БІОЛОГІЧНІ ДОСЛІДЖЕННЯ

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ACCUMULATION OF HEAVY METALS (CU, CR, PB, CD) IN AQUATIC INVERTEBRATES FROM THE HYPERHALINE KUYALNYK ESTUARY (UKRAINE, NORTH-WESTERN BLACK SEA REGION)

Purpose. To investigate the content of heavy metals (Cu, Cr, Pb, Cd) and the features of their migration and accumulation in the components (*Artemia salina:* biomass of individuals of and eggs (cysts), *Chironomus salinarius* larvae, water, bottom sediments) of the Kuyalnyk Estuary ecosystem.

Methods. Atomic absorption spectrophotometry, hydrochemical, biological, automated analysis methods and static analyses.

Results. This is the first study of the accumulation of Cr, Cu, Pb, Cd in the biomass of gill crustaceans A. salina and larvae of Ch. salinarius, extracted from the hyperhaline Kuyalnyk estuary. It was determined that the average heavy metal content in the components of the aquatic environment is represented by the following regression series: Water: Pb > Cu > Cr > Cd ; Bottom sediments: Cu > Pb > Cr > Cd; Crustaceans and cysts of A. salina: Pb > Cu > Cr > Cd; Larvae of Ch. salinarius: Cr > Cu > Pb > Cd. Intensive accumulation of heavy metals in bottom sediments, crustaceans A. salina and larvae of Ch. salinarius compared to water is shown, which is confirmed by the coefficients of bottom accumulation and bioaccumulation. A. salina and larvae of chironomids Ch. salinarius are effective concentrators of the studied heavy metals, especially lead and chromium. It was found that A. salina accumulates Pb and Cd more intensively, while Ch. salinarius accumulates Cr the most.

Conclusions. Hydrobionts of the Kuyalnyk estuary (A. salina and Ch. salinarius) are informative bioindicators of chemical pollution of water bodies with heavy metals, with the effective transfer of these metals to the biota mainly from water. The concentrations of Cd and Pb in these organisms do not exceed the permissible levels for animal feed, which indicates their potential safety for use. The obtained data are important for the scientific and biological justification of the use of bioresources of the Kuyalnytskyi estuary and saline water bodies in general.

KEY WORDS: *Kuyalnyk estuary, heavy metals, aquatic invertebrate organisms, concentration, coefficient of biological absorption, bioindication*

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Introduction

The hypersaline conditions of the Kuyalnyk Estuary (KE) severely limit biodiversity [1-2], as in many saline water bodies [3-5].

It should be noted that saline ecosystems are widespread on all continents and have distinct geochemical properties and biodiversity of extremophiles, which provides invaluable economic value (such as salt, Artemia cyst and nauplii, chironomid larvae as a product of aquaculture, Artemia as a promising new agent for biomedical applications, etc.) [6-10].

In salt lakes and in particular in the KE, *A. salina* is the dominant macrozooplankton [6, 11, 12]. Artemia populations are adapted to a widele range of salinity (45-300 pps), temperature (<6 to 35°C), and to different ionic compositions of brine. Moreover, pH values which they can tolerate vary from neutral to highly alkaline During periods of higher water availability in the estuary (2004-2005), of *Ch. salinarius* larvae were recorded at a depth of 0.3-0.5 m in the coastal zone.

It should be noted that since the KE is a shallow water body, *A. salina* is mainly found in bottom sediments, as well as chironomid larvae (bloodworms) [12].

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp Artemia constitute the most widely used food item. Chironomid larvae are also excellent food items for both vertebrates and invertebrates [6, 8, 10, 13-15].

Today, hypersaline ecosystems are increasingly threatened by anthropogenic pressures, such as pollution. Among the most harmful pollutants are heavy metals, which are of particular concern due to their toxicity, potential to accumulate in the biomass of aquatic species and in the food chain, causing ecological damage and posing a threat to biota and human health through bioaccumulation over time [16-19].

They include essential elements such as copper, chromium, iron, an others, as well as toxic metals such as lead, cadmium and mercury.

Heavy metal pollution of the aquatic environment is determined by measuring their concentration in water, sediments and living organisms.

Our research has shown that in recent decades, the content of heavy metals in the water and sediments of the KE almost always reaches excessive levels [20-21]. Some results for heavy levels of metals in water plants from the KE are presented in our work [22].

