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ORIGINAL PAPERS

Heavy Metal Content in the Seagrass *Zostera marina* Linnaeus, 1753 in Voevoda Bay (Amur Bay, Sea of Japan)

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Abstract—Concentrations of heavy metals in live and dead leaves, stems, rhizomes and roots of the seagrass *Zostera marina* Linnaeus, 1753 are analyzed in the study. On the basis of the concentration, the metals are arranged in the following order: Fe > Mn > Zn > Cu > Pb > Ni >Cd. Differences in Fe, Pb, Ni, and Cd for live and dead leaves are revealed. The factor of sediment enrichment in heavy metals from dead leaves is calculated. The enrichment factor for the studied metals changed in the order Cu > Pb > Zn > Ni > Mn, and the value of solubility product [–log(SP)] for sulfides of the above-listed metals changed in the same order.

Keywords: *Zostera marina*, heavy metals, Voevoda Bay, Amur Bay

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INTRODUCTION

Heavy metals (HM) invariably remain one of the leading groups among contaminating agents entering the environment. Metals enter the aquatic environment with atmospheric precipitation, through chemical rock weathering, and leaching of sorption forms from the soil, with surface runoff and waste (technical, agricultural, and household) waters. Moreover, pollution can be caused by cargo tankers transferring oil and petrochemicals, as well as other vessels [12]. HM are considered serious pollutants due to their toxicity, stability in the environment, and ability to accumulate in organisms.

The ability of HMs for bioaccumulation has been determined for various marine organisms: animals [14, 19, 28], algae [5, 11, 16, 39], and also seagrasses. *Zostera marina* Linnaeus, 1753 is among the latter and, as a possible indicator of the water condition, is one of the subjects of researchers' increased focus [15, 18, 20, 22–24, 26, 29, 30, 34, 42].

The main advantage of the *Z. marina* as an integral indicator of the littoral ecosystem condition consists of it being a key element of the ecosystem influencing the condition of many organisms in the water, at the water–bottom interface, and in the upper layer of the sediments [32]. Seagrass, as well as seaweeds, take up metals from the water absorbing them with the leaf blade, but, in contrast to algae, they have developed a root system through which they also take up metals from the pore water between sediment particles. One of the important *Z. marina* characteristics is its extremely high productivity [1, 2, 37] which leads to enrichment of sediments in organic matter [10]. It is clear that with such a high *Z. marina* production and bioaccumulation ability, we can expect HM magnification in the sediments covered by *Z. marina* beds. Moreover, a linear relationship between the content of organic matter and the concentration of some HM in the sediments covered by seagrass was found [6].

A distinctive feature of the sediments in Voevoda Bay is the high organic matter content and processes of sulfate reduction that occur there [6, 9].

The aim of this of this study is as follows: determine the concentrations of HM (Fe, Mn, Zn, Cu, Pb, Ni, and Cd) and organic carbon in different *Z. marina* organs (live and dead leaves, stems, rhizomes, roots); and assess the probability of seagrass enrichment of the upper sediment layer in HM. To achieve them, the following objectives were set: collection of *Z. marina* specimens and subsequent sample preparation; analyses for HM and organic carbon contents; and calculation of HM enrichment factor for the upper sediment layer.

MATERIALS AND METHODS

Voevoda Bay is located in the eastern part of the offshore area of Amur Bay (Peter the Great Bay, Sea of Japan) and deeply cuts into the western coast of Russky Island (Fig. 1). It consists of two shallow waterbodies: Kruglaya and Melkovodnaya bays. The water area of Voevoda Bay is about 4.0 km^2; Melkovodnaya Bay, about 2.4 km^2; and Kruglaya Bay,

