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Microbiological water quality of Lake Baikal: a review

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ABSTRACT. The article provides information about the sanitary-bacteriological studies conducted in the water area of Lake Baikal. We show the data on the long-term observations of the spread and abundance of fecal indicator bacteria and potentially pathogenic bacteria in the pelagic and littoral waters of the lake. We also present a review of the standards and methods for the sanitary-bacteriological water quality worldwide, techniques for detecting fecal indicator bacteria, including those specific for human microbiota, methods for microbial source tracking, as well as studies of the spread and retaining of fecal indicator bacteria in various ecotopes, such as bottom sediments and biofilms. For the sanitary-bacteriological monitoring of Lake Baikal, we propose an integrated approach based on the application of modern techniques that correspond to world practice. This approach would allow identification of the sanitary adverse sites and more reliable and standardized assessment of the Baikal water quality.

Keywords: monitoring, surface water, water pollution, fecal indicator bacteria, coliforms, enterococci, Lake Baikal

Sanitary and microbiological characteristics of Lake Baikal: history and current state

Water is an essential condition of life as well as a necessary resource for the implementation of economic activities. The surface waters of the planet are subject to the intensive anthropogenic impact, since the population uses them for many purposes, such as recreation, water consumption, irrigation, fishery, wastewater discharge, reservoir construction, etc. Nowadays, the ecological state of freshwater bodies as the main sources of drinking water is deteriorating throughout the world, which leads to the disruption of the evolutionary formed microcenoses and the development of the pathogenic and opportunistic bacteria in them (Thevenon et al., 2012; Sales-Ortells et al., 2015; Strathmann et al., 2016; Lenart-Boron et al., 2017; Korajkic et al., 2018). The problem of chronic fecal pollution has long been recorded for the complexes of Great Lakes of North America and African Great Lakes that serve as a source of drinking water for hundreds of millions of people. Thus, during the past decade waters and beaches of Lake Huron and Upper Lake are considered as dangerous for swimming and recreation due to high concentrations of coliform bacteria and enterococci (Kon et al., 2007; Newton et al., 2013; Ran et al., 2013; Harwood et al., 2017). The water quality of Victoria and Kivu lakes also have poor sanitary state (Byamukama et al., 2000; Olapade, 2012). Chronic fecal pollution of freshwater bodies has also become one of the primary problems in European countries. Monitoring of the sanitary state of Lithuanian and Belgian lakes and rivers indicated the low microbial quality of waters (Ouattara et al., 2011; Staradumskyte and Paulauskas, 2012). Sediments of Lake Geneva and tributaries of Lake Onega showed a high abundance of opportunistic microflora (Thevenon et al., 2012; Tekanova et al., 2015).

In recent years, the water quality of the world's oldest and deepest Lake Baikal has been also evidently deteriorating. The volume of fresh water in the lake is 20 % of all fresh water in lakes and rivers of our planet (Atlas of Lake Baikal, 1993). It is a source of water consumption and recreation for locals. The large settlements located directly on the Baikal coast, where the industrial and agricultural activities are carried out, are significant sources of pollution. These are the Slyudyanka and Baikalsk towns, the Kultuk settlement, the Severobaikalsk town, the Nizhneangarsk town, the Goryachinsk settlement, and the Babushkin town. The Selenga River, which places the capital of the Republic of Buryatiya, a city of Ulan-Ude, also poses a potential danger as a source of pollution (Grachev, 2002).

Microbiological studies of Lake Baikal have been conducting for more than a hundred years (Parfenova et al., 2016; Belykh and Drucker, 2018). According to long-term observations, microbial indicators in the pelagic zone of Lake Baikal were rather constant for a long time. The pelagic waters of Lake Baikal contained mainly single quantities of fecal indicator bacteria (FIB).

