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Old Sulfidic Ore Tailing Dump: Ground Features, Mineralogy, Biodiversity—A Case Study from Sibay, Russia

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Abstract: The Urals (Russia) are among the largest mining areas in the world, with millions of tons of mine waste deposited. An old sulfidic tailing dump formed over decades of mining activities at the Sibay ore-processing plant is a typical cause of acid mine drainage (AMD) formation, posing a threat to ecosystems of neighboring environments. In this study, the formation of oxidized surface soil layers in four zones of the Sibay tailing dump was revealed, and their chemical–mineralogical and physical–mechanical characteristics were analyzed. According to the results of the metabarcoding of hypervariable regions of the 16S rRNA genes, oxidation in soil layers was associated with the activity of sulfur- and iron-oxidizing acidophiles represented by a few genera: *Ferroacidibacillus*, *Sulfoacidibacillus*, *Sulfobacillus*, and *Ferroplasma*. The structure of the microbial communities in soil layers differed depending on the zone and depth of sampling. In the samples characterized by the weak oxidation of sulfide minerals, microbial communities were dominated by bacteria of the genus *Pseudomonas*. The data obtained in this research are of importance to predict the oxidation/leaching processes in mine wastes and their negative environmental impacts in the mining region, as well as to develop technologies for processing these raw materials.

Keywords: tailing dump; bioleaching; acidophilic microorganisms; microbial community; sulfides

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1. Introduction

The acidification of soil leading to a decrease in pH below 5.5 and considerably lower values (~1.0–2.0) may pose a serious problem to ecosystems at abandoned mine areas and neighboring environments. Many abandoned mine areas are left unattended for decades, thus aggravating the acidification. In addition to a decrease in pH that has negative impacts on soil productivity and plant growth, the leaching of toxic metals contributes to more severe pollution [1,2]. Acid mine drainage (AMD) is one of the common consequences of the bio-oxidation of sulfides by acidophilic microorganisms during mining activities and the deposition of mine wastes, including mine tailing dumps [3].

The Urals (Russia) are among the largest mining regions in the world. Since the beginning of the development of the Urals sulfide deposits in 1635, millions of tons of various types of waste from the mining and processing of sulfide ores have been accumulated [4]. Several studies have proven the harmful effects of accumulation and acidification of waste from mines and tailing dumps [5,6]. At the same time, special attention is paid to the study of the processes of migration and accumulation of metals in the aquatic landscape [7].

Apart from the negative environmental impact, the Urals tailing dumps contain non-ferrous, rare, and precious metals. Therefore, these mine wastes are considered objects of re-mining for the extraction of valuable components [8–10]. However, within the same tailing dump, the properties of tailings may differ. They depend on the conditions and period of deposition of tailing zones, the duration of storage (with and without flooding), humidity, climatic conditions of the region (including temperature changes), and other factors [11]. Within the same dump containing sulfidic ore flotation tailings, zones are usually characterized by different pH and Eh values, as well as varying elemental composition [12]. Therefore, characteristics of the near-surface section of the tailing dump, especially a zone of relatively active aeration, may be expected to promote the development of microbial communities, the structure of which depends on the conditions described above. In turn, the structure of the community is predicted to affect the elemental composition of the tailings stored.

The presence and composition of acidophilic microbial communities can predict the oxidation/leaching processes in mine wastes, as well as probable negative environmental impacts. It has been previously shown that iron and sulfur oxidizers contribute to the highest acidity values in the iron mine dump in Eastern China. The microbial communities present there are dominated by the genera *Acidiferrobacter* and *Sulfobacillus*. In old bare samples, *Acidibacter*, *Metallibacterium*, and *Cyanobacteria* are abundant, while the phylum *Acidobacteriota* prevails in the vegetated samples and promotes nutrient enrichment and plant growth significantly [13]. The assessment of the microbiological and chemical spatial distribution within two tailing basins from a tungsten mine has shown that the tailings sediments core microbiome contained members of the family *Anaerolineacea* and the following genera: *Acinetobacter*, *Bacillus*, *Cellulomonas*, *Pseudomonas*, *Streptococcus*, and *Rothia* [14]. Acidic contaminated soils of antimony mine (Lengshuijiang, China) are dominated by *Rhodobium*, *Sphingopyxis*, *Streptomyces*, *Burkholderia*, *Mucilaginibacter*, *Phenylobacterium*, *Flavobacterium*, and *Arthrobacter.* The presence of these genera positively correlates with one or more heavy metals in the soil samples: antimony, arsenic, cadmium, and zinc [15].

