

Adaptation of cryomicrobiocenoses to mercury pollution

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ABSTRACT. This article presents the results of a layer-by-layer study of the ice thickness in the main channel of the Amur River (Khabarovsk city area) and Pemzenskaya and Amurskaya channels. Spectral and microbiological methods were used to assess the nature of ice contamination with organic compounds. The study of suspensions from ice melts by mass spectrometry (ICP MS) confirmed mercury pollution. Among the cultivated heterotrophic bacteria from the ice mass (cryomicrobiocenoses), there were communities adapted to high mercury concentrations (up to 0.001 mg/L). In some layers of the ice, we recorded a high portion of sulfate-reducing bacteria involved in mercury methylation. In the mainstream of the Amur River, a layer of ice (70-117 cm) had a high content of mercury, dissolved organic matter, plant detritus, and the maximum number of bacteria resistant to mercury pollution. This ice layer was formed during the period of technological discharges from reservoirs to combat the consequences of a major flood in the Amur River Basin. The indicator of resistance of cryomicrobiocenoses to high mercury concentrations was due to the adaptive potential of heterotrophic microorganisms, including active mercury methylators, i.e. sulfate-reducing bacteria. This indicator can be used for a retrospective assessment of mercury pollution of rivers during the freeze-up period.

Keywords: mercury pollution, cryomicrobiocenoses, methylation, sulfate reduction, organic matter

1. Introduction

Mercury is a dangerous toxicant for humans and aquatic organisms due to its high accumulation capacity. There are numerous data on its accumulation in bottom sediments and living organisms (Wiener et al., 2006). The risk of mercury pollution depends on many factors, including its occurrence in the environment. The migration ability of mercury is due to its transformation into a toxic form, methylmercury (CH_3Hg^+), resulted from microbial activity in the presence of organic substances (OS) (Ermakov, 2010; Liu et al., 2012). Dissolved OS play a more significant role in mercury methylation than pH or Eh (Feyte et al., 2010). The main mechanism of resistance of microorganisms to mercury, including cryomicrobiocenoses (CM) developing in the ice mass, is associated with the metabolism of specialized groups of microorganisms (iron- and sulfate-reducing bacteria) (Kerin et al., 2006). These bacteria from the Arctic ice and permafrost were adapted to mercury pollution. They can maintain their metabolic activity at low temperatures (Rivkina et al., 2000; Moller et al., 2011).

Previously, an increased resistance of CM to mercury ions was recorded in the transboundary section of the Amur River downstream the mouth of the Sungari River (right-bank tributary from the territory of China). Heterotrophic bacteria isolated from the ice in the study of river runoff from the territory of China were resistant to high concentrations of mercury, lead and cadmium salts (up to 0.1 g/L). In the control section, CM growth was inhibited by a lower content of these metals (0.001 g/L).

This article presents the results of experimental studies of the cryomicrobiocenoses resistance from the Amur River near Khabarovsk to mercury pollution after a major flood.

2. Materials and methods

The ice cores were sampled in March 2014 during a complex expedition by specialists from Institute of water and ecology problems, Far Eastern Branch, Russian Academy of Sciences. Ice was sampled using an annular drill with an inner diameter of 16 cm. Melts

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of different layers of ice sampled along the transverse profile of the Amur River near the city of Khabarovsk and in large tributaries (Amurskaya and Pemzenskaya) were used for chemical and microbiological analyzes. The thickness of the ice layers was determined by its heterogeneity: transparency, turbidity, presence of detritus, etc.

For microbiological studies, the ice samples were melted at room temperature according to asepsis rules. The abundance of cultivated heterotrophic bacteria (CHB) was determined on a dense nutrient medium (fish-peptone agar diluted 10 times); that of sulfate-reducing bacteria (SRB) – by deep inoculation of 1 mL of ice melt on Postgate agar medium. The growth activity and adaptation of SRB to mercury pollution were assessed by the change in the optical density (OD) of the culture liquid at 600 nm on a KFK-3-01 photometer. Cultivation was carried out in a liquid medium with calcium lactate. Water-soluble mercury salt (HgNO_3) was added at concentrations of 0.0005 (Hg1) and 0.001 mg/L (Hg2).

Total concentrations of dissolved OS in the ice melts samples after separation of suspensions was determined by the spectrophotometric method at 254 nm (Shimadzu UV-3600) and expressed as a spectral absorption coefficient (SAC_{254} , abs. units). Suspended substances were separated from the melts by filtration through a double paper filter (blue ribbon treated with freshly prepared 1% HNO_3). The solid precipitate was washed off the filters with an acidified nitric acid solution into glassy carbon crucibles and decomposed. The elemental composition in ice melts and suspended matter was determined according to Federal environmental normative document (PND F 16.1:2.3:3.11-98) by inductively coupled plasma spectrometry on a Perkin Elmer instrument (USA) at the Khabarovsk Innovation and Analytical Center for collective use at the Yu.A. Kosygin Institute of tectonics and geophysics FEB RAS.

