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COMPLEX ASSESSMENT OF CHEMICAL POLLUTION OF SMALL RIVERS ON THE EXAMPLE OF THE RIVER SERET

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Abstract – Contamination of the aquatic environment at the present stage is one of the main factors of the pathology of hydrobionts. Especially this influence is noticeable on small rivers of Ukraine, very closely connected with the surrounding landscape. On small rivers located in the same landscape and having small water outlays, the productive effect of natural and anthropogenic factors manifests itself more quickly and distinctly. Small rivers are particularly sensitive to pollution by sewage from industrial enterprises, agricultural and communal industries. Therefore, each type of impact on the catchment landscapes leads to a change in the ecological state of the rivers.

Taking into account the above, the actual problem of modern hydrochemistry and hydroecology is the estimation of anthropogenic load on small rivers of Ukraine. We used chemical and biological methods (generally accepted methods for sampling water and biological material, centrifugation, titrimetry, spectrophotometry, atomic absorption spectrophotometry) for a comprehensive assessment of the water quality of the Seret River within the Ternopil-city. After all, any change in the chemical composition of the aquatic environment inevitably leads to a change in the functional indices of metabolism in the organism of aquatic animals in general and the mollusks *Unio pictorum L.* in particular.

It is established that the Seret River undergoes significant anthropogenic impact. In the summer season, there was an increase in the amount of organic substances, ammonium cations, nitrite ions, chloride ions, phosphate ions, metals (Mn, Cu and Pb) and a decrease in the oxygen concentration. Positive correlation between the content of metals in the liver of bivalve mollusks *Unio pictorum L.* and their content in water has been noted, which makes it possible to use them as bioindicators of water quality. Mollusks serve as the main objects of biomonitoring, which is due to their prevalence in the bottom biocenoses, the ability to accumulate a number of components from water and resistance to contamination. One of the important indicators of metabolism in mollusks with changing environmental conditions is the level of activity of enzymes involved in adaptive rearrangements. These include transamination enzymes - alanine and aspartate aminotransferase (ALAT and ASAT). As shown by the results of the studies, in spring the activity of the transamination processes in the liver of freshwater mollusks is higher in comparison with the summer season. High activity of aminotransferases hydrobionts in the spring, perhaps, is an adaptation of the enzyme apparatus of cells of this organ to unfavorable conditions of existence, because in the winter and spring period the organism uses protein reserves as a source of energy. The increase in the activity of transaminases in the liver of mollusks, associated with the action of anthropogenic and abiotic factors, can be used to assess the quality of water in general.

As a result of the study, data were obtained on a comprehensive assessment of the water quality of the Seret River within the city of Ternopil. The prospects of using

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biochemical indicators (bioaccumulation of metals, activity of transaminases) of bivalve mollusks *Unio pictorum* L for the evaluation of water quality are shown.

Introduction

Contamination of the aquatic environment at the modern period is one of the main factors of the pathology of hydrobionts. This influence is especially noticeable on the small rivers of Ukraine, which are very closely connected with the surrounding landscape. Effective action of natural and anthropogenic factors manifests itself more quickly and distinctly on small rivers that are located in the same landscape and have a small water flow. Small rivers are particularly sensitive to pollution by sewage from industrial enterprises, agricultural and communal industries. Therefore, each type of impact on the catchment landscapes leads to a change in the ecological state of the rivers. Water quality is a limiting factor of water use against the backdrop of a sharp increase in demand for fresh water in general. Scientists believe that heavy metals are the most dangerous for biota due to toxicity and the ability to accumulate in hydrobionts [6]. They belong to the class of conservative pollutants, they are not used and do not decompose during migration through trophic chains, have a mutagenic and toxic effect, significantly reduce the intensity of biochemical processes in aquatic organisms [1, 4]. Some of the metals are toxic even at very low concentrations, and such important trace elements as Fe, Cu and Zn, at high concentrations, can also be biologically hazardous [4].

Taking into account the above, the actual problem of modern hydrochemistry and hydroecology is the estimation of anthropogenic load on small rivers of Ukraine.