This article is the first to consider the peculiarities of migration and accumulation of a number of metals (Cu, Cr, Pb, Cd) in the components (the invertebrates – *A. salina (*adult brine shrimp, eggs) and *Ch. salinarius* larvae, water, botton sediments) of the hypersaline KE ecosystem.

Materials and methods

Materials for the study were simultaneously collected samples of *A. salina* salt shrimp and eggs (cysts), *Ch. salinarius* larvae, water and bottom sediments in the central part of the KE in autumn 2004-2005 (Fig. 1).

The coordinates of the observation stations were determined using GPS navigator.

Representatives of invertebrates – *A. salina* and *Ch. salinarius* belong to the type *Arthropoda*, classes *Branchiopoda* and *Insecta* and families *Artemiidae* and *Chironomidae* respectively.

Collection, fixation and processing of the collected material was carried out according to standard hydrobiological methods [23].

The water temperature, dissolved oxygen, pH, salinity were measured in situ.

A portable conductometer ‘Sension 5’ and an oximeter ‘Sension 6’ were used to determine the mineralisation and dissolved oxygen in water respectively. Water temperature was measured using a TL-4 glass thermometer with the scale of 0,1°C, pH – using a self-powered pH meter pH-150 MI at the time of sampling.

The content of heavy metals in water, botton sediments and biological objects was assessed by atomic absorption spectrophotometry (electrothermal variant) using a «Saturn 3P» spectrophotometer with a «Graphite 2».

For this purpose, appropriate sample preparation was carried out: samples of water, bottom sediments and hydrobionts were dried to a dry residue at a temperature of +105°C. After that, dry mineralization of the samples was carried out in a muffle furnace at a temperature of

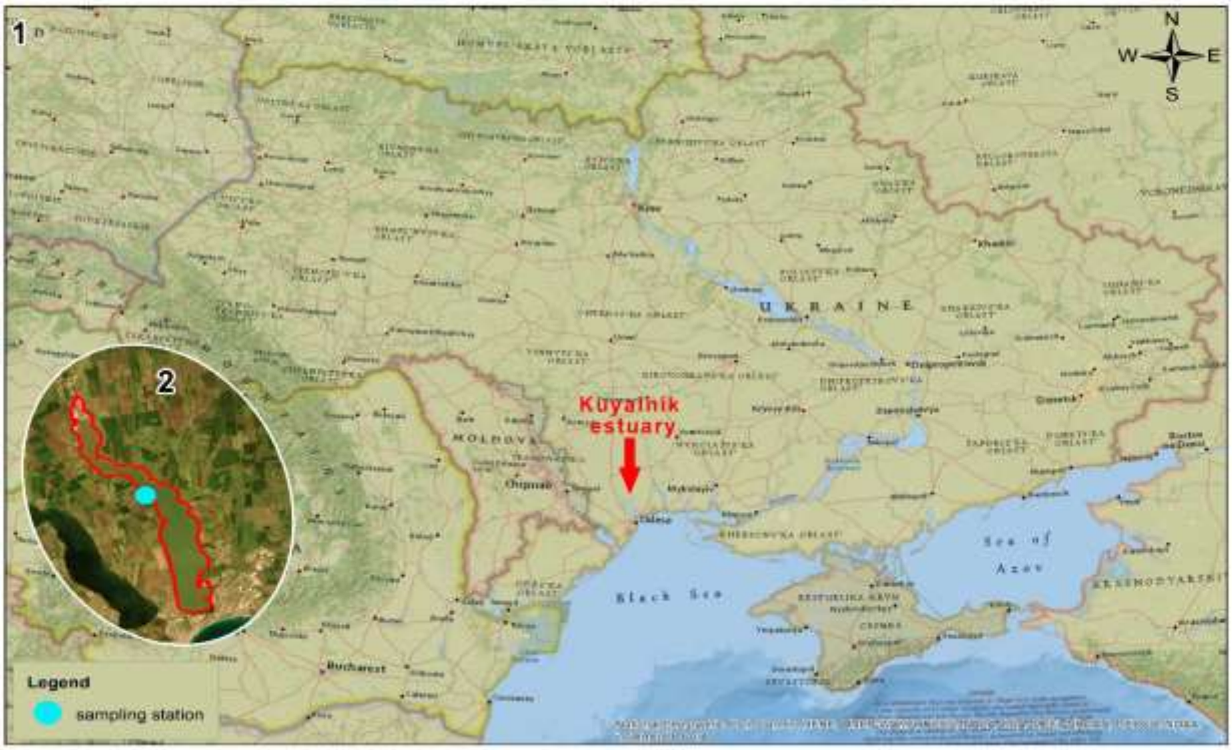

Fig. 1 – Location of Kuyalnik estuary on the territory of Ukraine: 1 – Map of Ukraine (red arrow shows the geographical location of the KE); 2 – Kuyalnik Estuary and sampling station

450°C, followed by dissolution of the ash residue in a nitric acid solution.

