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PRELIMINARY RESULTS OF THE COMPREHENSIVE ASSESSMENT OF CONTAMINATION AND TOXIC EFFECTS OF BOTTOM SEDIMENTS IN SHALLOW BIGHTS OF AMUR BAY (SEA OF JAPAN)

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In August 2023, surface bottom sediments (top 5-cm layer) were sampled in three bights of Amur Bay: Melkovodnaya Bight (V1), Kruglaya Bight (V2) (both within Voevoda Bight), and Uglovoy Bight (U1). Despite being neighboring bays, V1 and V2 differ significantly in granulometric composition and distribution of both heavy metal concentration and organic carbon content. Elevated levels of heavy metals and organic carbon were revealed in Kruglaya Bight (at station V2). In its bottom sediments, values of the enrichment factor (EF) for each analyzed metal, except for Co, exceeded levels attributable to natural weathering processes. A high level of enrichment was registered for Cd (EF = 24.08); a moderately high one, for Cu (EF = 6.15) and Pb (EF = 5.51); and a moderate one, for Ni, Zn, and Cr (EF accounted for 4.5, 4.3, and 3.21, respectively). The calculation of the modified degree of contamination index (mCd) showed a moderate degree of contamination at sta. V2 (mCd = 4.38). In bottom sediments from sta. V1, most metals were characterized by a low level of enrichment, except for Cd, with its moderate level (EF = 4.07). EF values for bottom sediments from sta. U1 were significantly below 1.5 providing evidence for the natural-origin input of heavy metals from weathering processes. In the studied bays, biotesting was carried out for the first time, with larvae of the sea urchin *Scaphechinus mirabilis* serving as a test object. During bioassays, the highest abundance of abnormally developed larvae was found at sta. V2. In aqueous extracts of bottom sediments from sta. V2, none of larvae reached the gastrula stage, and the value of the integral toxicity index (ITI) was 9.84 points. In aqueous extracts from sta. V1 and U1, normally developed larvae at the late gastrula stage predominated 18 h after the start of the experiment. In extracts of bottom sediments from Melkovodnaya Bight (sta. V1), the overwhelming majority of larvae developed with severe morphological abnormalities. Larvae at the prism and early pluteus stages were characterized by poorly developed skeletal rods or their lack. Moreover, we found exoforms of larvae with no intestine in their body cavity (it was protruded outward). ITI for bottom sediments from sta. V1 (7.72 points) was twice as high as for samples from sta. U1 (3.5 points). The obtained data indicate that the accumulation of heavy metals in Kruglaya Bight is driven by a combination of factors. In their turn, high concentrations of heavy metals and organic carbon may mediate the formation of unfavorable conditions for benthic organisms.

Keywords: Amur Bay, biotesting, bottom sediments, organic carbon, heavy metals, *Scaphechinus mirabilis*

Sediments play a significant role in marine ecosystem functioning, as those serve as a habitat for benthos. Moreover, in sediments, accumulation and transformation of nutrients occur, as well as of a wide range of pollutants delivered to marine systems *via* terrestrial runoff and precipitation [Roberts, 2012]. Monitoring surveys and research on environmental pollution often target heavy metals currently defined as metals with an atomic mass exceeding 50 atomic units: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Sn, Hg, Pb, Bi, *etc.* [Naleta et al., 2015]. These contaminants have significant toxic and cumulative effects [Rouchon, Phillips, 2017]. Consequently, an analysis of heavy metal concentrations in marine ecosystems remains an integral component of environmental quality assessments[Marine Water Pollution, 2022; Wilbers et al., 2014].

The toxicity of sediments can be evaluated by comparing concentrations of certain heavy metals and international regulatory values (sediment quality guidelines, SQGs [MacDonald et al., 2004]), and by calculating various individual and comprehensive indices. These approaches only provide information about potential toxic risks to hydrobionts. To fully assess risks associated with pollutant exposure, biological methods are additionally applied. One of them is bioassays on marine sediment extracts involving different test organisms. In this context, echinoderms are widely used as model organisms in ecotoxicological studies, as their responses to environmental stressors can be observed at various levels of biological organization: from the molecular genetic level to the ecosystem one [Canty, 2009]. The use of sea urchin larvae allows for the estimation of teratogenic effect of heavy metals [Chiarelli et al., 2019; Mazur et al., 2020; Rouchon, Phillips, 2017]. Furthermore, these test organisms are listed in numerous regulatory documents as the ones recommended for assessing the toxicity of marine sediments [ABNT-NBR 15350 DE 03/2023, 2025; ASTM, 2012].

Kruglaya and Melkovodnaya bights, located on the western side of Russky Island, are adjacent areas exhibiting pronounced differences in grain size distribution, and also in distribution of heavy metals and organic carbon [Ryumina et al., 2023]. Kruglaya Bight hosts extensive meadows of a seagrass *Zostera marina* Linnaeus, 1753. Moreover, there is an aquaculture farm, where the Yesso scallop *Mizuhopecten yessoensis* (Jay, 1857) and the Pacific blue mussel *Mytilus trossulus* A. Gould, 1850 are cultured [Gavrilova, Kucheryavenko, 2011]. The “Bely Lebed” sanatorium renowned for its therapeutic muds is located on Melkovodnaya Bight shores. Hydrological and hydrochemical studies in Voevoda Bight have shown that its offshore and middle sites (excluding Kruglaya Bight) are the areas that freely exchange waters with Amur Bay [Barabanshchikov et al., 2015]. In Voevoda Bight, the nature of biogeochemical processes is determined by the intensity of the production and decomposition of organic matter derived from three sources: phytoplankton, benthic macrophytes, and periphyton. In Melkovodnaya Bight, its specific coastal morphology, when combined with the effect of river runoff peaking in summer, results in closed cyclonic circulation. Consequently, water exchange between Melkovodnaya Bight and Amur Bay is weakened. Hydrochemical parameters in the bight have local features. Its circulation pattern can facilitate summer phytoplankton blooms that are primarily driven by an increased supply of nutrients from the Russkaya River runoff [Barabanshchikov et al., 2015]. The resulting excess of phytoplankton biomass, not eaten by zooplankton, is decomposed consuming oxygen and releasing carbon dioxide. Along with this, dead fragments of *Z. marina* are decomposed.

