Microbiota and Hydrochemistry of Thermal Groundwaters of the Kuldur Spa (Far East, Russia)

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Abstract. Chemical composition, distribution, structure, and numbers of different physiological groups of bacteria as well as their biodiversity have been researched. It has been shown that thermal waters are alkaline with low-TDS, Na-HCO₃ type, and enriched in boron, lithium, arsenic, and strontium. The content of organic carbon in groundwaters from studied wells is low. They show uneven distribution, low diversity, and numbers of physiological groups of bacteria. The most insufficient numbers and the least diverse composition of physiological bacterial groups were registered in the groundwaters of higher temperatures. The study discovered the prevalence of chemolithotrophic thionic bacteria in the community structure, thus indicating the prevailing bacteria-induced oxidation of reduced sulfur compounds in the groundwaters. Silicate, iron- and manganese-reducing microorganisms also prevailed in groundwaters of wells No.3-87 and No.3-51. Low biodiversity of microorganisms with a significant prevalence of Bacillus sp. (50-60%) spore-forming bacteria in the taxonomic structure has been revealed. Thermophilic bacilli mostly included the species of B. flexus, B. licheniformis, B. subtilis, B. cereus, and B. cohnii.

1. Introduction

The Kuldur field of thermal waters is situated in the continental part of the southern Far East, near the Kuldur settlement of the Jewish Autonomous Region of Russia. The studied area is the most high-output and high-temperature among the thermal fields of the continental part of the Russian Far East. The Kuldur thermal springs are typical pressure fissure-vein groundwaters ascending from the depth of about 3 km. They are formed by the infiltration of meteoric waters into the deep layers of the Earth’s crust and heated by the normal geothermic gradient [1].

The abyssal subterranean biosphere is one of the least studied biospheres of the Earth that contain communities of extremophilic microorganisms. It is known that microorganisms are able to actively modify the chemical and gas composition of groundwaters [2-4]. On the one hand, microorganisms use the substances of ground and waters in their life cycles; on the other hand, they create new ones. The life activity of microorganisms generates different compounds and gases: CO₂, CH₄, NO₃, NO₂, N₂, NH₃, H₂, H₂S, SO₄. Heterotrophic microorganisms that destruct organic matters play an especially significant role in transforming the chemical composition of groundwaters. In the process of their lifecycle, these bacteria produce organic acids, which are active chelatisers and initiate passing into the solution of metals and other ore elements. The water diversity in terms of chemical and gas
composition, temperature and other indicators create the water-ground-gas-organic matter system with the conditions that both stimulate and restrict the bacterial life.

As the composition of bacteria in the Kuldur groundwaters was not sufficiently studied [5-7], the present research work was aimed at investigating the chemical composition, distribution, structure, and numbers of physiological groups of cultivated bacteria and their biodiversity in the thermal groundwaters of Kuldur field.

2. Material and methods

2.1. Sampling

The Kuldur thermal field is located within the Khingan basin of the Amur-Okhotsk hydrogeological fold area [1]. The thermal site is located on the right bank of the Kuldur River and includes two operational (No.1-87 and No.2-87) and three observational (No.3-51, No.5-51 and No.3-87) wells. The discharge of thermal waters occurs in the tectonic fracturing zones. The main operated wells are No.1-87 and 2-87 because the withdrawal waters are remarkably suitable for the spa treatment. However, the water from wells No.3-87, No.3-51 and No.5-51 is not used for the spa procedures due to its lower temperature.

Samples of groundwaters were collected in sterile conditions into a sterile 500-ml glass bottle. The samples were collected in August 2015. Prior to the analysis, the samples were stored in the refrigerator for no longer than 12 hours.

2.2. Sample preparation and analysis

Water samples for analysis of cations, anions and microelements were prepared with standard methods. Unstable parameters (temperature, pH, conductivity, HCO₃⁻ concentration) were measured during sampling. Chemical analysis of samples was performed at the analytical center of FEGI FEB RAS.

Traditional methods of practical microbiology were applied for revealing and cultivating the bacteria [8]. The quantity of different physiological bacterial groups was determined by limiting dilution and Koch’s method [9]. Due to the low amount of bacteria, the colony-forming forms of microorganisms were separated using membrane filtration with subsequent cultivation in different selective media [10]. Anaerobic forms of bacteria were cultivated in an aerostat using GasPak EZ gas generating sachets. Bacteria were cultivated in a thermostat at temperatures 25°C and 60°C for 3-7 days. The types of the cell wall were identified with Gram’s method. The morphology, size, mobility and spore formation of separated pure cultures were studied with AxioStar plus light microscope (Carl Zeiss, Germany) [9]. The separated heterotrophic bacteria were identified with molecular-genetic methods and Bergey’s manual [11-12].

3. Results and discussion

3.1. Chemical composition of Kuldur thermal groundwaters

Results of the chemical analysis have shown that the distinctive features of the surveyed thermal springs are as follows: high temperature (28.0–71.4 °C), low mineralization (152.2-399.8 mg/l), alkaline conditions (pH between 7.82 and 9.70), and reducing environment (Eh from -96 to -375 mV). The amount of total carbon (C₉ₒ₉) is not high and can go up to 17.0 mg/l. The share of organic carbon (C орг.) in nitric thermal springs is low, never exceeding 2.5 mg/l (table 1).

