Microorganisms in the deposits of cold carbon mineral waters of the Russian Far East and their habitats

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Abstract. Study of the chemical composition of carbon mineral waters has shown the prevalence of calcium, magnesium and sodium among the cations, sulfate, nitrate and chloride ions among the anions, and ferric iron, strontium and manganese in the microelement composition. Results of the microbiological studies have revealed that carbon mineral waters contain various microorganisms that can transform the physical and chemical composition of mineral waters by interfering with geochemical cycles. The sanitary and microbiological properties of carbon mineral waters have been evaluated thus proving that the waters of Medvezhii (Shmakovskoe deposit) are microbiologically clean.

1. Introduction

The geologically complex territory of the Russian Far East is home to extensive resources of mineral ground waters. With respect to chemical and gas composition, and their temperature range, the region’s mineral waters fall into the groups: carbon, nitric or nitric-methane by their chemical composition, and thermal or cold temperature-wise [1]. In recent decades the chemical composition of mineral waters as well as their genesis has been sufficiently studied including the works by O.V. Chudaev, G.A. Chelnokov, and N.A. Kharitonova [2-5]; however, the formation mechanisms of many water types remain undiscovered. Recent years have brought many works that show show that microbiological processes actively change the hydrogeochemical system thus partially transforming the physical and chemical properties of ground waters and porous space of water-bearing rocks [6-8].

Microorganisms are a huge group of life forms; however, bacteria are most relevant for ground waters. They can be suspended in the ground waters ranging from 0.2 to 1.0 µm in size. In certain physical and chemical conditions bacteria form large agglomerates and become part of the porous medium. Bacteria often become lodged with the surface of water-bearing rocks thus staying in place and not travelling together with the water medium. It is adherent bacteria that cause the major transformations of the chemical composition of water-bearing rocks [9-10]. The most important biochemical processes in the ground waters that involve microorganisms include the production of methane, sulfate reduction, biogenic generation of CO₂, nitrification and denitrification [11-15]. Mineral waters can also contain contaminant pathogenic and potentially pathogenic microorganisms that can survive for long periods of time and cause a serious threat to human health [16]. Since mineral waters are actively used in balneology, they have to be monitored both for their chemical and microbiological composition in order to reveal the natural and disease-causing organisms. Study of the microorganisms residing in ground waters is required for predicting possible biochemical processes in the water-rock system that involve microorganisms as well as for assessing the sanitary and microbiological quality of ground drinking waters.

Since there is no data on the qualitative composition of microorganisms in the ground mineral carbon waters of the Russian Far East, the present research is aimed at studying the distribution, structure and quantity of various physiological groups of bacteria and indicative microorganisms, as well as at defining their habitat conditions.
2. Material and methods

2.1. Sampling
Carbon mineral waters of four major deposits – Lastochka, Shmakovskoe (Medvezhii and Vostochno-Ussurskii locations), Pokrovskoe and Nizhnie Luzhki have been selected as objects of research (fig.1). The deposits are located in various hydrogeological conditions and have different types of water-bearing rocks and geochemical types of waters. Water samples were collected in conditions of sterility in July 2014 into 1500 ml sterile glass vials, in triplicate.

![Figure 1. Location of the surveyed deposits of carbon mineral waters in the Russian Far East (1 – Lastochka; 2- Shmakovskoe; 3 - Pokrovskoe; 4 – Nizhnie Luzhki).](image)

2.2. Sample preparation and analysis
Water samples for analysis of cations and sulfates were filtered through membrane filters (cellulose nitrate, 0.45 μm, Sartorius) at the site of sampling for removing the suspended matter and were subsequently acidified with nitric acid. Water samples for analysis of anions were also filtered through membrane filters (cellulose nitrate, 0.45 μm, Sartorius) and then placed into plastic vials (without acidification). The cations and anions were separated via the method of liquid ion chromatography (HPLC-10AVp, SHIMADZU); the microelements were analyzed via plasma optical emission spectrometry (ICP-AES, Plasmaquant-110) and inductively-coupled plasma mass spectrometry (ICP-MS, Agilent 7500c).

The quantity of bacteria of different physiological groups was calculated via the method of limiting dilution in different selection media [17]. Mac-Credi tables were used to identify the most probable number of bacteria cells. The Koch’s technique was used to count the colony-forming types of indicative microorganisms [18]. Saprophytic bacteria (QMA&OAMO – quantity of mesophyll aerobic and optional-anaerobic microorganisms) were seeded on meat-peptone agar with incubation at 30°C for 48-72 hours. Total coliforms and thermostolerant coliforms were identified in accordance with methodological guidelines № 96/225-1997 via the method of membrane filtration [19]. The bacteria *Pseudomonas aeruginosa* were separated in the Endo selective and differential medium with their prior
accumulation in the lactose peptone broth [20]. Bergey’s manual of bacteria classification was used to identify the bacteria.