The above-described mechanism cannot be applied to Kruglaya Bight, as its waters are not affected by river runoff. There, the oxygen deficiency and elevated partial pressure of carbon dioxide are driven by the decomposition of organic matter produced mainly by a seagrass *Z. marina*.

Hydrochemical studies of Uglovoy Bight have revealed its ongoing ecosystem degradation [Tishchenko et al., 2021]. Its visible manifestations include the disappearance of *Z. marina* meadows, the occurrence of hypoxic events, and the fact that bight waters became a source of carbon dioxide for the atmosphere. Pollution of sediments with heavy metals occurs constantly as a result of economic activity on the bight shores. Pollutants mostly come with river runoff, due to sheet erosion from the urbanized landscape, and because of the operation of a low-water bridge [Petukhov et al., 2023]. The construction of this bridge (De Frieze–Sedanka) in 2009–2012 also noticeably affected sediments in the bay (now De Frieze Peninsula and Muravyov-Amursky Peninsula are connected by a four-lane highway). Hydraulic engineering construction in the coastal zone is typically accompanied by a significant rise in water turbidity and pollutant concentrations. The volume of suspended matter entering the sea can be comparable to the discharge and annual runoff of large rivers [Mishukov et al., 2009].

The input of heavy metals into sediments of Voevoda Bight is poorly studied. Bioindication research on pollution in coastal waters of Russky Island revealed elevated concentrations of heavy metals in brown algae of the genus *Sargassum*, *S. miyabei* Yendo, 1907 and *S. pallidum* (Turner) C. Agardh, 1820, at stations on the Amur Bay side [Khristoforova et al., 2018]. These toxicants usually enter the aquatic environment through precipitation, chemical weathering of rocks, leaching of sorbed forms from soils, surface runoff, and wastewater (industrial, agricultural, and domestic). Crude oil tankers, product tankers, and other vessels can also contribute to pollution [Khristoforova et al., 2015]. According to previous geochemical surveys, the highest content of zinc and copper was registered in sediment samples from Kruglaya Bight, while the highest concentrations of chromium, nickel, and cobalt were recorded at stations in Uglovoy Bight [Ryumina et al., 2023].

The aim of this study was to carry out a preliminary comprehensive assessment of heavy metal contamination and toxic effects on hydrobionts in surface sediments (top 5-cm layer) from several sites in Amur Bay. The assessment integrates chemical analysis with biotesting involving embryos and larvae of the sand dollar *Scaphechinus mirabilis* (Agassiz, 1864). This test organism is used for bioassay in Amur Bay areas for the first time.

MATERIAL AND METHODS

Uglovoy Bight and Voevoda Bight are shallow second-order basins of Amur Bay (Peter the Great Bay, Sea of Japan) (Fig. 1). The area of Uglovoy Bight is 42 km^2. Numerous small streams and rivers flow into its eastern and northeastern shores; the largest rivers are Chernaya, Bogataya, Vesennyaya, Pyaty Klyuch, Peschanka, Saperka, Gryaznukha, and Aerodromnaya [Losev, 2019]. Voevoda Bight consists of two shallow basins: Kruglaya Bight and Melkovodnaya Bight. The water area of Voevoda Bight is approximately 4.0 km^2 (Melkovodnaya is about 2.4 km^2, and Kruglaya is about 1.6 km^2). The only river on the island, Russkaya (a small one), flows into the bight from the east. Detailed hydrological descriptions of these basins are provided in [Barabanshchikov et al., 2015; Rakov, 2008]. Voevoda Bight is partially covered with *Z. marina* meadows. In Uglovoy Bight, *Z. marina* formed meadows in the 1980s [Rakov, 2008]; by now, they have disappeared [Tishchenko et al., 2021].

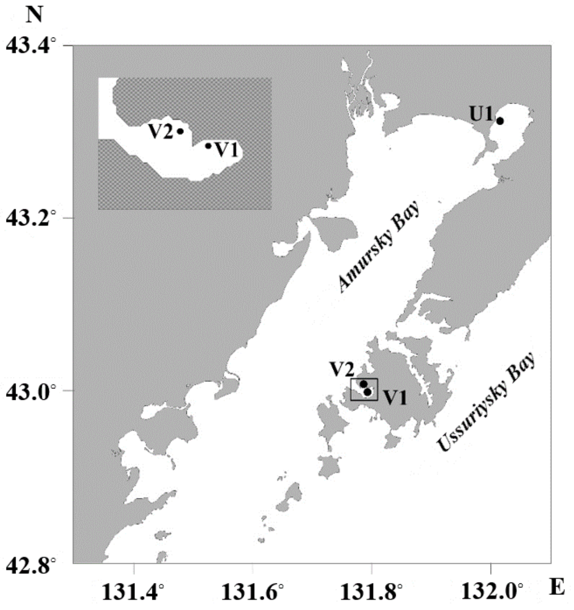


Fig. 1. Geographical location of the study area and sampling stations (hereinafter, V1, Melkovodnaya Bight; V2, Kruglaya Bight; U1, Uglovoy Bight)

In August 2023, three samples of surface sediments (top 5-cm layer) were taken by scuba diving. The sampling covered an area of 1 m^2 and depths of 1.5–3.5 m. A plastic container was used. Three sites within Amur Bay were surveyed: Melkovodnaya Bight (V1), Kruglaya Bight (V2), and Uglovoy Bight (U1). Location of stations is shown in Fig. 1. At sta. V2, sediments were covered with *Z. marina* meadows. The sampled material was delivered to a laboratory for subsequent analysis. The portion of sediments used for bioassay was sorted, frozen, and stored at −18 °C.

Concentrations of heavy metals (Fe, Mn, Zn, Cu, Pb, Cr, Ni, Co, and Cd) in the samples were determined by flame atomic absorption spectrometry on a Shimadzu AA-3600 spectrophotometer (Japan). The samples were prepared in accordance with the methodology for the quantitative chemical analysis of soils, sediments, sewage sludge, and waste (federal environmental regulation PND F 16.1:2.2:2.3:3.36-2002) involving acid digestion. Considering the cumulative errors at all stages of sample preparation and the measurement error inherent to the flame atomic absorption method, the total relative error was estimated to be 5%.

