RESEARCH ARTICLE

Mercury in the world's largest hypersaline lagoon Bay Sivash, the Sea of Azov

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Abstract

There are few studies on mercury content in hypersaline waters. Mercury content was studied in Bay Sivash (the Sea of Azov), the world's largest hypersaline lagoon with a strong salinity gradient from 36 to 90 g l^{-1} . The dissolved mercury compounds ranged from 120 to 250 ng l^{−1}, Hg varied from 60 to 450 ng l^{−1} in the suspended matter, and total mercury in the water ranged from 200 to 600 ng l⁻¹. Salinity and the total suspended matter had practically no effect on the amount of dissolved and suspended forms of mercury separately, but their growth significantly increased total mercury content in water. Only the concentration of dissolved forms of mercury in water significantly correlated with dissolved organic matter. The Hg concentration in the bottom sediments averaged 13.8 ng g^{-1} wet weight. Both high salinity and human activities on the Sivash drainage area are responsible for high Hg content in lagoon water.

Keywords Hypersaline waters . Mercury . Lagoon . Total suspended matter . Dissolved organic matter . Ecosystem

Introduction

Heavy metal pollution of the biosphere is accelerated now. Their release into the atmosphere from human activity significantly exceeded that from natural sources, for individual metals this excess was from 2 to 335 times (Norton et al. [1990](#page-7-0); Likens [1992](#page-7-0); Pirrone et al. [2010](#page-7-0); Mason [2013](#page-7-0)). Mercury (Hg) degassing from the World Ocean and soils are probably the most important natural contributions to the Hg global atmospheric content (Pirrone et al. [2010](#page-7-0)). Mercury compounds were widely used as fungicides, in the manufacture of paper, but now, in most agricultural and pharmaceutical products, the use of inorganic mercury has been discontinued in most countries around the world. It serves as catalysts in the production of plastics, etc., and so, their different forms fall into industrial effluents or the air (Norton et al. [1990;](#page-7-0) Schmidt [1991](#page-7-0); Likens [1992;](#page-7-0) Pirrone et al. [2010](#page-7-0);

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Eagles-Smith et al. [2016;](#page-6-0) Gębka et al. [2016\)](#page-6-0). Currently, the estimated contribution from natural sources is only about 10% of a total annual emitted Hg to the atmosphere from all sources of 5500–8900 t (Zillioux [2015\)](#page-8-0). Global and regional Hg emissions both lead to an increase of Hg content in the atmosphere and water bodies (Norton et al. [1990;](#page-7-0) Bank [2012](#page-6-0); Eagles-Smith et al. [2016\)](#page-6-0). Direct atmospheric fallout and income from watershed contribute to the pollution of water bodies by Hg (Norton et al. [1990](#page-7-0); Van Metre and Fuller [2009;](#page-7-0) Eagles-Smith et al. [2016](#page-6-0)). Coastal erosion is also a source of Hg coming into water bodies, and there is a trend of its increase due to natural and anthropogenic causes (Zhang et al. [2004;](#page-8-0) Shadrin and Anufriieva [2013](#page-7-0); Bełdowska et al. [2016\)](#page-6-0). Analysis of the bottom sediment depth profiles in different water bodies showed a strong enrichment of mercury concentrations in the past decades (Arnason and Fletcher [2003](#page-6-0); Díaz-Asencio et al. [2009\)](#page-6-0).

Mercury can enter water bodies in a wide variety of forms, and in the aquatic environment, every form of mercury is converted to methyl mercury (MMHg), which is a highly toxic and persistent compound (Schmidt [1991;](#page-7-0) Canário et al. [2008;](#page-6-0) Johnson et al. [2015](#page-6-0); Zillioux [2015](#page-8-0); Boyd et al. [2017](#page-6-0)). Aquatic ecosystems are the most sensitive to MMHg pollution, as they are the main repositories of natural and anthropogenic mercury and the habitats for active populations of methylating mercury bacteria (Fitzgerald et al. [2007\)](#page-6-0). Mercury was accumulated by

