Earth`s Cryosphere, 2017, vol. XXI, No. 2, pp. 21–27 http://earthcryosphere.ru/

DOI: 10.21782/EC2541-9994-2017-1(21-27)

BIOGEOCHEMICAL ASPECTS OF MERCURY METHYLATION IN THE ICE OF THE AMUR RIVER

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The results of studying the content of heterotrophic bacteria, mercury and volatile organic compounds, including ethyl acetate, in the ice layer of the Amur River and the Pemzenskaya Channel are discussed. The maximum amount of cultivated heterotrophic bacteria participating in biotransformation of organic compounds of different origin was found in the Amur River near the left bank in the 30–40 cm layer of ice containing particles of detritus. Ethyl acetate was established to be the main substance contained in different layers of ice; its maximum concentration (18.2 mg/L) was recorded in the middle of the Amur River in the 40–70 cm layer of ice. In the 20–30 cm ice layer from the Pemzenskaya Channel, the following pollutants were found near the right bank: acetaldehyde (4.1 mg/L), benzene (0.5 mg/L) and styrene (2.0 mg/L). Higher mercury concentrations ranging from 0.13 to 0.14 μg/L characterized the upper layers of ice near the right bank of the Amur River and the left bank of the Pemzenskaya Channel. The combination of these components in the ice layer preconditions methylation of mercury.

Methylmercury, ice, volatile organic matter, biotransformation

INTRODUCTION

Previously mercury contamination of the Amur River was discussed due to the operation of the Amur pulp and cardboard factory and accumulation of mercury-containing waste. In the 1990s, the workers of the Institute of Water and Ecological Problems, FEB RAS, carried out detailed studies of the geochemical background and contamination of the territory of the Amur basin with heavy metals. Industrial centers (Khabarovsk, Amursk, Komsomolsk-on-the-Amur) and the Songhua River were referred to as sources of industrial pollution. Averaged data were presented on the content of mercury in the bed silt for the middle and lower Amur River – 0.20 mg/kg (0.10–0.46 mg/kg). Increased content of mercury (without indicating concentrations) was found in the fish caught in that period. Significant accumulation of heavy metals and mercury was recorded in the surface layer of the bed silt of the mouth zone of the Amur River, where active sedimentation of suspended matter takes place [*Kot, 1994*]. The seasonal studies conducted in 2012–2014 by the Krai Center of Environmental Monitoring and Emergency Prediction of Khabarovsk krai demonstrated the increase in the content of mercury to 2–3 maximum permissible concentration values in the spring period. Many authors note an increase in the migration ability of mercury, which is mostly caused by transformation of mercury into methylmercury, due to the microbiological activity in the presence of organic matter [*Ermakov, 2010; Moiseenko, 2010; Liu et al., 2012*].

The factors contributing to methylation of mercury in the Amur River in the winter period are the limit of oxygen, discharge of insufficiently treated wastewater, recharge of underground iron-containing waters and activation of the processes of sulfate reduction in the bed silt. The problem of mercury becomes especially important for the entire river basin due to trans-borderline contamination of the Amur River due to its tributary the Songhua River (China), winter discharge from water reservoirs and the spring outflow of ice into the coastal waters of the Far Eastern seas [*Kondratyeva et al., 2013; Kondratyeva and Zhukov, 2014*].

Several sources of the mercury contamination of the Amur River may be identified.

1. Trans-borderline supply of mercury from the territory of China with the waters of the Songhua River, in which, according to Chinese researchers, the content of mercury was 0.009–0.069 μg/dm^3. It is known that in the period from 1958 to 1982, the river was contaminated by discharge of wastewater from the Jinlin Chemical Co chemical factory (a large producer of acetaldehyde from acetylene), in which mercury sulfate (II) was used as a catalyst. A large part of this mercury was accumulated in bottom sediments and gradually migrated into the water medium [*Jiang et al., 2006; Zhang et al., 2010b*].