The heavy metals contents were measured in the biomass of mature individuals of the brine shrimp and eggs *A. salina* and *Ch. salinarius* larvae.

Metal content was calculated in terms of dry matter. The concentration (C) of metals in water was expressed in mg/dm³, and in sediments and biological objects in mg/kg dry weight.

The degree of metal accumulation in bioobjects was assessed by using the biological absorption faktor (BAF) [24-26]. Acumulation factors, were considered in order to estimate heavy metal contamination loads in biota.

BAF is the ratio of the concentration of heavy metals in biota to the concentration of heavy metals in the surrounding aquatic environment (water, bottom sediments), which is determined by the formula given below [26]:

$$BAFA(B) = Cbiota/CA(B)$$

where Cbiota – concentration of heavy metals in a bioobject,
CA(B) – concentration of heavy metals in water (bottom sediments).

Biota-water (BAFA) and biota-botton sediment (BAFB) accumulation factors, commonly used for the evalution of water and sediments role in aquatic systems contami-nation, were determined for the considered metals.

An important indicator in studying the mechanism of redistribution of pollutants from the aquatic environment to bottom sediments is the bottom accumulation coefficient (CBA), which is calculated using the formula [27]:

$$CBA = CB / CA$$

where CB – concentration of heavy metals in a bottom sediments,
CA – concentration of heavy metals in a water.

A correlation has also been found between levels of heavy metals in the aquatic organism and in water and in botton sediments.

Results and discussion

A feature of hyperhaline KE is the significant variability of the water regime. It is characterized by quick fluctuations in water level and salinity. In terms of hydrochemistry, the KE is a reservoir sodium-chloride type reservoir. The main cations are Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}, and the main anions are Cl^{-}, SO_4^{2-} and HCO_3^{-}.

During the sampling, the salinity of the water in the central part of the KE water area ranged from 109 to 160 ‰, the pH value ranged from neutral to slightly alkaline (7.3 to 7.8), and the concentration of dissolved oxygen in the water ranged from 6.6 to 10.1 mg/dm³, the temperatura – ranged from 16 to 18 °C.

The maximum density of the shrimp *A. salina* in plankton and eggs in the bottom sediments during this period was 33600 units/m³ and over 6 million units/m², respectively, and of *Ch. salinarius* larvae – 33600 units/m².

The content of heavy metals in aquatic organisms and their habitat (water, bottom sediments) is presented in the figure 2.

In the water, the concentrations of heavy metals were the lowest (Fig. 2).