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FIB in surface waters were found mostly in summer, near settlements and the mouths of large tributaries of the lake (Drucker et al., 1993; Parfenova et al., 2009a). Observations conducted in the second half of the 20th century detected significant number of FIB mainly in the southern basin of the lake, in the surface waters of the littoral zone near the wastewater discharge of Baikalsk Pulp and Paper Mill (BPPM) (Goman, 1973; Maksimova and Maksimov, 1989; Maksimova et al., 1998; Shchetinina et al., 2003; 2013; Verkhozina et al., 2003). In the littoral zone of the same wastewater discharge area, there was contamination with hepatitis A viruses and rotaviruses. In deep areas, there were no anthroponotic viruses and FIB (Maksimov et al., 2003; Shchetinina et al., 2013). For 47 years of the BPPM operation, the waters of the Southern Baikal have been subject to constant anthropogenic load in the form of the BPPM wastewaters as well as domestic sewage of the Baikalsk town, which is fed to the treatment facilities until now. From 2000 to 2009, there was an increase in the sanitary-bacteriological indicators near the BPPM wastewater discharge (Shchetinina et al., 2013). The deterioration of the sanitary situation initiated studies of the diversity and antibiotic resistance of bacteria isolated from the lake areas with a high anthropogenic impact (Shchetinina and Maksimov, 1999; Verkhozina, 2003; Savilov et al., 2008). The distribution and determination of species composition of potentially pathogenic bacteria (PPB): bacteria of Enterobacteriaceae family and non-enzymatic group (Panasyuk, 2002) as well as bacteria of the genus *Enterococcus* (Kravchenko, 2009), were of special attention. The PPB distribution throughout the water area of the lake was uneven and associated with the settlements, the mouths of the main tributaries and the sites discharging insufficiently treated domestic sewage. They showed the highest abundance in the south of Baikal (the Bolshiye Koty, Listyanka, Port Baikal settlements and Baikalsk town) as well as in the central part of Baikal (Barguzin and Chivyrkuy bays and the Selenga delta). The PPB number naturally increased during the summer and autumn and decreased in the winter months. The isolated PPB showed poly-resistance to antibiotics, haemolytic activity and ability to adapt to low-temperature environmental conditions; hence, they have a potential epidemic hazard to human health. In addition, the results of the research implied using extra FIB for control of the water quality. They can be considered as specific indicators of the fecal water influx. These FIB are *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Citrobacter freundii*, *Burkhalderia cepacia* and bacteria of the genus *Enterococcus* (*E. faecium, E. avium, E. faecalis, E. mundtii, E. hirae, E. durans,* and *E. gallinarum*), which were detected in the water area of the lake (Panasyuk, 2002; Panasyuk et al., 2002; Drucker and Panasyuk, 2006; Parfenova et al., 2008; 2010; Kravchenko, 2009; Parfenova, 2009).

In recent years, the Baikal ecosystem has been experiencing a serious ecological crisis. The coastal areas have signs of eutrophication, i.e. intensive development of algae that are non-specific for the littoral zone of Lake Baikal, a mass death of sponges and toxic cyanobacterial blooms (Kravtsova et al., 2012; 2014; Belykh et al., 2016; Khanaev et al., 2016; Kobanova et al., 2016; Potapskaya et al., 2016; Suturin et al., 2016; Timoshkin et al., 2014a; 2014b; 2015; 2016; 2018). The poorly treated sewage from the settlements located on the coast are discharged to the lake waters; the tourist load and the number of vessels increase. The number of tourists visiting the Baikal coast has increased manifold over the period of 2012-2015. The annual number of the officially registered tourists at Lake Baikal is approximately 2.2 million people (2015), which is associated with the active development of the infrastructure in this industry, particularly, the accommodation sector. More than 40 recreational development zones have formed directly on the Baikal coast. They concentrate most of the tourist accommodation facilities, i.e. tourist camps, hotels, holiday houses, etc. (Evstropyeva, 2016). The sanitary-bacterial monitoring from 2010 to 2017 indicated FIB in the waters of the lake for the entire research period. There were near-mouth sites of the Turka, Barguzin, Buguldeika, Goloustnaya, and Sukhaya Anga rivers as well as the Selenga delta, where a high FIB abundance was detected most frequently during this period. In 2011, there was the exceeding the RF standards throughout the pelagic zone of Lake Baikal and in most of its major tributaries. Furthermore, in 2012 pelagic water in the southern part of the lake had an unfavourable microbiological and sanitary state. The littoral zone showed significant number of FIB near the Listvyanka settlement and the Baikalsk town in the southern basin of Baikal, in the waters of the Maloye More and Olkhonskiye Vorota straits and the Selenga delta – in the central basin, as well as, in the coastal waters of the Severobaikalsk town and the Zarechny settlement – in the northern basin (Kovadlo and Drucker, 2010; Timoshkin et al., 2014a; Verkhozina et al., 2014a; Bondarenko et al., 2015; Shtykova et al., 2016; 2017; 2018a; 2018c; Suslova et al., 2017a; 2017b; 2018a). PPB characterized by resistance to a wide range of antibiotics were present and accumulated in various ecotopes of the Baikal coastal zone (in the water column, epilithic and neuston biofilms, sponges and bottom sediments) (Verkhozina et al., 2014b; Shtykova et al., 2018b).