Abandoned gold mine tailings and adjacent soil samples (Krugersdorp, South Africa) characterized by low pH and high metal concentrations have been shown to contain mainly acidophilic sulfur and iron-oxidizing bacteria *Acidithiobacillus* spp. and *Acidiphilium* spp. [16]. Other microorganisms, such as *Bacillus* spp., *Arthrobacter* spp., *Pseudomonas* spp., *Achromobacter* spp., and *Streptomyces* spp., have also been found to thrive under the conditions of high acidity and metal content in abandoned mine areas and tailings [17,18].

The study of the microbial diversity in tailing dumps and its impact on the characteristics of tailings can be used not only to assess their environmental impact in the mining region but also to develop technologies for processing these raw materials.

Thus, the goal of this research was to study the chemical–mineralogical and physical–mechanical properties, as well as microbial diversity, in the surface layers of an old sulfidic tailing dump.

2. Materials and Methods

2.1. Tailing Dump

The type of waste from the mining and processing of pyrite ores obtained at the Sibay ore concentrator (ore-dressing plant) is a typical cause of the formation of AMD. Therefore, the object of the study was an old tailing dump of the concentrator located in Sibay (Republic of Bashkortostan, Russia; Figure 1).

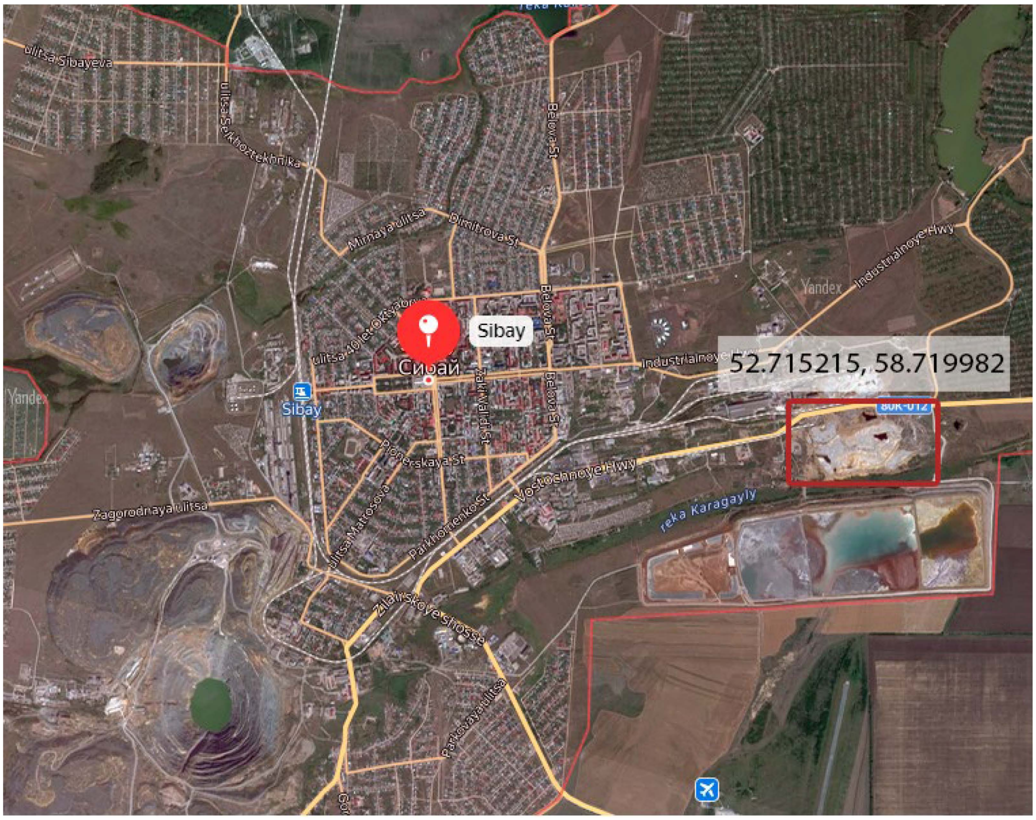


Figure 1. Location of the old tailing dump of the Sibay concentrator within the city of Sibay (the object is in the red frame).

At the Sibay concentrator, operating since 1959, ores from neighbor sulfide deposits (Sibayskoye, Yubileinoye, Mayskoye, and several small deposits in the Baymak district) have been processed. The old tailing dump of the Sibay concentrator was formed from the beginning of its operation until 1965.

The old tailing dump is located 1.0 km to the south of the industrial site of the Sibay concentrator, ~0.15 km from the right bank of the river Karagayly. The coordinates of the central part of the old tailing dump are 52.715215, 58.719982.