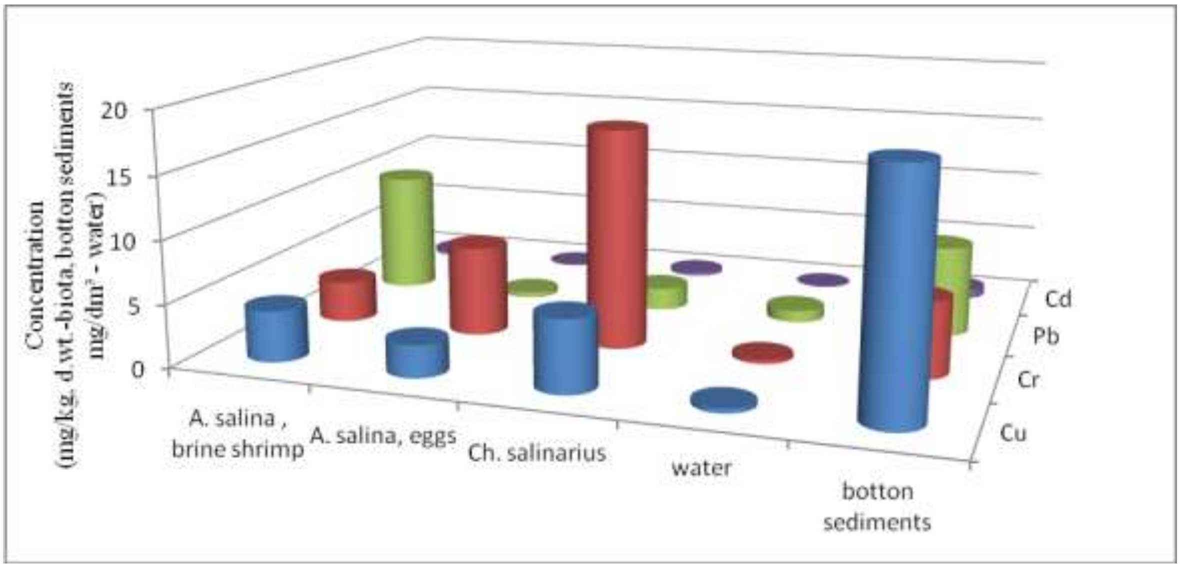

Fig. 2 – Heavy metal concentrations in biota, botton sediments and estuary water

The levels of all trace elements tested were significantly higher in the bioobject samples than in water. It should be noted that the heavy metal content in the bottom sediments of the KE was significantly higher than in water or aquatic organisms (with the exception of lead in biomass *A. salina* and chromium in *A. salina* eggs and *Ch. salinarius* larvae). The accumulation of heavy metals in bottom sediments is usually caused by solubility in water, the tendency of metals to adsorb on solid phases and enter into complexation reactions. Which is primarily related to the oxidising potential, temperature, pH of the environment, organic matter content etc.

Heavy metals in aquatic organisms also varied depending on the species and the developmental stage of the organism. Moreover, the concentrations of Pb and Cd were highest in the sea shrimp *A. salina*, and Cr – in the larvae of *Ch. salinarius*. Different species have varying sensitivities to heavy metals, and the impact can also differ greatly at various stages of developme. For example, Pb, Cu and Cd were more abundant in the brine shrimp *A. salina*; Cr – in the eggs (Fig. 2).

The obtained average indicators of heavy metals in the components of the aquatic environment of the KE are the following regressive series: Pb>Cu>Cr>Cd (water); Cu>Pb>Cr>Cd (bottom sediments); Pb>Cu>Cr>Cd (brine shrimps and eggs of *A. salina*); Cr>Cu>Pb>Cd (larvae of *Ch. salinarius).*

For water KE, which is used for recreational purposes, excess content of lead and cadmium.

The concentrations of toxic lead and cadmium in biota do remain below limits European Union standards for animal feed [28].

The biological accumulation factors biota-water (BAFA) and biota-bottom sediments