The studies have shown that the waters of Lake Baikal experience an intensive anthropogenic load, which has been steadily increasing recently. The FIB distribution is uneven and local in nature mainly due to the association with the sites of the anthropogenic impact on the lake, which the data on chemical analysis also confirm (Khodzher et al., 2017). They show the maximum abundance in the estuarine zones of the rivers, and it gradually decreases with the river waters flowing to the lake (Parfenova et al., 2009b). The results of the studies suggest that under the influence of the anthropogenic factors in the coastal areas of the lake, there is a shift of the autochthonous microbiota towards the allochthonous one, which is highly resistant to antibiotics. The presence of the additional factors that contribute to the PPB persistence and reproduction would reduce the ability of the coastal Baikal waters to self-purification to such a degree that their use would be epidemiologically dangerous. This leads to the need to develop a water quality management strategy for Lake Baikal.

Review of modern criteria and methods for assessing microbiological water quality

Sanitary and bacteriological assessment of water quality imply determination of a set of sanitary indicators, i.e. criteria that reflect compliance or non-compliance of the sanitary state of the studied water body with the requirements of regulatory documents. FIB serve as these sanitary indicators. They are used throughout the world to detect and prevent fecal contamination and its associated human health risk, since they indicate the probable presence of pathogenic bacteria and viruses. FIB include such bacteria as total coliforms, thermotolerant coliforms, fecal coliforms, *E. coli*, enterococci, *Bifidobacterium*, *Bacteroides*, and *Clostridium* spp. These FIB are widespread in faeces of humans and most animals. Their levels in wastewater and faeces are rather high; therefore, with fecal contamination, they are usually present in surface waters (Harwood et al., 2017; Aguirre et al., 2019). The surface water monitoring includes using FIB abundances. Various regulations establish FIB standards to determine water quality (Table 1).

In the Russian Federation, sanitary rules SanPiN 2.1.5.980-00 «Water disposal of the populated areas and sanitary protection of water bodies. Hygienic requirements for the protection of surface water» regulate the compliance of the quality of surface water sources with sanitary standards. This regulatory document divides water bodies into two categories. The category I includes water bodies or their sites used as a source of drinking and domestic water consumption as well as water supply for food industries. The category II includes water bodies or their sites used for recreational purposes. The water quality requirements established for the water use category II applies, inter alia, to all sites of water bodies within the boundaries of the populated areas (SanPiN 2.1.5.980-00). Indicator of total coliforms, which reflects the water treatment quality, and indices of thermotolerant coliforms and coliphages, which reflect the degree of fecal contamination, are the main standardized indicators in the Russian Federation that used in assessing the sanitary and microbiological state of the water body (MUK 4.2.1884-04; SanPiN 2.1.5.980-00; Tymchuk et al., 2013). In the presence of epidemiological evidence, the indicators of the abundance of *Clostridium* spp., enterococci, *Staphylococcus* spp., and *E. coli* are additionally used, and the coefficient of self-purification is determined through calculating the total number of mesophilic aerobic and facultative anaerobic microorganisms (total microbial number) capable of forming colonies on meat-peptone agar (MPA) at 37 ˚ C for 24 hours and at 22 ˚ C for 72 hours.

The Russian system of sanitary and microbiological standards differs in many aspects from its counterparts abroad. The analysis of the standards stipulated by the regulations listed above has shown that the Russian standards of water quality for recreation are rather strict compared to the European and American regulations (Table 1). They do not allow the presence of any pathogens of intestinal infections and even twice limit the total number of coliform bacteria than the water EU standard for the classification «Good». Unlike SanPiN2.1.5.980-00, its counterpart regulation does not standardize physical and chemical indicators as well as the level of radioactive contamination at all (Tsoupikova, 2016). However, it should be noted that in the EU countries and USA, FIB (*Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens*), as well as bacterial genera *Bacteroides* and *Bifidobacterium*, which are more specific to human body and warm-blooded animals, have long been directly detected, since they typically present in human faeces in higher quantities compared to *Clostridium* spp., enterococci, *E. coli* and other coliforms (Newton et al., 2011; Tymchuk et al., 2013; Harwood et al., 2017). This makes the water quality assessment more standardized and reliable.