As a result of the study, data were obtained on a comprehensive assessment of the water quality in Seret-river within Ternopil-city. The use of biochemical indicators (bioaccumulation of metals, activity of transaminases) of bivalve mollusks *Unio pictorum* L. has proved promising for assessing of the quality of aquatic environment.

Materials and methods

Samples of water for the study were selected in spring (in April) and summer (in July) 2015 from the Seret-river at two points: higher and lower than Ternopil, which makes it possible to assess the level of anthropogenic pressure and chemical pollution of the river. Samples of water after the selection were fixed and transported to the laboratory for research. Determination of hydrochemical indicators was carried out according to the generally accepted methods [9]. To determine the content of manganese, zinc, copper, lead and cadmium, water samples were evaporated and burned in nitric acid. Determination of content of manganese, zinc, cuprum, lead and cadmium was carried out by atomic absorption spectrophotometry at appropriate wavelengths, which correspond to the maximum absorption of each of the metals studied according to standard methods [13]. The content of metals was measured in mg / dm^3 of water.

Collection of bivalve mollusks *Unio pictorum* L. was carried out in the same places in the spring and summer seasons. Bivalve mollusks, as bioindicator organisms, were chosen by us for research because of their prevalence and low migration activity. The selected mollusks were at the age of 6 years (average length 95 ± 5 mm and weight 82 ± 3 g). All selected for experiment mollusks were healthy, without visible mechanical damage and parasites.

To study the metal content and enzyme activity, the liver was taken from the bivalve mollusk. After preparation, the tissue samples were immediately frozen, transported to the laboratory, ground and used to prepare homogenates. Determination of transaminase activity in the liver of mollusks was determined according to Paskhina T.S. [14].

The protein content in the liver homogenates of the investigated mollusks was determined by the method of Lowry et al. [3].

To determine the content of manganese, zinc, copper, lead and cadmium ions in tissues, the samples were burned in distilled nitric acid in a ratio of 1:5 (mass:volume). The content of manganese, zinc and copper was determined on an atomic-adsorption spectrophotometer C-115 at an appropriate wavelength for a certain metal ion and expressed in grams per kilogram of wet weight in tissues. Statistical processing of the data was carried out using the "Microsoft Excel 2003" package.

Results

The total content of metals in the Seret-river. Complex forms of metals are less toxic than free metals, so to understand the factors regulating the concentration of heavy metals, their bioavailability and toxicity, it is important to know the proportion of free and bound forms of metals [11]. However, the definition of metal forms in natural waters is associated with difficulties due to the small absolute concentration and diversity of complex forms. We conducted a determination of the total concentrations of manganese, zinc, copper, lead and cadmium in the Seret-river before and after Ternopil-city.

Analysis of the studies results showed that the level of zinc in water varies within fairly narrow limits from 0.025 to 0.030 mg / dm^3 of water (Table 1). At the same time there is an excess of maximum permissible concentrations for zinc, which may pose a risk of metal accumulation by hydrobionts and their chronic poisoning. Its toxicity is due to antagonism with other heavy metals.

The concentration of manganese in the water of the Seret-river varied from 0.019 to 0.045 mg / dm^3. A sharp increasing of Mn content in the summer below Ternopil was observed. The high manganese content can be caused by the relatively low oxygen content during this period, which causes the metal to flow from the bottom sediments as a result of the reduction (in the conditions of oxygen deficiency), the high solubility in water of its compounds, their low ability to complex formation and high migration ability [11].

Table 1 – Total content of metals in the water of the Seret-river, mg / dm^3 (M±m, n=5).

Metal	Fish-economic max.permissible concentration of metal, mg/l	Spring		Summer	
		Above of the Ternopil-city	Below of the Ternopil-city	Above of the Ternopil-city	Below of the Ternopil-city
Zn	0,01	0,015±0,006	0,019±0,004	0,020±0,005	0,018±0,002
Mn	0,01	0,019±0,003	0,025±0,003	0,023±0,003	0,045±0,003
Cu	0,001	0,0008±0,0002	0,0009± 0,0002	0,0006±0,002	0,0015±0,001
Pb	0,01	0,009±0,002	0,011±0,003	0,009±0,002	0,015±0,002
Cd	0,005	0,003±0,001	0,007±0,002	0,007±0,001	0,009±0,002

Manganese can pose a significant danger to hydrobionts due to the high mobility of the metal and the relatively high level of "free" ions that have the greatest toxicity.

