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VARIABILITY OF METALLOTHIONEINS IN FRESHWATER BIVALVE ANODONTA CYGNEA (UNIONIDAE) FROM TWO FERAL POPULATIONS

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The metallothioneins (MTs) from the digestive gland of the bivalve mollusc *Anodonta cygnea* were compared in May, July and September at two sites of Dnister River Basin, defined as rural (R) and urbanized (U). The dramatic declining of Cu content in MTs and in the tissue from May till September was accompanied by the appearance of MTs form with molecular weight of about 20 kDa (MT20) in addition to constantly presented MT10 under the size-exclusive chromatography. The chromatography on DEAE-cellulose demonstrated constant MT-1 and variable MT-2/2a forms. The higher levels of Cu or Cd in the MTs reflected their concentrations in the tissue in the R site and the U site correspondingly. The lower metal-binding ability of MTs was reflected at the R site. In spring, the excess of unbound with MTs Zn coupled with the appearance of abundant chromatographic form MT-2a was indicated at the U site.

Key words: Freshwater bivalve; Metallothioneins; Feral population.

Metallothioneins (MTs) are low molecular weight, soluble, thermostable and sulfhydryl-rich proteins, which can act as buffers for essential metals zinc (Zn) and copper (Cu) and non-essential metal cadmium (Cd) to protect cells from their toxicity [13, 18]. The MTs content is proposed as a specific "biomarker" response to metal pollution because individuals collected from environments rich in metals can exhibit an elevated MTs content [18, 23]. Among different aquatic animals, the utilization of MTs of bivalve molluscs in biomonitoring is often connected to their capacity to accumulate a large amount of trace metals [1, 23]. Even in the comparatively unpolluted area in the Western Ukraine, we confirm the sensitivity of this marker of pollution by Cd in the urban area in three consequent seasons despite Cd level in the water was close to the limit of detection [7]. This sensitivity was more remarkable than that of the MTs of fish and frog [8].

However, utilization of the MTs concentration as the single their characteristic in biomonitoring seems to restrict the ability of this biomarker. The molluscs' MTs are known for a particularly high variation of functions and inducibility of different isoforms with variable molecular weights, structure, metal binding properties and molecular charges [5, 6, 11]. However, the studies in the field are limited [1, 2, 5, 18]. In addition, MTs might be expressed by the administration of a variety of factors, among them being oxidative stress agents [4, 6, 15]. This circumstance seems to be very important for the field conditions of complex pollution. Therefore the ascertaining of the MTs features was the part of our multi-biomarkers study of bivalve molluscs *Anodonta cygnea* (Unionidae) widely distributed in the Dnister basin (Ukraine). First part of this study was devoted to the analysis of MTs and metal contents in the tissues jointly with the determining of the oxidative defense and some specific markers in molluscs [7]. It demonstrated that if Cd and MTs contents in the tissues of the mollusc were always higher at the U site, the pollution by organic substances was reflected at the R site. The oxidative stress in the mollusc was observed at the U site during spring. In the present report, the digestive gland was selected for the study as the sensitive to the environmental conditions tissue [7].

The experiments were carried out during the middle of May, July and September of 2007 year. Bivalve molluscs *Anodonta cygnea* (Unionidae) were collected manually from the depth 0,5–1 m in two sites of the basin of the river Dnister in the Western Ukraine. The rural (R) site is situated close to the mouth of the river Nichlava in the rural vicinity. The urban (U) site is located in the mid stream in the river Seret in the central park of city Ternopil. The samplings were carried out simultaneously in both sites. Individuals were transported to the laboratory in cages with native water and treated within a day after the sampling procedure. For each biochemical parameters six digestive gland and gills were prepared individually. The six individuals in each group were dissected and the digestive gland was immediately removed.

Chymotrypsinogen, myoglobin, cytohrome *c*, trypsin inhibitor, phenylmethylsulfonyl fluoride (PMSF), β-mercaptoethanol, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), metallothionein from rabbit liver, DEAE-cellulose, and Sephadex G-50 were purchased from Sigma. All other chemicals were of analytical grade.