The concentration of organic carbon (C_{org}) in the solid phase of sediments was determined on a TOC-VCPH analyzer coupled with a Shimadzu SSM-5000A solid sample combustion unit (Japan). The analyzer was calibrated with certified reference material: sediment standard "Limestone Silt" (state standard sample GSO 1758-80). Analytical precision was based on parallel measurements, and it was ±3% of the determined value. The result is given as the percentage of C_{org} in dry sediments.

Grain size distribution was assessed on an ANALYSETTE 22 NanoTec laser particle analyzer (Fritsch, Germany). Natural moisture samples were homogenized. To create a dispersion medium, a surfactant 901 (Fritsch) was used. The analyzer was calibrated using a certified standard F-500 (Fritsch).

Pollution and environmental risk indices. Heavy metal concentrations determined in this study were compared with background levels for Peter the Great Bay [Kovekovdova, Simokon, 2004], permissible values [Marine Water Pollution, 2022], and threshold ones [MacDonald et al., 2004] for marine sediments. Also, individual pollution and environmental risk indices were calculated [enrichment factor (EF) and modified hazard quotient (mHQ)], as well as comprehensive ones [modified contamination degree (mCd) and toxic risk index (TRI)].

Individual indices. Enrichment factor (EF) is an effective tool for quantifying the contribution of each analyzed toxicant to the overall sediment pollution load. Its calculation involves the comparison of obtained heavy metal concentrations to those of conservative elements (Al or Fe), less susceptible to anthropogenic load [Li et al., 2015]. In this study, Fe was selected as a reference element. EF value higher than 1.5 indicates an anthropogenic source of heavy metals, whereas a value lower than 1.5 suggests a predominantly natural origin from weathering processes [Li et al., 2015]. Obtained factor values are classified into seven enrichment categories, as detailed in Table 1. EF was calculated by the formula as follows:

$$EF = \frac{(C_n/C_{Fe})_{sample}}{(B_n/B_{Fe})_{background}} ,$$

where $(C_n/CF_e)_{sample}$ is the ratio of a heavy metal content to Fe concentration in an analyzed sediment sample;

$(B_n/BF_e)_{background}$ is the natural background ratio of heavy metal content to Fe concentration in bottom sediment samples at a reference site (Table 2).

Table 1. Classification of indices

Class	Enrichment factor, EF		Modified hazard quotient, mHQ		Modified contamination degree, mCd		Toxic risk index, TRI	
	Value	Degree of enrichment	Value	Contamination severity	Value	Contamination degree	Value	Risk level
1	EF ≤ 1	depletion	mHQ ≤ 0.5	nil to very low	mCd ≤ 1.5	very low	TRI ≤ 5	no toxic risk
2	1 < EF ≤ 3	minor	0.5 < mHQ ≤ 1.0	very low	1.5 < mCd ≤ 2	low	5 < TRI ≤ 10	low
3	3 < EF ≤ 5	moderate	1.0 < mHQ ≤ 1.5	low	2 < mCd ≤ 4	moderate	10 < TRI ≤ 15	moderate
4	5 < EF ≤ 10	significant	1.5 < mHQ ≤ 2.0	moderate	4 < mCd ≤ 8	high	15 < TRI ≤ 20	considerable
5	10 < EF ≤ 25	high	2.0 < mHQ ≤ 2.5	significant	8 < mCd ≤ 16	very high	TRI > 20	very high
6	25 < EF ≤ 50	very high	2.5 < mHQ ≤ 3.0	high	16 < mCd ≤ 32	extremely high		
7	EF > 50	extremely high	3.0 < mHQ ≤ 3.5	very high	mCd > 32	ultra-high		
8			mHQ > 3.5	extreme				

Table 2. Content of heavy metals (mg·kg^{-1}) and organic carbon (%) in the surface layer of bottom sediments of Melkovodnaya Bight (V1), Kruglaya Bight (V2), and Uglovoy Bight (U1); values of background (BC) and permissible (PC) concentrations; and values of threshold exposure levels (TEL), probabilistic exposure levels (PEL), and severe exposure levels (SEL) for heavy metals analyzed

Element	Bights (n = 3)			BC^1	PC^2	TEL^3	PEL^3	SEL^3
	V1	V2	U3					
Fe	14,800	34,500	15,500	–	–	–	–	–
Mn	147	207	170	–	–	–	–	–
Zn	44.4	134	43.3	50	140	124	271	820
Cu	13.3	38.8	5.9	10	35	18.7	108	110
Pb	13	34.3	13.3	10	85	30	112	250
Cr	17	40	24	20	100	52.3	160	110
Ni	9	28	18	10	35	15.9	42.8	75
Co	4.3	7.3	7.5	8	20	–	–	–
Cd	0.3	1.5	0.3	0.1	0.8	0.68	4.21	10
C$_{org}$	0.83	4.83	1.03					

Note: *n*, number of samples; ^{1}, [Kovekovdova, Simokon, 2004]; ^{2}, [Warmer, van Dokkum, 2002]; ^{3}, [MacDonald et al., 2004].

Modified hazard quotient (mHQ) characterizes the degree of risk of toxic effect from each studied heavy metal on marine hydrobionts [MacDonald et al., 2004]. The calculation is based on the ratio of the pollutant concentration in sediments to SQGs values for this toxicant: threshold effect level (TEL), probable effect level (PEL), and severe effect level (SEL) (Table 2). To determine mHQ, the following formula is used:

$$mHQ = \sqrt{\frac{C_i}{TEL_i} + \frac{C_i}{PEL_i} + \frac{C_i}{SEL_i}}\,,$$

where C_i is the concentration of the toxicant *i*;

TEL_i, PEL_i, and SEL_i are values of TEL, PEL, and SEL for the toxicant *i*.

The obtained values are ranked according to eight hazard levels (Table 1).

Comprehensive indices. Modified contamination degree (mCd) characterizes the sediment contamination degree and is defined as the sum of all contamination factors (Cf) [Brady et al., 2014]. To calculate mCd, the following formula is applied:

$$mC_d = \frac{\sum_{i=1}^{n} Cf^i}{n}\,,$$

where Cf^i is the contamination factor;

n is the total number of analyzed heavy metals.

