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CONTENT OF Ba, B, Sr AND As IN WATER AND FISH LARVAE OF THE GENUS ATHERINIDAE L. SAMPLED IN THREE BAYS IN THE SEVASTOPOL COASTAL AREA*

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ABSTRACT

One of the most reliable methods of the environmental monitoring of marine ecosystems, especially the ones characterized by sizeable populations, is the use of populations of vertebrate animals, and fish in particular. Many authors point to the specific suitability of fish larvae for environmental monitoring. Fish larvae have rapid metabolism, which translates to more intensive toxicological effects. The aim of the study was to assess the quality of the aquatic environment in three bays of Sevastopol, based on the determined content of Ba, B, Sr and As and bioaccumulation of these elements in the water and in fish larvae of the genus *Atherinidae* L. Larvae were caught in July 2012, in the coastal shallow water, at a depth of 1 m, using a fishing net. Samples of fish larvae were subjected to wet digestion in a closed system with the use of microwave energy. The concentration of the analyzed elements in the samples was determined using atomic emission spectrometry with inductively coupled plasma. The results of mean element concentrations were compared using Student's *t*-test, at the significance level of $p = 0.01$. The element content in water was similar to that observed in unpolluted water bodies. The average concentration of Ba, B, Sr and As in water was, respectively: 46.52; 1428; 7737 and 1.821 $\mu\text{g dm}^{-3}$, whereas the mean content of these elements in the biomass of fish larvae was: 1.550 mg Ba kg^{-1} ; 70.90 mg B kg^{-1} ; 21.40 mg Sr kg^{-1} and 1.829 mg As kg^{-1} . The highest barium and strontium content in the studied fish larvae was found in samples taken in Omega Bay. The highest arsenic content was found in organisms from the bays Omega and Karantinnaya,

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while the largest content of boron was found in the larvae from the bays Omega and Golubaya. The values of the bioaccumulation coefficient for the analyzed elements in Atherinidae L. fish larvae can be ordered as follows: As >B> Ba> Sr. The most severely contaminated with Ba, Sr, As and B were the coastal parts of Golubaya, Karantinnaya, Omega Bays.

Keywords: fish larvae, Black Sea, barium, boron, strontium, arsenic, bioaccumulation, the bays of Sevastopol.

INTRODUCTION

Due to its geological and geographical location, the Black Sea is a body of water with specific properties. Its geographical location limits the exchange of water with other water bodies of the hydrosphere. The exchange takes place only with the Mediterranean Sea, through the Bosphorus. The chemistry of the coastal zone waters is shaped mainly by fluvial inputs, especially from the basin of the Danube, Dnieper and Dniester, as well as some smaller rivers. In urban areas and in densely populated regions, the quality of water is significantly impacted by municipal, agricultural and industrial sewage discharged directly into the sea (DINEVA 2011, ROJE-BUSATTO, UJEVIĆ 2014, NAPIÓRKOWSKA-KRZEBIETKE, DUNALSKA 2015). Due to a very strong influence of the land on the chemical properties of the Black Sea waters, a number of specific ecosystems developed within the sea. Also, the Black Sea demonstrates significant differences in the level of pollution between specific sites (NIEMIEC et al. 2015). This is characteristic of inland seas. It is difficult to monitor water bodies characterized by significant territorial variability in water chemistry because of the high risk of erroneous inference (NAPIÓRKOWSKA-KRZEBIETKE et al. 2015). The most common errors are due to the selection of sampling points, sampling dates, types of samples and the quality indicators on the basis of which the inference is made. One of the most reliable methods of environmental monitoring of marine ecosystems, especially those characterized by sizeable populations, is the use of populations of vertebrate animals, and fish in particular. Many authors point to the particular value of fish larvae in environmental monitoring (NIEMIEC et al. 2016). Despite the problems with obtaining the test material, the results related to the growth and development of fish larvae, as well as the level of accumulation of trace elements in their organisms substantiate more complete interpretation of the toxicological effects of pollution. Fish larvae have rapid metabolism, which translates to more intensive toxicological effect (MAJEWSKI et al. 2017). In addition, their mobility, which is lower than among adult specimens, facilitates more accurate reference of the results to a specific area. The enormous capacity of fish larvae of the genus Atherinidae L. to accumulate metals makes these parasites suited to be used as sensitive bioindicators for monitoring low levels of metal pollution in the environment. The study into concentrations of Ba, B, Sr and As in the water and in the fish larvae of the genus Atherinidae L. caught in three bays of Sevastopol and determination of the

levels of bioaccumulation of these elements in the larvae enabled us to assess the environmental condition of the selected research areas. The examined elements may have a significant influence on the functioning of aquatic ecosystems. These elements play an important role in lives of aquatic organisms. High content of these elements in the biotope has a negative impact on aquatic ecosystems.

