Chemical and Microbiological Composition of Technogenic Waters in the Tailing Dumps of Krasnorechensk Ore-dressing Plant (Primorsky Krai, Russia)

E G Kalitina¹, N A Kharitonova², T V Kuzmina¹

¹Far Eastern Geological Institute, FEB RAS, Vladivostok, 690022, Russia
²Lomonosov Moscow State University, Moscow, 119991, Russia

E-mail: microbiol@mail.ru

Abstract. Chemical composition, distribution and quantity of chemolytotrophic and heterotrophic microorganisms as well as some of their ecologo-physiological properties have been studied in the technogenic waters of tailing dumps. It has been shown that technogenic waters exhibit low values of pH (2.8 to 6.1), high mineralization (up to 5.6 g/l) and high content of trace elements. Microbiological analysis has shown that a wide spectrum of microorganisms is involved in destruction of sulfate-containing minerals. Technogenic waters of tailing dumps contain autotrophic thionic and sulfate-reducing bacteria, autotrophic and heterotrophic nitrifiers, ammonifiers, heterotrophic manganese and iron-oxidizing bacteria, saprophyte microorganisms and microscopic fungi. Thionic and sulfate-reducing bacteria were most numerous in the waters under study thus indicating the processes of sulfur bacterial oxidation and reduction in the tailing dumps. Microbiocoenosis of the tailing dumps has failed to show a high qualitative diversity. Among the thionic bacteria there were Thiobacillus thiooxidans, Th. intermedius, Th. ferooxidans and Th. acidophilus. Microscopic fungi included Aspergillus, Penicillum, Mucor. The heterotrophic saprophyte microorganisms were predominantly gram-positive Bacillus sp. The isolated heterotrophic bacteria grew at the wide temperature and pH ranges and showed high resistance to heavy metals.

1. Introduction
Tailing dumps of the currently non-operating Krasnorechensk ore-dressing plant [1] are a major source of anthropogenic environmental pollution in Primorsky Krai. Large-scale oxidation processes of residual sulfides and sulfide ores in the tailing dumps pose a serious threat to the nearby ecosystems by producing acidic drainage waters with high concentrations of heavy metal ions. When in the atmosphere, soil or water bodies, these pollutants get involved in the natural circulation of elements and take a long time to leave it [2]. Microorganisms contribute a lot to the oxidation processes in tailing dumps and extraction of metals into solution [3-6]. However, no research of microorganisms in the Krasnorechensk tailing dumps has been done yet.

This study is focused on the chemical composition, distribution, and quantity of different ecologo-trophic groups of microorganisms as well as some of their physiological and biochemical features in the technogenic waters of Krasnorechensk tailing dumps.
2. Material and methods

2.1. Sampling

The subjects under study included technogenic waters and subsoil of the old and new tailing dumps of the Krasnorechensk ore-dressing plant in Dalnegorsky district, Primorsky Krai (figure 1) where the tailings of complex tin-polymetallic and silver-metallic ores of Smirnovsky and Yuzhny deposits were dumped. Samples for chemical and microbiological analysis were collected during the 2013 field season. The samples were collected into sterile 200-ml. vials in triplicate.

![Figure 1. Sampling of water and soil at the old (a) and new (b) Krasnorechensk tailing dumps: 1 - contours of the tailing dish with a dry dyke; 2 - lake; 3 - sites of collecting “liquid” samples; 4 - sites of collecting “solid” samples.](image)

2.2. Sample preparation and analysis

The content of rare-earth and trace elements was identified by inductively coupled plasma mass-spectrometry (ICP-MS, Agilent 7700x). Unstable parameters were measured at the place of water sampling. Analysis of collected samples was conducted at the analytical center of FEB RAS Far East Geological Institute.

Quantitative composition of the tailing dump microorganisms was studied via culturing water and soil samples in special selective media [7-8]. The quantity of microorganisms of different ecologo-trophic groups was defined with a method of limiting dilution in triplicate [9-10]. The most probable quantity of bacteria was calculated through the McCreddie table. Microorganisms were identified through defining their morphological, cultural and physiological-biochemical properties [11-12]. Physiological-biochemical properties of bacteria were studied with standard methods [13-14]. Individual resistance of bacterial strains to heavy metal ions was measured based on determining the minimal inhibitory concentration (MIC) of each metal salt [15-16].