MTs were obtained as the thermostable proteins [3]. The tissue samples from fifth individual of group were pooled in aliquot quality (total mass 350 mg). The MTs were purified by size-exclusion chromatography on Sephadex G-50 to determine their molecular weigh, and by anion-exchange liquid chromatography on DEAE cellulose to measure metal content in separate forms as previously described [8]. The fractions of each peak with high absorbance at 254 nm were pooled for the ultraviolet (UV) absorption spectra and the metals determination.

The Zn, Cu and Cd content was measured in the samples of weighed tissue (250 mg) of each animal, and pooled eluate of each MTs fraction after chromatography on the DEAE-cellulose (15 ml) in each group of animals (in triplicate). The samples were dried for 24 h at $105^{o}C$, and then digested with 5 ml HNO_3 for 3 h at $105^{o}C$ under pressure, using an acid-cleaned Teflon bomb. The Cu and Zn content was analyzed by atomic absorption spectrophotometry against certified standards on spectrophotometer S-115, (“Lomo”, Russia) and Cd – on graphite furnace atomic absorption spectrophotometer S-600 (“Selmi”, Ukraine). Metal content in tissues and MTs was expressed as $\mu g \cdot g^{-1}$ fresh weight (FW), in MTs also – as $nmol \cdot g^{-1}$ FW.

MTs chemical analysis was carried out in triplicate and all the other measurements were performed on 6 animals. The results were expressed as means ± standard deviation (SD). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene’s tests, respectively. Two-way analysis of variance (ANOVA) with Post-hoc Tukey HSD test was used to determine whether there were differences in the individual biochemical variables among sites and seasons and the interaction between two factors. Data were subjected to a Principal component analysis (PCA) to determine the relation between metal levels in the tissues and MTs, significant at factorial weight > 0,7, and centroid grouping analysis to differentiate the individual specimens by the set of their indices. All statistical calculations were performed on the separate data from each individual with SPSS 15,0 software, Statistica v 6.0 and Excel for Windows-2000.

Gel-filtration of the thermostable solution from the digestive gland of molluscs revealed the low molecular weight peak, which had an apparent molecular weight of 10 kDa, and was identified as MTs-contained peak based upon its spectral features (comparative high density ratio D_{254}/D_{280}), thermostability, and molecular weight [13] (fig. 1). An intermediate peak with an apparent molecular weight of 20 kDa, reflected in summer in molluscs from the R site and in autumn in both groups, also revealed the spectral features of MT.

The anion-exchange chromatography of the thermostable supernatant demonstrated (fig. 2) typical for animals' MTs comprising of two peaks designated as MT-1 and MT-2 [13]. It was confirmed by the comparison with metallothionein from rabbit liver. MT-2 was much less abundant than MT-1, especially in the molluscs from the U site. An additional peak, MT-2a, was also seen, especially prominent at the U site in spring. The UV-spectra of MTs forms (fig 2C, 2D) exhibited an absorbance peak in the middle UV indicating the presence of characteristic metals-thiolate clusters [13]. In summer and autumn, the maximum of this peak was some shifted to a far ultraviolet as compare to spring.

The metal content analysis in the digestive gland and its MTs (Table, fig. 3) demonstrated the abrupt decreasing of the Cu content in the MTs (about fifty times) and in the tissue

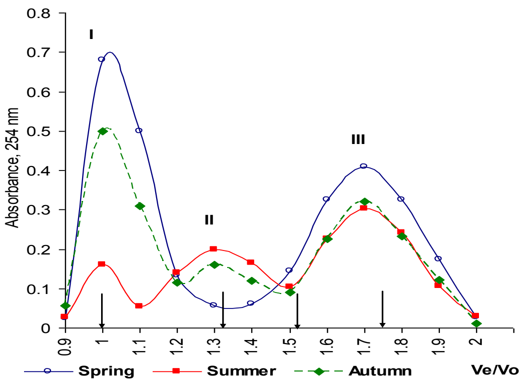


Figure 1(*A*)