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planktonic organisms, and in the aquatic food chains, methylmercury concentration increases from lower trophic level organisms to higher link. In the top-level predators (fish, birds, humans), it may reach the highest concentration endangering health and livehood (Schmidt [1991](#page-7-0); Sonesten [2003;](#page-7-0) Helgason et al. [2008](#page-6-0); Eagles-Smith et al. [2016\)](#page-6-0). So, every knowledge of Hg concentration and behavior in an aquatic environment is highly important to the sustainable use of water bodies. There are a lot of articles on the concentration of different Hg forms and their biological role in fresh and marine waters due to Hg health effects for humans and ecosystem disturbing (Schmidt [1991;](#page-7-0) Prato et al. [2006;](#page-7-0) Zillioux [2015;](#page-8-0) Boyd et al. [2017](#page-6-0)). However, there are only a few data on such issues in hypersaline waters despite that the highest concentration of Hg was recorded in some of them (Shumilin et al. [2002;](#page-7-0) Pietrelli and Biondi [2009](#page-7-0); Johnson et al. [2015](#page-6-0); Boyd et al. [2017\)](#page-6-0).

Bay Sivash (the Sea of Azov) is the world's largest hypersaline lagoon with a strong spatial salinity gradient from 30 to 35 to 100–110 g I^{-1} (Shadrin et al. [2018\)](#page-7-0). In 2018, mercury content was studied in water and bottom sediments of the lagoon along with salinity, total suspended solids, and dissolved organic matter. The goals of this paper are to present new data, to discuss them comparing with published data for other hypersaline water bodies, and to prove or disprove a hypothesis that salinity may influence Hg concentration in the lagoon.

Material and methods

Study area

In Europe, Bay Sivash is the largest lagoon with its area of about 2600 km^2 (Vorobyev [1940;](#page-8-0) Shadrin et al. 2018 ; Sergeeva et al. [2019\)](#page-7-0). The narrow sand Arabat Spit of 112–116 km long separates the lagoon from the Sea of Azov, and the Crimean peninsula, largest in the Black Sea, is a lagoon west coast. Two narrow straits connect the Sea of Azov with the lagoon on the north (Fig. 1). Maximum depth does not exceed 2 m in the shallow semiclosed lagoon. Before the construction of the North Crimean Canal (from 1963 to 1975), Bay Sivash was a highly productive shallow hypersaline lagoon (average salinity of about 140 g l^{-1} and more than 200 g Γ^1 in the southern part); the small salt lakes and pools were in depressions surrounded it. The North Crimean Canal was built to improve water supply to the Crimean Peninsula by waters of the Dnieper River. Irrigated agriculture occupying 4000 km², mainly rice cultivation, was developed on the Bay Sivash drainage area. A lot of different pesticides and fertilizers were used, especially, for rice cultivation. Drainage waters from agricultural lands began discharging into Bay Sivash in large volume, up to 1.1×10^6 m³ per year. As a result, in the lagoon, the salinity began to drop, and its average value had decreased up to 17–23 g Γ^{-1} . The Dnieper River collects

Fig. 1 Bay Sivash on the maps and distribution of the sampling sites in 2018

almost all Ukrainian industry and agriculture effluents, which are then supplied to Crimea through the North Crimean Canal. This led to an increase in the pollution level of many Crimean water bodies that were shown, as an example, for artificial radionuclides (Mirzoeva et al. [2020](#page-7-0)). In April 2014, the North Crimean Canal was blocked, and the supply of Dnieper water in Crimea was ceased. The discharge of drainage waters to the bay was also stopped. Salinity grew from 22 to 24 g l^{-1} up to 6– 75 g Γ^{-1} in 2015, and up to 100 g Γ^{-1} in 2019 (Shadrin et al. [2019;](#page-7-0) Anufriieva and Shadrin [2020](#page-6-0)). The lagoon again became hypersaline. Changes occurred in ecosystem biotic structure leading also to changes in the environment. As an example, Cladophora siwaschensis (C.J. Meyer, 1922), filamentous green algae, again creates floating mats with high wet weight biomass up to 2.5– 3.0 kg m^{-2} as before dropping salinity after the canal construction. Currently, they cover large areas leading often to hypoxic and even anoxic events below them near the bottom (Shadrin et al. [2018](#page-7-0), [2019](#page-7-0)). There is a range of bottom sediment types in the bay—silt and silty-sand with fragments of mollusk shells.