2. The land runoff from the territories of gold production by the amalgamation method [*Stepanov et al., 2003*], from the Lana vermilion field and from

abandoned storages of granosan pesticide, which was used in the period of 1963–1989 in the territory of the Amur region [*Koval, 2003*].

3. Discharge of technical water containing a large amount of detritus from reservoirs of the hydroelectric power plants built on the Zeya-Bureya plain [*Kondratyeva et al., 2013*].

4. In addition, atmospheric transfer of mercury over the territory of the Amur River basin is possible when coal is burnt and during forest fires [*Yudovich and Ketris, 2010*].

Despite the studies of many years, a number of questions related to mercury methylation remain unsolved [*Liu et al., 2012*]: the way of mercury transport to cells (whether active or facilitated transport); it is not clear yet by which chemical mechanism the methyl group is transported into mercury, what kind of contribution syntrophy makes in the process of mercury methylation; the mechanism of oxidative decomposition of methylmercury by microorganisms is at the stage of investigation; the influence of substances contained in individual components of ecosystems on mercury methylation has not yet been completely studied. Still open is the issue of the relationship between microbial communities and their potential for mercury methylation in different components of the aqueous ecosystems.

The rate of microbial mercury methylation depends on a number of environmental factors which may affect the bioavailability of mercury and the structure of the microbial community: the water temperature, pH, the oxidation-reduction potential, availability of nutrients and acceptors of electrons, as well as the presence of ligands and absorbing surfaces. These parameters cannot be considered independently from each other, as they often interact, with the resulting complex system of biogeochemical processes formed.

The methylation processes are especially intense in the upper layers of the bottom sediments, rich in organic matter (OM), in the substances suspended in water, as well as in slime covering fish [*Moiseenko, 2010*]. As a rule, chlorides boost the methylation process, whereas sulfates in large concentrations inhibit it [*Ermakov, 2010*].

The majority of microorganisms methylating mercury are referred to *Deltaproteobacteria*. Many studies are aimed at answering the question whether the methylating activity is randomly distributed among proteobacteria or whether it correlates with phylogenetic appurtenance [*Kerin et al., 2006*]. It is known that sulfate-reducing bacteria (SRB), capable of oxidizing different sources of carbon in a wide range of temperatures, are the key microbiological methylator of mercury in many aqueous systems [*Sokolova, 2010*]. Mercury-methylating SRB, including representatives of the *Desulfovibrionaceae* family, may oxidize carbon sources, with an intermediate product, acetate, formed [*Wasik et al., 2012*]. The study of the biochemical mechanism of mercury methylation with participation of SRB showed the presence of at least two ways of this process with acetyl-CoA used and without it [*Liu et al., 2012*]. It has been demonstrated in the studies of individual SRB strains that mercury methylation most often takes place in the bed silt. Yet, this process may occur not only in the bed silt but also in the water mass [*Eckley and Hintelmann, 2006*] and in the periphyton communities of fresh-water ecosystems [*Guimaraes et al., 2006*], where other groups of bacteria also play a large role in mineralization of carbon.

Iron-reducing and methane-producing bacteria may take part in the methylation of mercury [*Roh et al., 2006; Hamelin et al., 2011*]. Several strains of *Geobacter*, *Desulfuromonas* and *Shewanella* were tested for the methylating activity. The representatives of the former two geni produced methylmercury when cultivated with Fe(III), whereas *Shewanella* spp. did not methylate mercury under nitrate-reducing conditions and in the presence of Fe(III). In addition, *Geobacter metallireducens* and *Geobacter sulfurreducens* methylated mercury by using either fumarate or nitrate, thus indicating that reduction of iron is not obligatory for mercury methylation.

Humic acids play a significant role in the biogeochemical processes of mercury methylation. Formation of insoluble complexes with humic acids retards transformation of mercury and creates a possibility of its deposition, while formation of soluble complexes with fulvic acids boosts its migration. A high content of organic matter with prevalence of humic acids, oxygen deficit, and neutral or subacid medium contribute to the processes of mercury alkylation [*Ermakov, 2010*]. It was established that in the winter period the content of humic acids in the Amur River decreases to a minimum [*Levshina, 2006*]; under such conditions, concentration of mobile mercury may increase.

