сценарієм глобального потепління A1B).*Український гідрометеорологічний журнал.* 2014, №15.С.149–159.
4. Грубінко В.В. Екологічні проблеми річок в умовах зарегулювання стоку та маловоддя.Тернопільські біологічні читання – Ternopil Bioscience – 2017 : матеріали Всеукр. наук.-практ. конф. з міжнар. участю, присвяч. 20-річчю заснування наук. фахового видання України "Наукові записки ТНПУ ім. В. Гнатюка. Сер. Біологія". 20–22 квіт. 2017 р., Тернопіль : Терно-граф, 2017. С. 113–117
5. Природні умови та ресурси Тернопільщини / Сивий М.Я., ред. Тернопіль: ТзОВ “Терно-граф”, 2011. С. 512.
6. Сніжко С. І. Оцінка та прогнозування якості природних вод. – Київ: Ніка – Центр, 2001. С. 262.

УДК: 577.352.38:577.64

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

Khoma V.V. [1], Gnatyshyna L.L.[1,2], Martinyuk V.V. [1], Rarok Yu.S. [1], Mishchuk O.V., I. Ozoliņš D.[3], Kokorite I.[3], Skuja A.[3], Spriņģe G.H. [3], Stoliar O.B.[1*]

[1] Volodymyr Hnatyuk Ternopil National Pedagogical University, Ternopil,
[2] I.Ya. Horbachevsky Ternopil State Medical University, Ternopil
3 University of Latvia, Salaspils
E-mail: Oksana.Stolyar@tnpu.edu.ua

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 [4]. 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 metallothioneins towards the toxic metals or to activate their efflux from the organism [3,4]. These circumstances can have the serious limitations for the biological monitoring of pollution by industrial metals in the aquatic environment.

The present study aimed to compare the ability of bivalve mollusks and cyprinid fish to accumulate in their tissues Zn, Cu, Cd as the compounds of the industrial pollution and to buffering them in the metallothioneins in the conditions of realistic environment. For this study, we selected the typical areas that are characterized by the complex agricultural and municipal pollution. The sites in Latvia were represented by the artificial reservoir of Riga hydropower plant (HPP) on the river Daugava (R-group) in two consequent years and the pristine lake Kanieris (KL, referent site, in one year). In Ukraine, the areas in the basin of the river Dniester were selected, namely Ternopil lake (T-group), the reservoir of small Kasperivtci HPP (before and downstream of the dam, Kb and Kd correspondingly) on the river Seret and the sites on the small tributary Zhvanchyk (before and downstream of the dam of micro HPP, Zhb, Zhd correspondingly). The R- and T-groups were represented by the bivalve mollusk, zebra mussel *Dreissena polymorpha* (Pallas, 1771, Bivalvia), Zh groups were comprised by the swollen river mussel *Unio tumidus* (Philipsson, 1788, Bivalvia), and the Kb and Kd groups included both *U. tumidus* (Kbm, Kdm groups) and fish prussian carp *Carassius gibelio* (Bloch, 1782, Cyprinidae) (Kbf, Kdf groups).

For the analysis of the metals in the tissues (digestive gland and gonads), the six individuals of *U. tumidus* or *C. auratus* or six pools of the soft tissues of *D. polymorpha* (from at least five specimens each) in each group were dissected. For the metallothionein chromatography, tissue samples from five individuals of experimental group were pooled in aliquot quantity. The concentration of Cu, Zn

and Cd was measured in the samples of the tissues and pooled eluate of metallothionein-containing fractions after the size-exclusion chromatography. The metal concentration was analyzed by atomic absorption spectrophotometry against certified standards after the digesting of samples. Cu and Zn concentration was analysed on spectrometer C-115, ("Lomo", Russia) and Cd, on graphite furnace atomic absorption spectrometer S-600 ("Selmi", Ukraine). Quantification of metallothioneins associated thiols was accomplished spectrophotometrically after their ethanol/chloroform extraction and incubation with 5,5'-dithio-bis(2-nitrobenzoic acid).

When the mussels from different sites and in two years were compared, the lesser level of Zn was found in the KL specimens, whereas the level of Zn in the gonads in the Kbm group and the level of Cu in the digestive gland in this group where greatest. The lower Cd concentration was found in the digestive gland in Kdm group. In all other groups levels of these metals were similar. In fish the concentrations of metals in the tissues were not different between the sites, and only the level of Cd was lower in the Kdf group.

The assessment of the chromatographic profiles and UV-spectra of the metallothioneins from all studied groups of animals demonstrated the similarity of their molecular weight and spectral features. Among the metals in the composition of metallothioneins in the mollusks, the concentration of Zn was decreased in the order: Kb>R>KL>Kd groups. The concentrations of Cu and Cd in the metallothioneins of mollusks were similar in all groups. In the fish, we did not find differences between two groups for the level of metals in the metallothioneins. The level of metallothionein associated thiols (MT-SH) in the mussels was about 8 times higher in the swollen mussel than in the zebra mussel (probably due to the tissue specific location). In each group of comparison, the level of the SH groups in metallothioneins was lesser in the most polluted area: 1.9 and 3.4 times in T compare to R and Kbm compare to Kdm groups correspondingly. However, in the fish the opposite relation was shown: the level of MT-SH was 2.4 times greater in the Kbf than Kdf group.

Hence, the examined mollusks are more sensitive then the fish to metal accumulation in their tissues depending on the local site, even in the geographically close related areas. However, their

metallothioneins are highly vulnerable to the oxidation reflecting the pressure of common mixed pollution in the artificial reservoirs. The participation of metallothioneins in the oxidative stress response can be the reason for their SH groups depletion and was demonstrated for the mollusks from the Ternopil lake earlier [3]. These data reflect that the bivalve mollusks can be valuable bioindicative organisms for the toxic metals and a total press of the mixed pollution, whereas the fish has not such properties.

This work has been granted by the Ministry of Education and Science of Ukraine to Oksana Stoliar (Projects M/70-2017; M/35-2018 and 132B) and State *Education* Development *Agency* of Latvia to Gunta Springe (Project LV-UA/2016/5). The authors are grateful to the Director of the National Nature Park Dniester Canyon Mr. Mykhaylo Shkilniuk for his assistance in the sampling in the Kasperivtcy area, and to PhD St Oksana Horyn for the technical assistance in the trial.

1. Молекулярні реакції водних тварин на вплив пошкоджувальних чинників середовища : [монографія] / О. Б. Столяр, Г.І. Фальфушинська, Л.Л. Гнатишина, В.Г. Юкало – Тернопіль : Вид-во ТНТУ імені Івана Пулюя, 2016. – 352 с.
2. Dailianis S. Environmental impact of anthropogenic activities: the use of mussels as a reliable tool for monitoring marine pollution. Mussels anatomy, habitat and environmental impact. New York: Nova Science Publisher. 2011. P. 43–72.
3. Stoliar O. B., Mykhayliv R. L., Mishchuk Y. V. Influence of the environmental conditions on binding of heavy metals and oxidative decomposition of biomolecules in tissues of Anodonta cygnea (Bivalvia). Hydrobiological Journal. 2004. Vol. 40(2). P. 70–79.
4. Stoliar O. B., Lushchak V. I. Environmental Pollution and Oxidative Stress in Fish. Oxidative Stress - Environmental Induction and Dietary Antioxidants. 2012. P. 131–166. URL: http://www.intechopen.com/books/oxidative-stress-environmental-induction-and-dietaryantioxidants/environmental-pollution-and-oxidative-stress-in-fish