3. Results and discussion

3.1. Chemical composition of tailing dumps technogenic waters

Analysis of the chemical composition has shown that all waters fall into two types: Ca-Mg-SO₄ and Ca-Mg-SO₄·HCO₃. The first type waters (the right lake В-2 and the spring of the old tailing dump В-3) are highly mineralized (up to 5.6 g/l) and have a low pH level (2.9-3.0). These waters contain a lot of trace elements with many of them exceeding the maximum permissible concentrations: Fe total (up to 1908 mg/l; exceeded the maximum permissible concentrations by 6360 times), Mn (up to 364.7 mg/l; exceeded the maximal permissible concentrations by over 3000 times), Al (up to 54.3 mg/l; exceeded by over 270 times), Zn (up to 71 mg/l; exceeded by 12 times), Pb (up to 0.1; exceeded by 3 times). The content of total organic carbon is small (1.3-7.27 mg/l). The concentrations of rare-earth elements are quite high (table 1). The second type includes the lake waters of the old tailing dump (B-1), the Rudnaya River (B-5), and the new tailing dump waters (B-6). These waters are characterized by low
mineralization (0.2–0.250 g/l) and pH values within (5.1 – 6.1). The content of rare-earth elements is lower, as well as the concentration of trace elements (table 1). However, the samples from the Rudnaya contained higher than normal quantities of Mn and Fe_total, though within the maximum permissible concentrations. The concentrations of total organic carbon were low (table 1).

Table 1. The chemical composition of technogenic waters of the of the Krasnorechensk tailing dumps and surface waters of the Rudnaya River.

| Sample name | Old tailing dump, far lake (B-1) | Old tailing dump, right lake (B-2) | Old tailing dump, spring (B-3) | The Rudnaya River (B-5) | New tailing dump, lake (B-6) |
|-------------|-------------------------------|---------------------------------|--------------------------------|-------------------------|-----------------------------|
| pH          | 6.1                           | 2.9                             | 3.0                            | 6.7                     | 5.15                        |
| Mn, мг/л    | 235                           | 5600                           | 1500                           | 205                     | 250                         |
| TOC         | 3.88                          | 7.27                            | 1.3                            | 1.6                     | 1.29                        |
| Fe          | 394.66                        | 1908000                        | 86280                          | 188.52                  | 1038.65                     |
| Mn          | 664.03                        | 364700                         | 80960                          | 915.30                  | 5142.00                     |
| Al          | 16.45                         | 54390                          | 26980                          | 32.97                   | 498.01                      |
| Si          | 3.70                          | 36.10                          | 20.45                          | 9.77                    | 7.62                        |
| La          | 0.03                          | 47.84                          | 43.80                          | 0.73                    | 1.41                        |
| Ce          | 0.05                          | 113.46                         | 107.43                         | 0.88                    | 2.20                        |
| Pr          | 0.02                          | 13.77                          | 16.44                          | 0.51                    | 0.25                        |
| Nd          | 0.03                          | 64.51                          | 82.84                          | 0.86                    | 1.01                        |
| Sm          | 0.02                          | 18.15                          | 26.46                          | 0.58                    | 0.24                        |
| Eu          | 0.02                          | 6.72                           | 7.81                           | 0.50                    | 0.09                        |
| Gd          | 0.02                          | 23.99                          | 36.11                          | 0.62                    | 0.38                        |
| Tb          | 0.01                          | 3.39                           | 5.67                           | 0.49                    | 0.07                        |
| Dy          | 0.02                          | 18.09                          | 30.60                          | 0.58                    | 0.31                        |
| Ho          | 0.01                          | 3.03                           | 4.86                           | 0.49                    | 0.07                        |
| Er          | 0.02                          | 7.69                           | 11.80                          | 0.54                    | 0.15                        |
| Tm          | 0.01                          | 0.88                           | 1.28                           | 0.48                    | 0.03                        |

M- mineralization

3.2. Distribution, quantity of physiological groups of microorganisms and their biodiversity in the technogenic waters and subsoil of tailing dumps