Sampling and analyses

To analyze the mercury content, six water samples (stations 1, 3–7) were collected in May 2018 and seven samples of bottom sediments (stations 1–7) in November 2019 at different stations in Bay Sivash (Fig. [1,](#page-1-0) Table 1). To study the mercury content, 1-l water samples were taken at each station by bathometer. The upper layer of bottom sediments (4–5 cm) was taken by a manual grab with an area of 0.01 m^2 . In sampling stations, the concentrations of total suspended solids (TSS) and dissolved organic matter (DOM) were determined using the Condor complex (Akvastandart-Yug, Sevastopol, Russia) as described (Shadrin et al. [2019\)](#page-7-0). Salinity was measured by a WZ212 refractometer (Kelilong Electron Co. Ltd, Fuan, Fujian, China), and temperature by РНН-830 electronic thermometer (OMEGA Engineering, INC., Norwalk, CT). To separate the forms of mercury, water samples, fixed by concentrated nitric acid (10 ml of $HNO₃$ per 1 l of water), were filtered through pre-weighed nuclear 0.45-μm pore size filters.

The dissolved form of mercury was determined in the filtrate and the suspended form on the filters. Potassium permanganate was added to the filtered water sample for oxidation in a volume of $15-20$ ml and then 5 ml $\frac{1}{2}$ sulfuric acid, thereby destroying all dissolved forms of mercury to ions. Suspension and bottom sediments were acid burned (10 ml $\frac{1}{2}H_2SO_4$ and 5 ml HNO3 per sample). Next, the samples were subjected to destruction at no more than 60 °C, and then cooled. A solution of potassium permanganate was added to the samples in an amount ensuring complete oxidation of the test sample (from 15 to 20 ml). After 10–15 min, the samples were filtered. For analysis on a device, sulfuric acid (1:1) in a volume of 5 ml was added to 100 ml of the filtered sample. A hydroxylamine solution (up to 5 ml) was poured into all samples to remove

28706 Environ Sci Pollut Res (2021) 28:28704–28712

excess potassium permanganate. Before the determination, a reducing agent $(SnCl₂)$ was added in a volume of 10 ml. The prepared samples were analyzed by the flameless atomic absorption method on a Hiranuma-1 mercury analyzer (Hiranuma Sangyo Co. Ltd, Mito, Ibaraki, Japan). Mercury ions were reduced by tin dichloride (5 ml per sample) to gaseous form and then immediately introduced into the aerator of the device. The amount of mercury was determined on the scale of the device at a wavelength of 253.7 nm. To calibrate the Hiranuma-1 mercury analyzer, a standard sample of solutions of mercury (II) ions was used. First, a "blank calibration" was carried out (100 ml of distilled water + 5 ml of H_2SO_4 (1:1)), then calibration using a series of calibration solutions: 0.2; 0.4; 0.6; 0.8; 1 μ g dm⁻³ (10 replicates for each concentration). The sensitivity of the Hiranuma-1 mercury analyzer is 0.01; the detection limit is 0.5 ng 1^{-1} of mercury with high sensitivity 1/1000 of standard 0.5 ppb.

Coefficients of mercury accumulation by a suspended phase (also known as distribution coefficient) were calculated by the equation (Polikarpov and Egorov [1986\)](#page-7-0):

$$
Kss = (1000 \times Css)/Cw,
$$
 (1)

where Kss – coefficients of mercury accumulation by TSS; Css – the concentration of mercury in TSS, ng g^{-1} ; Cw is the concentration of mercury in the dissolved phase, ng kg^{-1} .

Data analysis

Statistical processing of the results was carried out in MS Excel 2007 and Statistica 6.0, where mean values, standard deviations (SD), coefficients of variability (CV), correlation (R) , determination (R^2) , parameters of regression equations,

Table 1 Coordinates and characteristics of sampling stations in Bay Sivash

S salinity, T temperature, TSS total suspended solids, DOM and dissolved organic matter

and dendrograms were calculated. The significance of differences was assessed by Student's t-test after normality tests (Thode 2002) were done. The confidence level (p) of the correlation coefficients was found (Muller et al. [1979\)](#page-7-0).