The classification of contamination degrees is given in Table 1.

Toxic risk index (TRI) allows for the comprehensive assess of the toxicity for the sediments with TEL and PEL taken into account. The criterion is used to normalize the toxicity of various pollutants. Also, it is applied for further comparison of effects on biological systems [Li et al., 2015]. TRI is calculated as follows:

$$TRI_i = \sqrt{\frac{\left(\frac{C_i}{TEL_i}\right)^2 + \left(\frac{C_i}{PEL_i}\right)^2}{2}}\,; \quad TRI = \sum_{i=1}^{n} TRI_i\,,$$

where C_i is the concentration of the analyzed pollutant *i*;

n is the total number of pollutants;

TEL_i and PEL_i are values of TEL and PEL for the toxicant *i* (Table 2).

The results of TRI calculation are ranked according to five risk levels (see Table 1).

Ecotoxicological assessment. Bioassays with the sand dollar *S. mirabilis* were carried out at the “Vostok” marine biological station (NSCMB FEB RAS). For the experiment, we used seawater sampled in Vostok Bay from a 6-m depth *via* a supply system. Seawater was filtered through a three-stage gravel filter and subsequently treated with ultraviolet light.

Aqueous extracts from sediments were prepared following a standard technique [Beiras et al., 2003]. Pre-thawed sediment samples (100 g) were placed in conical flasks and filled with 400 mL of sterile seawater. The mixtures were agitated on an orbital shaker Biosan PSU-20i (Latvia) for 2 h. Subsequently, the samples were allowed to settle, and then filtered through a membrane filter (pore size of 2–3 μm).

Adult mature *S. mirabilis* were sampled along the western coast of Vostok Bay (Peter the Great Bay, Sea of Japan) at a depth of 4–4.5 m. The sand dollars were placed in containers with ice packs to ensure their safe transport to the laboratory. *Prior* to the experiment, sea urchins were acclimated in aquariums with flow-through seawater for 48 h. The temperature was +18…+19 °C.

Spawning of the animals was induced by injecting 0.2 mL of 0.5 M potassium chloride solution into perivisceral cavity. Gametes were collected and prepared following a standard method [Beiras et al., 2003]. For a control fertilization series, 1 mL of egg suspension was placed in beakers filled with 50 mL of sterile seawater. After eggs had settled to bottom, 200 µL of diluted sperm suspension was added. Fertilization success was assessed in 20 min under an Axio Imager A1 microscope (Carl Zeiss, Germany). Batches with a fertilization rate lower than 95% were excluded from the assay.

Throughout the experiment, the temperature was maintained at +18…+19 °C. Bioassays were performed in four replicates using gametes from three parental pairs ($N = 3$). Fertilization was carried out in filtered sterile seawater. Then, zygotes were transferred to test extracts for further embryonic and larval development [Beiras et al., 2003].

The toxicity of the studied samples was determined by evaluating the developmental status of larvae at the gastrula (18 h) and pluteus (48 h) stages; integrated toxicity index (ITI) was applied [Morroni et al., 2016]. For each parental pair, 400 larvae were examined at each controlled developmental stage. To calculate ITI, the toxic effect was quantified by analyzing the frequency of developmental delays and/or morphological abnormalities; those were ranked on a scale from 0 to 10 points according to the severity of their implications for subsequent larval development. Lower scores were assigned to larvae exhibiting developmental delays without malformations, whereas higher ones, to those with delays accompanied with non-specific morphological changes.

ITI is calculated using the following formula:

$$ITI = \sum_{i=10}^{n} \frac{S_i \times F_I}{100},$$

where S_i is the score assigned to each category of anomalies;

F_i the number of anomalies;

100 the number of larvae counted.

RESULTS

Grain size distribution. According to the results of the analysis of grain size distribution (Fig. 2), at sta. V1 (Melkovodnaya Bight), sediments consisted chiefly of fine-grained material. Thus, the psammitic fraction amounted to 70%, while the aleuritic and pelitic fractions constituted 20% and 7.5%, respectively. The proportion of the gravel fraction did not exceed 2.5%. At sta. V2 (Kruglaya Bight), fine-grained fractions also predominated: the proportion of the psammitic one was 43%; the aleuritic, 44.1%; and the pelitic, 12.5%. The gravel fraction amounted to 0.4% only. At sta. U1 (Uglovoy Bight), sediments were characterized by a predominance of the psammitic fraction: its proportion was 49.9%. The aleuritic and pelitic fractions constituted 37 and 13%, respectively. The proportion of the gravel fraction was 0.1%.

