoproteins	
	2 MPC	5 MPC	2 MPC	5 MPC
Control	72,22±0,74	77,63±1,3	27,77±0,74	22,37±1,3
Manganese	86,18±0,83*	89,31±0,7*	13,82±0,66*	10,69±0,7*
Zinc	86,18±0,06*	89,48±1,5*	11,82±0,07*	10,52±1,15*
Copper	85,68±1,02*	90,73±0,7*	14,32±1,02*	9,27±0,7*
Lead	68,47±1,27	83,08±1,88	31,53±1,27	16,92±1,88*

Note. * – significant difference compared to the control, P < 0,05

The growth of *α*-lipoproteins may be explained by the fact that this fraction is quite easily formed from very low density lipoproteins and chylomicrons the decay of which is accompanied by the increase in the number of phospholipids, free cholesterol and apolipoproteins [7]. Biosynthesis of *α*-lipoproteins takes place in the liver and small intestine and the main function of this fraction is to maintain the transformation processes of lipids. Alongside with this the level of high-density lipoproteins (*α*-lipoprotein) in blood serum is an integral indicator of lipoproteins exchange and characterizes the efficiency of the transport systems functioning and transformation of lipids in the body as a whole.

Conclusion

Thus, the study of carp protein system under intoxication of its body by heavy metal ions made it possible to learn the mechanisms of functional homeostasis and adaptive responses of fish blood, which may serve as a prerequisite for identifying of the integrated indicators, that point to the key changes in the organisms of the aquatic environment under the extreme conditions.

1. Беспамятнов Г. П., Кротов Ю. А. Предельно допустимые концентрации химических веществ в окружающей среде: справочник. Л.: Химия, 1985. 240 с.
2. Галкин В. В., Бердышев Г. Д. К вопросу о количественном определении нуклеиновых кислот биохимическими методами в тканях различных животных. *Биохимия*. 1968. Т. 33, № 1. С. 66-76.
3. Калачнюк Г. І., Гжицький С. З. Визначення концентрації білка у вмісті рубця за принципом виявлення пептидних зв'язків. ДАН УРСР. 1974 Б. №4. С. 353–355.
4. Кольман Я., Рём К.-Г. Наглядная биохимия. М.: Мир, 2000. 470 с.
5. Лакин Г. Ф. Биометрия. М.: Высшая школа, 1990. 351 с.

6. Ноздрюхина Л. Р. Биологическая роль микроэлементов в организме животных и человека. М.: Наука, 1977. 184 с.
7. Холодова Ю. Д., Чаяло П. П. Липопротеины крови. К.: Наукова думка, 1990. 208 с.
8. Цанев Р. Г., Марков Г. Г. К вопросу о количественном спектрофотометрическом определении нуклеиновых кислот. Биохимия. 1960. Т. 25, № 1. С. 151–159.
9. Чегер С. И. Транспортная функция сывороточного альбумина. Бухарест: Изд-во Академии соц. респ. Румынии, 1975. 183 с.
10. Hochachka P. W., Somero G. N. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford: Oxford University Press, 2002. 466 p.
11. Lowry O. H., Rosenbrough N. J., Farr A., Randall R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951. Vol. 193, № 1. P.265–275.

References

1. Bespamiatnov G.P., Krotov Iu.A. Predel'no dopustimye kontsentratsii khimicheskikh veshchestv v okruzhaiushchey srede: spravochnik. L.: Khimiia, 1985. 240 s. (in Russian).
2. Galkin V.V., Berdyshev G.D. K voprosu o kolichestvennom opredelenii nukleinovykh kislot biokhimicheskimi metodami v tkaniakh razlichnykh zhivotnykh Biokhimiia. 1968. T. 33, No 1. S. 66 76. (in Russian).
3. Kalachniuk H.I., Hzhyts'kyy S.Z. Vyznachennia kontsentratsii bilka u vmisti rubtsia za pryntsypom vyiavlennia peptydnykh zv'iazkiv. DAN URSR. 1974 B. No 4. S. 353-355. (in Ukrainian).
4. Kol'man Ia., Rem K.-G. Nagliadnaia biokhimiia. M.: Mir, 2000. 470 s. (in Russian).
5. Lakin G.F. Biometriia. M.: Vysshaia shkola, 1990. 351 s. (in Russian).
6. Nozdriukhina L.R. Biologicheskaia rol' mikroelementov v organizme zhivotnykh i cheloveka. M.: Nauka, 1977. 184 s. (in Russian).
7. Kholodova Iu.D., Chaialo P.P. Lipoproteiny krovi. K.: Naukova dumka, 1990. 208 s. (in Russian).
8. Tsanev R.G., Markov G.G. K voprosu o kolichestvennom spektrofotometricheskom opredelenii nukleinovykh kislot. Biokhimiia. 1960. T. 25, No 1. S. 151-159. (in Russian).
9. Cheger S.I. Transportnaia funktsiia syvorotochnogo al'bumina. Bukharest: Izd-vo Akademii sots. resp. Rumynii, 1975. 183 s. (in Russian).
10. Hochachka P. W., Somero G. N. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford : Oxford University Press, 2002. 466 p.
11. Lowry O.H., Rosenbrough N.J., Farr A., Randall R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951. Vol. 193, № 1. P. 265-275.