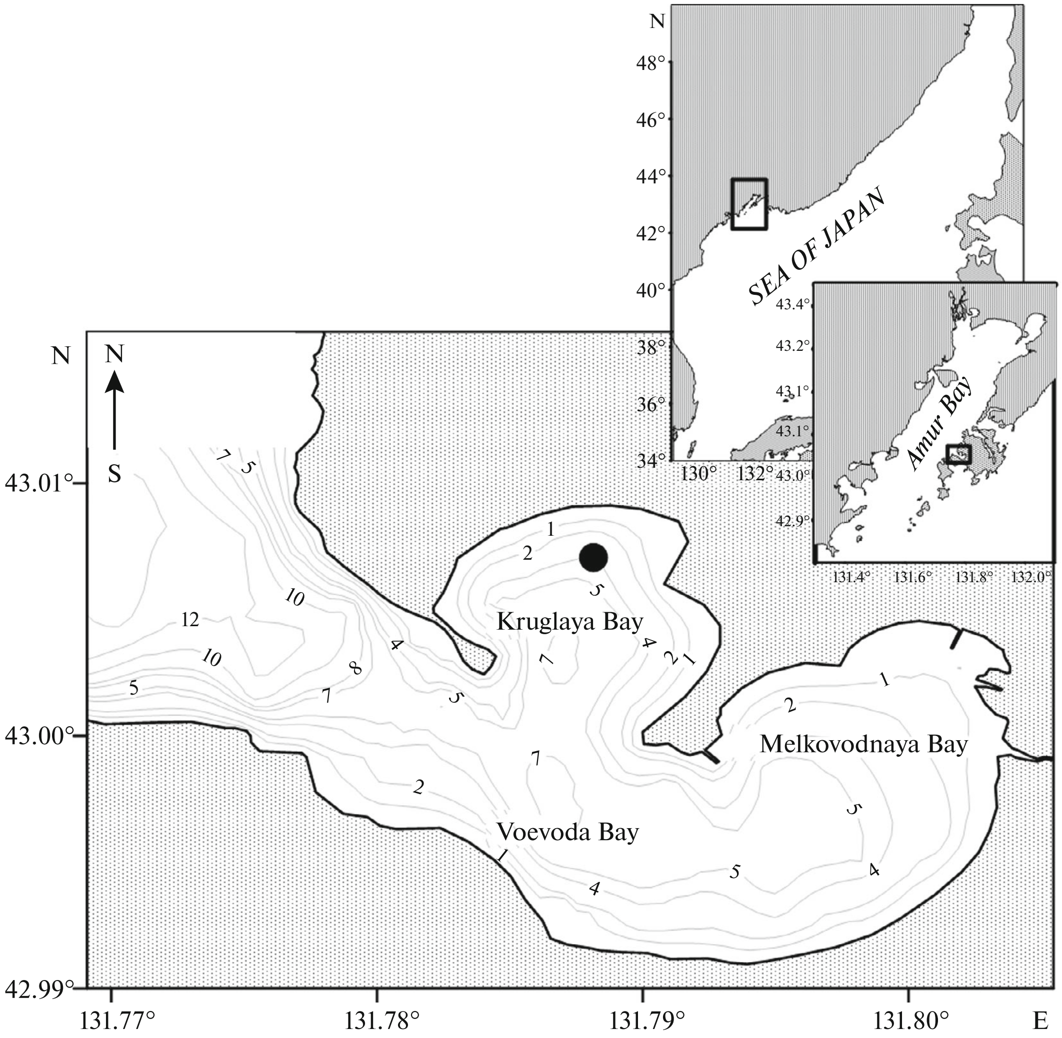

Fig. 1. Map of the sampling station located in Voevoda Bay.

about 1.6 km^2. The only small river, the Russkaya River, empties into the bay from the east.

Seagrass samples were collected in September 2022 in the enclosed shallow part of Voevoda Bay where extensive seagrass beds were found (Fig. 1). Core samples of bottom sediments had previously been collected here, and the contents of HM and organic carbon in them studied [6].