The climatic conditions of Sibay are characterized by moderate continental climate and aridity throughout the year typical for this climate. Precipitation is especially rare in summer and more frequent in winter. Winters are frosty and long. The average temperature in January is −13 °C. Summer is hot and short. The average temperature in July is +21 °C.

2.2. Sampling

Samples were collected from various zones of the tailing dump, taking into account the features of their surface topography and local characteristics of water circulation.

Based on the presence of visibly oxidized crust near the southern dam, sampling zone no. 1 was determined. In this zone, three samples were collected from a depth of 0.5, 1.0, and 1.5 m: samples 1.1, 1.2, and 1.3, respectively.

The presence of an artificial riverbed on the surface of the tailing dump, which ensures the outflow of accumulated precipitation from the southwest to the northeast due to the difference in the height of the tailing dump, determined the sites of sampling in zones nos. 2 and 3.

Zone no. 2 was chosen as the tallest location in the main part of the old tailing dump at the source of the artificial riverbed. In zone no. 2, three averaged samples were also collected every 0.5 m in depth (samples 2.1, 2.2, and 2.3).

Zone no. 3 is located in the zone of accumulation and release of engineered waters at the surface due to the lowered part of the relief. Three samples were also collected from this point at intervals of 0.5 m in depth (samples 3.1, 3.2, and 3.3).

Moreover, zone no. 4 located outside the tailing ponds in the northwestern section of the tailing dump was distinguished. It was located at a greater height compared to the main part of the dump (zones nos. 1, 2, and 3) and separated from it by a dam. Technogenic soils in zone no. 4 were represented by pyrite tailings characterized by longer storage periods and, consequently, more pronounced oxidation. In this zone, sample 4.1 was collected from a depth of 1.5 m to compare its properties with those of the samples from soils of the main part of the tailing dump.

The result of the zoning of the Sibay tailing dump, taking into account relief features, is shown in Figure 2.