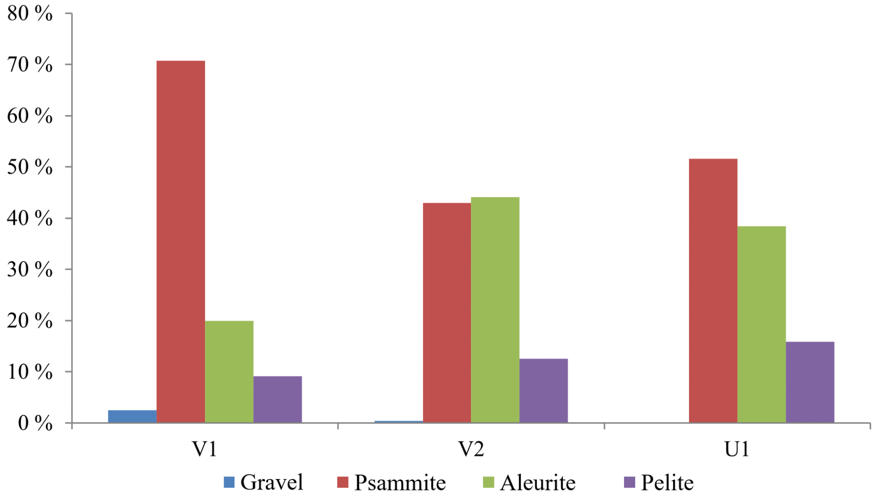


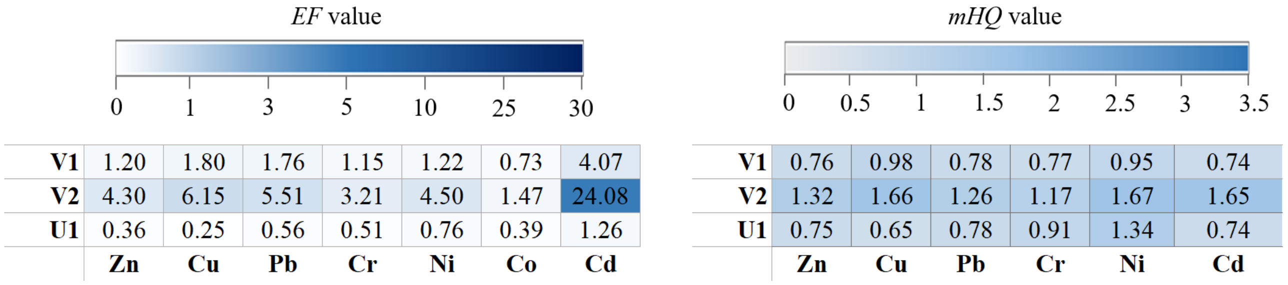
Fig. 2. Grain size distribution of the surface layer of bottom sediments from Melkovodnaya Bight, Kruglaya Bight, and Uglovoy Bight

Content of heavy metals and organic carbon in sediments. According to the data presented in Table 2, the surface layer of sediments at sta. V2 featured elevated concentrations of all measured elements, including organic carbon, compared to content at other stations. At this site, the background levels were exceeded for all analyzed elements, except for Co. Concentrations of Cu, Pb, and Cd at sta. V1 were higher than the background level, the same as content of Pb, Cr, Ni, and Cd at sta. U1.

The level of heavy metal pollution in sediments in the study water areas was compared with foreign regulatory levels for heavy metal content listed in the so-called Dutch Lists [Warmer, van Dokkum, 2002]. Importantly, these concentrations are not referenced in regulatory legal acts of any foreign country, and also have no legal force in the Russian Federation. Despite this fact, the levels given in Dutch Lists are used in works assessing the ecological state of sediments [Petukhov et al., 2023]. Moreover, those are used in the compiled reviews and annual reports on the quality of Russian marine waters [Marine Water Pollution, 2022]. In sediments of Kruglaya Bight (sta. V2), permissible concentrations were exceeded for Cu and Cd.

The potential toxic effects of heavy metals were analyzed by comparing their concentrations with SQGs values recommended in the Canadian guidelines for the protection of aquatic life [MacDonald et al., 2004]. Two criteria were used: the threshold effect level and the probable one (TEL and PEL). Their values are based on ecotoxicological studies on representatives of freshwater, marine, and estuarine fauna. TEL describes the permissible concentration of a contaminant for hydrobionts that is considered statistically safe. In contrast, PEL defines the level above which organisms can undergo toxicological effects [MacDonald et al., 2004]. As revealed, TEL was exceeded for Zn, Cu, Pb, Ni, and Cd in sediments at sta. V2, and for Ni at sta. U1.

The calculation of the enrichment factor, EF, allowed revealing similar accumulation patterns for toxic elements in sediments of Melkovodnaya Bight (V1) and Kruglaya Bight (V2): Cd > Cu > Pb > Ni > Cr > Zn > Co for sta. V1 and Cd > Cu > Pb > Ni > Zn > Cr > Co for sta. V2. In Uglovoy Bight (U1), the following pattern was observed: Cd > Ni > Pb > Cr > Co > Zn > Cu. The value of EF higher than 1.5 indicates an anthropogenic source of contaminants. At sta. V2, EF values for all analyzed metals, except for Co, exceeded this threshold (Fig. 3). High degree of enrichment was recorded for Cd (EF = 24.08); significant one, for Cu (EF = 6.15) and Pb (EF = 5.51); and moderate one, for Ni, Zn, and Cr (EF values were 4.5, 4.3, and 3.21, respectively). At sta. V1, most heavy metals featured minimum degree of enrichment; the exception was Cd, with its moderate degree (EF = 4.07). At sta. U1, all values for sediments were significantly lower than 1.5; this indicates a natural origin of heavy metals: from weathering processes.



V1	1.20	1.80	1.76	1.15	1.22	0.73	4.07
V2	4.30	6.15	5.51	3.21	4.50	1.47	24.08
U1	0.36	0.25	0.56	0.51	0.76	0.39	1.26
	Zn	**Cu**	**Pb**	**Cr**	**Ni**	**Co**	**Cd**

V1	0.76	0.98	0.78	0.77	0.95	0.74
V2	1.32	1.66	1.26	1.17	1.67	1.65
U1	0.75	0.65	0.78	0.91	1.34	0.74
	Zn	**Cu**	**Pb**	**Cr**	**Ni**	**Cd**

Fig. 3. Values of enrichment factor (EF) and modified hazard quotient (mHQ) for analyzed bottom sediments

The calculation of mCd showed as follows: sediments from sta. V1 and U1 were characterized by a very low contamination degree (mCd ≤ 1.5), while sediments from sta. V2 (Kruglaya Bight), by a moderate one (mCd = 4.38) (Fig. 4).