Results

General hydrographic properties

In both periods of this study, a strong spatial gradient of salinity was expressed. Salinity increased from north to south, changing at sampling points in May from 36 to 82 g l^{-1} , and from 38 to 90 g l^{-1} in November (Table [1\)](#page-2-0). Changes in TSS and DOM were correlated with changes in salinity (Table [1\)](#page-2-0). In May, TSS was determined by two methods, the average of these two values was used in further analysis. It varied from 2.6 to 18.2 mg l⁻¹, on average 12.4 mg l⁻¹ (SD = 6.5, CV = 0.52). Concentrations of TSS and DOM significantly positively correlated with salinity in May (Fig. 2a), but in November there were no such dependencies. In November, the concentration of TSS was 10% higher than in May (the differences were not significant), and the concentration of DOM was 2.6 times higher (the differences were significant, $p = 0.001$). There was a reliable positive correlation between TSS and DOM; its quantitative expression was different at different times. In May, it was best approximated by the equation (R) $= 0.918, p = 0.001$:

$$
DOM = 2.40 \text{ TSS}^{0.17} \tag{2}
$$

and in November ($R = 0.999$, $p = 0.0001$):

$$
DOM = 0.58 \text{ TSS} + 1.22 \tag{3}
$$

Mercury in water

In May, concentration of dissolved mercury compounds ranged from 120 to 250 ng l⁻¹, on average 170 ng l⁻¹ (SD = 46.9, $CV = 0.28$, and the amount of mercury in TSS varied from 60 to 450 ng l⁻¹, on average 165 ng l⁻¹ (SD = 112, CV = 0.68) (Table [2](#page-4-0)). The mercury content in 1 g of suspension ranged from 3×10^3 to 50×10^3 ng g⁻¹, on average of 23.4

Fig. 2 Correlation between a total suspended substrates (TSS) and dissolved organic matter (DOM) and b total content of Hg in water and salinity in Bay Sivash during June 2018

 \times 10³ ng g⁻¹ (SD = 17.8 \times 10³, CV = 0.76), and this value significantly negatively correlated with TSS ($R = -0.754$, $p =$ 0.05):

$$
C'ss = 6.1 \times 10^4 e^{-0.14TSS},
$$
 (4)

where C'ss – Hg in TSS, ng g^{-1} .

The total mercury content in the water varied from 200 to 600 ng l⁻¹, on average of 335 ng l⁻¹ (SD = 172, CV = 0.51). Salinity had practically no effect on the amount of dissolved and suspended forms of mercury separately, but significantly $(p = 0.05)$ influenced its total content (Fig. 2b). Salinity also did not affect the ratio of the contents of suspended and dissolved forms. TSS significantly positively affected only the total amount of mercury ($R = 0.740$, $p = 0.05$), and only the Hg concentration in the dissolved phase significantly correlated with DOM ($R = 0.755$, $p = 0.05$). Coefficients of mercury accumulation by TSS varying from 1.5×10^4 to 41.7×10^4 , averaging 16.7×10^4 (SD = 15.8×10^4 , CV = 0.95), did not reliably depend on salinity and DOM. It significantly negatively correlated with TSS ($R = -0.771$; $p = 0.05$):

$$
Kss = 50 \times 10^4 e^{-0.17TSS},
$$
 (5)

where Kss – coefficients of mercury accumulation in TSS (eq. [1](#page-2-0)).

Cluster analysis using indicators of dissolved, suspended and total mercury contents showed that all stations fall into two main groups (Fig. [3a\)](#page-4-0). Two stations located in the shallowest southernmost part were detached in a separate group. The content of dissolved forms of mercury practically did not differ in the two groups of stations, and the concentration of suspended matter in the group of shallow water stations was on average 5 times higher, while the total mercury content was 2 times higher. Cluster analysis was also carried out using salinity, TSS, and DOM, and it led to another grouping of stations (Fig. [3b](#page-4-0)).

Mercury in bottom sediments

There were a lot of empty shells of mollusks in the bottom sediments. From point to point, the concentration of mercury in the bottom sediments did not change much (Fig. [4\)](#page-5-0), averaging 13.8 ng g⁻¹ wet weight – Cww (SD = 3.1, CV = 0.23) or

Table 2 The concentration of Hg in the water at different sites of Bay Sivash during May 2018

Kss, coefficients of mercury accumulation by suspended matter

19.8 ng g^{-1} dry weight – Cdw (SD = 6.3, CV = 0.32). The Cdw/Cww ratio averaged 1.4 (SD = 0.15 , CV = 0.11).