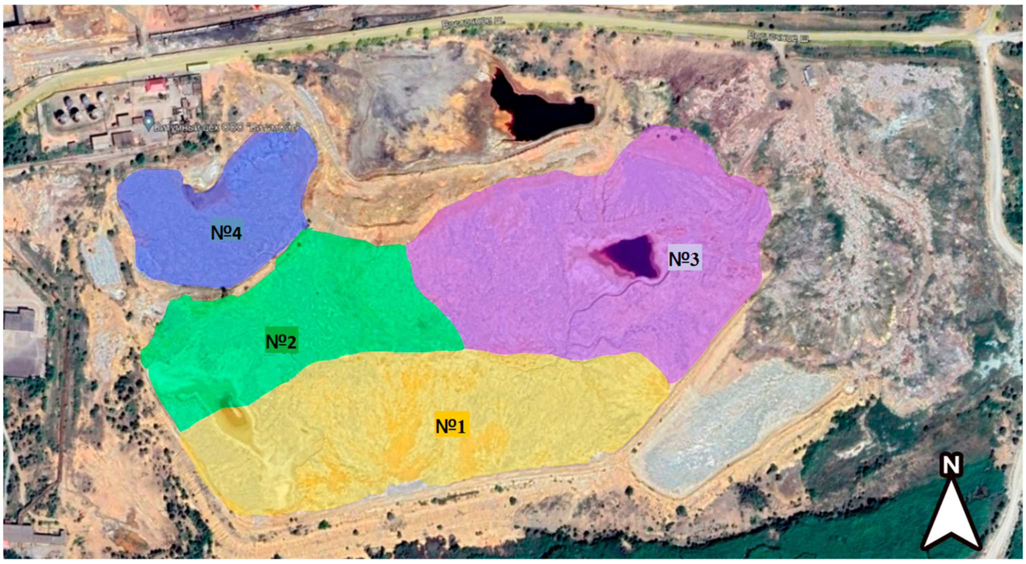


Figure 2. Zoning of the Sibay tailing dump considering the relief features and sampling sites: no. 1, southern zone; no. 2, southwestern zone; no. 3, northeastern zone; no. 4, northwestern zone.

The weight of each sample was 2 kg. Each sample was assigned a unique number and hermetically packed in plastic containers for storage at +5 °C.

2.3. Analytical Methods

The value of the pH of wet samples was measured with a pH-150MI pH meter–millivoltmeter (Izmeritelnaya tekhnika, Moscow, Russia).

The chemical composition of the samples was determined by X-ray fluorescence method using Innov-X Alpha Series analyzer (Innov-X Systems, Mountain View, CA, USA). The mineralogical composition of the samples was determined using powder X-ray diffraction with an XRD-6000 diffractometer with CuKα radiation (SHIMADZU, Kyoto, Japan). The relative mineral phase amounts were estimated using the Rietveld refinement of the XRD patterns by SIROQUANT V4 software (Sietronics, Mitchell, Maranoa, Queensland, Australia).

The physical and mechanical properties of the selected ore flotation tailings were assessed in their natural state under field conditions, as well as in the geomechanics laboratory in the state of their natural moisture, which was ensured by sealed packaging after sampling.

The bulk density of old tailings was determined on the field using a metal cylinder without a bottom and a lid with a sharp cutting edge. The volume of the samples was determined by the internal volume of the experimental cylinder.

The natural moisture content of technogenic soils was determined according to Russian Standard no. 5180-84 "Soils. Laboratory methods for determination of physical characteristics".

To determine the liquid limit, the Vasiliev cone was used. Determination of the plastic limit was carried out by the standard method of rolling out a thread of soil. Based on the values of the liquid and plastic limits, the plasticity index was determined, which was used to assess the soil clayiness. After that, the liquidity index was determined to classify soils (Russian Standard no. 25100-2011 "Soils. Classification".)

To determine the internal angle of friction and specific cohesion, soil tests using the single-plane shear test according to the scheme of consolidated–drained (slow) shear were carried out (Russian Standard no. 12248-2010 "Soils. Laboratory methods for determining the strength and strain characteristics."). The samples were pre-compacted under vertical stress of 0.1, 0.2, and 0.3 MPa for two days using a soil compaction device. The tests were carried out at natural soil moisture and in a water-saturated state when stress was applied.

Particle size distribution studies were carried out in the laboratory using combined sieve and sedimentation methods, according to current standard methods for determining the content of particles of a certain size class (Russian Standard no. 12536-2014 "Soils. Methods of laboratory granulometric (grain-size) and microaggregate distribution."). The sample was dispersed with distilled water to improve particle separation and eliminate soluble phases and subsequently placed into a drying oven and heated to a constant mass. The dried sample was dispersed using a standard set of sieves. Products sieved were weighed using scales with an average accuracy class.