There are reports that mercury may be methylated in the snow. The scientists of the Khanty-Mansi autonomous region discovered sevenfold presence of mercury in the places of snow deposition from territories of settlements versus the background mercury content [*Moskovchenko and Babushkin, 2012*]. A brief review was published on the role of mercury in the cryosphere [*Durnford and Dastoor, 2011*]. Mercury-resistant microorganisms were identified in the Arctic snow and ice [*Moller et al., 2011*]. Some scientists believe that the living activity of microbes stops at the temperatures below 0 °C [*Brouchkov et al., 2011*], but, as shown in the permafrost studies [*Rivkina et al., 2000*], the metabolic activity of microorganisms may be sustained at –20 °C.

The recent results of studying the genetic, evolutionary and biochemical aspects of mercury methylation have shown that different taxonomic groups may take part in this process (*Proteobacteria*, *Firmicutes*, *Archaea*), which occupy different ecological niches and which account for the global scales of mercury methylation both under aerobic and anaerobic conditions [*Acha et al., 2012; Podar et al., 2015*]. In accordance with metagenomics analysis, microbial communities of fresh-water ecosystems, tropic and Arctic seas, underground waters, soils of different regions, bogged areas the permafrost zone and rice fields have an environmental potential for mercury methylation [*Zhang et al., 2010a; Lehnherr et al., 2012; Wasik et al., 2012; Gilmour et al., 2013*].

Contamination of river ice with mercury is studied much rarer than pollution of the Arctic ice. The previously held investigations testify to mercury pollution of different components of the ecosystem of the Amur River, including bed silt and ice [*Kondratyeva, 2010; Kondratyeva et al., 2012, 2013*]. The study of the ice of the Amur and Songhua Rivers after the anthropogenic disaster in China in November 2005 showed that the amount of microorganisms belonging to different physiological groups essentially increased in the presence of various organic substances which accumulated in the ice mass [*Kondratyeva et al., 2011a; Kondratyeva and Fisher, 2012*].

It is to be emphasized that complex microbial consortia consisting of different taxonomic groups take part in the carbon cycle. Heterotrophic bacteria (aerobic and opportunistically anaerobic) play an important role in the transformation and mineralization of organic matter. Destroying highly molecular and stable organic substances of different origin, changing the oxidation-reduction potential of their habitats and supplying metabolic products as donors and acceptors of electrons, they create prerequisites for the growth of anaerobic groups of bacteria (sulfate reducing, iron-recovering, denitrifying and methanotrophic bacteria). The presence of heterotrophic bacteria in ice is one of the important prerequisites for forming conditions for mercury methylation by specialized groups of bacteria at the end of the freeze-up. Therefore, the following conditions are required for the process of mercury methylation: the presence of mercury compounds and of organic matter, ensuring functioning of microbial complexes; formation of sources of methyl radicals for mercury methylation.

The purpose of this study was to conduct a biogeochemical examination of ice cores and to analyze the conditions needed for mercury methylation in the period of the freeze-up in the main bed of the Amur River of the Pemzenskaya Channel in the area of the city of Khabarovsk.

OBJECTS AND METHODS OF STUDY

Ice cores were obtained by drilling at the end of the freeze-up period of 2011/12 by the researchers of the Institute of Water and Ecological Problems, FEB RAS, during the winter expedition to the Amur River guided by A.N. Makhinov. The ice cores were drilled with an auger (the internal diameter 16 cm) in the cross section (from the left bank to the right bank) in the main bed of the Amur River and the Pemzenskaya Channel in the area of the city of Khabarovsk. The ice cores were sawed into slabs considering its inhomogeneous structure on ice cleaned from snow, and then the slabs were stored in a freezing chamber with the temperature −18 °C.