Difficulties in the interpretation of results arise from a multidirectional impact of pollution on fish. The actual impact depends on a fish species, sex, age, physiological condition, nutritional level, individual traits and the locally developed population traits.

The aim of the study was to assess the quality of the aquatic environment in three bays of Sevastopol according to the content of Ba, B, Sr and As and their bioaccumulation in the water and in fish larvae of the genus *Atherinidae* L.

MATERIAL AND METHODS

Fish larvae of the genus *Atherinidae* were caught in July 2012 in three bays within the city of Sevastopol: Golubaya, Omega and Karantinnaya (Figure 1). The catch was carried out in the morning (between 7 and 8). Larvae were caught in the coastal shallow water, at a depth of 1 m, using fishing nets. A laboratory sample was composed of the aggregated primary samples collected in coastal areas around the surveyed bays. The mass of the laboratory sample, consisting of circa 20 original samples, was about 1 g DM. The mass of a single larva was 0.001 - 0.005 g. The collected material was transported immediately after collection to the laboratory, where it was preserved by drying. Before drying, the fish samples were washed thoroughly with distilled water. The samples of water were collected with a scoop from the same location; a 1 dm³ laboratory sample consisted of about 10 primary samples. The bulk sample was composed of aggregated primary samples. The collected water was preserved at the sampling site by adding nitric acid (V) in the amount of 2 cm³ per 100 cm³ of water; then the samples were transported to the laboratory, where they were concentrated twenty-fold by evaporation. Fish larval samples were subjected to wet digestion in a closed system with the use of microwave energy. The digestion was carried out using a microwave system Anton Paar Multivawe 3000. The analytical sample weight was approximately 0.5 g per dry matter. The biological material was dissolved in a mixture of HNO₃ and H₂O₂ at a ratio of 5: 1, v/v. Concentrations of the analyzed elements in the samples was determined using atomic emission spectrometry with inductively coupled plasma in an apparatus Optima 7600 DV Perkin Elmer. The wavelengths used in the analysis, as well as the quality parameters of the analytical methods are shown in (Table 1). To control the accuracy of the analysis certified reference materials

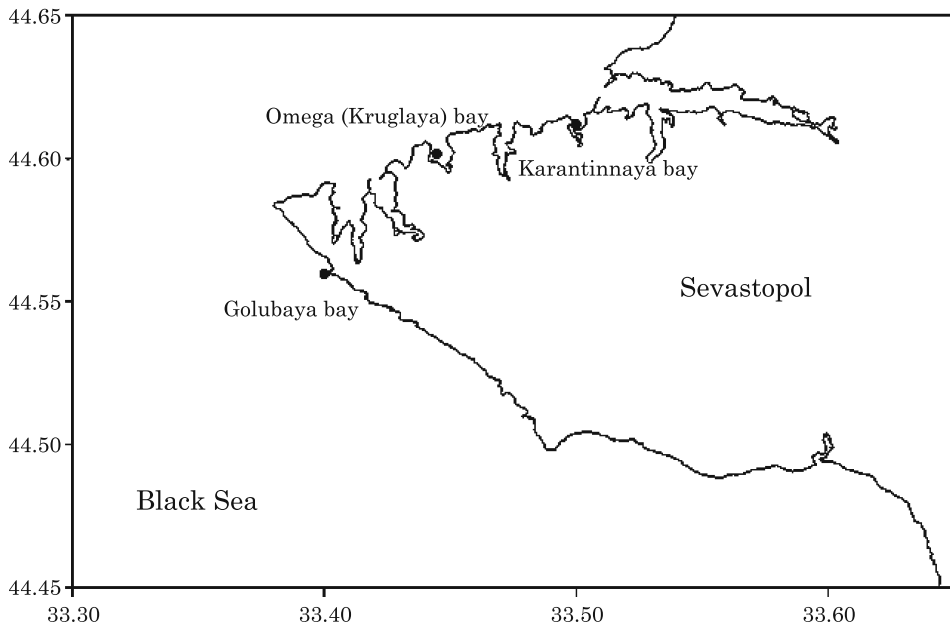


Fig. 1. Maps with sampling sites

Table 1

Parameters of the analytical method

Parameter	Ba	B	Sr	As
Wavelengths (nm)	233.527	249.677	407.777	188.979
Detection limit ($\mu\text{g dm}^{-3}$)	4	5.7	0.4	86
Content in the certificated material (mg kg^{-1})	2.46	55.67	130	12.6
Measured (mg kg^{-1})	2.386	53.86	135.7	11.07
Recovery (%)	97.0	96.7	104.4	95.0