The Krasnorechensk tailing dumps contain numerous physiological bacterial groups that in combination are involved in geochemical transformations of sulfide ores. The water showed predominance of chemolytrophic thionic bacteria (table 2) that were involved in oxidation of reduced sulfur and iron compounds and generation of sulfuric acid. The highest quantities of these microorganisms were found in the waters of B-6 (pH=5.15; 3.1×10³ c/ml) and in the acidic waters of B-2 (pH=2.9; 2.5×10³ c/ml). The isolated samples of B-1, B-6 showed predominance of neutrophilic Thiothrix intermediius, and Th. thiooxidans; the acidic waters B-2, B-3 mostly contained acidophilic bacteria Th. feroxidans and Th. acidophilus. The water also contained numerous ammonium-oxidizing bacteria Nitrosomonas sp. involved in oxidation of ammonium nitrogen to nitrates and nitrates as well as various associations of physiological groups of heterotrophic microorganisms. The heterotrophic bacteria were predominated by heterotrophic nitrifiers, saprophyte, silicate and iron-oxidizing microorganisms (table 2). The highest quantities of iron-oxidizing and silicate bacteria were found in the acidic waters of the right lake (B-2) and spring (B-3) at the old tailing dump that coincided with the highest concentrations of total iron (table 2). The Bacillus sp. of saprophyte microorganisms was predominant in the waters under study.

The subsoil of tailing dumps also contained a great variety of thionic bacteria of high quantities ranging from 9.5×10⁴ (T-8) to 1.1×10⁵ c/g (T-10) that exhibits the processes of iron and sulfur bacterial oxidation in the tailings. Samples T-1 and T-10 showed prevalence of neutrophilic thiobacteria Th.
intermedius and Th. thiooxidans; the acidic residuals T-5 and T-8 mostly contained acidophilic Th. ferooxidans and Th. acidophilus. Anaerobic sulfate-reducing bacteria also reached high quantities and peaked in the acidic subsoil of the spring (2.5×10⁶ cfu/g, T-8) and neutral residues of the far lake (1.5×10⁴ cfu/g, T-1). Sulfate-reducing bacteria in the tailing dumps were involved in reducing sulfates to sulfides and could influence on metal transfer.

In addition to sulfide waste, the weathered tailings contain high quantities of iron and manganese oxides (hydroxides). Iron- and manganese-reducing bacteria are known to be able to use these compounds as terminal electron acceptors in their respiratory processes; they are also instrumental in recovery of insoluble iron and manganese oxides with generation of active forms of metals [17-19]. In the oxidized residues of Krasnorechensk tailing dumps the highest quantity of iron- and manganese-oxidizing bacteria was typical of the subsoil with almost neutral pH-values (T-1, T-10) (table 2) that is indicative of the bacteria-involving processes of iron and manganese oxidation in the tailing dumps. The residues also contained numerous chemolytrotrophic ammonium-oxidizing, saprophyte and other physiological groups of heterotrophic microorganisms (ammonifiers, silicate, heterotrophic nitrifiers and denitrifiers) that being indicative of the intensive processes of organic matter decomposition, oxidation and reduction of nitrogen compounds, and destruction of silicate minerals. The most average quantities of ammonium-oxidizing bacteria as well as various groups of heterotrophic bacteria were found in the lake subsoil at the new tailing dump (T-10) that is indicative of the favorable conditions for bacterial growth. Ammonium-oxidizing bacteria were predominated by Nitrosomonas sp. and Nitrosococcus sp. The saprophyte microorganisms in the old tailing dump were mostly those of Bacillus sp. and Pseudomonas sp.; the subsoil of the new tailing dump also contain Mycobacterium sp.

It is known that in the process of organic matter decomposition the heterotrophic bacteria can discharge various byproducts (organic acids, polysaccharides etc.) that can influence the metal mobility and destruction of sulfide minerals [20]. Thus, heterotrophic microorganisms are instrumental in geochemical transformations of sulfide ores in tailing dumps.

The subsoil of the Krasnorechensk old and new tailing dumps contained numerous microscopic fungi ranging from 4.7×10⁵ (T-1) to 0.7×10⁶ cfu/g (T-8). The highest quantities of fungi were found in acidic subsoil of the right lake (T-5) and spring (T-8) at the old tailing dump (table 2). The fungi were involved in oxidation and reduction of iron and manganese; they were also active in the destruction of silicate minerals. Aspergillus, Penicillium and Mucor were mostly predominant among the fungi.