To conduct chemical and microbiological tests, melts of different ice layers were used. The ice specimens were melted at room temperature with all the aseptic rules observed. The ice was placed into sterilized containers covered with lids. The amount of cultivated heterotrophic bacteria (CHB) was determined by culturing 0.1 mL of melted ice onto 10 times diluted peptone agar by the limit dilutions method, with subsequent re-calculation per 1 mL of melt-water and was expressed in colony-forming units (CFU/mL). Microbiological cultures were grown three times. The diagrams demonstrate the average amounts of the microorganisms found. The content of volatile organic matter was determined by the gas chromatography method (the Shimadzu GC-2010 gas chromatograph), in accordance with the ISO 11423-1 standard (analyst A.G. Zhukov). Mercury concentration was determined with an inductively coupled plasma emission mass spectrometer ICP-MS Elan DRC II PerkinElmer (USA).

RESULTS AND DISCUSSION

Pollution of natural waters takes place all the year round. However, it is very difficult to monitor the quality of water in the rivers of Siberia and of the Russian Far East in the winter period. One of the possible ways of overcoming these difficulties is to carry our simultaneous examination of ice layer by layer, using spectral, chromatographic and microbiological methods. Layer-by-layer examination of ice at the end of the freeze-up period allows making retrospective analysis of contamination of the stream ecosystems in the period of formation of the ice cover [*Kondratyeva, 2010*]. Sampling ice cores in the longitudinal and latitudinal sections of the streams allows the character of their contamination in space to be evaluated, whereas layer-by-layer investigation of ice was carried out in time, over the period of ice cover formation. Organic substances of natural and anthropogenic origin may be identified in different ice layers, present in the water at the time of formation of the given ice layer, as well as products of bacterial metabolism.

Microbiological transformation of organic matter is an important factor which determines the format of mercury existence. Soluble organic matter usually boosts microbial activity and thus may con-

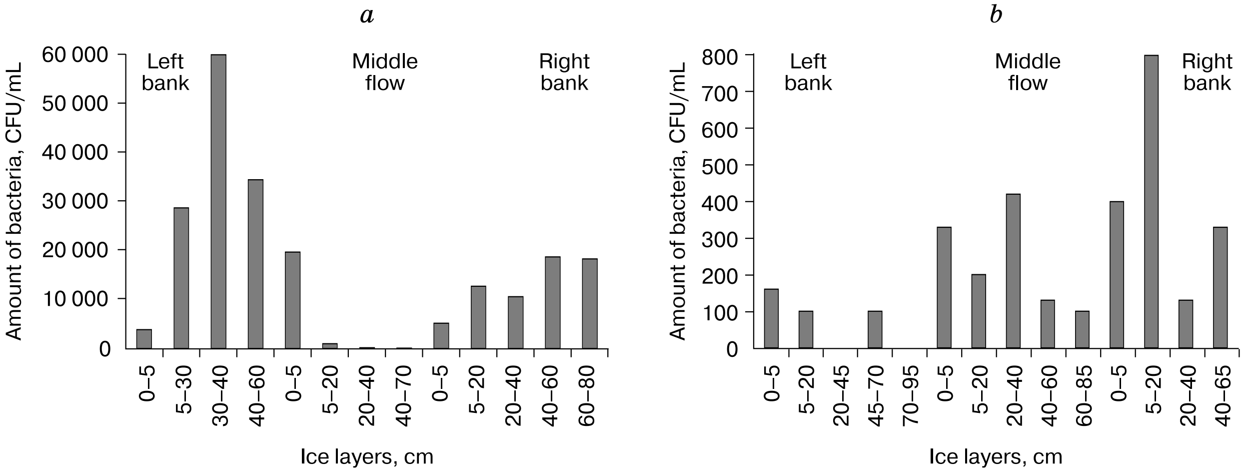

Fig. 1. Change in the amount of cultivated heterotrophic bacteria in different layers of ice of the Amur River (*a*) and the Pemzenskaya Channel (*b*) in the directions: left bank, middle flow, right bank (March 2012).

tribute to synthesis of methylmercury. Microbiological investigations of river ice specimens sampled in 2012 in the area of Khabarovsk showed that the high amount of cultured heterotrophic bacteria (HB) is observed in the main bed of the Amur River near the left and right banks, whereas the amount in the middle flow of the river is minimal (Fig. 1).