In the world practice of sanitary research, new methods and approaches to the assessment of the sanitary state of water bodies are constantly appearing and being introduced. To detect and count FIB, accelerated identification methods using chromogenic media (GOST 24849-2014, 2016) are used, and the studies focused on the direct detection of *E. coli* (litmus paper test «DipTest») are conducted (Gunda et al., 2017). However, the cultivation methods often yield false positive results and do not consider uncultivated bacteria; therefore, the development and use of sensitive methods for the rapid detection and counting of FIB in water are recently relevant. These rapid methods are the nucleic acid-based, immunology-based and biosensor-based detection (Deshmukh et al., 2016; Darkazanli et al., 2018). The U.S. Environmental Protection Agency has long ago developed and implemented standardized procedures for the accelerated identification of Enterococci and Bacteroidales using Quantitative Polymerase Chain Reaction (qPCR) (USEPA, 2010; 2012b; 2013).

Nevertheless, the detection of FIB in environmental waters does not provide the information about the pollution source, since many members of this group are ubiquitous in the environment. FIB can be both fecal and environmental (Leclerc et al., 2001). Identification of the source is crucial for risk assessment and its elimination, since not all fecal sources are equally hazardous to human health. For example, human fecal contamination generally has the greatest risk due to the possible presence of human viral and bacterial pathogens, whereas cattle fecal contamination indicates the possible presence of zoonotic pathogens. Therefore, the adequate assessment of the sanitary state of the water body and the protection of human health requires microbial source tracking (MST) of fecal contaminations. Such methods are being developed in many countries (Bower et al., 2005; Newton et al., 2011; 2013; Harwood et al., 2017). There are still no formal regulation or methodologies for conducting MST,

Table 1. The norms and standards of FIB in surface recreational waters

Area, state	Limit for fecal indicator bacteria	Guideline, norm, or standard
Russia	Main indicators: Absence of pathogenic bacteria; Coliphages 10 PFU/100 mL; Thermotolerant coliforms 100 CFU/100mL; Total coliforms 1000 CFU/100mL (water use category for drinking and domestic water supply), 500 CFU/100mL (water use category for recreational water, and within cities); Additional indicators: The ratio of the total microbial number (TMN) of 22 °C to TMN of 37 °C is 4 and above; *E. coli* 100 CFU/100mL; Enterococci 50 CFU/100mL; Staphylococci 10 CFU/100mL	Hygienic requirements for the protection of surface water (SanPiN 2.1.5.980-00); Sanitary-microbiological and sanitary-parasitological analysis of surface water bodies (MUK 4.2.1884-04)
EC (EU)	*Inland waters:* Classification «Excellent» (95th percentile of log10 densities): Enterococci 200 CFU/100mL, *E. coli* 500 CFU/100mL; Classification «Good» (95th percentile of log10 densities): Enterococci 400 CFU/100mL, *E. coli* 1000 CFU/100mL; Classification «Sufficient» (90th percentile of log10 densities): Enterococci 330 CFU/100mL, *E. coli* 900 CFU/100mL; *Coastal waters and transitional waters:* Classification «Excellent» (95th percentile of log10 densities): Enterococci 100 CFU/100mL, *E. coli* 250 CFU/100mL; Classification «Good» (95th percentile of log10 densities): Enterococci 200 CFU/100mL, *E. coli* 500 CFU/100mL; Classification «Sufficient» (90th percentile of log10 densities): Enterococci 185 CFU/100mL, *E. coli* 500 CFU/100mL	Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality (Directive 2006/7/EC)
USA	*Recommendation 1* (for an estimated illness rate of 36/1,000): Enterococci (marine and freshwater) 35 CFU/100mL (geometric mean), 130 CFU/100mL (10% statistical threshold value); E. coli (freshwater only) 126 CFU/100mL (geometric mean), 410 CFU/100mL (10% statistical threshold value); *Recommendation 2* (for an estimated illness rate of 32/1,000): Enterococci (marine and freshwater) 30 CFU/100mL (geometric mean), 110 CFU/100mL (10% statistical threshold value); E. coli (freshwater only) 100 CFU/100mL (geometric mean), 320 CFU/100mL (10% statistical threshold value)	Recreational Water Quality Criteria (USEPA, 2012a)
Brazil	Total coliforms < 5000 CFU/100mL in 80% of at least 5 monthly samples; Fecal coliforms < 1000 CFU/100mL in 80% of at least 5 monthly samples	Regulation/GM/No. 0013: Classifying domestic water courses in order to protect their quality (Brazilian Ministry of Health, 1976)
Japan	Fecal coliforms 1000 CFU/100mL	Environmental Quality Standards Regarding Water Pollution (Japan Environment Agency, 1986)
Kenya	Fecal coliforms < CFU/100mL, Total coliforms CFU/100mL	Environmental management and coordination (Water quality) regulations (Republic of Kenya, 2006)

PFU – plaque-forming units; CFU – colony-forming units

but in the U.S. Environmental Protection Agency the standardized procedures are already being developed (Harwood et al., 2017).