No significant effect of salinity, TSS, and DOM on mercury content was found. It should be noted that during sampling of bottom sediments in November, the weather was very windy, while the direction of the wind often changed and resuspended bottom sediments and increased spatial homogeneity. The concentration of mercury in sediments in November was on average 1600 times less than in suspended matter in May (per g).

Discussion

The total Hg content in water of different Black Sea areas varied between 1 and 120 ng l^{-1} and averaged between 7.2 and 37.0 ng l^{-1} in different seasons and years (Kostova and Popovichev [2002](#page-7-0); Stetsiuk and Egorov [2018\)](#page-7-0). However, in a highly polluted Bay Sevastopolskaya (Black Sea), a total Hg content in the most part was not higher than 155 ng l^{-1} (Kostova et al. [2001\)](#page-7-0) and episodically reached up to 460 ng 1^{-1} in the most polluted sites (Stetsiuk, unpublished data). Hence, the total Hg concentrations in water were on average

higher in Bay Sivash than in the Black Sea. There are also several hypersaline lakes nearby Bay Sivash with high Hg contents exceeding 500 ng 1^{-1} (Mirzoyeva et al. [2015;](#page-7-0) Stetsiuk et al. [2018](#page-7-0)). It is worth comparing our data with those from the available literature. The total Hg concentrations vary from 0.14 to 15.1 ng l^{-1} in the freshwater lakes and from 2 to 45 ng l⁻¹ in rivers (Petrisor [2006\)](#page-7-0) and from < 1 to 78.0 ng l⁻¹ in seawater (Fitzgerald et al. [2007](#page-6-0); Mason [2013\)](#page-7-0) with higher levels in some coastal heavy polluted areas (Mousavi et al. [2011\)](#page-7-0). It may be even higher, up to 145 ng l^{-1} , in polluted lagoons (Covelli et al. [2009\)](#page-6-0). In hypersaline Great Salt Lake (Utah, USA), the total Hg content in water is high reaching up to 100 ng l^{-1} (Naftz et al. [2008\)](#page-7-0) but 3–6 times lower compared to this study.

The contributions of different sources to high Hg content in Bay Sivash as well as the Crimean hypersaline lakes were not quantitatively assessed yet. In high salinity basins, the enhanced Hg transfer from atmosphere to water (Mason and Gill [2005\)](#page-7-0) can be one of the causes since an increase of the total Hg content was found along the salinity gradient in Bay Sivash. The significant correlation of total Hg with salinity and TSS with salinity could be the result of the total Hg association with TSS. However, the human activities in the Sivash

Fig. 4 The Hg content in bottom sediments on different stations in Bay Sivash during June 2018

drainage area are probably more responsible. An economically developed zone, located in Krasnoperokopsk and Armyansk in the bay watershed, has a diverse chemical industry and their dangerous emissions include also different Hg species. Since the 1970s, the development of irrigated agriculture, mainly rice, was started with intensive use of various pesticides and fertilizers. Most of the drainage waters from the agricultural land were regularly discharged into Bay Sivash (Shadrin et al. [2018](#page-7-0)). Also, the water in the North Crimean Canal was initially high polluted by various pollutants including Hg (Bashkirtseva [2003](#page-6-0); Mirzoyeva et al. [2015\)](#page-7-0).

In coastal areas around Crimea, except Bay Sevastopolskaya, a Hg content in surface sediments averaged 70.4 ng g^{-1} wet weight $(SD = 31.5, CV = 0.45)$ (Kostova et al. [2001;](#page-7-0) Raybushko et al. [2005\)](#page-7-0). In highly polluted Bay Sevastopolskaya, it was significantly higher averaging 942 ng g⁻¹ wet weight (SD = 457, CV = 0.50) for silty sediments and 96 ng g^{-1} wet weight (SD = 11.7, CV = 0.12) for sandy sediments (Kostova [2005](#page-6-0)). In the Bay Sivash sediments, there was a significantly lower Hg concentration (on average 13.8 ng g^{-1} wet weight) probably due to a high proportion of mollusk shells in sediments and the frequent anoxic near bottom conditions, as a result of the massive development of floating mats of filamentous green algae Cladophora (Shadrin et al. [2018](#page-7-0), [2019,](#page-7-0) [2020](#page-7-0)), enhancing the Hg release from sediments (Koron and Faganeli [2012\)](#page-6-0). The role of DOM, which most probably originates from the intense growth and decay of macrophytes, seems important in the complexation of dissolved Hg (Ravichandran [2004\)](#page-7-0).