Maximum amount of HB was found near the left bank of the river in the 30–40-cm layer of ice. Detritus was present in the melt of this layer of ice. The amount of HB near the right bank of the river gradually increased towards the lower layers of ice but it did not reach the maximum values near the left bank.

In accordance with the microbiological test of the ice melts sampled from Pemzenskaya Channel, the quality of water in it in the period of formation of the ice cover was much higher, which was reflected in the lower amount of bacteria in different layers, compared to the trunk of the Amur River.

The distribution of microorganisms by the layers of ice may be related to the specific character of the transport of organic matter with water masses in the period of formation of the ice cover. The high amount of HB in the 30–40-cm layer of ice near the left bank in the trunk of the Amur River may be related to technical discharge of water from the Bureya and Zeya water reservoirs located upstream on the left-bank territory of the basin. It was previously noted that water masses with a high content of HB were transported along the left bank of the river in the autumn period, especially at the time of floods in the basins of the Zeya and Bureya Rivers [*Kondratyeva et al., 2013*]. The amount of heterotrophic bacteria in different layers of ice reflects the content of nitrogen-containing and aromatic organic compounds of different origin. Microbial complexes create prerequisites for transformation of organic matter to products ensuring methylation processes. We can assume active microbiological processes to take place in these layers of ice with a high content of HB.

Earlier, in the mouth zones of the large Amur tributaries (the Zeya and Bureya Rivers), characterized by an increased content of humic substances, we discovered actively growing sulfate reducing bacteria, while the microbial community displayed resistance to mercury salts [*Kondratyeva et al., 2011b, 2013*].

Based on the many years' studies we conducted, we concluded that the composition of volatile organic substances in ice changes from year to year; yet, methylated compounds are practically always present there. For example, in 2005–2006, after an anthropogenic disaster in China, methylated derivatives of benzene dominated, which are potential sources of methyl radicals. In the winter period of 2010/11, all the ice samples taken from the water along the right bank of the Amur River contained dichloromethane and butyl acetate. In some lower layers of ice, together with the high concentrations of dichloromethane and butyl acetate, there were isopropylbenzene and methylated derivatives of benzene (*o*- and *p*-xylols). It is to be pointed out that dichloromethane was also present in the ice sampled from the Pemzenskaya Channel; however, its concentrations were lower than those in the trunk of the Amur River [*Kondratyeva et al., 2012*].

As the studies conducted in the Amur River in 2011–2012 showed, the qualitative and quantitative composition of volatile components in the under-ice water and in the lower layers of ice differed significantly (Table 1). Ethyl acetate was the dominant component in different layers of ice sampled from the cross sectional profile of the Amur River and the Pemzenskaya Channel. Higher concentrations of ethyl acetate were found in the lower layers of ice than in the under-ice water. We can suppose that this component common for the ice is a product of microbiological decomposition of high-molecular organic matter of different origin transported in the period of ice cover formation.

During studies of the layers of the ice cores, high concentrations of ethyl acetate were discovered in the trunk of the Amur River in the middle flow and near the right bank (Fig. 2). Maximum concentration (18.2 mg/L) of ethyl acetate was recorded in the lower 40–70-cm-thick layer of ice. High concentrations of ethyl acetate (8.0–11.1 mg/L) were also found in the middle flow of the Pemzenskaya Channel for the entire mass of ice. In the layers of ice formed at the beginning of the freeze-up near the river banks, its concentrations were 2–3 times lower. In addition, near the right bank of the river, acetaldehyde (4.1 mg/L), benzene (0.5 mg/L) and styrene (2.0 mg/L) were found near the right bank in the 20–30-cm-thick layer of ice. Near the left bank, in addition to ethyl acetate, cyanoethylene, which is found in the wastewater of the textile industry, hexane and

Table 1. **Content of volatile organic matter (mg/L) in the lower layer of ice and in the under-ice water of the Amur River in the area of Khabarovsk (March 2012)**

Component	Left bank		Middle flow		Right bank	
	Water	Ice	Water	Ice	Water	Ice
Hexane	4.6	bdl	16.8	3.0	6.4	3.2
Ethyl acetate	1.9	3.3	4.2	18.2	2.8	9.9
Styrene	7.4	3.6	6.1	3.7	5.3	1.7

Note. Bdl – below detection limit.

