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ORIGINAL PAPERS

The Metal Content in Tissues of the Ascidian *Halocynthia aurantium* Pallas, 1787 (Ascidiacea: Stolidobranchia) from Coastal Waters of the Sea of Japan

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Abstract—This article presents data on the contents of Fe, Mn, Cu, Zn, Ni, Pb, and Cd in the tissues of the tunic, muscular sac, stomach, digestive gland, and gonads of the ascidian *Halocynthia aurantium* Pallas, 1787 from Peter the Great Bay and Kievka Bay (Sea of Japan). The physiologically important elements Fe, Mn, and Zn prevailed in almost all organs of ascidians in terms of their concentration levels. Patterns of metal distribution in the organs and tissues of *H. aurantium* were determined depending on their age. The levels of content of Zn, Mn, and Cu were similar in the organs of 2- and 3-year-old individuals. The element concentrations were highest in the digestive gland (Zn), stomach (Zn, Cu), tunic (Mn), and gonads (Cu). The content of Mn in the tunic of *H. aurantium* from different biotopes was higher compared to other organs. The content of Pb in the tissues of *H. aurantium* from Kievka Bay was higher than that of the tissues of ascidians from the other studied localities.

Keywords: commercial species, ascidians, *Halocynthia aurantium*, elemental composition, coastal waters, Sea of Japan

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INTRODUCTION

Ascidians are sessile organisms that are represented by both solitary and colonial forms; after completion of the free-swimming larval stage they attach to the substrate and undergo metamorphosis. Being active filter feeders, ascidians are able to accumulate various trace elements, depending on their content in the environment and on the biological characteristics of the species. In the course of life, modern tunicates accumulate P, Pb, V, Ti, Zn, Ba, Ni, Be, Sn, Mo, and Ag [7]. In the available literature, there are no data on the variations in the metal content of ascidians at different stages of individual development. It was shown that tunicates accumulate both physiologically essential elements and metals with pronounced toxic properties. Due to their ability to bioaccumulate trace elements, ascidians are considered for use as bioindicator organisms [20, 22, 25].

† Deceased.

Two commercially important species of ascidians inhabit the Sea of Japan: *Halocynthia aurantium* and *H. roretzi*. They are used as raw materials for the production of medicines and are consumed as food in countries of Southeast Asia. The solitary ascidian *H. aurantium* occurs at a depth of 4 to 400 m in all Far-Eastern seas [5].

Information on the physiologically significant concentrations of chemical elements and concentrations that have toxic effects on living organisms is of great importance. New data have been published on the metal levels and inter-species differences in the contents of Ca, S, Fe, P, K, Ti, Ba, Br, Sr, Mn, Zn, As, Cu, Pb, Ni, Rb, and Se in the tunic and mantle of different species of ascidians from Peter the Great Bay [3, 4].

The aims of this work were to determine the content of chemical elements (Fe, Mn, Cu, Zn, Ni, Pb, and Cd) in different age groups of the ascidian *H. aurantium* from coastal waters of the northwestern part of the Sea of Japan and to reveal the pattern of the