Monitoring of the microbiological quality of surface waters is usually limited to testing the water column. At the same time, the studies show that fecal bacteria occur in the bottom sediments of freshwater bodies and can be resuspended from the sediments during human activities or natural processes, which would increase their number in the water column (Haller et al., 2009; Thevenon et al., 2012). Bottom sediments may contain from 100 to 1000 more FIB than the water column, since FIB can persist longer in bottom sediments due to a high content of organic compounds in them and protection from insolation (Davies et al., 1995). Moreover, FIB in bottom sediments may represent a more stable indicator of the water quality than in the water column (Thevenon et al., 2012). Epilithic biofilms in freshwater bodies can also serve as a reservoir for pathogenic bacteria (Ksoll et al., 2007; Balzer et al., 2010; Gubelit et al., 2011). The presence of coliform bacteria in biofilms and bottom sediments of Lake Baikal has been already recorded (Namsarayev and Zemskaya, 2000; Shtykova et al., 2018b; Suslova et al., 2018b; Sukhanova et al., 2019). The presence of fecal bacteria in biofilms and bottom sediments suggests that in such a way these bacteria adapt to adverse environmental factors and circulate in the autochtonous community. The study of these processes is especially important, since at present the antibiotic-resistant PPB are distributed in water bodies. The water area of Lake Baikal also shows an alarming trend in the detection of antibiotic-resistant strains (Parfenova et al., 2008; Verkhozina et al., 2014b). The ingress of antibiotic-resistant strains into the environmental objects maintains a pool of resistant strains due to the transfer of resistance genes to the autochtonous bacterial communities of the ecosystem. The transfer of antibiotic-resistance genes in the environmental communities is of special attention worldwide. The spread of antibiotic-resistant genes among bacteria increases morbidity and mortality from the infections they cause (Ashbolt et al., 2013; Berglund, 2015; Xu et al., 2018).

In addition to the anthropogenic factors, climatic, hydrochemical and hydrological conditions also influence the sanitary-bacteriological state of water bodies, which should be considered during monitoring (Gutierrez-Cacciabue et al., 2014). The effective monitoring of surface waters requires processing a large amount of data using various multivariate statistical techniques. Multivariate data processing is widely used to characterize and assess surface water quality and is useful for identifying temporal and spatial changes caused by natural and anthropogenic factors. The use of multivariate techniques allowed for optimizing monitoring tasks (Aguirre et al., 2019).

Conclusion

Based on the analysis of long-term studies and foreign methodologies for sanitary assessment of water quality, there is an obvious need to develop an algorithm for monitoring the sanitary-bacteriological state of Lake Baikal. Monitoring would include not only the identification of indicator levels and their comparison with standards according to the RF regulatory documents but also the detection of a wider range of FIB using modern methods for identifying and tracking the sources of fecal contamination, which correspond to the world practice. In order to determine the sites that are most susceptible to anthropogenic impact, monitoring should be supplemented with the study of the diversity and distribution of PPB isolated from various ecotopes of the lake (water, bottom sediments and biofilms) as well as determination of the proportion of the antibiotic-resistant strains among these bacteria and autochtonous microbiota. Such an approach would allow not only detection of the potentially pathogenic bacteria in the ecosystem but also a complex diagnostics of their state in order to determine a degree of the potential hazard of the Baikal waters to human health. A holistic approach to monitoring requires using multivariate statistic techniques, considering climatic, hydrochemical and hydrological data.

The assessment of the current sanitary-bacteriological state of Lake Baikal is increasingly relevant each year in order to preserve its uniqueness. It is important to consider key threats to the Baikal ecosystem, including anthropogenic impact, i.e. lack of the wastewater treatment plants, the spontaneous development of the lake coast, the lack of communal infrastructure of tourist places, and the increase in water transport. The analysis of water used by the population as a source of domestic, recreational and even drinking water supply is becoming important due to the cluster organization of tourist zones in the coastal area of Lake Baikal. The application of modern techniques that correspond to the world level would allow assessment of the sanitary state of Lake Baikal as well as its possible use as a source of drinking water and a recreational object. The monitoring results would contribute to the protection of the lake ecosystem and human health.

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