3. Results and discussion

3.1. Chemical composition of carbon mineral waters

In the Lastochka mineral waters, sodium and calcium are the prevailing cations; nitrate and sulfate ions dominate among the anions, and ferric iron and strontium are the major microelements (table 1). Carbon waters of the Shmakovskoe deposit show prevalence of calcium and magnesium among the cations, sulfate, chloride and nitrate ions among the anions, and ferric iron, strontium and manganese among the microelements (table 1). In the Pokrovskoe and Nizhnie Luzhki deposits the prevailing cations include calcium and sodium, the dominating anion is a nitrate ion; the carbon waters are significantly enriched in strontium, ferric iron and manganese (table 1).

**Table 1.** Content of cations, anions and microelements in the deposits of cold carbon mineral waters of the Russian Far East

| Components | Deposits of carbon mineral waters in the Russian Far East |
|------------|---------------------------------------------------------|
|            | Lastochka | Shmakovskoe | Pokrovskoe | Nizhnie Luzhki |
|            | Medvezhii | Vostochno-Ussurskii |         |               |
| F          | 0.80      | 0.61        | 0.64      | 0.20          | 0.35          |
| Cl⁻        | 1.71      | 1.09        | 1.18      | 0.68          | 1.32          |
| NO₃⁻       | 4.32      | 0.14        | 1.72      | 10.2          | 16.33         |
| SO₄²⁻      | 2.50      | 3.09        | 2.40      | 0.78          | 0.64          |
| Li⁺        | 1.18      | 0.09        | 0.12      | 0.23          | 0.60          |
| Na⁺        | 264.0     | 21.9        | 32.7      | 53.1          | 67.2          |
| NH₄⁺       | 0         | 0.28        | 0.26      | 0.52          | 1.10          |
| K⁺         | 29.1      | 3.40        | 2.52      | 3.80          | 2.96          |
| Ca²⁺       | 74.7      | 139         | 127.0     | 70.9          | 110.0         |
| Mg²⁺       | 60.8      | 54.6        | 48.6      | 6.13          | 10.3          |
| Be         | 1.52      | 2.79        | 6.83      | 6.37          | 10.3          |
| Al         | 6.0       | 157.0       | 100.0     | 93.0          | 19.3          |
| Cr         | 1.12      | 0.495       | 0.154     | 1.16          | 7.16          |
| Fe         | 7544.0    | 25030.0     | 2232.0    | 1073.0        | 2137.0        |
| Mn         | 214.0     | 427.0       | 732.0     | 539.0         | 1640.0        |
| Cu         | 1.45      | 3.6         | 6.18      | 0.271         | 1.55          |
| Sr         | 5332.0    | 487.0       | 620.0     | 1249.0        | 3408.0        |

| Microelements, µg/l |
|---------------------|
| Be                  | 1.52    |
| Al                  | 6.0     |
| Cr                  | 1.12    |
| Fe                  | 7544.0  |
| Mn                  | 214.0   |
| Cu                  | 1.45    |
| Sr                  | 5332.0  |
3.2. **Counts of bacteria functional groups in carbon waters**

Each source of mineral waters has a specific set of prevailing physiological groups of microorganisms that can be attributed to the difference in the chemical composition of ground mineral waters as well as to the different composition of water-bearing rocks.

Physiological groups of microorganisms in the carbon waters of Lastochka deposit are not numerous on average ranging from 0 (denitrifiers) to 2.5×10³ c/ml; as this takes place, the microbiocenosis includes a prevailing number of chemolithoautotrophic thionic bacteria *Thiobacillus denitrificans* that can anaerobically oxidize reduced sulfur compounds to elemental sulfur and subsequently to sulfuric acid. Earlier researchers showed the ability of *Thiobacillus denitrificans* to cause anaerobic oxidation of pyrite nanoparticles by using nitrates as electron acceptors [21]. The thionic bacteria activity may cause generation of sulfates in the Lastochka carbon waters by the following reaction equation:

$$\begin{align*}
5\text{Na}_2\text{SO}_3 + 4\text{O}_2 + \text{H}_2\text{O} \rightarrow 5\text{Na}_2\text{SO}_4 + \text{H}_2\text{SO}_4 + 4\text{S}
\end{align*}$$

(1)

High numbers of thionic bacteria identified only in the Lastochka carbon waters may be caused by the active processing of sulfide minerals (pyrite) that are found in sufficient quantity in the water-bearing rocks of the deposit [22]. The numbers of other ecolego-trophic groups of bacteria in the Lastochka reservoir are quite low, ranging from 0.1×10² to 0.4×10³ c/ml (table 2).