The Rudnaya River is characterized with a higher average quantity of bacteria (7.9×10³ cfu/ml) and a high quantity of silicate (6.9×10⁴ cfu/ml), nitrogen-fixing (2.3×10⁶ cfu/ml) and saprophyte microorganisms (1.5×10³ cfu/ml) that is indicative of the prevailing oxidizing of organic matter, transformation of nitrogen compounds and destruction of silicate minerals in the river.

3.3. Physiological and biochemical properties of the isolated heterotrophic bacteria
In the waters and subsoil of the Krasnorechensk tailing dump 25 pure cultures of the most active heterotrophic microorganisms have been isolated; these microorganisms are able to oxidize and reduce iron and manganese, or destroy silicate minerals. With respect for the morphological diversity, the isolated strains mostly contained non-pigmented grey and light beige colonies. Rod-shaped bacteria with cell length ranges of 1.05-6.31 μm were the prevailing morphotype. The strains under study were mostly gram-positive (81%), predominantly motile (75%), spore-forming (65%), catalase-positive (93%) and oxidase-negative (45%) rods. The isolated cultures mostly had a fermentative type of metabolism (O²/F²) (75%). With respect for subsistence type, the majority of strains were chemoorganotrophic. All strains under study grew at the wide temperature range of 25-42°C, pH 5.0-10.0; they showed growth at concentration in the medium NaCl 1-6%. The isolated bacteria showed high resistance to heavy metals. The MIC of heavy metals for the isolated bacteria was for Cd (10-150 mg/l), Co(100-500 mg/l), Cu (400-800 mg/l), Ni (500-1000 mg/l), Zn (500-3500 mg/l), and Pb (3500-5500 mg/l). The cultures were most resistant to zinc and lead.
Table 2. Average quantity of different ecologo-trophic groups of microorganisms in technogenic waters and subsoil of the Krasnorechensk tailing dumps.

| Physiological groups of microorganisms | B-1 | B-2 | B-3 | B-6 | The Radunaya River (B-5) |
|----------------------------------------|-----|-----|-----|-----|-------------------------|
| c/ml (water)                           | c/g (subsoil) |
| Saprophyte                             | 1.5×10^3 | 0   | 0.9×10^3 | 2.4×10^2 | 1.5×10^3 |
| Ammonium-oxidizing                     | 0.9×10^2 | 1.4×10^2 | 2.3×10^3 | 1.5×10^3 | 9.5×10^2 |
| Denitrifying                           | 0   | 0   | 0   | 0   | 2.3×10^4 |
| Nitrogen-fixing                        | 0   | 1.0×10^2 | 2.0×10^3 | 0   | 0   |
| Heterotrophic nitrifiers               | 2.6×10^2 | 1.1×10^2 | 2.1×10^2 | 2.4×10^2 | 2.0×10^2 |
| Ammonifiers                            | 1.3×10^2 | 0   | 1.0×10^2 | 0   | 0   |
| Silicate                               | 2.0×10^3 | 8.2×10^3 | 5.8×10^3 | 4.2×10^5 | 0   |
| Thionic bacteria                        | 3.2×10^3 | 8.1×10^3 | 2.2×10^3 | 2.1×10^5 | 6.9×10^4 |
| Sulfate-reducing                       | 5.0×10^3 | 2.5×10^3 | 7.4×10^2 | 3.1×10^3 | 1.0×10^2 |
| Heterotrophic iron-oxidizing           | 0   | 0   | 0   | 0   | 0   |
| Heterotrophic manganese-oxidizing      | 0   | 0.8×10^2 | 1.5×10^2 | 0   | 1.5×10^2 |
| Iron-reducing                          | 2.5×10^2 | 1.0×10^3 | 1.5×10^3 | 0   | 7.2×10^2 |
| Manganese-reducing                     | 7.4×10^3 | 1.8×10^2 | 1.2×10^2 | 6.7×10^3 | 0   |
| Microscopic fungi                      | 5.6×10^3 | 0.5×10^2 | 0.9×10^2 | 4.5×10^3 | 0   |