IAEA-407 and CRM 16-050 were used (Table 1). The results of mean element content were compared using Student's t-test at the significance level of $p = 0.01$. Based on the results, the bioaccumulation factors of individual elements were calculated. The bioaccumulation factor was derived by dividing the concentration of an element in the dry matter of larvae used in the experiment by the content of the same element in the water, according to the formula:

$$BF = C_l / C_w,$$

where: BF – bioaccumulation factor, C_l – element concentration in larva dry matter, C_w – element content of in water.

RESULT AND DISCUSSION

Barium in seawater comes from a variety of sources; of which the most frequently mentioned are: leaching from minerals, river transport and mixing of the deep ocean waters, which contain significant amounts of this element. The supply of barium from land is only relevant in coastal areas (SINGH et al. 2013). This element does not remain long in water in its dissolved form because it is strongly bonded with the bottom sediments. The soluble barium compounds are toxic to living organisms. The content of this element in the ocean waters ranges from several to dozens of $\mu\text{g Ba dm}^{-3}$, and increases with depth. In the studied samples, the concentration of this element ranged from 43.65 to 50.52 $\mu\text{g Ba dm}^{-3}$ (Table 2). The largest amount

Table 2

The content of elements in water from the bays ($\mu\text{g dm}^{-3}$)

Points of sampling	Ba	B	Sr	As
Karantinnaya	50.52 b	1156 a	7678 a	2.180 b
Omega	43.65 a	1391 b	7546 a	1.852 b
Golubaya	45.40 ab	1737 c	7989 a	1.432 a
Detection limit	0.40	0.57	0.04	0.86

Different letters at mean values indicate statistically significant differences between the samples from each of the bays at $p = 0.01$.

of this element was found in the water of Karantinnaya Bay, and the least amount of barium was observed in the samples in Omega Bay. Statistically significant differences were found only between samples taken from the bays Omega and Golubaya.

Arsenic is one of the most significant pollutants in aquatic environment due to its prevalence and high toxicity. Toxicological effects of arsenic in the aquatic environment are associated with its amount and the forms in which it is present. In the aquatic environment, much of the element is present in organic compounds; they are, however, less toxic in comparison to the inorganic forms of the element. In the biogeochemical cycle of this element, an important role is played by micro-organisms and phytoplankton, which absorb the largest amount of the element from water (SHARMA, SOHN 2009). The concentration of arsenic in the studied water samples ranged from 1.432 to 2,180 $\mu\text{g As dm}^{-3}$ (Table 2). The least amount of this element was found in the water taken in from Golubaya Bay. There was half more of it in the samples taken from Karantinnaya Bay. Arsenic is an element, the presence of which in aquatic ecosystems is very strongly correlated with the level of human pressure. The natural content of this element in marine waters is ca 1 $\mu\text{g dm}^{-3}$ (SHARMA, SOHN 2009). In polluted bodies of water, the content of this element may even reach above several thousand $\mu\text{g dm}^{-3}$ (RUIZ-CHANCHO et al. 2013). This element bioaccumulates significantly, thus has a high po-

tential of posing a toxicological risk for humans. The arsenic content in the water of polluted Lake Machar ranged from 60 to 101 $\mu\text{g dm}^{-3}$ (SHAH et al. 2009). A very high content of arsenic in fish biomass was also found in this lake. Depending on a species and organ of a fish, it ranged from about 1 to 15 mg kg^{-1} DM. On the other hand, the concentration of this element in the water of Daya Bay was only 1.4 $\mu\text{g dm}^{-3}$ (QIU et al. 2011).