Analysis of the results showed an increase in the concentration of copper in the Seret-river section below Ternopil in the summer season. At the same time the maximum permissible concentrations of copper was excessed 1.5 times. In other period, the copper content did not exceed the maximum allowable concentration for a given metal.

The lead content in the river water was in the range of 0.009÷0.015 mg / dm^3. The maximum concentration of metal was observed in the summer season below the city, where there was a 1.5-fold increase in the background level.

The factors that contribute to the pollution of water bodies by cadmium compounds include intensive farming (the use of mineral fertilizers and pesticides, the use of sewage for irrigation works) and the accumulation of household waste [12].

The concentration of cadmium in the Seret River varied from 0.003 to 0.009 mg / dm^3. The minimum concentration of metal was noted in the spring season above Ternopil, and the maximum - in the summer in the river below the city.

Features of the accumulation of metals by mollusks. The penetration of heavy metal ions into the body of aquatic animals and their accumulation depend on many external and internal factors. The molecular mechanisms of this process have not been sufficiently studied. It is known only that in general the penetration of heavy metal ions into the body of hydrobionts includes the following stages: binding to the mucous epithelium; transporting through the apical membrane; the penetration of metal through the basolateral membrane, at the level of which the intake regulates; transportation of metals by the current of blood to all parts of the body [15].

It should be noted that there are no seasonal features of zinc accumulation in the liver of mollusks (Fig. 1).

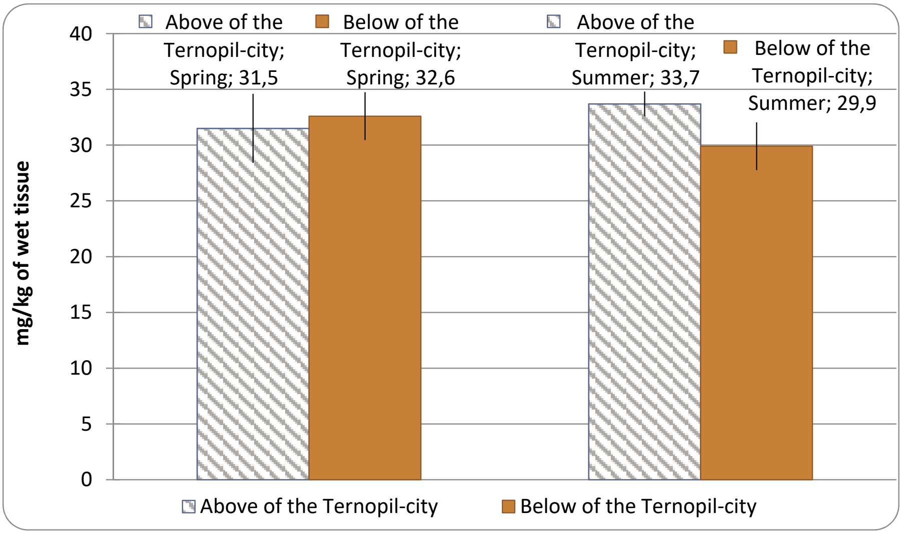

Figure 1 – The content of zinc in the liver of the bivalve mollusks *Unio pictorum* L. (mg/kg of wet tissue, M±m, n=5).

When analyzing the content of manganese in the liver of mollusks, we note a somewhat smaller amount of accumulated metal compared to zinc (Fig. 2). It should also be noted a positive correlation between the concentration of metal in water and tissues of *Unio pictorum* L. The maximum concentrations of Mn in both water and liver of mollusks were noted in samples collected in the Seret River below Ternopil. The amount of accumulated metal bivalve mollusks and bioaccumulation factors can be used to assess the contamination of the aquatic environment by manganese ions.

