Bioindication of mercury pollution of the Amur River during the ice-covered period

D V Andreeva

Institute of Water and Ecology Problems of the Far Eastern Branch of the Russian Academy of Sciences, 56 Dikopoltsev Street, Khabarovsk, 680021, Russia

E-mail: freckles2008@yandex.ru
ORCID ID: https://orcid.org/0000-0003-1548-5066

Abstract. The results of the layer-by-layer research of a river ice cores by using spectral and microbiological methods were presented. The impact of the mercury on sulfate-reducing bacteria activity from different layers of ice that had been sampled in March 2016 in the lower Amur River in Khabarovsk city was found. It was found that mercury concentration range of 0.0005–0.001 mg/l stimulated the sulfate-reducing bacteria activity, which were into the ice throughout the study area of the Amur River. To a large extent, this effect was typical for the ice that was sampled in the right bank of the Amur River near Khabarovsk city, where mercury pollution of the aquatic environment had been detected repeatedly. The activity of biogeochemical processes into the ice determined by high concentrations of organic substances, by the pollution of the aquatic environment with mercury during the ice cover formation, and by the abundance of cultivated heterotrophic and sulfate-reducing bacteria. During the ice drift and ice melting, the secondary pollution with toxic substances of various genesis of the aquatic environment occurs, which can have a negative impact on a hydrobionts.

1. Introduction
The spatiotemporal integrity of aquatic ecosystems, as well as the seasonality of the impact of individual factors, determine the relevance of studying glaciochemical and biochemical processes occurring in ice, which can act as accumulators of toxic substances [1]. In the ice, despite the extreme conditions, biogeochemical transformation of organic matter (OM) with the participation of various ecological and physiological groups of microorganisms is a rather active [2]. Cryophilic microbial communities are involved in the destruction of autochthonous and allochthonous OM, they promote the migration of nutrients, affect changes in the structure of biocenoses, and act as indicators of anthropogenic pollution of aquatic ecosystems [3; 4].

Intra-water body processes occurring in winter are largely determined by the functioning of biological complexes in the water-ice contact zone. The biogeochemical processes occurring in the thickness of river ice and the features of its pollution in various sections of rivers have been little studied. Many ideas about cryogenic processes change significantly when using bioindication methods to assess the state of aquatic ecosystems in winter.

The peculiarity of the ice cover of the Amur River is the presence of a significant amount of terrigenous material of various granulometric composition in the ice mass. The presence of weakly expressed interlayers or irregularly shaped accumulations of dispersed loamy material is also noted...
inside ice cores, which is probably associated with an increase in water turbidity during releases from reservoirs, accompanied by an increase in flow rates [5].

In various layers of ice, it is possible to identify organic substances of natural and anthropogenic origin that were present in the water during the formation of a particular layer of ice, as well as products of bacterial metabolism.

The presence of sulfate-reducing bacteria (SRB) in ice is used to indicate reducing conditions in the ice mass, an increased content of readily available OM and sulfates.

For the first time, the bioindication role of cryomicrobocenoses was shown when assessing transboundary pollution of the Amur River during the freeze-up period of 2000–2001 [6]. Pronounced responses of microbial communities of ice to the complex impact of various factors were identified: biogenic (the development of algae) and abiotic (the presence of toxic micro-admixtures of organic substances and heavy metals). Increased resistance of microorganisms of the ice-water contact zone to mercury was noted near the right bank near Leninskoe village, where the influence of polluted water masses of the Sungari River is manifested [6].

In the Amur region, the sources of mercury pollution include industrial centers (the cities of Khabarovsk, Amursk, Komsomolsk-on-Amur) and the runoff of the Sungari river (territory of China). A significant accumulation of heavy metals and mercury was recorded in the surface layer of bottom sediments in the estuarine zones of the Amur, the Bureya and the Zeya Rivers, in the basins of which reservoirs were created [7].

Mercury and its compounds are hazardous toxic substances that pose a threat to human health. The risk of mercury pollution depends on many factors, including the form of this heavy metal in the aquatic environment and bottom sediments [8; 9]. As a result of biogeochemical processes, including microbiological destruction of plant residues and humic substances of soils, mercury transforms into a more toxic form – methylmercury (CH$_3$Hg$^+$). This increases its migratory capacity, entry into the water column and accumulation by aquatic organisms.

Microbiological transformation is an important factor which determines the form of existence of mercury. Soluble organic matter usually stimulates microbial activity, and thus can promote the synthesis of methylmercury.

It is known that sulfate-reducing bacteria (SRB), which are capable of oxidizing various carbon sources at 0°C, act as a key microbiological mercury methylator in many water systems [10]. Mercury methylating SRB, including members of the Desulfovibrionaceae family, oxidize carbon sources to form an intermediate product – acetate. The ability to methylate mercury does not depend on the genus or species of bacteria. For strains representing two genera Desulfovibrio and Desulfomicrobium, it was shown that methylmercury production in natural ecosystems depends on the presence of SRB or genes encoding sulfate reduction [11]. Sulfate-reducing bacteria have a pair of HgcAB genes that encode proteins required for mercury methylation [12].