Boron is an element commonly found in the hydrosphere and lithosphere. It belongs to a group of elements essential for living organisms. In animals, it affects the metabolism of other elements such as calcium, magnesium, phosphorus, copper and nitrogen. Because of the prevalence of this element and good solubility of its compounds, the toxicological effects of high concentrations of boron are observed in the environment under certain conditions (SCHODERBOECK et al. 2011). Boron demonstrates a high affinity for oxygen and it is present in the sea water in the form of acid B(OH)_3 , or as an anion B(OH)_4^- . The quantitative ratio of both forms is mainly dependent on the salinity of water. The content of this element in the surface water ranges from 10 to over several thousand $\mu\text{g B dm}^{-3}$ (WYNESS et al. 2003). In freshwater, the content of this element is a hundred times lower than in marine waters. The main source of boron in the natural environment is the weathering of rocks, and the leaching of brine. An increased content of this element is often observed in industrial areas and in areas of intensive agricultural activity (WYNESS et al. 2003). The boron content in the water of several rivers in the United States was from 20 to 800 $\mu\text{g B dm}^{-3}$ (HOLT et al. 2003). A very high content is found in marine waters, ranging from 0.52 mg of B dm^{-3} in the Baltic Sea to over 10 mg B dm^{-3} in highly salinated seas (WOLSKA, BRYJAK 2013). In the seas and oceans, boron comes mainly from leaching of the rocks forming the bottom. The concentration of the soluble forms of this element over 10 $\mu\text{g B dm}^{-3}$ causes toxicological effects in the most sensitive species, e.g. *Xyrauchen texanus*. The fish that are the most resistant to a high content of boron in water, e.g., *Carassius auratus*, can live in water containing the soluble forms of boron even at over 10 mg B dm^{-3} (SCHODERBOECK et al. 2011). The boron content in the water taken from the selected bays of Sevastopol averaged 1428 $\mu\text{g B dm}^{-3}$ and ranged widely from 1156 to 1737 $\mu\text{g B dm}^{-3}$ (Table 2). The highest concentration of this element was found in the water taken from Golubaya Bay, while the lowest was in the water from Karantinaya Bay.

Strontium is a common element in the aquatic environment. The natural content of this element in the sea water is 7-8 mg dm^{-3} . The amount of strontium accumulated in aquatic organisms is dependent on the salinity of water (UDDIN et al. 2013).

The content of this element in the water from the three bays did not differ statistically. Its average content was 7737 $\mu\text{g Sr dm}^{-3}$ and ranged from 7546 to 7989 $\mu\text{g Sr dm}^{-3}$.

Assessment of the level of accumulation of chemical elements in living organisms is a very important element of environmental monitoring.

The accumulation of xenobiotics in different parts of the biocenosis is a very important source of knowledge about the actual dangers associated with it. The content of elements which are not toxic for the environment also may indicate the emergence of a stressor in the environment, which may result in disturbed ion levels. Due to the synergism or antagonism between the individual elements, evaluation of bioaccumulation of the elements such as boron, strontium or barium is particularly valuable for assessment of the functioning of an ecosystem. The results of the study indicate significant differences in the content of barium, strontium and arsenic in the biomass of fish larva Atherinae L. The contents of barium ranged from 0.522 to 3.128 mg Ba kg⁻¹ DM. The largest amount of this element was found in the water of Karantinnaya Bay, and the least amount was observed in the organisms caught in Golubaya Bay (Table 3). Between the samples from different sampling points statistically significant differences at $p = 0.01$ were found. Very small relative standard deviations, which ranged from 8 to 14%, were found in the samples from the different sampling sites (Table 2). Signifi-

Table 3

Statistic parameters of the results (mg kg⁻¹)

Parameter	Ba	B	Sr	As
Karantinnaya				
Mean	1.375 <i>b</i>	51.69 <i>a</i>	18.24 <i>a</i>	2.232 <i>b</i>
Min. value	1.250	41.05	13.61	1.269
Max. value	1.482	61.18	22.57	4.317
SD	0.113	8.815	3.544	1.216
(%)*	8.241	17.05	19.43	54.48
Omega				
Mean	2.696 <i>c</i>	78.60 <i>b</i>	28.39 <i>b</i>	1.923 <i>b</i>
Min. value	2.329	57.83	24.78	0.946
Max. value	3.128	98.25	36.26	2.663
SD	0.377	16.40	4.607	0.659
(%)*	14.0	20.87	16.23	34.25
Golubaya				
Mean	0.579 <i>a</i>	82.42 <i>b</i>	17.58 <i>a</i>	1.334 <i>a</i>
Min. value	0.522	64.74	14.63	0.882
Max. value	0.659	94.05	21.90	1.879
SD	0.063	11.31	3.059	0.405
(%)*	10.82	13.72	17.40	30.37

Different letters at mean values indicate statistically significant differences between the samples from each of the bays at $p = 0.01$.