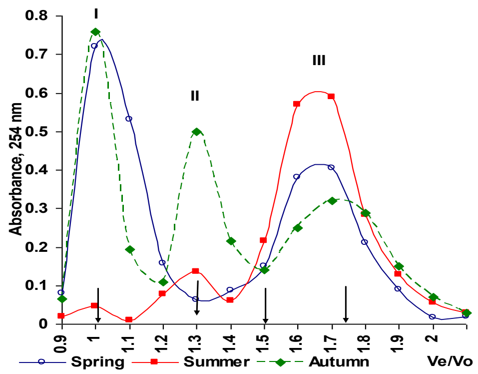


Figure 1(*B*)

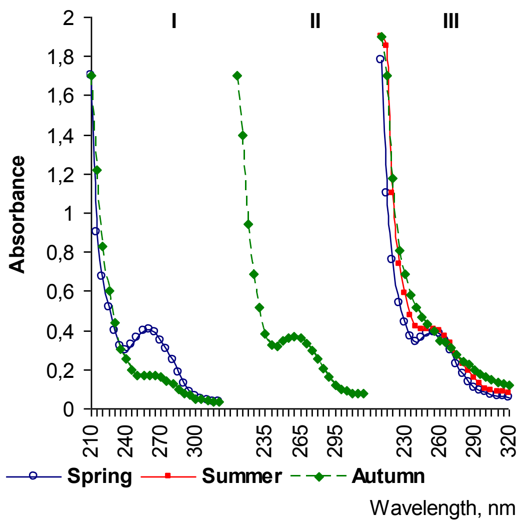


Figure 1(*C*)

Fig. 1. The elution profiles on Sephadex G-50 of the thermostable extract from the molluscs digestive gland at the R site (*A*) and U site (*B*) and typical UV-spectra of the fractions (*C*). Arrows highlight the elution volume of markers: 25.8 kDa, 17.0 kDa, 12.3 kDa, 8.4 kDa. I, II, III, protein peaks; Ve, elution volume; Vo, void volume of the column.

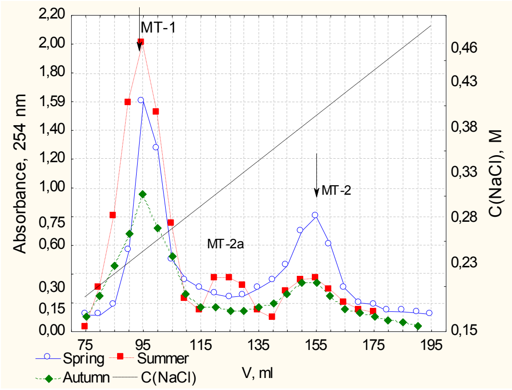
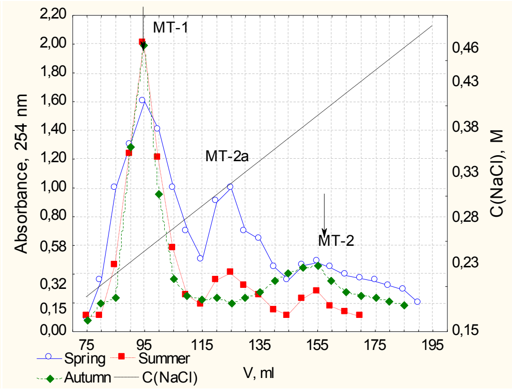


Figure 2(*A*)



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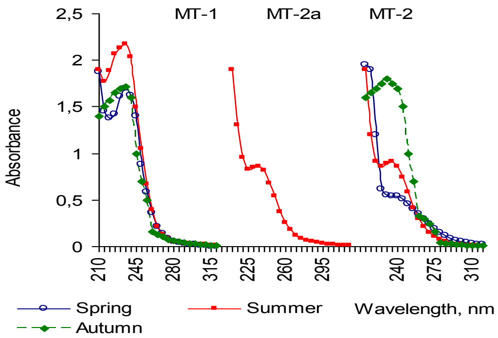