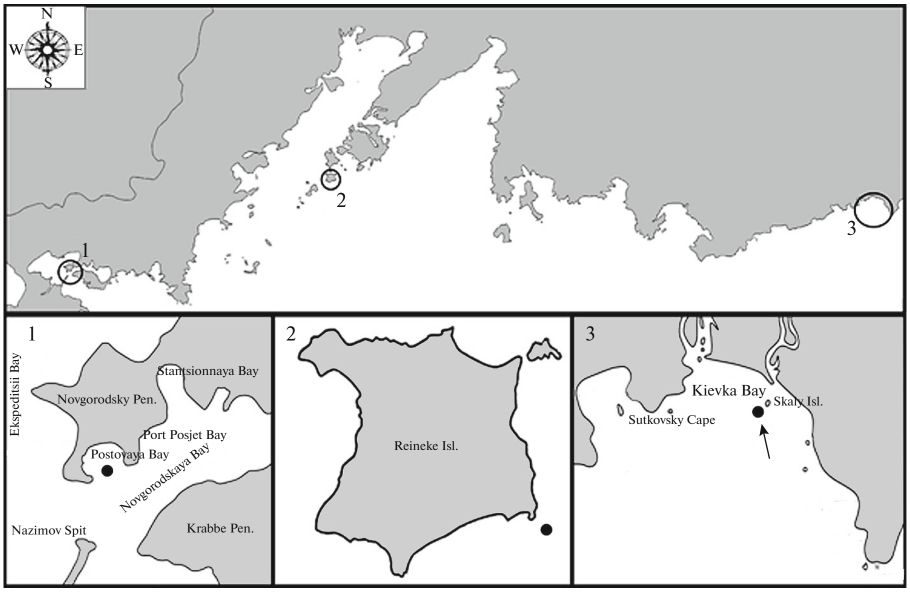

Fig. 1. A schematic map of the sampling.

element distribution in ascidian organs and tissues and their underlying causes.

MATERIALS AND METHODS

The study areas were located in the coastal waters of Primorye within and outside Peter the Great Bay: Postovaya Bay (in Possjet Bay) and a site south of Reineke Island (Amurskiy Bay), as well as Kievka Bay, respectively (Fig. 1). The material was collected in April 2010 at Reineke Island, in May 2011 in Postovaya Bay, and in August 2018 in Kievka Bay (Table 1).

The shallow Kievka Bay is open on the southern side and is influenced by a rather large river; there is free water exchange and a significant coastal runoff [6]. This area has virtually no sources of anthropogenic pollution and can be considered as a background site for a comparative environmental assessment [1]. The background concentrations of Fe, Mn, Zn, Cu, Pb, Cd, and Ni that were determined in a sample of the brown alga *Sargassum pallidum* from Kievka Bay in 1995–2008 [18] can serve as a basis for a chemical environmental assessment of water bodies. However, in the catchment basin of the river, which determines the chemical composition of waters in the bay, industrial felling of the forest is carried out using gasoline and diesel fuel. This can affect the concentration of metals in the tissues of aquatic organisms.

Postovaya Bay is situated in Possjet Bay (in Peter the Great Bay). It is a small shallow open bay; its area is approximately 5000 m^2 and the average depth is 3.6 m [2]. In the town of Possjet on the shores of the bay a large enterprise, the Commercial Port of Possjet, is engaged in coal transshipment. In 2007, in connection with the expansion of the scale of its activities, the port began bottom-dredging works, dumping of ground, and construction of berths. Wastewater from the town of Possjet that enters Postovaya Bay is classified as “insufficiently purified” and contains heavy metals Al, Fe, Zn, Cu, Pb, Ni, Cr, and Cd (total weight 0.117 t/year) [11].

Reineke Island is located in the southern part of Amurskiy Bay and is not directly affected by the continental runoff. The water area around Reineke Island is considered a background site for evaluation of heavy metal contents in marine sediments and organisms.

Table 1. The size–weight characteristics of the ascidian *Halocynthia aurantium* from coastal waters of the northwestern Sea of Japan

Sampling area	Sampling date	Number of specimens, *n*	Age, years	Size, mm	Weight, g
Reineke Island	09.04.2010	10	2	49–105	16–212.4
Reineke Island	09.04.2010	10	3	100–135	217–510
Kievka Bay	06.08.2018	10	2	85–115	82–292
Postovaya Bay	04.05.2011	10	2	65–85	25–115.4
Postovaya Bay	04.05.2011	10	3	85–105	110–293

Table 2. The average metal concentrations in the muscle (M) and tunic (T) of the ascidian *Halocynthia aurantium* collected near Reineke Island (Peter the Great Bay, Sea of Japan) (mean value ± standard deviation)