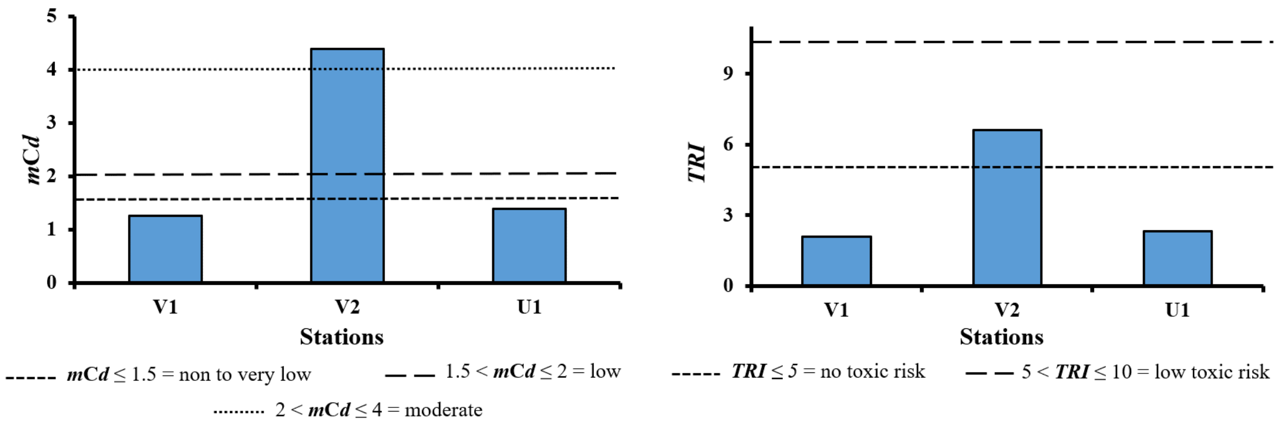


Fig. 4. Values of modified contamination degree (mCd) and toxic risk index (TRI) for analyzed bottom sediments

To assess the contribution of each heavy metal to the total potential toxic effect on hydrobionts, mHQ was calculated (see Fig. 3). In sediments from sta. V2, mHQ values for Ni, Cu, and Cd (1.67, 1.66, and 1.65, respectively) indicated moderate contamination severity, while values for Zn, Pb, and Cr (1.32, 1.26, and 1.17), low one. The values of mHQ for toxic elements in sediments from sta. V1 and U1 allowed to characterize contamination severity as very low. The exception was Ni at sta. U1 (mHQ = 1.34).

To determine the level of integrated effect of heavy metals on benthic hydrobionts, TRI was calculated (Fig. 4). Sediments from sta. V1 and U1 showed no potential toxic risks. The highest value (TRI = 6.62) was obtained for sediments at sta. V2; it corresponded to a low risk level. Importantly, although mHQ calculated for Ni, Cu, and Cd at this station indicated an individual moderate risk level, the value of the integrated index was low.

Ecotoxicological assessment. Aqueous extracts from sediments were biotested on larvae of the sand dollar *S. mirabilis*. The subsequent calculation of ITI yielded the following results. In 18 h after fertilization, the maximum abundance of abnormally developed larvae was observed at sta. V2 (Fig. 5). Specifically, in sediment extracts from this station, the overwhelming majority of embryos stopped their development at the abnormal morula stage, having an uneven surface and larger cells than those of normally developed embryos. A small number of larvae was classified as abnormal blastulae, whose blastocoel exhibited uneven cellular filling. Thus, in aqueous extracts of sediments from sta. V2, not a single larva developed to the gastrula stage, and ITI value was 9.84 points.

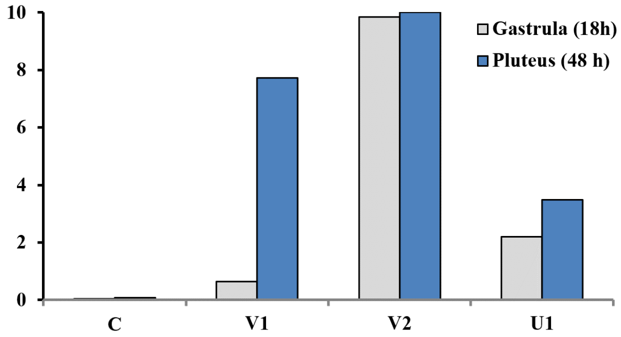


Fig. 5. Values of the integrated toxicity index (ITI) for analyzed bottom sediments 18 and 48 h after the start of the experiment

In 18 h after the start of the experiment, normally developed larvae at the late gastrula stage predominated in aqueous extracts from sta. V1 and U1. Interestingly, extracts from sta. U1 also contained normally formed but developmentally delayed larvae at the early gastrula and mesenchymal blastula stages, as well as abnormally developed blastulae. Accordingly, ITI values at this stage of the bioassay were higher for sediments from sta. U1 (2.2 points), while the value for sta. V1 was minimum (0.64 points).

In 48 h after fertilization, the maximum value of ITI (10 points) was also obtained for extracts from sediments of sta. V2 (Fig. 5). In blastulae developing in these extracts, the morphological abnormalities were noted, and the latter ones prevented larvae from reaching the mid-pluteus stage. Importantly, ITI values at this stage of the experiment for sta. V1 were significantly higher than those for sta. U1. In sediment extracts from Melkovodnaya Bight (V1), the vast majority of larvae developed with severe morphological impairments. Larvae at the prism and early pluteus stages were characterized by the presence of poorly developed skeletal rods or their absence. Furthermore, we found exoforms of larvae with no intestine in their body cavity (it was protruded outward). Although such an intestine has all three anatomical constrictions corresponding to gut subdivisions in normally developed larvae, it is non-functional [Mazur et al., 2020]. For sediments from sta. V1, ITI value (7.72 points) was twice as high as for samples from sta. U1 (3.5 points).

DISCUSSION

At sta. V1 and V2, background concentrations for copper, lead, and cadmium were exceeded. At sta. V2, background values for zinc, chromium, and nickel were exceeded as well. Zn and Cu input to water bodies is typically associated with mining, ore processing, metal smelting, with operation of electroplating shops, or with communal and household effects (both elements are common components of domestic wastewater). At the same time, Cd, Ni, and Pb serve as indicators of anthropogenic load on the environment [Khristoforova et al., 2018].

The enrichment of sediments with heavy metals is a complex and multifactorial process. Their concentrations can result from sorption by mineral and organic matter, complexation, coagulation, formation and sedimentation of poorly soluble compounds, *etc.* The intensity of these processes is governed by the morphometric and hydrological characteristics, and also by the hydrochemical regime of a water body. Post-sedimentation reactions can lead to an increase in content of elements in pore waters, establishing a concentration gradient between pore waters and the overlying bottom ones. If physicochemical conditions (pH, Eh, mineralization, and so on) change, heavy metals can be remobilized from sediments and diffuse from pore waters into the bottom layers; this is a potential threat of secondary pollution of the water area by these chemical elements [Dauvalter, 2012].