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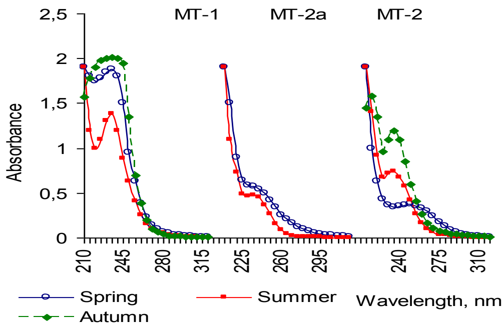

Figure 2(*D*)

Fig. 2. DEAE-cellulose chromatography profiles of metallothioneins from molluscs digestive gland at the R site (*A*) and U site (*B*) and UV-spectra of the MT forms at the R site (*C*) and U site (*D*). A gradient of elution buffer is shown by the diagonal line. Arrows indicate the elution volume of standard metallothionein from rabbit liver.

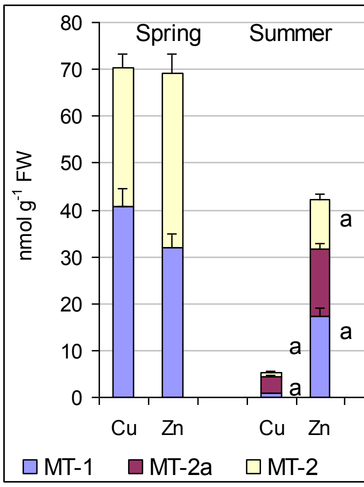

Figure 3(*A*)

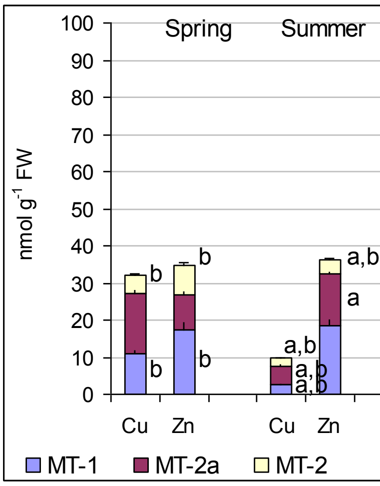

Figure 3(*B*)

Fig 3. The distribution of Cu and Zn between the metallothioneins isoforms in the molluscs digestive gland at the R site (*A*) and U site (*B*), nmol/g FW, M±S.D, n=3. [a] – temporal changing compare to spring; [b] – spatial changing significantly differed, $p < 0.05$.

in general from spring to summer–autumn. For Zn content in the tissue, the decreasing from the spring to summer–autumn was also significant at both sites, especially at the U site where the vernal level of Zn in the tissue was extraordinary high (by an order of magnitude) as compare to other values. Zn content in the MTs and its rate to the total Zn content in the tissue were comparative stable at the R site but changed significantly at the U site, where MTs bound about 1.3 % of total Zn content in the tissue in spring and 50,6% in autumn.

Total and metallothioneins (MTs) bound metal concentrations in the digestive gland of mollusc, μg·g^{-1} FW, M±S.D (% of total content)

Metal	Site	Spring		Summer		Autumn	
		Total content	MTs	Total content	MTs	Total content	MTs
Cu	R	20,72±0,31	4,52±0,40 (21,7%)	1,31±0,11^{a}	0,48±0,05^{a} (36,9%)	3,61±0,30^{a}	0,10±0,01^{a} (2,7%)
	U	6,73±1,60^{b}	2,30±0,21^{b} (34.3%)	0,90±0,10^{a,b}	0,64±0,06^{a,b} (71,1%)	1,00±0,18^{a,b}	0,05±0,00^{a,b} (5,0%)
Zn	R	26,84±0,79	4,41±0,43 (16.4%)	19,32±3,71^{a}	2,81±0,20^{a} (14,5%)	20,01±1,71^{a}	2,3±0,2^{a} (11,5%)
	U	166,34±30,29^{b}	2,12±0,23^{b} (1,3%)	17,42±1,91^{a}	2,62±0,21^{a} (14.9%)	14,42±0,71^{a,b}	7,3±0,7^{a,b} (50,6%)
Cd	R	0,33±0,04	0,27±0,02 (81,8%)	0,70±0,09^{a}	0,20±0,00^{a} (28,6%)	0,22±0,02^{a}	0,20±0,00^{a} (90,9%)
	U	0,45±0,05^{b}	0,37±0,03^{b} (82,2%)	0,85±0,09^{a,b}	0,62±0,05^{a,b} (72,9%)	0,22±0,02^{a}	0,20±0,00^{a} (90,9%)

^{a} – temporal changing compare to spring; ^{b} – spatial changing significantly differed, p<0.05.