* coefficient of variation

cantly different content of barium was found in fish in individual bays, as compared to the differences in the concentration of the element in water. The coefficient of bioaccumulation of barium calculated for the samples in individual bays ranged between 12.75 and 61.77. Such large differences in the values of this parameter indicate that the level of accumulation of this element affects not only its content in the water but also other factors influencing the absorption of this element. TRAVIS et al. (2003) found a significant correlation between the content of barium in living organisms and in water.

The average boron content in the studied samples was 70.90 mg kg⁻¹ DM and ranged from 41.05 to 98.25 mg B kg⁻¹ DM (Table 2). The least amount of boron was accumulated in the fish larvae caught in the Karantinnaya bay. The content of this element in the samples from the bays Omega and Golubaya do not differ significantly, and it was approx. half higher than that found in fish in the Karantinnaya bay. The coefficient of bioaccumulation of boron ranged in fish biomass ranged from 44.71 to 56.50, and reached the largest value in the samples of fish larvae from the Omega bay (Table 4).

Table 4

The bioaccumulation coefficient in larval bodies

Points of sampling	Ba	B	Sr	As
Karantinnaya	27.21	44.71	2.375	1024
Omega	61.77	56.50	3.763	1038
Golubaya	12.75	47.43	2.200	931

The content of strontium in the water, sediments and living organisms of marine ecosystems is more often used as an indicator of the quality of marine ecosystems (BECK et al. 2013). The average content of strontium in the larvae of the Atherinae fish caught in the Omega bay was 28 mg Sr kg⁻¹ DM (Table 3). In the organisms caught in the other locations, the content of the element was approx. 40% lower. The differences between the contents of the element in the laboratory samples from the individual sampling points was approximately 20%. The value of the bioaccumulation coefficient of the analyzed elements in the fish larvae was from 2.200 to 3.763 (Table 4). The largest value of the coefficient was noted in the larvae from the Omega Bay. The strontium concentration in the water did not affect the content of this element in the biomass of fish studied. TRAVIS et al. (2003) drew other conclusions of their research; they found a strong dependence between the contents of this element in the water and in fish.

The average arsenic content in the studied organisms was 1.829 As kg⁻¹ DM. The largest amount of this element (2.232 and 1.923 As kg⁻¹ DM.) was determined in organisms of the bays Omega and Karantinnaya (Table 3). Approximately 30% less of the element was accumulated in the larvae caught in Golubaya Bay. The values of the bioaccumulation coefficients in the studied

organisms ranged from 931 to 1038 (Table 4). Small differences in the bioaccumulation coefficient of arsenic in organisms from the individual sampling sites indicate a relationship between the content of this element in the water and in elements of a biocenosis. Fish and shellfish are an important source of arsenic for people because of their strong bioaccumulation potential of this element. Arsenic is absorbed by fish through the gills, directly from the water, and through the gastrointestinal tract. Quantitative relationships between the both intake routes of the element depend on properties of the abiotic part of the environment and on the species of an organism.