Divers gathered seagrass into plastic bags from a depth of approximately 4 m, washed it with seawater, and transported it to the laboratory in clean plastic bags. The seagrass was divided into five categories: live leaves, dead leaves, stems, rhizomes, and roots. The specimens were dried in a drying cabinet at 60°C. The plant material was powdered by grinding in a porcelain mortar. Sample preparation and analysis of the HM content were carried out according to the method by Brix and Lyngby [25]. A 1-g portion of the powdered plant material, additionally dried to a constant weight at 105°C, was placed in a 100-mL conical flask. Concentrated HNO_3 of the extra pure grade (20 mL) was added carefully in 5-mL portions while avoiding heavy foaming and splashing and then it was covered with a watch glass and left overnight. The next day, the samples were carefully heated, without removing the watch glass from the flasks, on an electric stove covered with an asbestos blanket until the solution cleared. Then 1 mL of H_2O_2 was added dropwise and heated for about 1 h once again. Afterwards, the solutions were evaporated to moist salts, supplemented with 1 mL of distilled water, evaporated to dryness, and again supplemented with 1 mL of water, and dried again. The dry residue was dissolved in 12 mL of 2% nitric acid. The HM content (Fe, Mn, Zn, Cu, Pb, Ni, and Cd) in different seagrass organs was determined by the flame atomic absorption spectrometry method on an AA-3600 atomic absorption spectrometer (Shimadzu, Japan).

Organic carbon (C_{org}) in the *Z. marina* organs was measured using a TOC-VCPN analyzer equipped with

Table 1. Heavy metal and organic carbon contents in different *Z. marina* organs and in the surface layer of bottom sediments

Substrate	Metal content (μg/g dry weight)							C_{org}, %	*n*
	Fe	Mn	Zn	Cu	Pb	Ni	Cd		
Live leaves	101 ± 10	285 ± 3	22.3 ± 1.1	3.0 ± 0.1	2.0 ± 0.5	1.6 ± 0.1	1.2 ± 0.3	35.03	5
Dead leaves	742 ± 1	245 ± 4	22.7 ± 1.2	3.1 ± 0.03	4.1 ± 0.2	3.2 ± 0.2	0.4 ± 0.2	33.23	3
Stems	70 ± 4	7.5 ± 0.3	41.3 ± 0.5	4.1 ± 0.8	1.5 ± 0.5	0.8 ± 0.1	0.5 ± 0.1	32.25	5
Rhizomes	119 ± 2	2.3 ± 0.1	18.0 ± 0.5	1.9 ± 0.6	2.3 ± 0.1	0.7 ± 0.3	0.3 ± 0.1	29.89	4
Roots	500 ± 20	32.8 ± 0.1	38.4 ± 1.7	4.1 ± 0.6	4.3 ± 0.4	1.4 ± 0.1	0.5 ± 0.1	34.43	2
Bottom sediments	–	208 ± 3	204 ± 4	91 ± 2	37 ± 2	27 ± 1.5	–	6.01	3
–log[SP(MeS)]	18.43	15.15	23.35	37.5	28.45	20.52	28.44	–	–
F_i	–	4.69	49.67	163.12	50.01	46.12	–	–	–

Average values of HM content and standard deviation from the mean are given. *n* is the number of specimens in the sample; –log[SP(MeS)] is the solubility product for metal sulfides according to the *Chemist's Handbook* [7]; F_i is the factor of sediment enrichment in HM for dead leaves.

an SSM-5000A solid sample combustion unit (Shimadzu, Japan). The analyzer's principle is based on the measurement of the infrared radiation absorption by the carbon dioxide that is formed during the combustion of organic and inorganic compounds containing carbon. Dry grinded weighed portions of the sediment were burnt in a flow of extra pure oxygen (99.995%) at 900°C to determine the total carbon content. To determine the inorganic carbon content, samples were acidified by orthophosphoric acid and burnt at 200°C. The quantity of organic carbon was calculated as the difference between the total and inorganic carbon.

The factor of bottom sediment enrichment (F_i) in HM was calculated using the following equation:

$$F_i = \frac{C_{i(\mathrm{sed})} C_{\mathrm{card}(ZM)}}{C_{\mathrm{eard(sed)}} C_{\mathrm{i}(ZM)}}, \qquad (1)$$

where $C_{i(\mathrm{sed})}$ and $C_{i(ZM)}$ are the concentration of the *i*th metal in the bottom sediment (μg/g) and in the dead *Z. marina* leaves (μg/g); $C_{\mathrm{carb(sed)}}$ and $C_{\mathrm{carb}(ZM)}$ are the concentration of organic carbon in the bottom sediment (gC/g) and dead leaves (gC/g) [3, 21].