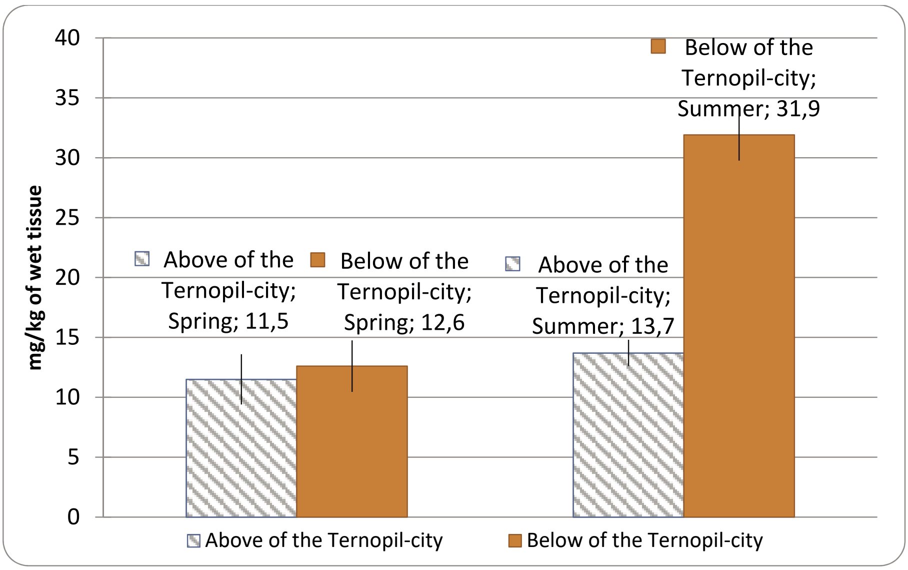

Figure 2 – The content of manganese in the liver of the bivalve mollusks *Unio pictorum* L. (mg/kg of wet tissue, M±m, n=5).

Copper is primarily accumulated in the liver - organ-depot for a given metal [15]. The data obtained as a result of experimental studies indicate that an increase in the concentration of copper ions in water leads to an increase in its content in the liver of bivalve mollusks (Fig. 3). Thus, the maximum amount of metal was accumulated by mollusks caught in the Seret-river in summer under Ternopil-city. By the nature of interaction with ligands, lead is classified as an intermediate acceptor between hard and soft acids. Anthropogenic incomes of lead significantly exceed natural, which is due to its wide application.

In the course of the studies, a relatively small amount of accumulated plumbum was found in the liver of mollusks (Figure 4). The metal content ranges from 0.5 to 1.4 mg / kg wet tissue. Analysis of the obtained results showed that the amount of cadmium in the liver of *Unio pictorum* L. has the tends to insignificant growth in mollusks caught below Ternopil (Figure 5). The content of metal in the liver of hydrobionics ranged from 0.55÷0.80 mg / kg.