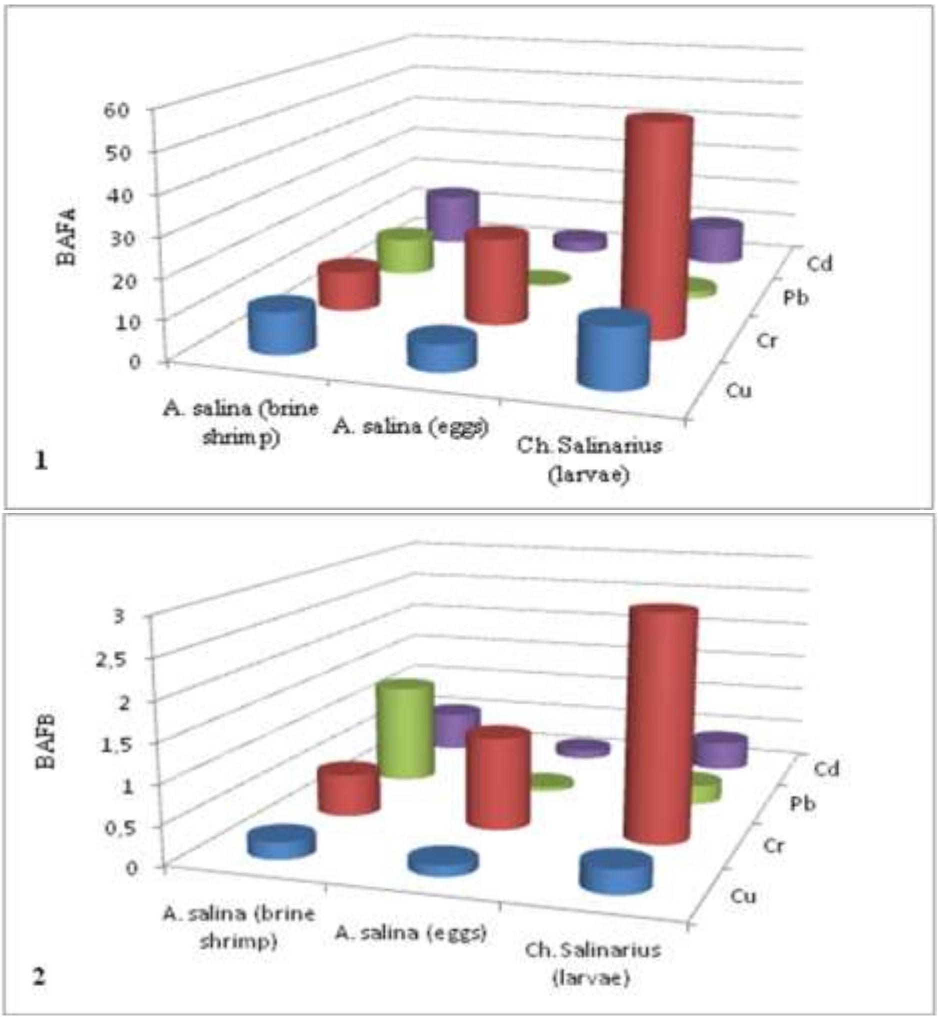

Fig. 3 – The coefficient of biological accumulation of heavy metals by the *A. salina* (brine shrimp, eggs) and the *Ch. salinarius* (larvae): 1 – BAFA, 2 – BAFB

(BAFB) of different aquatic invertebrate groups for the four metals is presented in Fig. 3.

The biological uptake coefficients (Fig. 3) show that the bulk of the pollutants migrate to the biota from water.

The *A. salina* brine shrimp can accumulate the most Cd from water, where as eggs *A. salina* and *Ch. salinarius* larvae can accumulate the most Cr from water (see Fig. 3.1).

The BAFB of Pb and Cr in *A. salina* (brine shrimp, eggs) was greater than 1. The BAFB of Cr in *Ch. salinarius* larvae was even greater than 2 and BAFB of all other metals was less than 1. (see in fig. 3.2).

Overal, BAFA and BAFB value greater than 1 indicates that of metal may be accumulated in biota.

According to [29], the BAF > 1000 – a very high level of accumulation;

100-1000 – a high level of accumulation;

30-100 – a medium/moderate level of accumulation;

< 30 – a low level of accumulation.

The higher the value of the BAF in an organism, the higher the accumulation of heavy metals in that organism.

Biological accumulation coefficients (Fig. 3) show that the bulk of pollutants migrate into biota from water.

The calculations of bioaccumulation coefficients (Fig. 3) and bottom accumulation (Fig. 4) show that bottom sediments accumulate copper and cadmium more, and the studied hydrobionts – lead and chromium, which indicates the ability to selectively accumulate certain elements from water.

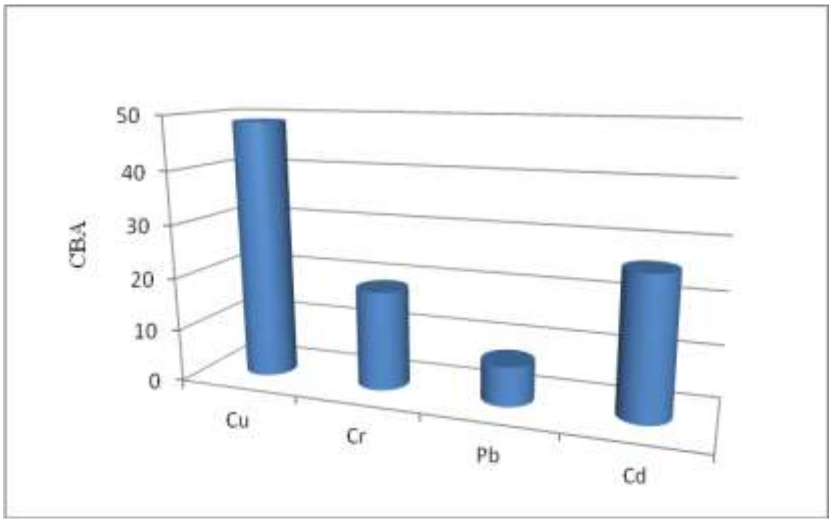

Fig. 4 – The coefficient of botton accumulation (CBA) of heavy metals in the central part of the KE

There fore, the obtained results allow stating that the ecosystem KE is significantly polluted by heavy metals, especially lead, cadmium and chromium. This was adequately reflected in the levels of heavy metals accumulation in aquatic organisms.