The carbon waters of Medvezhii (Shmakovskoe deposit) are characterized by low total numbers of all physiological groups of microorganisms that comprise 0.6×10² c/ml on average. Chemoorganotrophic heterotrophic nitrifiers are more widespread here; they oxidize nitrogen compounds to nitrates, nitrates and byproducts (table 2). The carbon waters of Vostochno-Ussurskii location (Shmakovskoe deposit) contain high numbers of colorless sulfur bacteria (4.5×10³ c/ml), heterotrophic nitrifiers (1.3×10³ c/ml) and an insignificant amount of manganese-oxidizing microorganisms (1.4×10² c/ml) (table 2). High numbers of sulfur bacteria can probably be attributed to the favorable conditions for propagation of microorganisms (presence of organic substances, oxygen, metallic sulfides and hydrogen sulfide), as well as to the specific hydrogeological structure of ground waters. High amount of heterotrophic nitrifiers found in the Vostochno-Ussurskii spring is indicative of the ammonia and organic nitrogen compounds oxidation processes. Growth of manganese-oxidizing bacteria only in the carbon waters of Vostochno-Ussurskii is probably caused by a higher oxygenation of ground waters (6.15 mg/l), higher concentrations of manganese (732 µg/l) combined with an average amount of organic carbon (3.0 mg/l) that altogether create favorable conditions for bacteria propagation. The identified manganese-oxidizing bacteria were morphologically similar to *Metallogenium*.

The bacterial count of microorganism physiological groups in the Pokrovskoe deposit ranges from 0 to 4.5×10³ c/ml with iron-oxidizing chemoautotrophs prevailing (table 2). High content of iron-oxidizing microorganisms in the mineral waters and lower concentrations of ferric iron in the ground waters may be indicative of oxidation processes of Fe²⁺ to Fe³⁺ and its subsequent sedimentation as goethite or lepidocrocite:

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ = 4\text{Fe}^{3+} + 2\text{H}_2\text{O}.$$ 

(2)

The isolated iron-oxidizing microorganisms were identified as *Thiobacillus ferrooxidans*, *Leptospirillum*, and *Siderococcus sp*.

The carbon mineral waters of Nizhnie Luzhki contain prevailing numbers of iron-oxidizing autotrophs (4.5×10³ c/ml), sulfate-reducing microorganisms (2.5×10³ c/ml) and heterotrophic nitrifiers (2.7×10³ c/ml) (table 2). Iron-oxidizing bacteria are morphologically similar to *Siderocapsa sp*. Anaerobic sulfate-reducing bacteria that reduce sulfates to hydrogen sulfide are found in high numbers only in the carbon waters of Nizhnie Luzhki which is probably related to the more favorable conditions for propagation of anaerobic bacteria (lower concentrations of dissolved oxygen (3.7 mg/l), presence of organic substances and sulfates (0.8 mg/l and 0.64 mg/l respectively), as well as to the specific hydrogeological structure of ground waters. The prevailing amount of ammonia-nitrogen (1.10 mg/l) in the ground waters of Nizhnie Luzhki triggers the growth of heterotrophic nitrifiers; however, the low amount of organic carbon (0.8 mg/l) does not let the bacterial count reach high numbers (table 2).
Table 2. Average amounts of different ecologo-trophic groups of bacteria in cold carbon mineral waters of the Russian Far East.