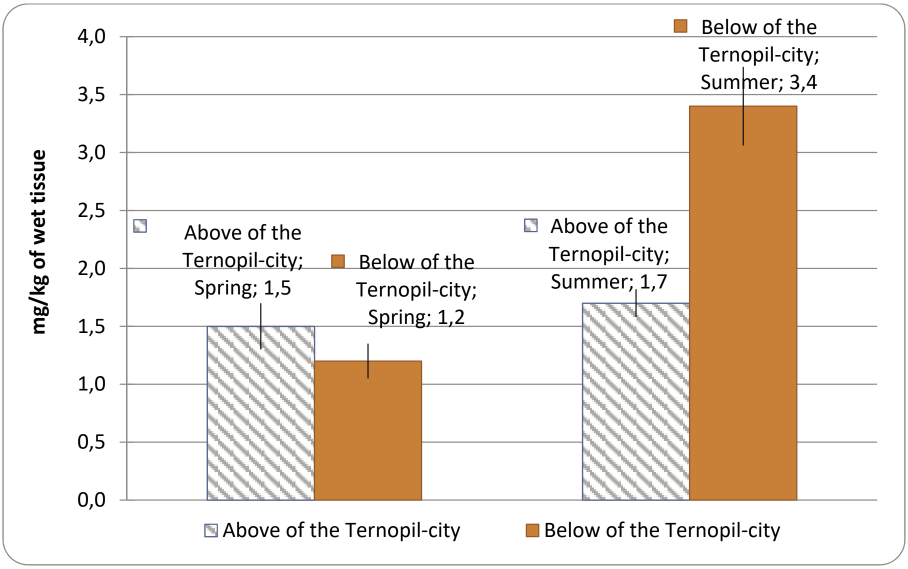

Figure 3 – The content of copper in the liver of the bivalve mollusks *Unio pictorum* L. (mg/kg of wet tissue, M±m, n=5).

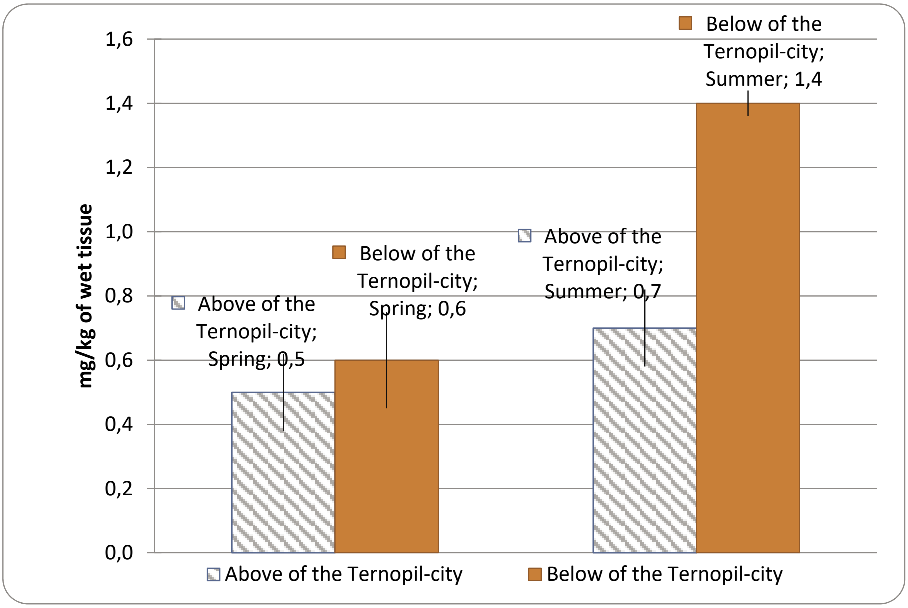

Figure 4 – The content of plumbum in the liver of the bivalve mollusks *Unio pictorum* L. (mg/kg of wet tissue, M±m, n=5).