To make a comparison of Hg content in the water column and bottom deposits, we calculated the total content in both reservoirs. We assumed that the density of sediment is 2 g cm^{-3} and the average depth of Bay Sivash is 0.7 m. The calculation showed that there was on average 0.235 mg m^{-2} in the water column and 2.8 mg m⁻² in the surface (10 cm) sediment layer. The total Hg reservoir in the surface sediment was, therefore, more than 100 times higher than in the water column. The resuspension of surface sediment also may contribute to high total Hg content in this shallow water column.

The food webs are the main pathway in the Hg flux in aquatic ecosystems (Jones and Wurtsbaugh [2014](#page-6-0); Walters et al. [2020](#page-8-0)). The high Hg concentration leads to disruption of the normal functioning of aquatic ecosystems and their use by humans. Artemia cysts are the most valuable bioresource in Bay Sivash which can become the largest Artemia habitat in the world (Anufriieva and Shadrin [2020\)](#page-6-0). Adult Artemia, as well cysts, can accumulate relatively high Hg up to 0.34 μg g^{-1} (Jones and Wurtsbaugh [2014;](#page-6-0) Stetsiuk et al. [2018](#page-7-0)). There are reports that salinity may affect the concentration and behavior of Hg in the aquatic environment as well as on toxicity of different Hg species on Artemia and other crustaceans (Jones [1973](#page-6-0); Okasako and Siegel [1980;](#page-7-0) Ullrich et al. [2001](#page-7-0); Jones and Wurtsbaugh [2014](#page-6-0); Johnson et al. [2015](#page-6-0); Boyd et al. [2017](#page-6-0)). Various Hg species, e.g., MeHg, may affect the processes and damaging the organism (Gebhardt [1976;](#page-6-0) Go et al. [1990;](#page-6-0) Sarabia et al. [1998;](#page-7-0) Jones and Wurtsbaugh [2014\)](#page-6-0) and, for example, reducing the brine shrimp lifespan (Pandey and MacRae [1991\)](#page-7-0). Since Artemia is a keystone species in the Bay Sivash ecosystem (Anufriieva and Shadrin [2020\)](#page-6-0), and increased Hg concentration may disturb not only the normal functioning of the *Artemia* population but also the whole bay ecosystem. Since Artemia and other invertebrates are the main food source for birds, the high Hg content can adversely affect them (Schmidt [1991;](#page-7-0) Helgason et al. [2008](#page-6-0); Pietrelli and Biondi [2009](#page-7-0)). Bay Sivash is an important area for many species of birds, which use it for nesting, wintering, and resting during transcontinental migrations (Havrylenko [2000](#page-6-0); Verkuil et al. [2003\)](#page-7-0). The bay is a crossroad of bird migratory routes between Europe, Asia, and Africa. Is the high content of Hg in Bay Sivash a real risk for all birds living there? Is this contamination also a significant risk for the Artemia cysts harvest and planned aquaculture development in the lagoon? The quantitative assessment of the impacts of this contamination on the ecosystem functioning, bird diversity, aquaculture perspectives, and Artemia cyst harvest is an urgent goal. Additional deeper studies in the bay are needed to plan the environmental management.

Conclusion

The concentration of the total mercury content is high in the water of Bay Sivash ranging from 200 to 600 ng l^{-1} due to its hypersalinity and heavy anthropogenic pollution. After the closing of the North Crimean Canal, the salinity growth significantly increased total mercury content in water. Currently, there are not enough data to quantitatively assess the salinity effects on the behavior of Hg in the aquatic environment as on the toxicity of different Hg forms for different aquatic organisms. New studies of these issues are very important for the development of general aquatic ecology to better understand the role of a salinity factor in geochemical and biological

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Authors' Contributions Nickolai Shadrin: conceptualization, formal analysis, field investigation, methodology, writing—original draft and final text. Aleksandra Stetsiuk: Hg content assessment, formal analysis, writing—review and editing. Alexander Latushkin: field investigation, methodology, formal analysis, writing—review and editing. Elena Anufriieva: methodology, formal analysis, writing—review and editing.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate The authors declare consent to participate.

Consent to Publish The authors declare consent to publish.

Competing interests The authors declare that they have no competing interests.

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