| Physiological groups of microorganisms: c/ml | Lastochka | Deposits of carbon mineral waters: | Medvezhii | Vostochno-Ussurskii | Pokrovskoe | Nizhnie Luzhki |
|---------------------------------------------|-----------|-----------------------------------|----------|---------------------|------------|---------------|
| Saprophytes-copiotrophs                     | 0.5×10^2  | 0.9×10^2                          | 0.8×10^2 | 0.3×10^2            | 0.7×10^2  | 0             |
| Saprophytes-oligotrophs                     | 0.7×10^2  | 0.4×10^1                          | 0.3×10^1 | 0                   | 0          | 0             |
| Ammonia-oxidizing bacteria                  | 0.2×10^2  | 0                                  | 0        | 0                   | 0          | 0             |
| Nitrite-oxidizing bacteria                  | 0.4×10^1  | 0                                  | 0        | 0                   | 0          | 0             |
| Denitrifiers                                | 0         | 0                                  | 0.2×10^2 | 0                   | 0          | 0             |
| Nitrogen fixers                             | 0.1×10^2  | 0                                  | 0.2×10^2 | 0                   | 0.5×10^2  | 0             |
| Ammonia nitrifiers                          | 0.2×10^2  | 0.7×10^3                          | 1.3×10^3 | 0.8×10^2            | 2.7×10^2  | 0             |
| Thionic bacteria                            | 0         | 0                                  | 0        | 0                   | 0          | 0             |
| Sulfate-reducing bacteria                   | 2.5×10^3  | 0.4×10^2                          | 0        | 0                   | 0          | 0             |
| Colorless sulfur bacteria                    | 0.2×10^2  | 0.2×10^2                          | 0        | 0                   | 2.5×10^2  | 0             |
| Iron-oxidizing autotrophs                   | 0.4×10^2  | 0.4×10^2                          | 4.5×10^3 | 0                   | 0          | 0             |
| Iron-oxidizing heterotrophs                 | 0         | 0                                  | 0        | 4.5×10^4            | 4.5×10^3  | 0             |
| Heterotrophic manganese-oxidizing bacteria  | 0         | 0                                  | 0        | 0                   | 0          | 0             |

### 3.3. Sanitary-microbiological analysis of carbon mineral waters

The total count of mesophyll aerobic and optional-anaerobic microorganisms in the carbon mineral waters is not high ranging from 0 CFU/ml (Medvezhii spring, Shmakovskoe deposit) up to 0.9×10^2 CFU/ml (Vostochno-Ussurskii spring, Shmakovskoe deposit) with the quantity of bacteria remaining below standard values (table 3). The count of total coliforms varied between 0.2×10^2±0.63 CFU/100 ml (Lastochka) and 2.1×10^2±5.7 CFU/100 ml (Nizhnie Luzhki) exceeding the maximum permissible values by 20-210 times that shows an anthropogenic pollution of the ground carbon waters (table 3). Total coliforms were not found in the carbon mineral waters of the Medvezhii spring (Shmakovskoe deposit) that shows the purity of ground waters. Thermotolerant coliform bacteria that are indicative of recent fecal pollution and also show the presence of bacteria *Escherichia coli* were not found in the carbon mineral waters (table 3). Pathogenic bacteria *Pseudomonas aeruginosa* were also absent in the samples of cold carbon ground waters thus proving their purity.
Table 3. Quantity of sanitary-indicative microorganisms in the ground cold carbon waters in the Russian Far East.

| Sanitary-indicative microorganisms: | Deposits of carbon mineral waters | Standard |
|-----------------------------------|----------------------------------|----------|
|                                   | Lastochka | Shmakovskoe | Pokrovskoe | Nizhnie Luzhki |                  |
| QMA&OAMO*, CFU/ml                 | 0.5×10^2±1.62 | 0         | 0.8×10^2±0.63 | Not > 1×10^2 CFU/ml |                  |
| Total coliform bacteria, CFU/100ml | 0.2×10^2±0.63 | 0         | 1.5×10^2±4.2 | None in 100 ml |                  |
| Thermotolerant coliform bacteria, CFU/100ml | 0         | 0         | 2.1×10^2±5.7 | None in 100 ml |                  |
| Pseudomonas aeruginosa CFU/1000ml | 0         | 0         | 0           | None in 1000 ml |                  |

*QMA&OAMO – quantity of mesophyll aerobic and optional-anaerobic microorganisms.

1Standardization in accordance with [19].

4. Conclusions

- The waters that have been studied are characterized by the prevalence of calcium, magnesium and sodium among the cations, sulfate, nitrate and chloride ions among the anions, and ferric iron, strontium and manganese in the microelement composition.
- The carbon mineral waters contain various physiological groups of microorganisms that can transform the physical and chemical composition of mineral waters by interfering with geochemical cycles. However, each source of mineral waters has its specific prevalent ecologo-trophic groups of microorganisms related to the differences in chemical composition of carbon waters and water-bearing rocks as well as to the specific hydrogeological structures of ground waters. The carbon waters of Lastochka, Medvezhii, Vostochno-Ussurskii (Shmakovskoe) showed the prevalence of microorganisms of sulfur and nitrogen cycles; iron-oxidizing chemolithotrophic bacteria were predominant in Pokrovskoe and Nizhnie Luzhki.
- Microbiological indicators of the carbon mineral waters have been evaluated through the analysis of indicator microorganisms. Total coliforms have been found in the ground waters of Lastochka, Vostochno-Ussurskii (Shmakovskoe), Pokrovskoe, Nizhnie Luzhki thus showing the evidence of their anthropogenic pollution. Neither thermotolerant coliform bacteria nor the pathogenic bacteria *Pseudomonas aeruginosa* have been found in the ground waters.