In spring, the Zn:Cu content ratio in MTs was close to 1:1, in summer, less than 10:1; and, in autumn, at the R site, the Zn content in MT was hundreds of times higher than Cu. The molar rate of Cd, in comparison to Cu and Zn in MTs, was highest at the U site in summer, but it was not higher than about 1 atom per 1 MTs molecule according to its metal binding stoichiometry [18]. The comparison of the MTs metal-binding relative abundance revealed its worsening for Cu (constantly), Cd (in summer) and Zn (in autumn) at the R site. No distinct advantage in the metal binding to MTs separate isoforms was found (fig. 3).

PCA was applied in order to define the relations between different metal distribution characteristics (fig. 4A). The set involved the metal concentrations in the tissue, in the MTs and the MTs concentrations, determined by differential pulse polarography and reported for the same groups of molluscs [7]. The first two components controlled about 63% of the parameter variability. The relation of Cu and Cd concentrations in the tissue and in its MTs was demonstrated as well as the independence of the Cu and Cd characteristics. The Zn and MTs indexes were not related significantly to both components. Centroid grouping analysis defined both temporal (especially for the R site in spring and for the U site in autumn) and spatial (especially in spring and summer) specificity of the set of indices of each specimen (fig. 4B).

It appears that if in fish MTs should be considered as a kind of stress protein which is particularly responsive to heavy metals, in molluscs, MTs seem more specifically involved in responses to heavy metals [23]. However, it is unknown the preference for what metal is supplied in the freshwater molluscs adapted to the ecologically realistic complex field pollution. The moiety of invertebrates unlike vertebrates demonstrates the well-regulated homeostasis of

Cu with the participation of MTs related to the metabolism of the respiratory pigment hemocyanin [2]. The ability of bivalve molluscs to accumulate Cu from environments contaminated by a variety of point and non-point sources is described for the marine species[12, 17]. At the same time, though well-regulated in vertebrates, Zn [16] could be toxic in bivalve molluscs [10]. PCA confirms this difference between the ability of MTs to regulate Cu and Zn distribution in the tissue (Fig. 4A). Concerning Cd, the ubiquitous priority in its binding for the MTs is well known (Roesijiady, 1999).

As it was established in our study, the highest Cu and Zn content in the digestive gland in spring reflected the vernal pollution of the area explained by the leaching of pollutants from adjoining lands with thawing water and seasonal rains. The establishment of Cu pollution at the sites with agricultural activity by its accumulation in the deposit tissues, particularly in spring, was confirmed by our recent studies on frogs and fish [7, 8, 21]. Moreover, in this study, the inter-site difference in the Cu levels in the tissue was observed throw three seasons despite its prominent temporal decline, confirming this kind of pollution to be typical for agricultural area in the Dnister bazin [19]. The comparison of the MTs-bound and total content of metals in the tissue demonstrates that the relation of these indices is observed for Cu and Cd but not for Zn. The higher levels of Cd and Zn in individuals from the U site may indicate the specific pollution intrinsic to an urban area. Certain data demonstrates that Zn is the most common metal associated with runoff waters [9, 22].

Comparison of molluscs at two sites and in different seasons demonstrated better buffering ability of MTs in the urban area (except for Zn in spring). It may be explained by the activation of MTs expression by the sub-toxic concentration of well known MTs inducer Cd in the tissue [1, 15]. On the other hand, the higher metal levels in the unbound with MTs form at the R site might be connected to the complex pollution of this agricultural area observed especially in summer and autumn [7]. Consequently, MTs may be involved in other than metal buffering functions in the tissue, for example, in the scavenging of the reactive oxygen species [4, 6, 15] that agree with the results of PCA (fig. 4A).