Organ	Concentration of metal, μg/g, dry weight						
	Fe	Zn	Mn	Cu	Ni	Pb	Cd
2-year-old individuals, n = 10							
T	22.1 ± 1.9	23.0 ± 1.73	53.5 ± 7.40	2.73 ± 0.33	0.60 ± 0.27	< 0.05	0.10 ± 0.01
M	10.0 ± 0.6	56.3 ± 9.10	4.4 ± 0.10	2.21 ± 0.30	1.05 ± 0.11	0.51 ± 0.06	0.10 ± 0.01
3-year old-individuals, n = 10							
T	48.2 ± 8.8	42.5 ± 7.51	50.0 ± 4.26	3.14 ± 0.34	0.51 ± 0.02	0.20 ± 0.02	0.21 ± 0.01
M	16.4 ± 1.6	44.1 ± 6.01	3.6 ± 0.90	2.95 ± 0.27	1.13 ± 0.14	0.32 ± 0.03	0.53 ± 0.02

Commercial ascidians *Halocynthia aurantium* of different ages (2 and 3 years old) were sampled using scuba at a depth of 15–20 m. In Postovaya Bay, ascidians were collected before the start of reconstruction of the port Possjet. Each sample consisted of ten individuals of the same age. Age was determined by the body height and weight of an individual. Earlier, four groups of ascidian generations of different years were identified based on these indices during observations of the growth of ascidians at a mariculture farm in Minonosok Bay (Peter the Great Bay) [6]. In our study, the size and weight characteristics of *H. aurantium* corresponded to those of 2- and 3-year-old individuals: the body weight of 2-year-olds varied from 23 to 200 g and the height varied from 40 to 90 mm; for 3-year-olds this was from 200 to 450 g and from 90 to 150 mm, respectively,

For analysis of the trace element composition of ascidians, we used tissues of the tunic (T), mantle (M), stomach (S), digestive gland (Dg), and gonads (G).

Sample preparation and heavy metal analysis were carried out at the Laboratory of Marine Ecotoxicology of the Ilyichev Oceanological Institute of the Far Eastern Branch of the Russian Academy of Sciences. The concentrations of the metals Fe, Mn, Cu, Zn, Ni, Pb, and Cd in the tissues of *H. aurantium* were determined on a Shimadzu AA-6800 atomic absorption spectrophotometer. The atomizer was a graphite cell; the background corrector was a deuterium lamp [12]. For atomic absorption measurements of heavy metals, tissue samples were prepared by the wet-acid mineralization method: tissue samples were dried to constant weight (complete moisture removal) at a temperature of 85°C. A sample of tissue (0.2–1.0 g) in a glassy carbon glass was then mineralized with a mixture of acids 16 M $HN0_3$ and 11.3 M $HClO_4$ (volume ratio of 3 : 1). The samples were maintained at room temperature for 24 hours. Further mineralization was carried out at a temperature of 90ºC until the solution became transparent, then the acid mixture was evaporated to a volume of 2.0–2.5 mL and was brought to a volume of 25 mL with bidistilled water [12].

The data were statistically processed using Microsoft Excel.

RESULTS

In the mantle of *Halocynthia aurantium* collected near Reineke Island (Table 2), the metal concentrations were arranged in decreasing order as follows: Zn > Fe > Mn > Cu > Ni > Pb > Cd in 2-year-old individuals and Zn > Fe > Mn > Cu > Ni > Cd > Pb in 3-year-old individuals. A tendency for biophilic elements to prevail in a series of decreasing concentrations was also observed for the tunic: Mn > Zn ≥ Fe > Cu > Ni > Cd > Pb in 2-year-old individuals; Mn > Fe ≥ Zn > Cu > Ni > Cd = Pb in 3-year-old individuals. Mn was in the first place in the content in tunic. In general, the highest content of Fe, Mn, and Cu was found in the tunic, while Zn, Ni, Pb, and Cd contents were highest in the mantle. The differences between samples were significant (Student's t-test, $p \leq 0.05$) for Fe, Mn, Zn, and Ni in 2-year-olds and for Fe, Mn, Zn, Ni, and Cd in 3-year-olds (differences were analyzed for the tunic and mantle of ascidians of each age group).