References

[1] Chudaev O V, Kharitonova N A, Chelnokov G A, Bragin I V 2016 Water and mineral resources of Primorsky Krai Bulletin FEB RAS. 5 11-20
[2] Chelnokov G A, Chepkaya N A, Karabtsov A A, et al 2006 Geochemistry of carbon mineral waters and water-enclosing rocks of the Lastochka deposit Russian journal of Pacific geology 25(3) 89-97
[3] Kharitonova N A, Chelnokov G A, Vach E A, Goryachev V A 2011 Geochemistry of Nizhniye Luzhki carbonaceous mineral deposit (Primorye) Russian journal of Pacific geology 30(1) 108-118
[4] Chelnokov G A, Kharitonova N A, Bragin I V, Vasileva M 2013 Deuterium, oxygen-18 and tritium in precipitation, surface and groundwater in the Far East of Russia Procedia earth and planetary science 7 151-154
[5] Kharitonova N A, Chelnokov G A, Chudaev O V, et al 2017 The fate of rare earth elements and yttrium in groundwater and bedrocks from Sikhote-Alin Ridge, Primorye Procedia earth and planetary science 17 412-415
[6] Flynn T M, Sanford R A, Bethke C M, et al 2013 Functional microbial diversity explains groundwater chemistry in a pristine aquifer BMC microbiology 13 146-161
[7] Sirisena K A, Daughney C J, Moreau-Fournier, et al 2013 National survey of molecular bacterial diversity of New Zeland groundwater: relationships between biodiversity, groundwater chemistry and aquifer characteristics FEMS microbiology ecology 86 490-504
[8] Chelnokov G A, Kalitina E G, Bragin I V, Kharitonova N A 2014 Hydrochemistry and genesis of thermal waters of the Goryachii Klyuch spring in Primorski Krai (Far East of Russia) Russian journal of Pacific geology 8(6) 475-488
[9] Lehmar R M, Roberto E F, Earley D, et al 2001 Attached and unattached bacterial communities in a 120-meter corehole in an acidic, crystalline rock aquifer Applied and environmental microbiology 67 2095-2106
[10] Griebler C, Mindi B, Slezak D, Geiger-Kaiser M 2002 Distribution patterns of attached and suspended bacteria in pristine and contaminated shallow aquifers studied with in situ sediment exposure microcosm Aquatic microbiology 28 117-129
[11] Grundger F, Jimenez N, Thielemann T, et al 2015 Microbial methan formation in deep aquifers of a coal-bearing sedimentary basin, Germany Frontiers in microbiology 6 200
[12] Miao Z, Brusseau M L, Carroll K C, et al 2012 Sulfate reduction in groundwater: characterization and applications for remediation Environ. Geochem. Health. 34(4) 539-550
[13] Moore T A, Xing Y, Lazenby B, et al 2011 Prevalence of anaerobic ammonium-oxidizing bacteria in contaminated groundwater Environ. Sci. Technol. 45(17) 7217-7225
[14] Soares M I 2000 Biological denitrification of groundwater Water, air and soil pollution 123(1-4) 183-193
[15] Vodyanitskii Y N 2016 Biochemical processes in soil and groundwater contaminated by leachates from municipal landfills (mini review) Annals of agrarian science 14(3) 249-256
[16] Aditi F Y, Rahman S S, Hossain M M 2017 A study on the microbiological status of mineral drinking water Open microbiology journal 11 31-44
[17] Kuznetsov S I, Dubinina G A 1989 Methods of studying aquatic microorganisms Moscow: Nauka 285 p.
[18] Egorov N S 1995 Guide to practical exercises on microbiology Moscow: Moscow State University 224 p.
[19] Methodical manual No. 96/225 1997 Control of the quality and safety of mineral waters by chemical and microbialological indicators Moscow Ministry of Health of Russia 18 p
[20] Methodical instructions 1986 Detection and identification of Pseudomonas aeruginosa in environmental objects (food, water, sewage) Moscow publisher of standards 22 p
[21] Bosch J, Lee K Y, Jordan G, et al 2012 Anaerobic, nitrate-dependent oxidation of pyrite nanoparticles by Thiobacillus denitrificans Environmental science and technology 46 2095-2101
[22] Kharitonova N A, Chelnokov G A, Karabtsov A A, Kiselev V I 2007 Geochemistry of Na-HCO3 groundwater and sedimentary bedrocks from the central part of Sikhote-Aline - 44 - mountain region (Far East of Russia) Applied geochemistry 22 1764-1776