Table 1. Field characteristics and chemical analysis of the thermal groundwaters of the Kuldur field.

| Well No. | t °C | pH | Na⁺ | Ca²⁺ | Mg²⁺ | K⁺ | Cl⁻ | SO₄²⁻ | HCO₃⁻ | F⁻ |
|----------|-----|----|-----|------|------|----|-----|-------|-------|----|
| 1-87     | 71.0| 8.02| 89.08| 1.76  | 0.12 | 4.17| 22.20| 11.20 | 152.90| 13.50|
The temperature conditions of the Kuldur field are stable, which shows that the thermal system is not exposed to the direct influence of the atmosphere, has a stable source of water supply and that the depth of its circulation is significant. According to their chemical composition, the waters are predominantly sodium bicarbonate (chloride-fluoride-sodium-bicarbonate). No significant macromolecular differences have been found between the water samples collected from different temperature zones. The amount of Na\(^+\) is quite high, reaching 94.20 mg/l; the amount of K\(^+\) is quite low (down to 8.84 mg/l). HCO\(_3\)\(^-\) is the most common among the anions, followed by Cl\(^-\) and then by F\(^-\) and SO\(_4\)\(^2-\) (table 1). The amount of cation Mg\(^2+\) is insignificant and in maximum comprises 0.03–0.17 mg/l that shows the absence of mixing between thermal and fresh groundwaters. The waters are enriched in boron (> 300.80 μg/l), lithium (> 211.0 μg/l), arsenic (> 87.2 μg/l), strontium (> 64.0 μg/l), silicone (> 34.4 μg/l), and aluminum (> 21.70 μg/l) (table 1). The analysis of the chemical composition of the gaseous phase has shown that nitrogen is the main component of both dissolved and spontaneous gases (up to 98 total %); the other gases (CH\(_4\), CO\(_2\), O\(_2\)) are, as a rule, insignificant. Nitrogen mostly comes from the atmosphere while CH\(_4\) and CO\(_2\) are of biogenic origin. Argon and oxygen also come from the atmosphere; helium is radiogenic-crustal.

### 3.2. Composition, structure, number of physiological groups of bacteria and their biodiversity in underground thermal water of the Kuldur deposit

Microbiological analysis has shown that groundwaters contain low numbers of physiological groups of bacteria that varied between 0 and 3.3×10\(^3\) CFU/ml. These low amounts of bacteria were previously discovered in different groundwaters of the Far East [13-15]. Low numbers of bacteria in groundwaters can be explained by the low content of organic matter (C\(_{org}\)=8.12–16.1 mg/l) and a high temperature of water (table 1) that restricted the bacterial growth. All studied wells have shown a prevalence of chemolithotrophic thionic bacteria (0.56×10\(^3\) CFU/ml) (table 2) that indicates the processes of microorganism-induced oxidation of reduced sulfur compounds.

### Table 2. The number of physiological groups of bacteria in the thermal waters of the Kuldur field.

| Well No. | TDS mg/l | Eh mV | SiO\(_2\) mg/l | C tot. \(\mu g /l\) | C org. \(\mu g /l\) | B µg/l | Li mg/l | As mg/l | Al mg/l | Fe mg/l |
|---------|----------|-------|---------------|-------------------|-------------------|-------|-------|-------|-------|-------|
| 1-87    | 399.8    | -     | 104.76        | 16.1              | 2.50              | 517.4 | 223.1 | 102.2 | 52.71 | 13.26 |
| 2-87    | 397.5    | -375.0| 106.07        | 13.4              | 0.50              | 506.1 | 215.3 | 95.02 | 33.17 | 6.61 |
| 3-87    | 375.6    | -290.0| 89.06         | 17.0              | 1.00              | 469.6 | 231.3 | 109.8 | 43.89 | 50.39 |
| 3-51    | 174.0    | -96.0 | 34.4          | 8.12              | 0.30              | 300.8 | 214.0 | 87.2  | 28.10 | 105.0 |
| 5-51    | 152.2    | -149.0| 43.4          | 6.43              | 0.10              | 363.0 | 211.0 | 111.0 | 21.70 | 3.28 |

| 1-87   | 2-87    | 3-87   | 3-51   | 5-51   |
|--------|--------|--------|--------|--------|
| 28\(^{1}\)C | 44\(^{1}\)C | 68\(^{1}\)C | 42\(^{1}\)C | 28\(^{1}\)C |

| Bacteria growth temperature 60\(^{1}\)C/25\(^{1}\)C | cell×10\(^2\) |
|-----------------------------------------------|---------------|