The distribution of heavy metals in sediments is also mediated by forms of their compounds with the organic and inorganic matrix. One group of elements closely associated with the organic matrix (Zn, Cu, Pb, and Cd) is characterized by high bioaccumulation coefficients in hydrobionts, and they enter sediments primarily with detritus. Another group includes metals that enter aquatic ecosystems bound within highly dispersed mineral particles, which are carried by allochthonous surface runoff (Cr, Ni, and Co). Also, this group includes typomorphic elements (Fe and Mn) [Ivanov et al., 2020]. In sediments, Fe and Mn commonly occur as hydroxides and oxides. The same as organic matter, those are very effective at accumulating and concentrating heavy metals [Bazilevskaya, Skolotnev, 2019].

In the present study, overall background pollution levels are similar for Kruglaya and Melkovodnaya bights; however, the extent of heavy metal accumulation in their sediments is different. In Kruglaya Bight, an analysis of the vertical distribution of organic carbon and heavy metals in a sediment core revealed a linear correlation between content of C_{org} and concentrations of zinc, lead, copper, and nickel [Ryumina et al., 2023]. The same study examined sediment cores from Melkovodnaya and Uglovoy bights as well, but found no correlation between organic carbon and heavy metals. These facts suggest that the concentration of heavy metals in sediments is more efficient at sites covered with *Z. marina* meadows.

Based on data on grain size distribution, the highest C_{org} content in sediments would be expected at stations where the pelitic fraction prevails [Romankevich, Vetrov, 2021]. Conversely, decline in organic carbon concentration is characteristic of sediments dominated by the psammitic fraction. This pattern is attributed to the mechanical sorting of different-sized particles. In the present study, C_{org} content at sta. V2 was 4.83% (see Table 2), which is almost 5 times higher than the value at sta. U1, 1.03%. C_{org} concentration was 0.83% at sta. V1, where the psammitic fraction clearly predominated; the value was close to that at sta. U1. At sta. V2 and U1, proportions of the pelitic fraction were 12.5 and 13%, respectively; proportions of the aleuritic fraction were 44.1 and 37%. It can be concluded that the distribution of organic carbon is not governed by the mechanical sorting of different-sized particles.

In the absence of allochthonous inputs, the primary source of organic matter for sediments of Kruglaya Bight is suspended matter derived from a seagrass *Z. marina*. A key component can be zosterates: salts of a polysaccharide zosterin, which also acts as a natural ligand for heavy metals [Khozhaenko et al., 2015]. Upon plant senescence, these organic complexes enrich sediments with both organic carbon and heavy metals [Hart, 1982]. The total content of carbohydrates produced by *Z. marina* can be over 35% of seagrass dry mass, with zosterin comprising approximately 20% [Tishchenko et al., 2020].

As already mentioned, sta. V2 area, where sediments were sampled, hosts *Z. marina* meadows. There, a very high degree of enrichment was noted for cadmium (EF = 24.08); a significant, for copper (EF = 6.15) and lead (EF = 5.51); and a moderate, for nickel (EF = 4.5), zinc (EF = 4.3), and chromium (EF = 3.21).

At sta. V2, Fe content (34,500 mg·kg^{-1}) was twice as high as at sta. V1 (14,800 mg·kg^{-1}) and U1 (15,500 mg·kg^{-1}). This also indicates the possible concentration of heavy metals by iron hydroxides, and not just by organic matter.

The calculation of mCd showed that contamination degree at sta. V2 can be characterized as moderate (mCd = 4.38). The determination of mHQ allowed for assessing the contribution of each analyzed heavy metal to the total potential toxic effect on hydrobionts. Thus, in sediments from sta. V2, mHQ values for nickel, copper, and cadmium (1.67, 1.66, and 1.65, respectively) indicated moderate contamination severity, and for zinc, lead, and chromium (1.32, 1.26, and 1.17), low one. The highest TRI value (6.62) was recorded at sta. V2; this corresponds to a low risk level (see Table 1). Interestingly, at this station, mHQ for nickel, copper, and cadmium indicated an individual moderate risk level, but the value of the integrated index was low.

Judging by values of the enrichment factor, in sediments of sta. V1, most heavy metals were characterized by insignificant levels; the exception was Cd which showed a moderate degree of enrichment (EF = 4.07). EF values for sediments from sta. U1 were significantly lower than 1.5 indicating that the input of heavy metals is associated with natural weathering processes.

The calculation of mCd showed that sediments from sta. V1 and U1 are characterized by a very low contamination degree (mCd ≤ 1.5). The values of mHQ for toxic elements in sediments from sta. V1 and U1 reflected very low contamination severity, except for Ni at sta. U1 (mHQ = 1.34). Sediments of these two stations were characterized by the lack of potential toxic risk.

In the water areas studied, the toxic effect of exposure to extracts from sediments can result from either high content of certain pollutants/compounds or the combined action of several contaminants [Gorbacheva, 2018]. In our survey, the highest abundance of abnormally developed larvae was observed in extracts from sta. V2; importantly, it is in sediments from this site that the highest Cu and Cd concentrations (exceeding the permissible ones) were found. According to [Rouchon, Phillips, 2017], exposure to ionic copper at concentrations of 5 and 10 mg·L^{-1} caused growth and developmental delays in larvae of a sea urchin *Evechinus chloroticus* (Valenciennes, 1846), while increasing content to 20 mg·L^{-1} led to a complete cessation of larval development at the gastrula stage. The effective concentration (EC_{50}) of cadmium ions in an experiment with larvae of *Paracentrotus lividus* (Lamarck, 1816) was 1.003 mg·L^{-1}, with developmental defects being primarily skeletal abnormalities and impaired gut differentiation [Manzo et al., 2010]. Notably, the percentage of abnormally developed larvae was high in extracts from sediments of Melkovodnaya Bight (sta. V1). Despite low level of heavy metal pollution and the absence of potential toxic risk to hydrobionts, these extracts had a noticeable effect on sea urchin larval development. As known, sediments accumulate not only the pollutants we analyzed, but also a wide range of contaminants. Those can have potential inhibitory, additive, and synergistic effects on each other, thereby regulating the toxic effect on larvae.