2.4. Isolation and Analysis of Microbial Communities

Microbial communities were washed out from the soil samples (10 g wet *wt*) collected at different depths of four zones of the tailing dump with the standard acidified 9K medium base containing no energy sources (pH 1.8) [19]. Cell numbers were determined using direct counting with a Mikmed-2 microscope equipped with a phase-contrast device (LOMO, Moscow, Russia).

Solids in the studied samples were precipitated by centrifugation (100× *g*, 2 min) and subsequent filtering through paper filters. Cell pellets were collected by centrifugation (5000× *g*, 15 min) of the obtained supernatant. The biomass was sequentially washed with the iron-free 9K medium base (pH 1.5) and with the same medium with a neutral pH by centrifugation (10,000× *g*, 5 min). The structure of the microbial communities from mine tailing samples was determined by the metabarcoding method using the 16S rRNA gene hypervariable region (V3–V4) as described [20]. Sequencing of DNA libraries was carried out with a MiSeq instrument (Illumina, San Diego, CA, USA) and the Miseq reagent kit v3 (Illumina, San Diego, CA, USA). To choose operational taxonomic units (OTU) and to carry out the taxon-based assignment, the QIIME open-source software pipeline [21] and the Silva132 database [22] were used. The analysis included ≈12,000–30,000 fragments (an average length, 449 nucleotides). Analysis of the similarity of nucleotide sequences of the 16S rRNA gene fragments was carried out to identify microorganisms at the species level using the Nucleotide Basic Local Alignment Search Tool (BLAST; Bethesda, MD, USA, https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 11 October 2023).

3. Results and Discussion

During a preliminary assessment work, it was found that the upper part of the tailing dump was formed by an oxidation zone (Figure 3a) represented by brown and gray soils (Figure 3b). Brown soils in the form of loose and dense masses indicate the predominance of secondary sulfates of the jarosite group, the formation of which occurs under conditions of acidic waters (pH < 3) and high oxidative potential of the environment during the oxidation of iron sulfides (pyrite, pyrrhotite, marcasite, and others) [3,23]. Gray soils include mineral forms characteristic of the intermediate step of sulfide oxidation in the form of secondary sulfates of the melanterite group (Figure 3c). The high acidity of solutions (pH < 3) and low oxidative potential of the environment (<0.2 V vs. SHE) are among the conditions required for the stability of these sulfates.

(**a**) (**b**) (**c**)

Figure 3. Soil from the oxidation zone of the tailing dump: excavation section (**a**), gray and brown soil (**b**), and secondary mineral forms: readily soluble sulfates (**c**).

3.1. Composition and Properties of Soils

The results of determining the pH of the material of samples collected from the soils of the tailing dump are shown in Table 1. In all samples, an acidic environment was observed, the formation of which was associated with the oxidation of sulfide minerals of flotation tailings. The simplified chemistry of sulfide oxidation can be expressed as the following reactions:

$$4Fe^{2+} + O_2 + 4H^+ = 4Fe^{3+} + 2H_2O, \quad (1)$$

$$MeS + 2Fe^{3+} = S^0 + Me^{2+} + 2Fe^{2+}, \quad (2)$$

where Me—metal of sulfide minerals.

$$2S^0 + 2H_2O + 3O_2 = 2H_2SO_4. \quad (3)$$

Table 1. Results of pH measurements in wet material of samples.

Zone of Tailing Dump	Sample	Sampling Depth (m)	pH
Southern (no. 1)	1.1	0.5	1.97
	1.2	1.0	2.40
	1.3	1.5	2.79
Southwestern (no. 2)	2.1	0.5	0.93
	2.2	1.0	2.27
	2.3	1.5	2.23
Northeastern (no. 3)	3.1	0.5	0.83
	3.2	1.0	1.54
	3.3	1.5	2.33
Northwestern (no. 4)	4.1	1.5	1.95

Due to the natural decrease in the concentration of dissolved oxygen with increasing soil depth due to a decrease in its permeability, an increase in pH values was observed for all studied zones of the tailing dump from the soil surface to depth.