RESULTS

The concentrations of HM and organic carbon in different organs of *Z. marina* that we obtained are presented in Table 1. The concentrations of HM (μg/g) in the upper layer of sediments (0–5 cm) and the value of sediment HM enrichment factor (F_i) for dead leaves are also provided in this table.

Dead leaves and roots of seagrass contained the highest amount of iron, while stems had the lowest amount. The concentrations of the metal in rhizomes and leaves were comparable. The highest content of manganese was found in live and dead leaves and the lowest content was detected in the stems and rhizomes. A much lower manganese content was found in the roots than in leaves, but it was much higher than in the stems and rhizomes. The highest amount of zinc and copper was detected in the stems and roots. Substantially lower amounts of these metals were in live and dead leaves. The highest lead content was recorded from roots and dead leaves. In rhizomes and live leaves and stems, its concentration decreased. The highest nickel content was observed in dead leaves; the nickel concentration decreased in roots and live leaves. Its lowest content was found in stems and rhizomes. The highest cadmium concentration was observed in live leaves (Table 1).

The factor of enrichment of the upper sediment layer in the toxic metals ranged from 4.69 (Mn) to 163.1 (Cu) (Table 1).

DISCUSSION

Comparison of the Obtained Heavy Metal Concentrations with the Published Data

Background HM concentrations in sediments [19] and seawater for the littoral zone of the Sea of Japan [17] are presented in Table 2. The HM content is commonly provided in the literature for the aboveground and the belowground parts of *Z. marina*. In the latter case, all organs of the seagrass are examined separately. The published results are summarized in Table 3.

As follows from Table 3, the iron concentrations in the aboveground and belowground *Z. marina* parts vary within a wide range. The lowest iron content corresponds to the stems; the highest content can be found in the roots and dead leaves; the live leaves and rhizomes contain intermediate concentrations. The obtained results are consistent with the data obtained by Shishlova [18] for Vostok Bay in terms of the pat-

Table 2. Background concentrations of heavy metals in coastal waters of the Sea of Japan

Substrate	Fe	Mn	Zn	Cu	Pb	Ni	Cd
1	3.5	350 ± 80	100 ± 10	28 ± 3	35 ± 5	45 ± 5	0.2 ± 0.1
2	1–10	2.6–20	0.5–0.8	0.3–1.2	0.05–0.1	–	0.005–0.04

(1) In bottom sediments, where Fe is expressed in % and other metals in μg/g [19]; (2) in seawater, μg/L [17].

terns of the iron contents in the *Z. marina* organs rather than in the order of magnitude.

The manganese concentration data that we obtained are also consistent with the data from the published sources. The manganese content in the *Z. marina* aboveground part is commonly higher compared to the belowground part.

The zinc and copper concentrations that we recorded from the aboveground part of *Z. marina* are comparable with the concentration of these metals in *Z. marina* growing near the port of Posyet [27] in the order of magnitude. In addition, we noted a lower content of these metals in rhizomes compared to those in roots, which is consistent with the data obtained earlier [18, 35].

The contents of lead and nickel in the *Z. marina* roots are almost twice as high as those in rhizome. The same pattern is observed for Vostok Bay [18]. The results for lead content in *Z. marina* agree with the study for the Black Sea coast of Turkey [20], and the data on the nickel content is consistent with those from the port of Posyet [27].

The cadmium concentrations obtained in the study are consistent with the data for the aboveground and belowground *Z. marina* parts from Denmark [35] and South Korea [33].

Distribution of Heavy Metals in the Seagrass Zostera Marina

Iron

The iron content in macrophytes such as *Z. marina* is usually higher than in other heavy metals [17]. It is known that ferrous hydroxides exist in seawater in the form of fine suspended particles [13]. Evidently, the abundance of fine suspended matter enriched in Fe is the main cause of the sharp increase in concentrations of this element in leaves, especially in dead ones.

The elevated levels of iron in the roots (500 ± 20 μg/g dry weight, d.w.) can be explained by their immediate proximity to the sediments and the fact that they contained small granules inside that were not completely washed out.