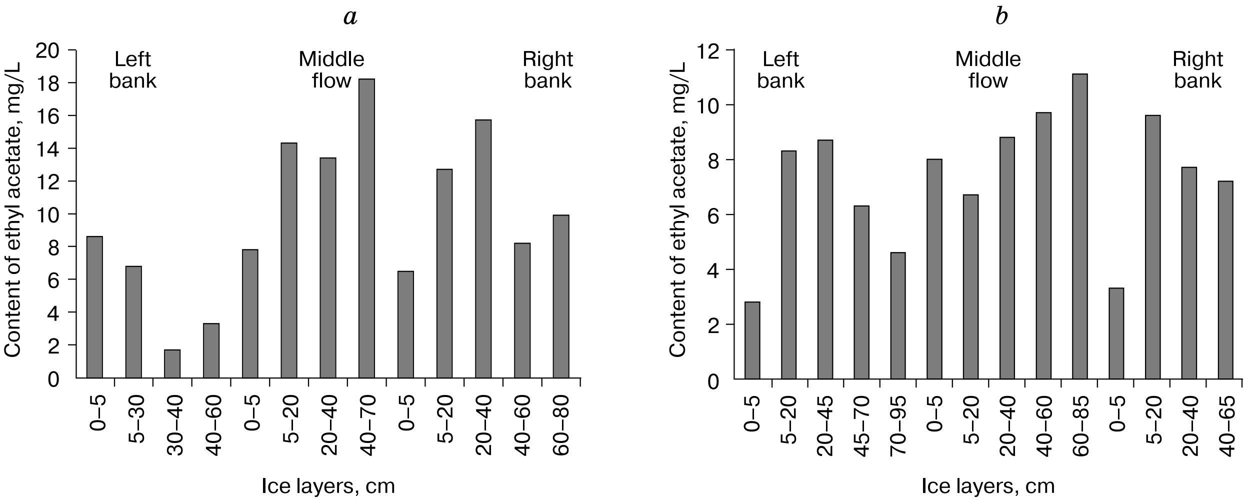

Fig. 2. The concentration of ethyl acetate in different layers of ice of the Amur River (*a*) and of the Pemzenskaya Channel (*b*) in the directions: left bank, middle flow, right bank (March 2012).