Table 2 shows the results of the analysis of metal content in the sample materials from the Sibay tailing dump. It can be concluded that in all cases, the copper and zinc content in the sample material was relatively low, except for the copper content in the soil at a depth of 1.0 and 1.5 m in the southwestern zone of the tailing dump (samples 2.2 and 2.3). When analyzing the content of copper and zinc, its increase with an increase in depth was observed in all zones of the tailing dump. This pattern is explained by the more pronounced oxidation of sulfides in the surface soil layers, which is accompanied by (bio)leaching of metals, as well as metal release from the tailing dump with precipitation.

Table 2. Content of main chemical elements in samples from the Sibay tailing dump.

Zone of Tailing Dump	Sample No.	Content (wt.%)					
		Cu	Zn	Pb	As	Ti	Fe
Southern (no. 1)	1.1	0	0.1	0.1	0.1	0.4	14.9
	1.2	0.2	0.2	0.1	0	0	25.6
	1.3	0.1	0.2	0	0	0.3	17.2
Southwestern (no. 2)	2.1	0.1	0.1	0.1	0.3	0	35.2
	2.2	0.6	0.3	0.1	0	0	22.9
	2.3	0.7	0.4	0.1	0	0	22.9
Northeastern (no. 3)	3.1	0	0.1	0.2	0	0	20.1
	3.2	0.1	0.1	0.1	0	0	29.8
	3.3	0.2	0.4	0.1	0	0	25.8
Northwestern (no. 4)	4.1	0.06	0.1	0.02	0.03	0	6.6

Data from the X-ray diffraction analysis (Table 3) allowed an assessment of the mineral composition of the material of soil samples collected from the tailing dump.

Table 3. Mineral composition of the samples from the Sibay tailing dump.

Zone of Tailing Dump	Sample No.	Content (wt.%)						
		Py *	Jr	Ml	Rz	Gp	Qz	Al-O-Si Minerals
Southern (no. 1)	1.1	10	10	0	4	10	38	28
	1.2	39	1	0	10	5	18	27
	1.3	18	1	4	0	8	24	45
Southwestern (no. 2)	2.1	56	0	25	3	4	7	5
	2.2	39	0	10	0	4	21	26
	2.3	36	0	8	0	4	21	31
Northeastern (no. 3)	3.1	32	6	3	4	8	28	19
	3.2	25	2	37	7	5	13	11
	3.3	48	1	3	0	3	17	28
Northwestern (no. 4)	4.1	6	3	0	0	17	55	19

* Py, pyrite; Jr, jarosite; Ml, melanterite; Rz, rozenite; Gp, gypsum; Qz, quarts; Al-O-Si, aluminosilicates.

According to the data in Table 3, the only sulfide mineral determined in all samples was pyrite (FeS_2, 6–56%). This was due to its high refractoriness to oxidation processes (including bio-oxidation), as compared to sphalerite (ZnS) and pyrrhotite (FeS) composing processed sulfide ores. It is also noteworthy that no carbonate minerals were present, which eliminates the neutralization of sulfuric acid formed during the oxidation of sulfides.

Products of secondary mineral formation were represented by jarosite ($KFe_3(SO_4)_2(OH)_6$, 1–11%), rozenite ($FeSO_4{\cdot}4H_2O$, 1–10%), and melanterite ($FeSO_4{\cdot}7H_2O$, 2–37%). The presence of secondary soluble mineral forms, such as rozenite and melanterite, is primarily associated with crystallization from solutions.

Jarosite is known to be a precipitation product of ferric iron:

$$3Fe_2(SO_4)_3 + 12H_2O + K_2SO_4 = 2K[Fe_3(SO_4)_2(OH)_6]\downarrow + 6H_2SO_4. \quad (4)$$

Thus, the presence of jarosite in the samples, as well as iron sulfate minerals, which are products of the transformation of iron-containing sulfides under aerobic conditions, indicated the presence of oxidative processes in the soils of the tailing dump. It is also noteworthy that melanterite is a relatively rare mineral. During hot weather, the crystal growth of melanterite occurs on pyrite aggregates [24], which are oxidized by aerobic microorganisms upon contact with oxygen.