In ascidians of both age groups from Postovaya Bay (Table 3), the highest values of the average concentrations were found for Fe and Zn in all organs and tissues, with the exception of the tunic. In the tunic of both age groups of ascidians, Fe was followed by manganese in terms of quantity: Fe > Mn > Zn > Cu > Pb = Ni > Cd. In the tissues of other organs, metal concentrations were distributed as follows: in the gonads—Fe > Zn > Mn > Cu > Pb > Ni > Cd in 2-year-olds and Fe > Zn > Mn > Cu > Ni > Pb > Cd in 3-year-olds; in the digestive gland—Fe > Zn> Mn> Cu> Ni> Pb = Cd in 2-year-olds and Fe > Zn > Mn > Cu > Pb > Ni > Cd in 3-year-olds. Age differences in the content of metals in the mantle and stomach are of interest. Unlike 2-year-olds, in which the series of concentrations began with iron, in 3-year-old ascidians it began with zinc: in the mantle—Fe > Zn > Mn > Cu > Pb > Ni > Cd in 2-year-olds and Zn > Fe > Mn > Cu > Ni > Pb > Cd in 3-year-olds; in the stomach—Fe > Zn > Cu > Mn > Ni > Pb > Cd in 2-year-olds and Zn > Fe > Cu > Mn > Pb > Ni > Cd in 3-year-olds.

In the organs and tissues of *H. aurantium* from Kievka Bay (Table 4), as in ascidians from Peter the Great Bay, Fe and Zn were predominant: in the

Table 3. The average metal concentrations in the organs and tissues of the ascidian *Halocynthia aurantium* from Postovaya Bay (Peter the Great Bay, Sea of Japan) (mean value ± standard deviation)

Organ	Concentration of metal, μg/g, dry weight						
	Fe	Zn	Mn	Cu	Ni	Pb	Cd
2-year-old individuals, $n = 10$							
G	844.1 ± 20.5	68.2 ± 3.5	18.5 ± 0.9	8.91 ± 0.30	1.07 ± 0.02	1.30 ± 0.10	< 0.05
T	463.0 ± 10.2	17.6 ± 0.8	42.1 ± 2.5	1.82 ± 0.02	0.58 ± 0.02	0.61 ± 0.02	0.25 ± 0.001
Dg	259.4 ± 12.9	135.5 ± 6.8	6.6 ± 0.4	2.70 ± 0.05	0.31 ± 0.01	0.18 ± 0.01	0.12 ± 0.01
M	178.0 ± 9.1	111.0 ± 5.6	4.4 ± 0.3	1.92 ± 0.01	0.56 ± 0.01	0.74 ± 0.02	< 0.05
S	144.1 ± 7.2	176.2 ± 8.8	10.0 ± 0.5	25.70 ± 1.30	0.54 ± 0.01	0.21 ± 0.01	0.10 ± 0.01
3-year-old individuals, $n = 10$							
G	141.0 ± 7.1	57.5 ± 2.8	12.5 ± 0.6	8.20 ± 0.40	0.70 ± 0.01	0.33 ± 0.01	0.14 ± 0.01
T	349.0 ± 15.3	17.2 ± 0.8	32.1 ± 1.7	1.95 ± 0.07	0.62 ± 0.02	0.61 ± 0.01	0.18 ± 0.01
Dg	846.0 ± 20.2	245.1 ± 12.3	10.1 ± 0.5	5.50 ± 0.10	0.21 ± 0.02	0.92 ± 0.02	0.11 ± 0.01
M	80.7 ± 4.1	114.2 ± 5.7	2.3 ± 0.1	1.72 ± 0.03	0.73 ± 0.02	0.40 ± 0.02	0.32 ± 0.01
S	94.3 ± 4.7	210.0 ± 10.5	7.7 ± 0.4	17.61 ± 0.80	0.60 ± 0.01	0.70 ± 0.02	0.41 ± 0.01

Here and in Tables 4, 5: T, tunic; M, mantle; S, stomach; Dg, digestive gland; G, gonads.