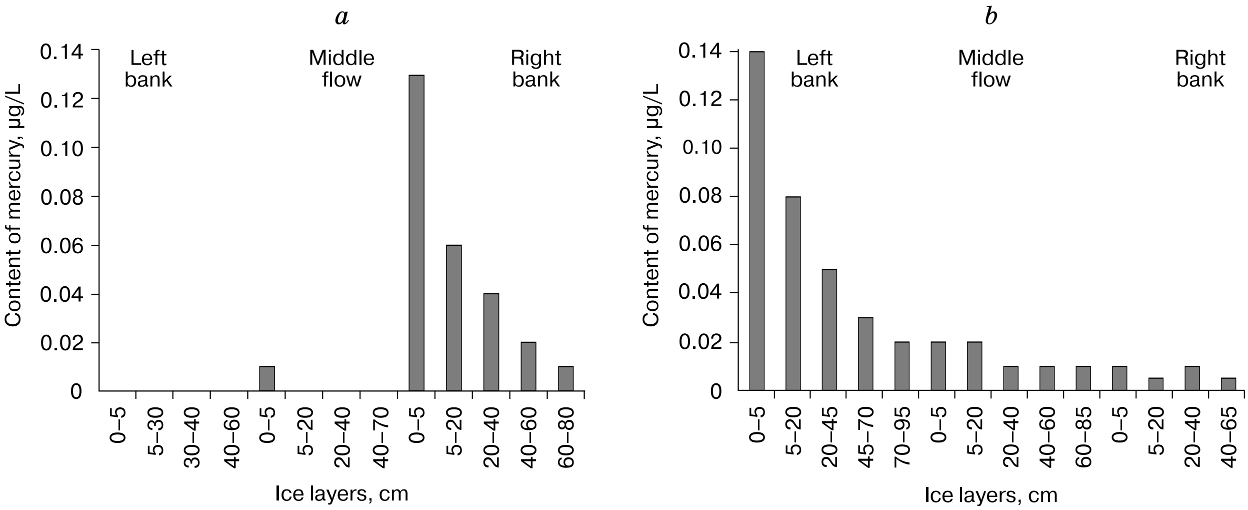

Fig. 3. The concentration of mercury in different layers of ice of the Amur River (*a*) and the Pemzenskaya Channel (*b*) in the directions: left bank, middle flow, right bank (March 2012).

styrene were identified in the ice layer 30–45 cm thick. We can suppose that in the period of the freeze-up, components of wastewater come from the trunk of the Amur River, illegally discharged by the industrial enterprises located along the Pemzenskaya Channel.

In accordance with the literature data [*Plata et al., 2005; Il'chenko and Shcherbakova, 2008*], ethyl acetate may be formed as a result of microbiological transformation of natural organic matter. Ethyl acetate we found in the river ice may be both of anthropogenic origin (from wastewater) and of microbiological origin.

The studies of ice in March 2012 (Fig. 3) showed the highest concentration of mercury in the upper layers of ice near the right bank of the Amur River (0.13 μg/L) and near the left bank of the Pemzenskaya Channel (0.14 μg/L). In the middle of these streams, mercury concentration in all the layers of ice was either minimal (0.01 or 0.02 μg/L) or below the detection limit (less than 0.001 μg/L).

In analyzing different layers of ice, we observed gradual reduction of mercury concentration from the upper layer to the lower layer. This may be related to the increased mercury concentration in the water at the beginning of formation of the ice cover due to the autumn water discharge from water reservoirs during preparation for the winter season as the influence of the surface runoff ends. High mercury concentrations in the near-surface layer of ice near the right bank may be caused by the transport of mercury with the runoff of the Songhua River from the rice-growing territories of China. Freeze-up in the lower reaches of the Songhua River occurs later than on the Amur River; therefore, the influence of the surface runoff may continue even when the upper layers of the Amur ice are formed.

CONCLUSIONS

The study conducted allowed us to conclude that in the winter period conditions for microbiological methylation of mercury are formed in the ice of the Amur River: contamination of water by organic matter (including substances of the humic nature) and mercury-containing compounds and the presence of mercury-resistant microbial complexes in the ice. The high amount of cultivated heterotrophic bacteria taking part in biotransformation of organic matter was found in the layers of ice containing particles of detritus. Unequal distribution of volatile organic substances over the layers of ice, including ethyl acetate and methylated derivatives of benzene, may result from differing fermenting activity of cryomicrobiocenoses.

In the absence of additional sources of carbon coming into ice and under conditions of oxygen shortage, insoluble organic substances of detritus become involved in the processes of transformation. Different representatives of heterotrophic psychrophilic microorganisms creating conditions for the growth of specialized groups of bacteria methylating mercury may take part in them.

Considering the regional specifics of seasonal changes in the content of humic substances in the Amur River, the presence of accessible organic matter in ice and development of mercury-resistant cryomicrobiocenoses in them, it is possible to predict formation of conditions for microbiological methylation of mercury and its inclusion into biogeochemical cycles. During the spring ice drift and during ice melting, methylated mercury, which has increased bioavailability, may be included into the trophic networks of the Amur River and be drifted to the coastal areas of the Sea of Okhotsk and of the Sea of Japan.

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Received November 12, 2015