- Saprophytic, aerobes CFU/50 ml: 0.01/0.01, 0.03/0.03, 0.05/0.09, 0.6/0.8, 0.05/2.8
- Saprophytic, anaerobes CFU/50 ml: 0/0, 0/0, 0/08, 0.4/0.6, 0.6/1.9
- Nitrogen fixing CFU/50 ml: 0/0, 0/0, 0.2/0, 0/0, 0/0
- Ammonifiers CFU/50 ml: 0/0, 0/0, 0.05/0, 0/0, 0/0
- Autotrophic nitrification cells/ml: 0/0, 0.5/0, 0.14/0, 0.5/0, 0/3.2
- Thionic bacteria cells/ml: 2.8/0, 4.3/0, 33/12, 3.4/1.2, 0/56
- Iron oxidizing CFU/50 ml: 0/0, 0/0, 0/0, 1.3/0, 0/0
- Iron reducing CFU/50 ml: 0/0, 0/0, 5.1/0, 1.9/0.6, 0/2.8
Thus, in the process of their life activity, thionic bacteria can contribute to the accumulation of sulfates in groundwaters. The most significant amount of thionic bacteria have been found in more low-temperature waters of drill holes 5-51 that are obviously connected with more favorable temperatures. The lowest diversity of physiological groups of bacteria and their low amounts were found in the waters of central drill holes No.1-87 and No.2-87 (table 2) that have the highest temperature 71-71.4°C. Thionic, silicate, autotrophic nitrification and heterotrophic saprophyte bacteria have been found (table 2); therefore, these bacteria are involved in the process of sulfur compound oxidation, silicate minerals decomposition, and organic matter destruction. Groundwaters of wells No.3-87 and No.3-51 contain large amounts of different physiological groups of microorganisms connected with the lower temperature of thermal waters (42-64°C), which is more favorable for bacterial activity. In addition to thionic bacteria, the waters of these drill holes contain the most significant amounts of microorganisms involved in the destruction of silicates and reduction of iron and manganese (table 2) that corresponded to the highest concentrations of iron (50.39-105.0 μg/l) discovered in these waters. The highest numbers of physiological bacterial groups in waters sampled from wells No.1-87, No.2-87, No.3-87, and No.3-51 were registered at cultivation temperature 60°C that allows classifying them as obligate thermophiles. More low-temperature waters from wells No.5-51 (28°C) generally contain higher numbers of bacteria that peak at cultivation temperature 25°C; it allows classifying these microorganisms as mesophilic forms. The highest amount of bacteria found in the waters of this drill hole correlated with the lowest concentrations of total and organic carbon (table 1) that shows active consumption of organic matter by bacteria at the temperature favorable for their development. The heterotrophic bacteria separated from groundwaters formed matt light-beige (c), pigmented light-yellow (b) and orange arching glossy colonies (a) on selective media (figure 1).

![Figure 1](image-url)

**Figure 1.** The visual appearance of colonies of heterotrophic bacteria separated from Kuldur thermal waters (a - *Williamsia muralis*; b - *Kocuria salsicia*; c - *Bacillus licheniformis*).

Separated cultures mostly contained motile, spore-forming, catalase- and gram-positive rods of different length (a, c), as well as catalase- and gram-positive non-motile cocci (b) (Figure 2).
Figure 2. Morphological forms of heterotrophic bacteria separated from thermal groundwaters of Kuldur (a- big rods Bacillus sp., b- cocci Kocuria sp., c- long rods Paenibacillus sp.).

Molecular-genetic identification of cultures has shown low diversity of bacteria in the surveyed drill holes with a significant prevalence of spore-carrying Bacillus sp. (50-60%) in the taxonomic structure. These bacteria are widespread in nature; they are found in different thermal and mineral springs and soils [16-18]. Having an extensive metabolic potential, this group of microorganisms is involved in major destructive processes in thermal springs and is the main component of the saprophyte bacterial complex. Thermophilic bacilli mostly include B. flexus, B. licheniformis, B. subtilis, B. cereus and B. cohnii. The groundwaters also contain the bacteria of species Kocuria salsicia, Williamsia muralis and more widespread Paenibacillus ehimensis. Bacteria of Paenibacillus sp. were previously found in the thermal springs of Kamchatka and Baikal region; it is reported that these bacteria carry a significant biological potential as they can synthesize different ferments [19-20].

4. Conclusions
The studied groundwaters are alkaline with low-TDS and belong to the Na-HCO₃ type with the prevalence of B, Li, As, Sr, Al and low amount of organic carbon. These waters are characterized by the uneven distribution of bacteria, low diversity of physiological groups, and their low numbers, which were limited by high temperatures of the water and low concentrations of organic carbon. Chemolithotrophic thionic bacteria involved in the oxidation of reduced sulfur compounds have prevailed in the community structure of waters from different drill holes. In the thermal groundwaters of drill holes No.3-87, No.3-51, No.5-51 that have more favorable temperatures (28-64°C) for bacterial development, the composition of physiological bacterial groups and their numbers were higher though not exceeding 5.6x10³ c/ml (drill hole No.5-51). The bacterial diversity is very low in the thermal waters of all studied wells. Among the separated heterotrophic bacteria, there were mostly gram-positive, catalase-positive, spore-forming rods of different kinds of Bacillus sp.

5. References
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