Analysis of the mineral composition of the samples collected from the main part of the tailing dump allowed the following conclusion. The largest amount of highly and moderately soluble sulfates characterizing the level of soil salinity was found in the samples from the southwestern (no. 2) and northeastern (no. 3) zones of the tailing dump, with the melanterite content reaching 25 and 37%, respectively. In general, the content of iron sulfate minerals varied at different soil depths without any explicit pattern.

Changes in the jarosite content in the soils of the southern (no. 2) and northeastern (no. 3) zones were characterized by a pronounced decrease in the jarosite content with an increase in depth. These data were in agreement with greater intensity of sulfide oxidation processes on the surface and a decreased amount of oxygen penetrating deep soil layers.

It is also worth mentioning that the pyrite content varied in the soil samples. This could be due to the heterogeneity of the ore flotation waste deposited in the tailing dump, which was confirmed by the significant variation in the content of insoluble and non-oxidizable quartz and aluminosilicates in the sample material. For instance, the quartz content in sample 4.1 was 55% and only 7% in sample 2.1.

The main results of determining the physical and mechanical characteristics of the tailing soils are summarized in Table 4.

Table 4. Physical and mechanical characteristics of tailing samples.

Tailing Dump Zone	Sample No.	Moisture (%)	Liquid-Limit Water Content (%)	Plastic-Limit Water Content (%)	Plasticity Index	Liquidity Index	Angle of Internal Friction (Degrees)	Specific Shear Cohesion (MPa)
Southern (no. 1)	1.1	14.6	24.5	24.0	0.5	−18.8	33.5	0.0217
	1.2	15.2	23.7	23.1	0.6	−13.2	29.9	0.0325
	1.3	8.50	22.2	21.5	0.7	−19.1	35.0	0.0050
Southwestern (no. 2)	2.1	25.0	27.4	22.7	4.7	0.53	33.0	0.0100
	2.2	16.4	22.0	21.0	1.0	−3.80	32.0	0.0430
	2.3	16.9	16.4	15.7	0.7	0	32.5	0.0330
Northeastern (no. 3)	3.1	26.9	27.3	21.5	5.8	0.910	19.2	0.0117
	3.2	28.1	27.4	22.8	4.6	0.650	- *	-
	3.3	19.9	26.7	19.3	7.4	−6.77	-	-
Northwestern (no. 4)	4.1	7.60	15.8	14.5	1.2	−5.72	41.0	0.0244

* could not be determined when studying the sample in its natural state.

Analysis of the data summarized in Tables 3 and 4 indicated the effect of the secondary mineral formation on the physical and mechanical properties of soils during storage.

For instance, in sample no. 4.1, characterized by complete pyrite leaching, the absence of intermediate forms (melanterite and rozenite), as well as the highest content of quartz and gypsum, were shown. These characteristics determined the highest value of the angle of internal friction (41.0 degrees) and a high value of the specific shear cohesion of soil, indicating the possibility of the development of this tailing dump zone: mining for metal extraction or land reclamation.

In the southern zone, sample no. 1.1 (an active aeration zone at a depth of 0.5 m) was represented by a jarosite crust, as in the case of zone no. 4 characterized by an increased content of the final products of the secondary mineral formation: jarosite and gypsum. The pyrite content in sample no. 1.1 was low—10%. The ongoing processes determined the formation of soil with high angles of internal friction and specific shear cohesion. Due to the high values of these indices, it would be possible to carry out the movement of the equipment along the surface of the tailing dump, excavation of soil, and its transportation for subsequent processing or disposal. At a depth of 1.0 m (below the well-aerated surface layer), active processes of secondary mineral formation occurred, resulting in complete melanterite dehydration and rozenite formation. At this depth, the pyrite content was higher than that in the aeration zone, and the secondarily formed rozenite reached 10%. Under such conditions, the value of the specific shear cohesion of soil was relatively high (0.0325 MPa), while the internal angle of friction was lower than that in the zone of the active aeration—29.9 degrees. At a depth of 1.5 m, pyrite was partially oxidized, with the formation of jarosite and melanterite.