В. З. Курант, В. О. Хоменчук, В. Я. Бияк, Н. Г. Зіньковська, В. С. Марків
Тернопільський національний педагогічний університет імені Володимира Гнатюка
Кременецька обласна гуманітарно-педагогічна академія імені Тараса Шевченка

ВПЛИВ ІОНІВ ВАЖКИХ МЕТАЛІВ НА ВМІСТ БІЛКІВ ТА НУКЛЕЇНОВИХ КИСЛОТ В ОРГАНІЗМІ ПРІСНОВОДНИХ РИБ

В дослідженнях одержано сукупність даних, які підтверджують і розширюють уяву про важливу роль білкового та нуклеїнового обмінів у процесах детоксикації іонів важких металів, у формуванні стійкості до них, а також отримані результати дають можливість здійснити комплексну оцінку біохімічної реакції організму риб на хронічну інтоксикацію.

За дії підвищених концентрацій іонів важких металів в тканинах коропа збільшується вміст нуклеїнових кислот. Більшою мірою зростає кількість РНК, особливо при 5 ГДК металів у воді. Виявлена зміна співвідношення РНК/ДНК за дії досліджуваних металів опосередковано свідчить про наявність експресії геному, що пов'язано із біосинтезом специфічних адаптивних білків, зокрема, металотіонеїнів. Значніші відхилення від норми спостерігали в печінці та крові риб, менші – у м'язах.

Інтоксикація організму коропа іонами важких металів приводить, як правило, до зростання в його тканинах загального вмісту білків. Збільшення кількості білків у печінці коропа свідчить про синтез у цьому органі металотіонеїнів, який більше активується іонами міді та свинцю і менше – іонами марганцю та цинку. Вміст білків у фракціях РНК та ДНК майже не змінюється, що вказує на стабільність нуклеопротеїдних комплексів тканин коропа за дії іонів досліджуваних металів.

Виявлено високу чутливість білкової системи сироватки крові коропа до підвищеного вмісту іонів важких металів у воді, яка проявляється у збільшенні сумарного вмісту білків та зростанні білкового коефіцієнту за дії всіх досліджених металів. На рівні фракційного складу білків сироватки крові помічено зростання вмісту альбумінів при обох вивчених концентраціях металів у воді та γ-глобулінів при їх рівні 5 ГДК. Відзначена тенденція до збільшення вмісту білків фракцій α_1- та β-глобулінів при 2 ГДК металів у воді та зниження цих показників при 5 ГДК. Вміст білків у фракції α_2-глобулінів, як правило, знижується, за винятком випадку впливу іонів міді та свинцю в кількості 5 ГДК.

Вплив підвищених концентрацій досліджених металів приводить до зростання кількості α-ліпопротеїдів в сироватці крові коропа та до зниження вмісту β-ліпопротеїдів, що свідчить про перебудову за інтоксикації механізмів гомеостатичної регуляції рівня ліпідів в крові риб та використання їх в адаптивних процесах.

Ключові слова: прісноводні риби, білки, нуклеїнові кислоти, важкі метали.

Надійшла 17.04.2019.

УДК: 577.352.38:577.64 doi: 10.25128/2078-2357.19.2.7

[1]V. V. KHOMA, [1,2]L. L. GNATYSHYNA, [1]V. V. MARTINYUK, [1]YU. S. ROROK, [3]D. OZOLIŅŠ, [3]I. KOKORITE, [3]A. SKUJA, [3]G. H. SPRIŅĢE, [1*]O. B. STOLIAR

[1]Volodymyr Hnatyuk Ternopil National Pedagogical University
M. Kryvonosa Str., 2, Ternopil, 46027, Ukraine
[2]I.Ya. Horbachevsky Ternopil State Medical University
Maidan Voli 1, Ternopil, 46001, Ukraine
[3]University of Latvia, Riga
e-mail: Oksana.Stolyar@tnpu.edu.ua

APPLICATION OF METAL-BINDING CHARACTERISTICS OF AQUATIC ANIMALS IN THE ASSESSMENT OF COMPLEX ENVIRONMENTAL POLLUTION

The present study aimed to compare the ability of bivalve mollusks and fish to accumulate some toxic metals in their tissues in the polluted environment. Populations of *Dreissena polymorpha, Unio tumidus* and *Carassius gibelio* in Latvia in Ukraine were investigated. In the mollusks from the polluted reservoirs, the accumulation of Zn, Cu, Cd in the tissues but decrease in metallothioneins concentration was detected, whereas the fish did not appropriate reflect the aquatic quality.

Key words: etallothioneins, bivalve mollusks, fisch, copper, zinc, cadmium, bioindicatson.

Freshwater mollusks are widely distributed in both natural and artificial aquatic bodies. They can serve as bio-indicators of aquatic pollution due to their ability to accumulate different substances from the environment [2]. Fish also is usually utilized as bioindicative species because of its major ecological role in the aquatic food-webs and sensitivity to stressful conditions [5]. The detection of the accumulation and compartmentalization of toxic metals in the tissues of the aquatic animals represents the valuable part of the exploring of these animals in the assessment of environmental health [2]. Metallothioneins are the ubiquitous cellular molecular targets for d-metals, mostly for cadmium (Cd), zinc (Zn) and copper (Cu). They serve as buffering proteins that keep these metals in the less toxic form (particularly Cd) and provide the distribution of essential metals Zn and Cu among the functional proteins of signaling and catalysis [1]. Therefore, the induction or increased levels of the metallothioneins in the organism or separated tissues are frequently using to justify metal exposure. However, according to the long-years' experience of the laboratory, the impact of complex pollution during life history can disturb the accumulative ability of the aquatic animals and their