Table 4. The average metal concentrations in the organs and tissues of the ascidian *Halocynthia aurantium* from Kievka Bay (Peter the Great Bay, Sea of Japan) (mean value ± standard deviation), $n = 10$ for Fe, Zn, Mn, Cu, Ni, and Cd; $n = 8$ for Pb)

Organ	Concentration of metal, μg/g, dry weight						
	Fe	Zn	Mn	Cu	Ni	Pb	Cd
G	41.2 ± 2.1	202.4 ± 10.1	12.2 ± 0.6	4.30 ± 0.02	0.74 ± 0.01	11.32 ± 1.5	< 0.05
T	141.5 ± 7.1	14.2 ± 0.8	31.4 ± 1.6	2.75 ± 0.2	0.94 ± 0.01	0.93 ± 0.01	0.10 ± 0.01
Dg	898.0 ± 22.3	29.0 ± 2.3	24.6 ± 1.5	7.11 ± 0.2	1.31 ± 0.01	1.41 ± 0.03	0.20 ± 0.01
M	49.5 ± 2.8	65.4 ± 3.3	4.9 ± 0.5	1.41 ± 0.01	0.10 ± 0.01	7.45 ± 1.2	< 0.05
S	344.3 ± 17.5	188.1 ± 9.4	21.1 ± 1.1	4.23 ± 0.2	0.99 ± 0.01	14.81 ± 1.5	0.10 ± 0.01

gonads—Zn > Fe > Mn > Pb > Cu > Ni > Cd; in the tunic—Fe > Mn > Zn > Cu > Pb = Ni> Cd; in the digestive gland—Fe > Zn > Mn > Cu > Pb > Ni > Cd; in the mantle—Zn > Fe > Pb > Mn > Cu > Ni > Cd; and in the stomach—Fe > Zn > Mn > Pb > Cu > Ni > Cd.

The average concentrations of metals were unevenly distributed in the organs and tissues of ascidians. According to the series of decreasing metal concentrations obtained for the organs of *H. aurantium* from Postovaya Bay, in 2-year-olds, the highest concentrations of Fe, Ni, and Pb were found in the gonads; Mn and Cd, in the tunic; and Zn and Cu, in the stomach. In 3-year-old individuals, high concentrations of Fe, Zn, and Pb were observed in the digestive gland; Mn and Cu, in the tunic; Cd, in the stomach tissues, and Ni, in the mantle and gonads (Table 5). The differences are significant at $p \leq 0.05$.

The levels of biophilic Zn, Mn, and Cu in the organs and tissues of *H. aurantium* were similar in both age groups from Postovaya Bay (Table 3). In 2- and 3-year-old ascidians from Postovaya Bay, the content of Zn was higher in the digestive gland and stomach than in other organs ($p \leq 0.05$). The concentration of Mn was higher in the tunic ($p \leq 0.05$) and Cu in the gonads and stomach ($p \leq 0.05$). In individuals of different ages, the concentrations of Fe ($p \leq 0.05$) and Pb ($p \leq 0.05$) differed significantly. In the organs and tissues of ascidians from Kievka Bay, the concentrations of Fe, Cu, Mn, and Zn were significantly different ($p \leq 0.05$).

The maximum concentrations of Fe, Cu, Ni, and Cd in the organs of ascidians from this locality were found in the digestive gland; Mn, in the tunic; and Zn and Pb, in the gonads and stomach tissues (Tables 4, 5).

The results indicated significant differences ($P \leq 0.05$) in the contents of Fe, Zn, and Pb in the organs of *H. aurantium*, depending on the locality. In most organs, Fe and Zn concentrations of ascidians from Postovaya Bay were higher than those in animals from Reineke Island and Kievka Bay. However, in the gonads of *H. aurantium* sampled in Kievka Bay, the concentration of Zn was almost 3 times higher than that in the reproductive organs of ascidians from Postovaya Bay. A many-fold excess (by 5–74 times) of the

Table 5. The series of decreasing metal concentrations in the organs of individuals of different ages of the ascidian *Halocynthia aurantium* from Postovaya and Kievka bays (Sea of Japan)