3. Results and discussion

Studies were carried out during the freeze-up period after the flood in the summer of 2013, when large reservoirs in the Russian territory (Zeyskoye and Bureyskoe reservoirs) were overfilled, and technological discharges were carried from them in winter. It is important to emphasize that during the period of ice

core sampling, the mercury content in the under-ice water and in ice melts was below the detection limits. A completely different situation was observed with suspended matter (SM) from different ice layers (Table 1). The maximum concentration of mercury was in the 70–117 cm layer, which was characterized by a high content of detritus and mineral suspensions. The finely dispersed part of SM from this layer was a complex conglomerate of diatoms, bacterial complexes and a lithogenic component on SEM images (Golubeva et al., 2020).

Microbiological studies were carried out with ice melts with a high concentration of dissolved OS sampled in the mainstream of the Amur River, Pemzenskaya and Amurskaya channels. A high abundance of CHB and SRB was characteristic of the melt of 70-117 cm ice layer that was brown in color, high in SAC_{254} , detritus, and aromatic compounds due to the presence of phenol-resistant bacteria (Table 2). High abundance of CHB and SRB in the ice is one of the important prerequisites for the formation of conditions for mercury methylation in ice.

Cryomicrobiocenoses from the ice layer of 70-117 cm sampled in the main channel of the Amur River, 272 m from the left bank, under the influence of the Bureya River, were distinguished by the maximum adaptive potential to mercury pollution. Growth on calcium lactate in the presence of mercury (0.0005 and 0.001 mg/L) did not actually differ from the control. In other parts of the main channel of the Amur River, the growth of CM on calcium lactate was weaker, but no inhibition by mercury was observed.

In the Pemzenskaya channel, with a lower concentration of dissolved OS, there were the maximum abundance of CHB and a high abundance of SRB in the ice layer of 60-85 cm. It was comparable to the abundance of the unique ice layer in the main channel of the Amur River. This may be due to the fact that part of the water masses of the Amur River enters the Pemzenskaya channel. Selected mercury concentrations inhibited the growth of cryomicrobiocenoses (Table 2).

Interesting pattern was noted in the study of ice in the Amurskaya channel, which receives the Ussuri River runoff from the territory of China. The OS concentrations in the ice melts were comparable to their values in the ice melts of the Pemzenskaya channel. However, the phenomenon of mercury stimulation of CM growth on calcium lactate from the surface layer

Table 1. Mercury concentrations in suspended matter of ice melts from the cores sampled in the mainstream of the Amur River in the winter of 2013-2014

Ice core, 20 m from the left bank		Ice core, 272 m from the left bank		Ice core, 80 m from the left bank	
Layer, cm	Concentration, $\mu\text{g/g}$	Layer, cm	Concentration, $\mu\text{g/g}$	Layer, cm	Concentration, $\mu\text{g/g}$
0-30	0.32	0-40	0.30	0-12	-
30-45	0.03	40-50	0.22	12-27	0.70
45-60	0.15	52-72	0.50	27-57	0.72
60-70	b.l.	70-117	0.91	57-82	0.11
120-132	b.l.	117-139	b.l.	-	-

Note: «b.l.» - below the detection limits of the device; «-» - only four ice layers.

Table 2. Integrated assessment of ice melt and resistance of microorganisms to different mercury concentrations (Hg1 = 0.0005 mg/L; Hg2 = 0.001 mg/L)

Sampling site of ice cores	Ice layer, cm	SAC ₂₅₄ , absorbance units	Abundance, CFU/mL		Growth activity on lactate, OD, 600 nm		
			CHB	SRB	Control	Hg1	Hg2
Mainstream of the Amur River, Khabarovsk city area					Control	Hg1	Hg2
Lb	70-117	0.982	68600	25070	0.85	1.1	0.8
Middle	190-200	0.127	6600	60	0.28	0.35	0.30
Rb	10-30	0.443	13800	7130	0.58	0.65	0.6
Penzenskaya channel							
Lb	10-35	0.319	23000	17800	0.35	0.42	0.15
Middle	60-85	0.352	83000	23700	0.65	0.55	0.36
Rb	90-102	0.192	360	120	0.20	0.25	0.20
Amurskaya channel							
Lb	90-100	0.312	12600	7560	0.25	0.30	0.34
Middle	0-20	0.256	2330	2257	0.32	0.36	0.35
Rb	0-10	0.264	10000	3500	0.45	0.60	0.67

Note: CFU/mL - number of colony-forming units in 1 mL of melted ice; Lb/Rb - on the left/ right bank.

of ice sampled from the right bank was revealed for both mercury concentrations. Previously, an elevated mercury concentration in water was repeatedly recorded on the right bank where the rice fields were located, which can be treated with mercury-containing pesticides. Such CM growth stimulation by elevated mercury concentrations may be associated with chronic pollution of the Amurskaya channel.

4. Conclusions

The adaptive potential of cryomicrobiocenoses that develop in the ice column can be used to indicate the contamination of river waters with organic substances and heavy metals, including mercury, during the ice formation on rivers. The study of layer-by-layer chemical characteristics of ice and the resistance of heterotrophic bacteria, including sulfate-reducing bacteria, to mercury pollution, allows us to assess the degree of environmental risk in case of chronic pollution of the water and bottom sediments of watercourses by toxic substances and to identify technological discharges from reservoirs and the impact of surface runoff from agricultural fields contaminated with mercury-containing pesticides.

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Conflict of interest

Authors declare no conflict of interest.

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