The analysis correlation showed that the tightest positive relationship was observed between heavy metal concentrations in water and biomass of the salt shrimp *A. salina* (r = 0.998). The correlation between the concentrations of heavy metals in water and larvae of *Ch. salinarius* was minus 0.134, and a weak relationship was observed between biota and botton sediments (r = 0.108-0.269). The correlation coefficient between the concentrations of heavy metals in the water and botton sediments of the KE was 0.245.

Conclusions

This is the first study of the accumulation of Cr, Cu, Pb, Cd in of biomass brine shrimps and cysts (eggs) of *A. salina* and the *Ch. salinarius* larvae from the Kuyalnyk Estuary.

Analysis of bioaccumulation coefficients indicates the efficient transfer of heavy metals to biota mainly from water.

It has been established that the brine shrimp *A. salina* are concentrators of lead and cadmium (BAFA – 9,8 and 13,8 respectively), and *Ch. salinarius* larvae are concentrators of chromium (BAFA – 54,2).

Our research shows that concentrations of toxic lead and cadmium do remain below limits European Union standards for animal feed. Thus, the biomass brine shrimp and eggs *A. salina* and *Ch. salinarius* larvae from the Kuyalnyk estuary are suitable as food for vertebrates and invertebrates and are effective as bioindicators of heavy metals in aquatic ecosystems.

The results obtained have theoretical and practical significance for the preparation of scientific and biological justifications for the use of biological resources of the Kl and saline water bodies in general.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

Project compliance

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НАКОПИЧЕННЯ ВАЖКИХ МЕТАЛІВ (Cu, Cr, Pb, Cd) У ВОДНИХ БЕЗХРЕБЕТНИХ З ГІПЕРГАЛИННОГО КУЯЛЬНИЦЬКОГО ЛИМАНУ (УКРАЇНА, ПІВНІЧНО-ЗАХІДНЕ ПРИЧОРНОМОР'Я)

Мета. Визначення вмісту ряду металів (Cu, Cr, Pb, Cd) та дослідження особливостей їх міграції і накопичення в компонентах (жаброногі рачки *Artemia salina*, хірономіди *Chironomus salinarius*, вода, донні відкладення) екосистеми Куяльницького лиману (Кл).

Методи. Гідробіологічні, атомно-абсорційної спектрофотометрії, автоматизовані методи аналізу, статистичні.

Результати: Це перше дослідження накопичення Cr, Cu, Pb, Cd у біомасі жаброві ракоподібних *A. salina* та личинок *Ch. salinarius*, вилучених з гіпергалінного Куяльницького лиману. Визначено, що середні показники вмісту важких металів у компонентах водного середовища представлені такими регресійними рядами: Вода: Pb > Cu > Cr > Cd ; Донні відкладення: Cu > Pb > Cr > Cd; Рачки та цисти *A. salina*: Pb > Cu > Cr > Cd; Личинки *Ch. salinarius*: Cr > Cu > Pb > Cd. Показано інтенсивне накопичення важких металів у донних відкладах, рачках *A. salina* та личинках *Ch. salinarius* порівняно з водою, що підтверджено коефіцієнтами донної акумуляції та біонакопичення. *A. salina* та личинки хірономід *Ch. salinarius* є ефективними концентраторами досліджених важких металів, особливо свинцю і хрому. Виявлено, що *A. salina* інтенсивніше накопичує Pb та Cd, тоді як *Ch. salinarius* найбільше акумулює Cr.

Висновки. Гідробіонти Куяльницького лиману (*A. salina* та *Ch. salinarius*) є **і**нформативними біоіндикаторами хімічного забруднення водойм важкими металами, з ефективним переходом цих металів до біоти переважно з води. Концентрації Cd та Pb у цих організмах не перевищують допустимих рівнів для кормів тварин, що вказує на їх потенційну безпечність для використання. Отримані дані мають значення для науково-біологічного обґрунтування використання біоресурсів Куяльницького лиману та засолених водойм загалом.

КЛЮЧОВІ СЛОВА: *гіпергалинний Куяльницький лиман, важкі метали, водні безхребетні організми, концентрація, коефіцієнт бологічного накопичення, біоіндикація*

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