The paper presents the results of experimental studies to assess the effect of mercury on the activity of sulfate-reducing bacteria isolated from ice formed in the winter period of 2015–2016 in the Amur downstream of Khabarovsk city.

2. Materials and methods

Ice cores were taken in March 2016 during a complex expedition of the staff of the Institute of Water and Environmental Problems of the Far Eastern Branch of the Russian Academy of Sciences under the leadership of Doctor of Geological Sciences A.N. Makhinov. Ice was sampled with an annular drill with an inner diameter of 16 cm. For chemical and microbiological analyzes, melts of different layers of ice were used, sampled along the transverse profile of the Amur River near Khabarovsk city. Ice samples were melted at room temperature in compliance with the rules of asepsis. The ice was placed in sterile glasses with lids.

The abundance of SRB was found by the method of submerged inoculation of 1 ml of melt on agarized Postgate medium. The activity of SRB growth on lactate was assessed photometrically by the
change in optical density (OD) of the culture fluid at 600 nm using KFK-3-01. In experimental studies, a water-soluble mercury salt (HgNO₃) was used at concentrations of 0.0005 and 0.001 mg/l.

The total content of dissolved OM in ice melts was found spectrophotometrically at 254 nm (Shimadzu UV-3600) and expressed as a spectral absorption coefficient (SAC₂₅₄, absorbance units).

All analytical studies were carried out at the Center for Shared Use of Scientific Equipment at the Institute of Water and Ecology Problems of the Far Eastern Branch of the Russian Academy of Sciences.

3. Results and discussion

To identify the role of SRB in the formation of redox conditions in the winter period 2015–2016, studies of their abundance and the content of dissolved OM in various layers of the ice of the Amur River were carried out downstream of Khabarovsk city (Table 1).

It was found that SRB involved in the sulfur cycle were contained in all investigated ice melts from the Amur River. Ice samples taken in the middle of the Amur downstream of Khabarovsk city in a layer of 37–53 cm contained the maximum abundance of SRB (2525.7 CFU/ml) which reflected the OM content in the ice (Table 1). The melt of this layer of ice was turbid and contained particles of detritus and biofilm. It is possible that during the formation of the ice cover there was an inflow of polluted water masses from the Sungari River.

| Sampling location | Layer, cm | Abundance SRB, CFU/ml | SAC₂₅₄, absorbance units |
|-------------------|-----------|-----------------------|-------------------------|
| Amur River, lower the railway bridge | 0–17 | 505.0±15.7 | 0.135 |
| Khabarovsk City | 34–47 | 723.7±18.2 | 0.234 |
| | 48–68 | **1258.7±23.2** | **0.404** |
| | 0–21 | 233.3±11.4 | 0.080 |
| | 37–53 | **2525.7±53.3** | **0.664** |
| | 73–91 | 567.7±16.4 | 0.143 |
| | 0–17 | 658.3±17.5 | 0.123 |
| | 18–34 | 956.7±20.6 | 0.247 |
| | 35–52 | **1827.3±41.6** | **0.468** |

Notice: maximum values are shown in bold.

In the lower layers of ice, sampled from the left and right banks of the Amur river, a high abundance of sulfate reducers and a high content of dissolved OM were recorded (Table 1). This may be due to the fact that the inflow of polluted water masses with the runoff of the Zeya, the Bureya and the Sungari Rivers occurred during the ice formation period.

Experimental studies carried out in 2016 showed that different layers of ice of the Amur contain SRB resistant to mercury ions at concentrations of 0.0005 (Hg1) and 0.001 mg/l (Hg2) (Table 2).

The maximum resistance to mercury at a concentration of 0.001 mg/l was exhibited by SRB from all ice layers sampled from the right bank downstream of Khabarovsk (Table 2). This data is consistent with the authors’ early studies of the resistance of the bacteriobenthos of the Amur River to mercury pollution [7; 13]. They indicate local pollution of water, ice and bottom sediments of the right bank of the Amur River with mercury, which can come from the runoff of the Ussuri and the Sungari Rivers and wastewater from Khabarovsk city.

Such resistance to mercury is characteristic of microbial complexes of bottom water layers in the dam section of reservoirs. Thus, microbiological and spectrophotometric studies of water quality in the Zeya reservoir in the summer of 2013 showed that active biogeochemical processes of OM transformation occur in the dam area [14].
Table 2. Effect of mercury on the growth of sulfate-reducing bacteria on lactate from different layers of the Amur River ice

| Sampling location                      | Layer, cm | Growth on a substrate, OD at 600 nm | Lactate | Lactate + Hg1 | Lactate + Hg2 |
|----------------------------------------|-----------|-----------------------------------|---------|---------------|---------------|
| Amur River, lower the railway bridge   | 0–17      |                                   | 0.46    | 0.35          | 0.21          |
|                                        | 34–47     |                                   | 0.54    | 0.46          | 0.32          |
|                                        | 48–68     |                                   | 0.62    | 0.58          | 0.44          |
|                                        | 0–21      |                                   | 0.23    | 0.24          | 0.21          |
|                                        | 37–53     |                                   | 0.92    | 0.85          | 0.83          |
|                                        | 73–91     |                                   | 0.34    | 0.27          | 0.23          |
| Khabarovsk City                        | 0–17      |                                   | 0.35    | 0.32          | 0.30          |
|                                        | 18–34     |                                   | 0.38    | 0.36          | 0.32          |
|                                        | 35–52     |                                   | 0.46    | 0.44          | 0.41          |

Notice: Hg1 – 0.0005 mg/L, Hg2 – 0.001 mg/L.