Metal	Postovaya Bay		Kievka Bay
	2-year-olds	3-year-olds	2-year-olds
Fe	G > T > Dg > M > S	Dg > T > G > S > M	Dg > S > T > M > G
Zn	S > Dg > M > G > T	Dg > S > M > G > T	G > S > M > Dg > T
Mn	T > G > S > Dg > M	T > G > Dg > S > M	T > Dg > S > G > M
Cu	S > G > Dg > M > T	S > G > Dg > T > M	Dg > G ~ S > T > M
Ni	G > T > M ~ S > Dg	M > G > T > S > Dg	Dg > S > T > G > M
Pb	G > M > T > S > Dg	Dg > S > T > M > G	S > G > M > Dg > T
Cd	T > Dg > S > M ~ G	S > M > T > G > Dg	Dg > T ~ S > M ~ G

Pb concentration was found in ascidians from Kievka Bay, compared to *H. aurantium* in coastal waters of Reineke Island and Postovaya Bay. In each locality, ascidians were sampled once and only in the spring–summer period; therefore, the data obviously only approximately reflect the difference in the levels of element concentrations at a different time of the year.

DISCUSSION

The ranking of the elements Fe, Mn, Cu, Zn, Ni, Pb, and Cd in terms of their levels in the organs and tissues of *Halocynthia aurantium* indicates the variability of their concentrations and reflects the individual and biological features of regulation of trace elements in the tissues of ascidians in response to a complex of environmental factors, as well as the physiological status of the animals (growth processes, preparation for reproduction, etc.).

Comparison of the series of decreasing average concentrations of metals in the organs of ascidians showed that iron usually ranked first in terms of its content in most tissues. However, in a number of cases, zinc predominated in terms of the average concentration: in the mantle and stomach tissues of ascidians from Postovaya Bay and in the gonads of animals from Kievka Bay. The predominance of iron and zinc, the most important elements involved in metabolism, was found in many living organisms. These elements are a part of some biomolecules, play an important role in electron transfer reactions, and are involved in the control of intracellular processes. Iron is a component of the blood of ascidians; it is part of hematocytes, in particular morular cells. The presence of independent markers, among them, bound iron, is characteristic of the specific organelles of these cells [16]. Copper is also one of the most common essential trace elements, but in terms of concentration in the tissues of the ascidian *H. aurantium,* it is in the fourth place after manganese. A similar pattern of distribution of elements (Fe, Mn > Zn > Cu) was earlier reported for the tunic and mantle of other ascidian species in Peter the Great Bay [3, 4]. The concentration series ended with Ni, as well as with elements with pronounced toxic properties, Pb and Cd.

Ascidians concentrate high levels of transition metals in their tissues; the reason for this is not fully understood [27]. It is known that the elemental composition of organs and tissues of living organisms is dynamic, closely associated with the metabolic process, and depends on the physiological functions of certain organs and tissues; it is determined by the presence and ratio of organic metal-binding ligands in tissues [13, 14].

The results of this research showed the predominance of Mn in the tunic of *H. aurantium* of all age groups from different localities. Manganese binds to lipids, proteins, and polysaccharides of the cell. The ascidian tunic is a cellulose-polysaccharide fabric that allows the binding of metal ions, including manganese. The molecular basis of many metal-dependent biochemical processes is still not clear, despite the long history of research in this area [24, 27].

In the body of ascidians, manganese can be bound not only to the polysaccharides of the tunic, but also to blood proteins. The tunic of ascidians is permeated with blood vessels; it is known that Mn present in living organisms in various chemical forms can bind to blood proteins. As an example, in the blood serum of mammals, Mn is predominantly associated with high-molecular weight compounds, and its excess in the blood serum of mammals causes an increase in the level of low-molecular ligands [13]. The blood of ascidians contains proteins of different molecular weight. The main type of hemocyte in ascidians (60–80% of the total number of blood cells) are morular cells that contain a large number of vacuoles. It is known that these cells play a significant role in the formation of the tunic [16, 19]. However, there is no direct evidence in the literature confirming the dependence of Mn binding to high- or low-molecular proteins of the blood in ascidians.

The results of the present work are consistent with the data on the Mn content in the dense tunic of *H. aurantium* and *Boltenia echinata* [4]. Nevertheless, the predominance of Mn in the tunic was not observed in the ascidians *Styela clava* and *Ciona intestinalis* in Peter the Great Bay [3]. A higher Mn content in the tunic compared to other tissues of ascidians was also reported for *Microcosmus exasperatus* from the Mediterranean and Red seas [25] and for the same species in polluted waters of the port of Paranagua located in southern Brazil [23].