4. Conclusions
The acidic technogenic waters of the Krasnorechensk tailing dumps contain high numbers of trace and rare-earth elements with many of them exceeding the maximum permissible concentrations. Concentrations of microelements and rare-earth elements were lower in the neutral waters of the tailing dumps and in the Rudunaya River. The technogenic waters mostly contained thionic bacteria; the residues showed prevalence of thionic, sulfate-reducing and various physiological groups of heterotrophic microorganisms that oxidized iron and metallic sulfides, reduced sulfites to sulfides, were involved in organic matter decomposition, oxidation of nitrogen compounds and destruction of silicate minerals. Thionic bacteria in the water and subsoil were predominated by Thiothrix intermedius, Th. thiooxidans, Th. ferooxidans and Th. acidophilus. Saprophyte microorganisms were mostly of the Bacillus sp. bacteria. The Krasnorechensk tailing dumps contain high quantities of microscopic fungi with prevalence of Aspergillus, Penicillium and Mucor. The isolated heterotrophic bacteria were mostly gram-positive, motile, spore-forming rods with fermentative metabolism. The microorganisms grew at the wide temperature range, pH, concentration of NaCl, and showed high resistance to heavy metals.
5. References

[1] Tarasenko I A, Kharitonova N A, Ovodova E V, Zinkov A V and Korzun A V 2017 Transformation of mineral and geochemical composition of ore-dressing tailings and its impact on formation of highly mineralized waters (Primorsky Krai) *Journal of Pacific Geology* **36**(2) 106-18

[2] Ovodova E V, Tarasenko I A, Nagornova N A and Salnikova L A 2016 Geochemistry of Krasnorechensk ore-dressing plant tailing dumps (Dalnegorsky district, Primorsky Krai) *Bulletin FEB RAS* **5** 43-51

[3] Koschorreck M 2008 Microbial sulphate reduction at a low pH *FEMS Microbiol. Ecol.* **64** 329-42

[4] Karnachuk O V et al 2009 Bacteria of the sulfur cycle in the sediments of gold mine tailings, Kuznetsk basin, Russia *Microbiology* **78**(4) 483-91

[5] Mardanov A V, Beletskii A V, Ravin N V, Ivasenko D A, Karnachuk O V and Pimenov N V 2017 Sulfate-reducing bacteria in the microbial community of acidic drainage from a gold deposit tailing storage *Microbiology* **86**(2) 286-88

[6] Kalitina E G, Shangina D A and Kharitonova N A 2016 Microbial composition of technogenic waters and residues of Krasnorechensk ore-dressing plant (Primorsky Krai, Russia) *Success of Modern Science and Education* 5 351

[7] Kuznetsov S I and Dubinina G A 1989 *Methods of studying aquatic microorganisms* (Moscow: Nauka) p 285

[8] Youchimizu M and Kimura T 1976 Study of intestinal microflora of Salmonids *Fish. Pathol.* **10**(2) 243

[9] Egorov N S 1995 *Guide to practical exercises on microbiology* (Moscow: Moscow State University Press) p 224

[10] Lysak V V, Zheldakova R A and Fomina O V 2015 *Microbiology, practicum* (Minsk) p 115

[11] *Bergey’s Classification of Bacteria* 1997 ed D Hoult, N Krig, P Snit, D Steili and S Yillyams (Moscow: Mir) vol 1 p 432

[12] *Bergey’s Classification of Bacteria* 1997 ed D Hoult, N Krig, P Snit, D Steili and S Yillyams (Moscow: Mir) vol 2 p 368

[13] Tepper E Z 2004 *Microbiology Practicum* (Moscow: Drofa) p 256

[14] Lisak V V and Ignatenko E I 2016 *Physiology of microorganisms* (Minsk: BSU) p 80

[15] Netrusov A I, Egorova M A and Zaharchuk L M 2005 *Microbiology Practicum* (Moscow: Academy Press) p 612

[16] Lambert R J and Pearson J 2000 Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values *J. of Appl. Microbiol.* **88** 784-90

[17] Wielinga B, Lucy J, Moore J N, Seastone O F and Gannon J 1999 Microbiological and geochemical characterization of fluvially deposited sulfidic mine tailing *Appl. Environ. Microbiol.* **65**(4) 1548-55

[18] Slobodkin A I 2005 Thermophilic microbe metal reduction *Microbiology* **74** 581-95

[19] Zavarzina D G 2004 Role of dissimilating iron-reducing bacteria in transformation of iron minerals *Paleontological Journal* **3** 3-10

[20] Philina N Yu and Verchovceva N V 2001 *Ecological Physiology of Microorganisms* (Yaroslavl: Yaroslavl State University) p 92

Acknowledgments

The present research was supported by FEB RAS grant № 18-5-089.