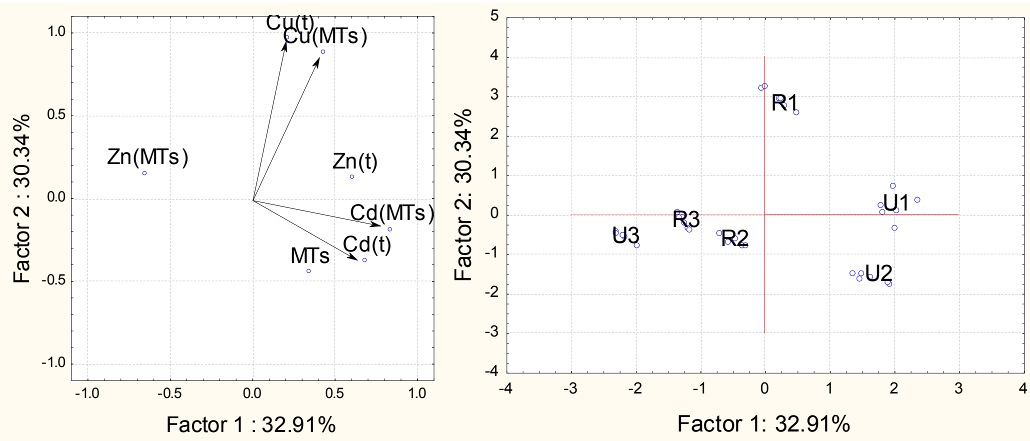

Figure 4(*A*) Figure 4(*B*)

Fig. 4. Principal component analysis of the content of metal in the tissue (Cu(t), Zn(t), Cd(t)) and in the metallothioneins (Cu(MTs), Zn(MTs), Cd(MTs) and metallothioneins content (MTs) (*A*); and the centroid grouping analysis of the data set from each specimen (*B*). A, the arrows indicate biomarkers having significant factorial weights > 0.7; B, the rural (R). and urban (U) sites in spring (R1 and U1), summer (R2 and U2), and autumn (R3 and U3). The data on metallothioneins content are taken from Falfushynska et al. (2009).

The diversity of bivalve molluscs' MTs connected to different molecular weights is well known. Two MTs forms, MT10 and MT20 and their genes were obtained from the digestive gland of the mussels *Mytilus edulis* and *Mytilus galloprovincialis* [2, 6]. While the MT10 represents the basal form, which is considered to be involved in essential metal regulation, the MT20 is induced by Cd probably to provide its sequestration and detoxification and by hydroxyl radicals to realize the antioxidant protection [5, 6]. Jenny et al. [11] propose the explanation of the presence of the high molecular weights MTs proteins in the molluscs by the multiplying of α- or β- domain as strategies for survival in certain metal-rich environments. In our study, the seasonal elevation of MT20 in both sites seems to be connected to the domination of Zn in the MTs. The aggregation of MTs in their functional state or of their Cd-containing α-domains unlike Cu-saturated β-domain was reported [14, 24].

Regarding MT isoforms, isolated by anion-exchange chromatography, some authors, mostly in laboratory experiments, reported the creation of multiple forms in molluscs and other invertebrates [3, 5]. In our study, the additional form MT 2a was the best recognized in spring at the U site accompanied with the extremely high Zn content in the tissue in the unbound with MTs form. Apparently, the toxic concentration of Zn in the tissue at the U site provoked the instability of MT-2 and the change in its properties. Our results confirm the elevation of lipid peroxidation as the main feature of oxidative stress only in spring at the U site [7] when Zn content in the unbound with MTs form reached 98,7%. This is going with the results of Geret and Bebianno [10] for mollusc *Ruditapes decussates.*

To summarize, the spatial and temporal differences of the MTs responses in the feral populations of bivalve molluscs were reflected and explained due to Zn, Cu and Cd content, essentiality and distribution in the tissues. The MTs of the freshwater bivalve mollusc *Anodonta cygnea* were sensitive to the elevated Zn:Cu rate in the environment and affected by the complex pollution in the agricultural area.