In the gonads of *H. aurantium* collected in May in Postovaya Bay, the concentrations of Fe and Cu were higher than those in the reproductive organs of ascidians from Kievka Bay collected in August. Perhaps this is due to seasonal differences, in particular, the development of gonads under conditions of accumulation or depletion of material and energy resources of cells, which are subject to change. In the gonads of *H. aurantium,* successive maturation of sex products, oocytes or spermatozoa, occurs several times during the year. In June, the size of mature oocytes in *H. aurantium* reaches a maximum of 274 μm [10].

Test cells are a type of iron-containing cell in ascidians that is not found in other animals. They transport substances that are necessary for oocyte maturation to oocytes and are probably responsible for the formation of the tunic during the larval period [19]. Iron may play a significant role in redox processes not only at the early stage of ontogeny, but also during the maturation of germ cells, which is reflected in the level of its concentration in the ascidian gonads.

Zn and Cu are also necessary elements for the reproductive system of aquatic organisms. The highest concentrations of these metals are characteristic of the stage of maximum maturity of gonads [15, 17, 25]. At the same time, it is known that zinc in high concentrations is a significant risk factor for the physiology of reproduction of ascidians [21]. Our data showed that in May the concentration of Zn in the gonads of *H. aurantium* from Postovaya Bay was lower than in other organs, while it was within the mean values reported for ascidians.

The predominance of biophilic elements in the stomach and digestive gland of *H. aurantium* from Postovaya Bay, compared to their content in other organs, reflects the paths of the intake and elimination of metals from the organism.

The uneven distribution of elements with pronounced toxic properties (Ni, Pb, and Cd) in the organs of *H. aurantium* depends on the physiological binding and excretion of metals, leading to the variability of these elements at the level of an individual and population as a whole.

As was shown based on the example of mollusks, the chemical composition of the body of aquatic organisms is formed due to the metabolism that developed during the evolution of the biosphere and the level of elements is connected with the habitat conditions and with the geochemical characteristics of the substrate [8].

The present results showed that the concentrations of Fe, Mn, and Zn in the tissues of *H. aurantium* from coastal waters of the Sea of Japan were within the range of values reported for different species of ascidians from other regions of the World Ocean [20, 23, 25]. The Cu and Ni contents were closer to the lower limits of the known concentration ranges for these elements. The Pb content in ascidians from waters near Reineke Island and Postovaya Bay was comparable to the value typical for the lower limits of concentration ranges. In contrast, in tissues of *H. aurantium* from Kievka Bay the concentration of Pb was closer to the upper limit.

Thus, biologically significant Fe, Mn, Zn, and Cu prevailed in the metal distribution among the organs and tissues of *H. aurantium*. The maximum concentrations of biophilic elements were found in organs that reflect the pathways of the input of elements into the body (stomach), as well as those responsible for metabolism (digestive gland) and reproductive function (gonads). The maximum concentrations of Mn were recorded in the tunic, which is an organ rich in biologically active substances. There was an uneven distribution of the concentrations of Ni, Pb, and Cd among the tissues of 2- and 3-year-old ascidians. Ascidians from Kievka Bay were characterized by the highest Pb content in tissues, compared to *H. aurantium* from the other studied localities. The concentrations of Fe, Mn, Zn, Cu, Pb, and Cd in the organs and tissues of *H. aurantium* from the studied areas were comparable to those in ascidians from Peter the Great Bay (Sea of Japan) and other regions of the World Ocean. Long-term observations are required to clarify the patterns of distribution of chemical elements among the organs and tissues of *H. aurantium* and to reveal both general tendencies and characteristic specific features of the content of elements in the organs of this species. The results we obtained indicated differences in the concentration of toxic elements in *H. aurantium* from different localities. This should be taken into account when organizing a mariculture farm. Based on the levels of metal concentrations in the organs and tissues of *H. aurantium*, this species can be proposed for use as a bioindicator organism.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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