Above the dam, in the bottom water, where the main sedimentation of suspended solids occurs, a high content of dissolved OM was recorded. The maximum resistance of SRB to contamination with mercury ions was also evidenced here. The activity of sulfate-reducing microorganisms at a Hg concentration of 0.0005 mg/l was 1.8 times higher than in the control sample, and at a Hg concentration of 0.001 mg/l the activity was comparable to that in the control sample [14].

4. Conclusion

Thus, there is reason to believe that the revealed resistance of SRB is due to their adaptation to mercury pollution of bottom sediments and the inclusion of these microorganisms in the ice mass as a part of detritus during discharges of water masses from reservoirs. The discovered resistance of sulfate-reducing bacteria to mercury downstream of Khabarovsk near the right bank may be associated with their direct participation in the formation of methylmercury in ice. In winter, the methylation of mercury in the Amur River can be promoted by the oxygen limit, the discharge of insufficiently treated wastewater, the supply of underground iron-containing waters, and the processes of sulfate reduction in bottom sediments.

Acknowledgments

The studies were carried out using the resources of the Center for Shared Use of Scientific Equipment "Center for Processing and Storage of Scientific Data of the Far Eastern Branch of the Russian Academy of Sciences", funded by the Russian Federation represented by the Ministry of Science and Higher Education of the Russian Federation under project No. 075-15-2021-663.

References

[1] Kondratyeva L M 2010 Geoecological studies of river ice Geoecol., Engin. Geol., Hydrogeol. 6 pp 511–20 (in Russian)
[2] Cameron K A, Stibal M, Hawkings J R, Mikkelsen A B, Telling J, Kohler T J, Gözdereğil E, Zarsky J D, Wadham J L and Jacobsen C S 2017 Meltwater export of prokaryotic cells from the Greenland ice sheet Environ. Microbiol. 19(2) pp 524–34
[3] Kondratyeva L M, Pan E V and Zhukov A G 2012 Pollution of the Amur River during an ice cover: primary factors of ecological risk Proc. 2nd Int. Meet. of Amur-Okhotsk Consortium ed Amur-Okhotsk Consortium (Sapporo: Institute of Low Temperature Science) pp 35–41
[4] Pandey R et al. 2016 Ice-nucleating bacteria control the order and dynamics of interfacial water Sci. Adv. 2(4) pp 1501–9
[5] Makhinov A N, Kim V I and Shmigirilov S A 2017 The structure of ice and ice cover in lower Amur reaches with branched channel Water Res. 44(4) pp 559–67
[6] Kondratyeva L M 2002 Ice as component of surface water pollution monitoring Proc. of Int.
Conf. «Enviromis-2002» vol 1 ed E P Gordova (Tomsk: GO Tomsk «CSTI») pp 174–9 (in Russian)

[7] Kondratyeva L M, Andreeva D V and Golubeva E M 2013 Influence of large tributaries on biogeochemical processes in the Amur River Geogr. Nat. Res. 34(2) pp 129–36

[8] Constant P, Poissant L, Villemur R, Yumvihozo E and Lean D 2007 Fate of inorganic mercury and methyl mercury within the snow cover in the low arctic tundra on the shore of Hudson Bay (Quebec, Canada) J. of Geophys. Res. Atmospheres 112(8) pp 1–10

[9] Durnford D and Dastoor A 2011 The behavior of mercury in the cryosphere: a review of what we know from observations J. of Geophys. Res. Oceans 116 pp 1–30

[10] Sokolova E A 2010 Effect of temperature on sulfatereducing bacteria under experimental and field conditions during winter Contemp. Probl. Ecol. 6 pp 865–9 (in Russian)

[11] RohY et al. 2006 Metal Reduction and Iron Biominalization by a Psychrotolerant Fe(III)-Reducing Bacterium, Shewanella sp. Strain PV–4 Appl. Environ. Microbiol. 72(5) pp 3236–44

[12] Regnell O and Watras C 2019 Microbial mercury methylation in aquatic environments: a critical review of published field and laboratory studies Environ. Sci. Technol. 53(1) pp 4–19

[13] Kondratyeva L M, Andreeva D V and Golubeva E M 2018 Factors affecting the conditions of sulfate reduction and mercury methylation in the River Amur ice Ice and Snow 58(1) pp 105–16 (in Russian)

[14] Andreeva D V, Kondrateva L M and Stukova O Yu 2014 Microbiological studies of the process of sulphate reduction in the Zeya reservoir Vladimir Ya. Levanidov’s Biennial Memorial Meetings vol 6 ed E A Makarchenko et al. (Vladivostok: Dalnauka) pp 32–7