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1. *Amiard J. C., Amiard-Triquet C., Barka S.* et al. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers // Aquat. Toxicol. 2006. Vol. 76. P. 160–202.
2. *Banni M., Dondero F., Jebali J.* et al. Assessment of heavy metal contamination using real-time PCR analysis of mussel metallothionein mt10 and mt20 expression: a validation along the Tunisian coast // Biomarkers. 2007. Vol. 12. P. 369–383.
3. *Brouwer M., Enghild J., Hoexum-Brouwer T.* et al. Primary structure and tissue-specific expression of blue crab (Callinectes sapidus) metallothionein isoforms // Biochem. J. 1995. Vol. 31. P. 617–622.
4. *Cavaletto M., Ghezzi A., Burlando B*. et al. Effect of hydrogen peroxide on antioxidant enzymes and metallothionein level in the digestive gland of Mytilus galloprovincialis // Comp. Biochem. Physiol. 2002. Vol. 131C. P. 447–455.
5. *Dallinger R., Berger B., Gruber C.* et al. Metallothioneins in terrestrial invertebrates: structural aspects, biological significance and implications for their use as biomarkers // Cell. Mol. Biol. 2000. Vol. 46. P. 331–346.

6. *Dondero F., Piacentini L., Banni M.* et al. Quantitative PCR analysis of two molluscan metallothionein genes unveils differential expression and regulation // Gene. 2005. Vol. 345. P. 259–270.
7. *Falfushynska H. I., Delahaut L., Stolyar O. B.* et al. Multi-Biomarkers Approach in Different Organs of Anodonta cygnea from the Dnister Basin (Ukraine) // Arch. Environ. Contam. Toxicol. 2009. Vol. 57. P. 86–95.
8. *Falfushynska H. I., Stoliar O. B.* Function of metallothioneins in carp *Cyprinus carpio* from two field sites in Western Ukraine // Ecotoxicol. Environ. Saf. 2009. Vol. 72. P. 1425–1432.
9. *Heijerick D. G., Janssen C. R., Carlen C. I.* et al. Bioavailability of zinc in runoff water from roofing materials // Chemosphere. 2002. Vol. 47. P. 1073–1080.
10. *Geret F., Bebianno M. J.* Does zinc produce reactive oxygen species in Ruditapes decussatus? // Ecotoxicol. Environ. Saf. 2004. Vol. 57. P. 399–409.
11. *Jenny M. J., Warr G. W., Ringwood A. H.* et al. Regulation of metallothionein genes in the American oyster (Crassostrea virginica): Ontogeny and differential expression in response to different stressors // Gene. 2006. Vol. 379. P. 156–165.
12. *Jing G., Li Y., Xie L., Zhang R.* Metal accumulation and enzyme activities in gills and digestive gland of pearl oyster (*Pinctada fucata*) exposed to copper // Comp. Biochem. Physiol. 2006. Vol. 144C. P. 184–190.
13. *Kagi J. H. R., Schaffer A.* Biochemistry of metallothionein // Biochem. 1988. Vol. 27. P. 8509–8515.
14. *Kim B. S., Kwon H. J., Choi M. U., Koh E. H.* Molecular Characteristics of Cysteinyl Groups of Metallothionein by 5,5'-Dithiobis(2-nitrobenzoic Acid) // Korean Biochem. J. 1987. Vol. 20. P. 223–229.
15. *Leung K. M., Furness R. W.* Metallothionein induction and condition index of dogwhelks Nucella lapillus (L.) exposed to cadmium and hydrogen peroxide // Chemosphere. 2001. Vol. 44. P. 321–325.
16. *Maret W.* The function of zinc metallothionein: A link between cellular zinc and redox state // J. Nutr. 2000. Vol. 130. P. 1455S–1458S.
17. *Ringwood A. H., Conners D. E., Di Novo A.* The effects of copper exposures on cellular responses in oysters // Mar. Environ. Res. 1998. Vol. 46. P. 591–595.
18. *Roesijadi G.* The basis for increased metallothionein in a natural population of *Crassostrea virginica* // Biomarkers. 1999. Vol. 4. P. 467–472.
19. *Sapoznikova Y., Zubcov N., Hungerford S.* et al. Evaluation of pesticides and metals in fish of the Dniester River, Moldova // Chemosphere. 2005. Vol. 60. P. 196–205.
20. *Stolyar O. B., Falfushinskaya G. I., Bazan O. G.* Seasonal peculiarities of properties of metallothioneins of the freshwater bivalve *Colletopterum piscinale* (Unionidae) // Hydrobiol. J. 2007. Vol. 43. P. 92–102.
21. *Stolyar O. B., Loumbourdis N. S., Falfushinska H. I., Romanchuk L. D.* Comparison of metal bioavailability in frogs from urban and rural sites of Western Ukraine // Arch. Environ. Contam. Toxicol. 2008. Vol. 54. P. 107–113.
22. *Taebi A., Droste R. L.* Pollution loads in urban runoff and sanitary wastewater // Sci. Total Environ. 2004. Vol. 327. P. 175–184.
23. *Viarengo A., Burlando B., Dondero F.* et al. Metallothionein as a tool in biomonitoring programmes // Biomarkers. 1999. Vol. 4. P. 455–466.
24. *Wilhelmsen T. W., Olsvik P. A., Hansen B. H., Andersen R. A.* Evidence for oligomerization of metallothioneins in their functional state // J. Chromatography. 2002. Vol. 979. P. 249–254.