Organic matter accumulated in sediments serves as a primary food source for most benthic organisms. However, its excessive concentrations can lead to community degradation, manifesting in both qualitative changes (decreased species richness) and quantitative ones (reduction in abundance and biomass). When C_{org} content exceeds 2.5%, a drop in abundance and biomass of the benthic community is observed, as well as the restructuring of its species composition [Orekhova et al., 2019]. In a study on the response of macrobenthos to organic matter enrichment in sediments, A. Petrov [2000] proposed a classification covering five threshold levels of C_{org} values. Based on this system, sediments at sta. V1 and U1 have a medium level of eutrophication (II class), whereas those at sta. V2 feature a very high level (V class). The organic matter concentration recorded in Kruglaya Bight is 4.83%. Following the above-mentioned classification, this value is expected to result in the near-complete degradation of benthic communities, with a predominance of opportunistic species. Such a high degree of sediment eutrophication is considered incompatible with favorable habitat conditions for most macrobenthic organisms; moreover, it is likely to facilitate the enhanced accumulation of various organic and inorganic pollutants [Petrov, 2000].

The results of this study, along with the ongoing extensive industrial development of the water areas, indicate the need for continued comprehensive assessments of heavy metal pollution in sediments and its potential toxicity to hydrobionts.

Conclusion. Analysis of heavy metal content in the surface layer of sediments from Melkovodnaya Bight (sta. V1), Kruglaya Bight (sta. V2), and Uglovoy Bight (sta. U1) revealed the highest values for sta. V2. The bottom of this site is extensively covered by *Zostera marina* meadows. Our results indicate that this seagrass contributes much to enriching sediments with both organic matter (4.83%) and heavy metals.

This enrichment has led to a lower overall ecological status for Kruglaya Bight compared to other two shallow basins. Values of individual and comprehensive pollution indices, and also ecological risk indices were the highest for sediments sampled at sta. V2.

Bioassay revealed the highest frequency of abnormally developed larvae of a sea urchin *Scaphechinus mirabilis* at sta. V2. In aqueous extracts of sediments from this station, no larvae developed to the gastrula stage, and the value of the integrated toxicity index (ITI) was 9.84 points. In contrast, aqueous extracts from sta. V1 and U1 supported normal development, with larvae at the late gastrula stage predominating 18 h after fertilization. Within 48 h after fertilization, the vast majority of larvae in extracts from sta. V1 featured severe morphological impairments. ITI value for sediments from sta. V1 (7.72 points) was more than twofold higher than that for samples from sta. U1 (3.5 points).

The results of the bioassay of sediments sampled in Kruglaya Bight were consistent with the calculation of indices of pollution and potential ecological risk for this site. Sediments from Melkovodnaya Bight featured a different pattern. Despite low levels of heavy metal pollution and the absence of any potential toxic risk to hydrobionts, sediment extracts significantly affected the development of the sand dollar larvae. This discrepancy suggests that sediments are contaminated with other pollutants that contribute to the cumulative toxic effect.

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ПРЕДВАРИТЕЛЬНЫЕ РЕЗУЛЬТАТЫ КОМПЛЕКСНОЙ ОЦЕНКИ ЗАГРЯЗНЕНИЯ И ТОКСИЧЕСКОГО ВОЗДЕЙСТВИЯ ДОННЫХ ОСАДКОВ МЕЛКОВОДНЫХ БУХТ АМУРСКОГО ЗАЛИВА (ЯПОНСКОЕ МОРЕ)

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В августе 2023 г. были отобраны образцы поверхностных донных осадков (верхние 5 см грунта) в трёх бухтах Амурского залива — в бухтах Мелководной (V1) и Круглой (V2), которые располагаются в бухте Воевода, а также в заливе Угловой (U1). V1 и V2, являясь соседними бухтами, имеют большие отличия в гранулометрическом составе и в распределении содержания тяжёлых металлов и органического углерода. Выявлено повышенное содержание тяжёлых металлов и органического углерода в бухте Круглой (на станции V2). В донных осадках на ст. V2 значения коэффициента обогащения (EF) для каждого из проанализированных металлов, за исключением Co, превышали уровень естественного выветривания тяжёлых металлов. Сильный уровень обогащения отмечен для Cd (EF = 24,08), умеренно сильный — для Cu (EF = 6,15) и Pb (EF = 5,51), умеренный — для Ni, Zn и Cr (значения EF составили 4,5, 4,3 и 3,21 соответственно). Расчёт модифицированного индекса степени загрязнения (mCd) показал, что на ст. V2 она является умеренной (mCd = 4,38). В донных осадках со ст. V1 большинство металлов характеризовались незначительным уровнем обогащения; исключение — Cd с умеренным уровнем (EF = 4,07). Величи́ны EF для донных осадков со ст. U1 были существенно ниже 1,5; это свидетельствует о поступлении тяжёлых металлов в результате естественных процессов выветривания. В исследуемых бухтах впервые проведено биотестирование с использованием личинок морского ежа *Scaphechinus mirabilis* в качестве тест-объекта. В ходе этого анализа максимальное число аномально развитых личинок выявлено на ст. V2. В водных вытяжках осадков со ст. V2 до стадии гаструлы не развилась ни одна из личинок, а значение интегрального индекса токсичности (ITI) составило 9,84 балла. В водных вытяжках со ст. V1 и U1 спустя 18 ч с начала эксперимента преобладали нормально развитые личинки на стадии поздней гаструлы. В экстрактах осадков из бухты Мелководной (ст. V1) подавляющее большинство личинок развивались с грубыми морфологическими нарушениями. Личинки на стадиях призмы и раннего плутеуса характеризовались наличием слаборазвитых скелетных стержней или их отсутствием. Кроме того, отмечены экзоформы личинок без кишечника в полости тела (он выпячен наружу). Выявленное значение ITI для донных осадков со ст. V1 (7,72 балла) оказалось вдвое выше, чем для проб со ст. U1 (3,5 балла). Полученные данные указывают на то, что к концентрированию тяжёлых металлов в бухте Круглой приводит совокупность факторов. В свою очередь, высокое содержание тяжёлых металлов и органического углерода может обуславливать формирование неблагоприятных условий жизни для бентосных организмов.

Ключевые слова: Амурский залив, биотестирование, донные осадки, органический углерод, тяжёлые металлы, *Scaphechinus mirabilis*