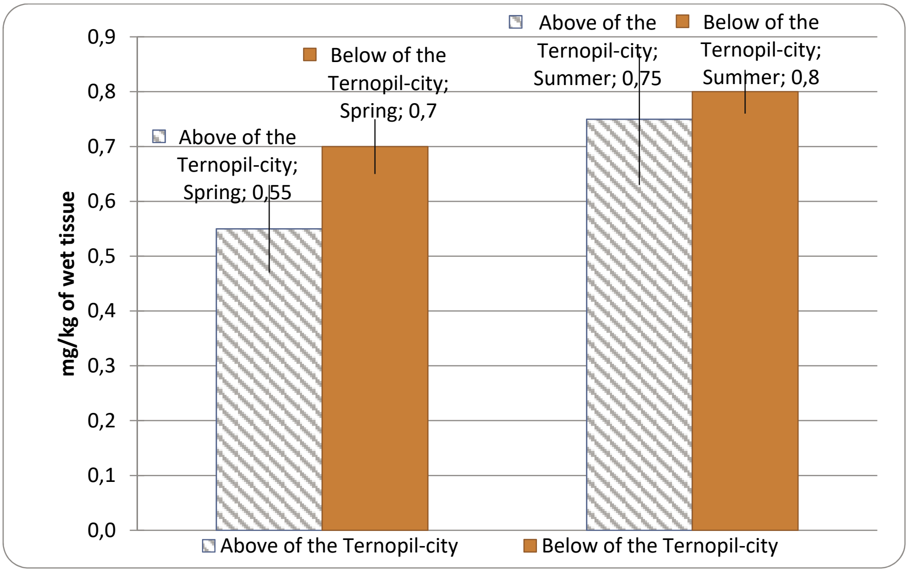

Figure 5 – The content of cadmium in the liver of the bivalve mollusks *Unio pictorum* L. (mg/kg of wet tissue, M±m, n=5).

Mollusks serve as the main objects of biomonitoring, which is due to their prevalence in the bottom biocenoses, the ability to accumulate a number of components from water and resistance to contamination. One of the important indicators of metabolism in mollusks with changing environmental conditions is the level of activity of enzymes involved in adaptive rearrangements. These include transamination enzymes – alanine- and aspartate aminotransferase (ALT and AST) [7].

One of the ways to adapt to the effects of a number of abiotic and biotic factors of the environment in mollusks is the high dynamics of exchange and renewal of nitrogen compounds, especially proteins [16]. Therefore, the parameters of protein metabolism are often used for the purpose of bioindication of the effect of various toxic compounds. The metabolism of most amino acids begins with the elimination of the amino group, mainly in the transamination reactions [10]. Transamination is carried out by transaminases [11]. Well-studied and most active transaminases in humans and animals, including hydrobionts, are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [2].

In this regard, we used indicators of the activity of transamination enzymes in mollusks to assess the water quality of the Seret-river.

As shown by the results of research, in spring the activity of the processes of transamination in the liver of freshwater mollusks is higher in comparison with the summer season (Fig. 6). The high activity of aminotransferases of hydrobionts in the spring may be an adaptation of the enzyme apparatus of the cells of this organ to unfavorable conditions of existence, because in the winter and spring the organism uses protein reserves as a source of energy [8].

The activity of alanine aminotransferase in the liver of mollusks caught below Ternopil was higher than that of caught before the city. So, in the spring season, the activity of the enzyme in the liver of mollusks caught in Seret-river below Ternopil, was in 1.3, and in summer in 1.4 times higher compared to *Unio pictorum* L. caught above the city (Fig. 6).

It is known that when heavy metals are poisoned, protein catabolism increases, which is accompanied by the breakdown of tissue proteins and an increase in the concentration of free amino acids, ammonia and other products of nitrogen metabolism in the body [7].

Activity of aspartate aminotransferase in the liver of *Unio pictorum* L. caught below Ternopil was in 1.5 times higher than in animals that were selected above the city, only in the spring (Fig. 7).

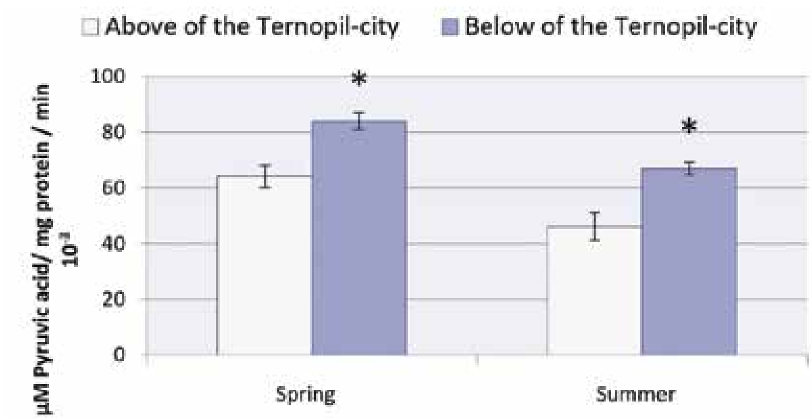

Figure 6 – The activity of alanine aminotransferase in the liver of the mollusk *Unio pictorum* L. (µM Pyruvic acid/ mg protein / min 10^{-3}, M ± m, n = 5) (*changes in comparison with the control are reliable (p <0.05).