Manganese

The elevated manganese content in leaves can be due to the fact that this element is part of the wide range of metalloenzymes and is involved in the photosynthesis process [18]. This explains its high amount in live leaves (285 ± 3 μg/g d.w.) and the decrease in content in dead leaves (245 ± 4 μg/g d.w.).

Zinc and Copper

Trace elements are involved in almost all processes taking place in the plant cell: energy metabolism, primary and secondary metabolism, hormonal regulation, and signal transmission. It is worth noting that 25–50% of all proteins work only in the presence of metal ions, of which the highest amount (more than 1200) are functionally related to zinc [8]. Copper, as a trace element, is an essential member of the electron transport chain of respiration, photosynthesis, oxidation reaction, and metabolism of proteins and carbohydrates [4]. The largest amount of zinc (41.3 ± 0.5 μg/g d.w.) and copper (4.1 ± 0.8 μg/g d.w.) was revealed in stems, whose main function is the transport of nutrients between the root system and leaves. High amounts of zinc (38.4 ± 1.7 μg/g d.w.) and copper (4.1 ± 0.6 μg/g d.w.) are also present in roots. There is no difference in the concentrations of the discussed trace elements between live and dead leaves. This indicates that these trace elements are present in the water in the dissolved form [13], not in the form of a suspension, and enter *Z. marina* through the root system [38].

Lead

The pattern of the lead distribution in plant organs and tissues is similar to that of iron. Both elements are in a suspended state in the water, easily precipitated and accumulated in sediments, and, therefore, they are accumulated mainly by *Z. marina* rhizomes and by dead leaves [18]. The same results are obtained for nickel. A study of the role of decomposing *Z. marina* leaves in the metal accumulation by surface sediments revealed that the presence of epiphytes on *Z. marina* leaves leads to an increase in the lead content [30]. The same pattern was observed in our study. It is assumed that lead can accumulate in decomposing leaves due to leaf fragmentation by invertebrates, which increases the surface area of the leaves [41], and the formation of biofilm leads to metal concentration [40]. The authors suggest that the water column is a source of metals during their accumulation in decomposing leaves.

Table 3. Published data on heavy metal content in *Z. marina* (μg/g dry weight)

Plant parts	Study area	Metals						
		Fe	Mn	Zn	Cu	Pb	Ni	Cd
Young leaves	Vostok Bay, Sea of Japan [18]	164	732	77.2	6.4	12.8	4.3	2.2
Old leaves		331	2118	28.9	4.9	8.4	5.8	3.8
Stems		505	288	31	5.3	9.4	3.6	1.8
Rhizomes		663	64.3	21.2	4.6	8.6	2.4	1.4
Roots		5214	555	29	9.4	17.4	8.5	1.9
Underground part	Port of Posyet							
	Cape Shelekh	268 ± 24	674 ± 43	31 ± 3.8	5.3 ± 2.1	9.1 ± 0.5	2.7 ± 0.3	2.3 ± 0.1
	Cape Ryazanov	297 ± 81	358 ± 29	26 ± 1.4	3.8 ± 0.7	11.4 ± 4.9	3.5 ± 0.3	1.9 ± 0.1
	Cape Morozov	235 ± 13	573 ± 88	25 ± 1.9	5.3 ± 0.1	8.2 ± 0.5	2.3 ± 0.1	1.6 ± 0.2
	Reid Pallada Bay Cape Mramorny [27]	214 ± 16	240 ± 27	21 ± 0.3	2.8 ± 0.2	8.9 ±4.5	2.3 ± 0.1	2.3 ± 0.1
Leaves	Peter the Great Bay, Sea of Japan [15]	23–537	58–925	12.6–35.8	2.3–6.2	–	1.8–4.5	1.1–3.4
Rhizomes		463–5742	16–142	12.5–40.5	3.5–9.1	–	0.4–5.3	0.5–2.6
Underground part	Onsan Bay	100.6	198.9	53.9	20.3	1	–	0.6
	Jaran Bay	68.9	187.9	26.1	20.8	2.1	–	0.6
	Koje Bay	83.1	174	20.1	17.1	1	–	0.2
Aboveground part	South Korea [33]	268.3	13.5	25.6	14.4	0.8	–	0.2
		291.5	15.7	13.1	12.1	1.5	–	0.5
		176	10.3	12.9	9.9	0.9	–	0.2
Underground part	Limfjord, Denmark [26]	80–2990	480–5770	41–175	2.7–0.7	0.5–3.1		0.1–2.9
Aboveground part		101–4080	74–1200	25–125	1.8–5.8	0.4–2.8		0.1–0.9
Live leaves	Limfjord, Denmark [35]	–	–	107–270	–	2.9–30.4	–	0.3–1.5
Dead leaves		–	–	148	–	31.4	–	0.5
Stem		–	–	62	–	2.9	–	0.3
Rhizomes		–	–	36–90	–	1.5–5.2	–	0.3
Roots		–	–	134	–	40.6	–	0.6
Underground part	Black Sea coast of Turkey [20]	1719.7	761.8	114.8	13.6	1.9	7.3	0.1
Aboveground part		4151	444	115.2	14	3.7	11.5	0.03