МІНЛИВІСТЬ МЕТАЛОТІОНЕЇНІВ ПРІСНОВОДНОГО МОЛЮСКА *ANODONTA CYGNEA* (UNIONIDAE) З ДВОХ ПРИРОДНИХ ПОПУЛЯЦІЙ

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Металотіонеїни (МТ) травної залози двостулкового молюска *Anodonta cygnea* з двох місцевостей у басейні ріки Дністер, сільській (R) та урбанізованій (U), порівнювали у травні, липні та вересні. Різке зменшення вмісту Cu в МТ та у тканині з травня до вересня супроводжувалося появою при гель-розподільчій хроматографії форми МТ з молекулярною масою близько 20 кДа (МТ20) додатково до постійно присутньої МТ10. За хроматографії на ДЕАЕ-целюлозі були виділені стабільна форма МТ-1 та мінлива МТ-2/2а. Високий вміст Cu або Cd в МТ відображав їх вміст у тканині в R та U групах відповідно. В R групі була відзначена нижча металозв'язувальна здатність МТ. Навесні в U групі було виявлено надлишок у тканині Zn у незв'язаній з МТ формі та появу масивної хроматографічної форми МТ-2а.

Ключові слова: прісноводний молюск, металотіонеїни, природна популяція.

ВАРИАБЕЛЬНОСТЬ МЕТАЛЛОТИОНЕИНОВ ПРЕСНОВОДНОГО МОЛЛЮСКА *ANODONTA CYGNEA* (UNIONIDAE) ИЗ ДВУХ ЕСТЕСТВЕННЫХ ПОПУЛЯЦИЙ

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Металлотионеины (МТ) пищеварительной железы двустворчатого моллюска *Anodonta cygnea* из двух местностей в бассейне реки Днестр, сельской (R) и урбанизированной (U), сравнивали в мае, июле и сентябре. Значительное снижение содержания Cu в МТ и в ткани с мая до сентября сопутствовало появлению при гель-распределительной хроматографии формы МТ с молекулярной массой около 20 кДа (МТ20) дополнительно к постоянно присутствующей МТ10. При хроматографии на ДЕАЕ-целлюлозе были выделены стабильная форма МТ-1 и вариабельная МТ-2/2а. Высокое содержание Cu или Cd в МТ отражало их содержание в ткани в R и U группах соответственно. В R группе металлосвязывающая способность МТ была сравнительно ниже. Весной в U группе выявлены избыток в ткани Zn в несвязанной с МТ форме и появление массивной хроматографической формы МТ-2а.

Ключевые слова: пресноводный моллюск, металлотионеины, природная популяция.

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