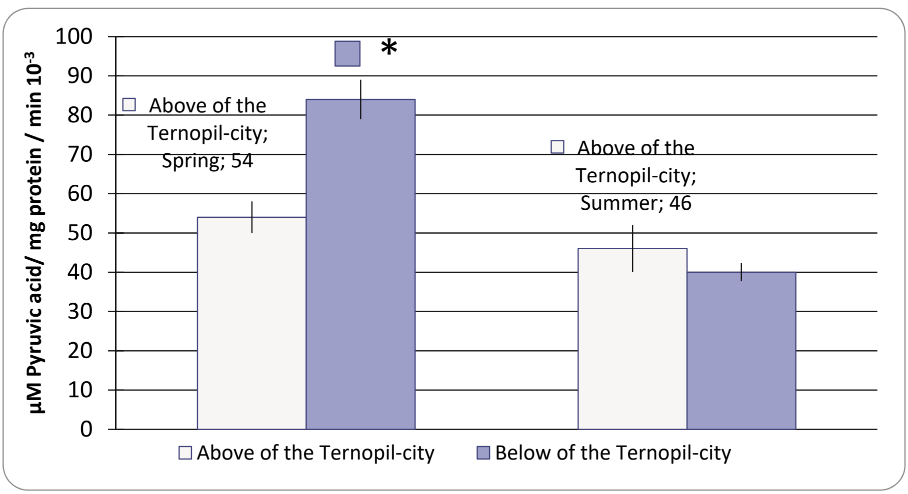

Figure 7 – The activity of aspartate aminotransferase in the liver of the mollusk *Unio pictorum* L. (µM Pyruvic acid/ mg protein / min 10^{-3}, M ± m, n = 5).

In the summer period, the enzymatic activity of mollusks collected in the Seret-river above and below of the city, practically did not differ.

Such changes in enzyme activity testify about the important role of the liver in providing homeostasis of investigated animals under the influence of unfavorable environmental factors.

Discussion

The distribution of metals in the water of the Seret-river is as follows: Mn → Zn → Pb → Cd → Cu. For the complex solution of the problems of the sanitary protection of surface water bodies from pollution by sewage and waste from the main industries, it is necessary to develop and introduce into practice the technological, sanitary and engineering measures that would reduce the ingress of heavy metals into the rivers.

There is a positive correlation between the content of metals in the liver of bivalve mollusks and their content in water. The organisms-filtrators are unable to counteract the excessive accumulation of metals (especially Mn, Cu and Pb), which allows them to be used as bioindicators of water quality.

The obtained data about the transamination processes in the mollusks *Unio pictorum* L. indicate that the modification of the activity of the transamination enzymes in the liver of the studied hydrobionts is related to the action of anthropogenic and abiotic factors, is able to informively reflect the state of the populations, which can be used to assess the quality of water as a whole. In this case, the transaminase system carries out both deamination of amino acids for the purpose of using their carbon skeletons in energy processes, and redistribution of the nitrogen reserves of the organism.

Conclusions

An increase in the concentration of metals (Mn, Cu and Pb) in the summer period below the Ternopil-city may be due to the discharge of insufficiently treated sewage. For zinc and manganese there is a slight excess of the maximum permissible concentrations. A number of metal concentrations in the water of the Seret-river are as follows: Mn → Zn → Pb → Cd → Cu. A positive correlation between the content of metals in the liver of bivalve molluscs and their content in water has been studied. The inability to counteract the excessive accumulation of metals (especially Mn, Cu and Pb) by organisms-filtrators makes it possible to use them as bioindicators of the quality of the aquatic environment. In the spring season, the activity of the transamination processes in the liver of freshwater mollusks is higher than in the summer season. The increase in the activity of transaminases in the liver of mollusks, associated with the effect of anthropogenic and abiotic factors (below Ternopil), which can be used to assess the quality of water in general. It is necessary to develop and put into practice technological, sanitary-technical and auxiliary measures that would reduce the chemical pollution of the Seret-river.

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