Nickel

It is known that nickel can be adsorbed by different minerals, e.g., sulfides and oxyhydroxides of manganese and iron [31]. Consequently, nickel can be coprecipitated together with iron and can stick to *Z. marina* leaves. This can explain the high content of this metal in leaves, especially in dead ones.

Cadmium

It was shown that during the decomposition of seagrass leaves by epiphytic organisms the cadmium content in leaves decreases [30]. The cadmium content decrease in decomposing leaves indicates a more pronounced leaching than accumulation despite the fact that the metal accumulation in leaves occurs under freshwater conditions [41]. Moreover, cadmium is known to be present at relatively low concentrations, compared to those of copper and lead, in dissolved organic substances containing humic acids [36, 43]. The properties of the humic substances can be responsible for the different tendencies of the accumulation of trace elements by decomposing leaves.

Factor of Sediment Enrichment in Heavy Metals

The microbiological degradation of organic matter of the *Z. marina* leaf litter leads to an increase in the ratio of the unit weight of the metal to the unit weight of organic matter, as the metal does not vanish and the organic matter is converted into inorganic form, mainly CO_2. Therefore, the metal concentration per unit organic carbon increases. The factor of sediment enrichment represented by Eq. (1) should be identical for all metals if only the following process occurs: microbiological degradation of organic matter. Actually, the enrichment factor varied from 4.69 (Mn) to 163.1 (Cu) (Table 1). From our point of view, when metals enter sediments together with organic matter residues, they begin splitting into two phases: stationary and mobile. The stationary phase is a metal sulfide that is formed as a result of sulfate reduction. This process occurs during the microbiological degradation of organic matter in bottom sediments with the absence of oxygen [9]. The HM organic complexes are the mobile phase. It should be noted that the enrichment factor for the studied metals changed in the following order: Cu > Pb > Zn > Ni > Mn (Table 1). The value of the solubility of the product [–log(SR)] according to the *Chemist's Handbook* [7] varied in the same order. It is evident that the less soluble the sulfide (CuS) the higher the proportion of the stationary phase becomes for each metal in the form of MeS. The mobile phase in the form of organic complexes can be removed from sediments by marine organisms (detritivores). Thus, some metals accumulate in sediments, while others are removed. Obviously, the removal of the mobile metal phase by marine organisms is also accompanied by fractionation.

We believe that this may be caused by the competition for the place of a complex formation between metal ions that are recycled together with organic substances by marine organisms. Relative to manganese, the ions of copper, zinc, and lead are inhibitors of a complex formation. Less stable complexes are excreted by marine organisms into the near-bottom water layer (e.g., manganese), which leads to the secondary pollution of the water layers above [44].

It has been noted that the stability of metalloorganic compounds with copper is higher than with nickel, which is comparable with the row of Irving—Williams stability constants ($Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$), which is based on a complex formation with simple organic ligands [45]. As follows from this order, more stable complexes with organic ligands are formed in the direction from manganese to copper. The higher the value of the stability constants the stronger the formed complex and the longer it remains in the mobile form of bottom sediments.

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ETHICS APPROVAL
AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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