The literature indicates significant differences in the content of the above elements in the fish biomass. NAWAZ et al. (2010) reported average levels of arsenic in the muscle of two species of edible freshwater fish (*Channa punctata* and *Labeo rohita*) in the Ravi River in Pakistan at 45.3 mg kg⁻¹ DM, ranging at 40.9 - 66.5 mg kg⁻¹. On the other hand, ONSANIT et al. (2010) investigated levels of arsenic in the tissues of farmed marine fish *Lateolabrax japonicus*, kept in different locations. The content of this element ranged between 0.86 and 6.47 mg kg⁻¹, similarly to our results. The cited authors found a large impact of the location on the level of arsenic accumulation in the tissues of fish. On the other hand, the content of arsenic in several species of fish caught in different places in coastal areas of southern China ranged from 0.1 to 7.3 mg kg⁻¹ DM, which is equivalent to ca 0.5 – over 35 mg kg⁻¹ DM (WANG et al. 2013). PERRAULT et al. (2014) reported the arsenic content in the liver of Atlantic *Mola mola* at 1.5 mg As kg⁻¹ DM. The arsenic content in the muscles of young fish of the species *Dicentrarchus labrax* (seabass) and *Chelon aurata* (golden grey mullet) from the estuary of the Ria de Aveiro ranged from 0.79 to 1.72 mg As kg⁻¹ DM (MIEIRO et al. 2012). These authors found almost twice as much of this element in muscles of fish from the estuary than in muscles of fish from the open sea. LEUNG et al. (2014) reported the concentration of arsenic in several species of fish caught in the Pearl Delta in China, which was similar to the one obtained in our study, ranging from 0.03 to 1.53 mg As kg⁻¹ DM. The quoted authors emphasized that the research area was heavily polluted. Differences in concentrations of the elements studied in fish from the same area but identified by LEUNG et al. (2014) were much larger than in our research. The arsenic content in the muscle of horse mackerels, sardines and mackerels caught in the Atlantic Ocean, northwest of the territorial waters of Portugal, ranged from about 4 to 6 mg As kg⁻¹ per dry weight (VIEIRA et al. 2011). The authors found significant differences in the content of this element depending on the species of fish. The largest accumulation was found in horse mackerel, and the lowest one - in mackerel. On the other hand, CULIOLI et al. (2009) reported the arsenic content in muscles of several species of river fish from polluted areas at circe 1.45 mg As kg⁻¹, with the content of the same element in water at 2330 µg As dm⁻³. The arsenic content was several times larger in the liver and gills of fish. The authors found a significant impact of the content of arsenic in water on the level of bioaccumulation of this element in the orga-

nisms studied. The value of the bioaccumulation factor reported by those authors is much lower than derived from the results of our research.

SCHENONE et al. (2014) reported the content of boron, barium, arsenic and strontium in different organs of fish from Lake Chascomus in Argentina at 0.36 - 0.75 mg B kg⁻¹, 0.21 - 0.95 mg Ba kg⁻¹, 0.13 - 0.78 mg As kg⁻¹ and 2 - 22 mg Sr kg⁻¹. In the studies of SUBOTIC et al. (2013), the content of boron, barium and strontium in the muscles of several species of fish caught in the Danube in Serbia ranged from 0.07 to 0.09 mg B kg⁻¹, from 0.66 to 2.18 mg Ba kg⁻¹ and from 0.57 to 6.44 mg Sr kg⁻¹ DM. The boron content in different organs of the freshwater fish *Leisicus cefalus* L., caught in the area of the city of Kirk, which is considered to be polluted by this element, was between 2 to 66 mg B kg⁻¹ DM. In general, a larger amount of this element was found in the muscle than in the gills and liver. The content of this element in the water ranged from 1610 to 3450 µg B dm⁻³ (EMIROĞLU et al. 2010). The coefficient of bioaccumulation of this element in the muscles of fish ranged from 2 to 30 (EMIROĞLU et al. 2010). The average content of barium in the muscles of white fish of the Mediterranean and South Africa regions was at 0.143 mg Ba kg⁻¹ DM (GONZÁLEZ-WELLERA et al. 2013). The strontium content in the liver and muscle of juvenile barramundi fish bred in an artificial hatchery was about 10 mg Sr kg⁻¹ DM. The barium content in the muscle of fish was about 1 mg Ba kg⁻¹ DM. Over four-fold more of this element was found in the liver (MILTON et al. 2001).

CONCLUSIONS

1. The highest barium and strontium content in the studied fish larvae was found in samples taken in Omega Bay. The largest arsenic content was found in the organisms from bays Omega and Karantinnaya, while the largest content of boron was found in the larvae from bays Omega and Golubaya.

2. The decreasing order of the concentrations of the elements in the water was as follows: Sr>B>Ba>As, compared to B>Sr>As>Ba in the larvae of fish Atherinidae L.

3. Fish larvae are a good indicator of the As content in the aquatic environment, but weaker indicators of B and Ba, and can only poorly reflect the Sr level in the environment.

4. The results showed small variations in the content of individual elements between the samples of water and organisms from the three bays. The arsenic content was an exception.

5. The content of the analyzed elements in the larvae of Atherinidae L. fish point to Omega Bay as the most polluted water body with such elements as Ba, B, Sr and As.

6. The content of the analyzed elements in the larvae of Atherinidae L. fish is characteristic for unpolluted waters.

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