Samples collected from zone no. 2 were characterized by the highest content of primary pyrite and secondary melanterite. Moreover, in the near-surface zone, melanterite dehydration and the formation of rozenite occurred. Jarosite, the final product of weathering, was not found in this zone; the gypsum content was also relatively low: 4% of the total mineral mass. The values of the angle of internal friction and specific shear cohesion were quite high, ensuring the stability of the slopes in this area.

Soil samples from zone no. 3 located in the pond area of the tailing dump with an artificial riverbed were characterized by pronounced secondary mineral formation and the presence of intermediate mineral forms. High values of moisture and liquidity index of the tailings in this zone would make it impossible to carry out extraction and loading operations by mechanical means without preliminary drainage.

Active leaching of pyrite from the aerated near-surface layers led to a change in the particle size distribution of the tailings. The granulometric composition of the soil from different zones of the tailing dump is shown in Table 5.

Table 5. Particle size distribution of soil from the Sibay tailing dump.

Zone of Tailing Dump	Sample No.	2–1 mm	1–0.5 mm	0.5–0.25 mm	0.25–0.10 mm	0.10–0.05 mm	0.05–0.01 mm	0.01–0.002 mm	<0.002 mm	Soil Type
Southern (no. 1)	1.1	0.19	8.45	36.4	30.4	7.50	7.50	5.20	4.36	Fine sand with clay
	1.2	0.32	9.50	40.7	29.5	8.54	3.56	5.11	2.76	
	1.3	0.15	9.94	37.6	34.5	7.23	5.40	4.67	0.54	
Southwestern (no. 2)	2.1	0	4.34	9.30	6.65	8.54	42.2	21.1	7.87	Silty sandy clay
	2.2	0	2.34	7.34	4.24	15.3	45.3	21.3	4.18	Silty sandy clay
	2.3	0.29	9.50	38.6	34.5	9.56	3.40	2.67	1.48	Fine sand with clay
Northeastern (no. 3)	3.1	0	3.56	6.70	8.70	9.40	44.2	20.4	7.04	Silty sandy clay
	3.2	0.36	5.43	5.84	9.94	7.56	39.5	27.4	3.93	
	3.3	0	0.94	2.24	3.85	8.01	47.7	25.8	11.5	Elastic silt
Northwestern (no. 4)	4.1	0.10	2.59	6.98	7.56	8.60	39.4	23.4	9.35	Silty sandy clay

Analysis of characteristics of the oxidized layers of the tailing material from different zones of the tailing dump showed the following.

The surface layers of the southern zone (no. 1) of the tailing dump were characterized by cemented soil containing secondary jarosite. The zone was composed of fine sands, the characteristics of which were similar to the sands from the southwestern zone.

At a depth of 1.0 m from the surface, the southwestern zone (no. 2) was composed of silty sandy clay, which swelled when moistened. Below 1.0 m, the zone contained loose fine sand with clay characterized by high water filtering. Moreover, the coarseness of sands of more than 0.10 mm (amount, >75%) indicates that they do not acquire the properties of quicksand when moistened.

Based on the nature of tailings deposition in the main accommodating volume of the tailing dump, the northeastern zone (no. 3) was a tailing pond. It was composed of water-saturated finely dispersed tailings, mainly fractions of the $-0.05 + 0.002$ mm class, which determine the instability of soils to moistening. As a result, the slopes of such soils can be easily eroded, which would complicate mining operations in this area.

In the northwestern part (no. 4) of the tailing dump, the soil was represented by silty sandy clay, with a particle content of -0.05 mm class of more than 50%, which determines higher cohesion of soils, compared to other zones (Table 4).

As mentioned above, microbial communities are known to participate actively in the processes of secondary mineral formation. Therefore, in addition to the determination of the composition and physical–mechanical characteristics of soils, it was interesting to study the structure of the microbial communities from each tailing dump zone.

3.2. Microbial Community Structure in Different Zones of Sibay Mine Tailing Dump

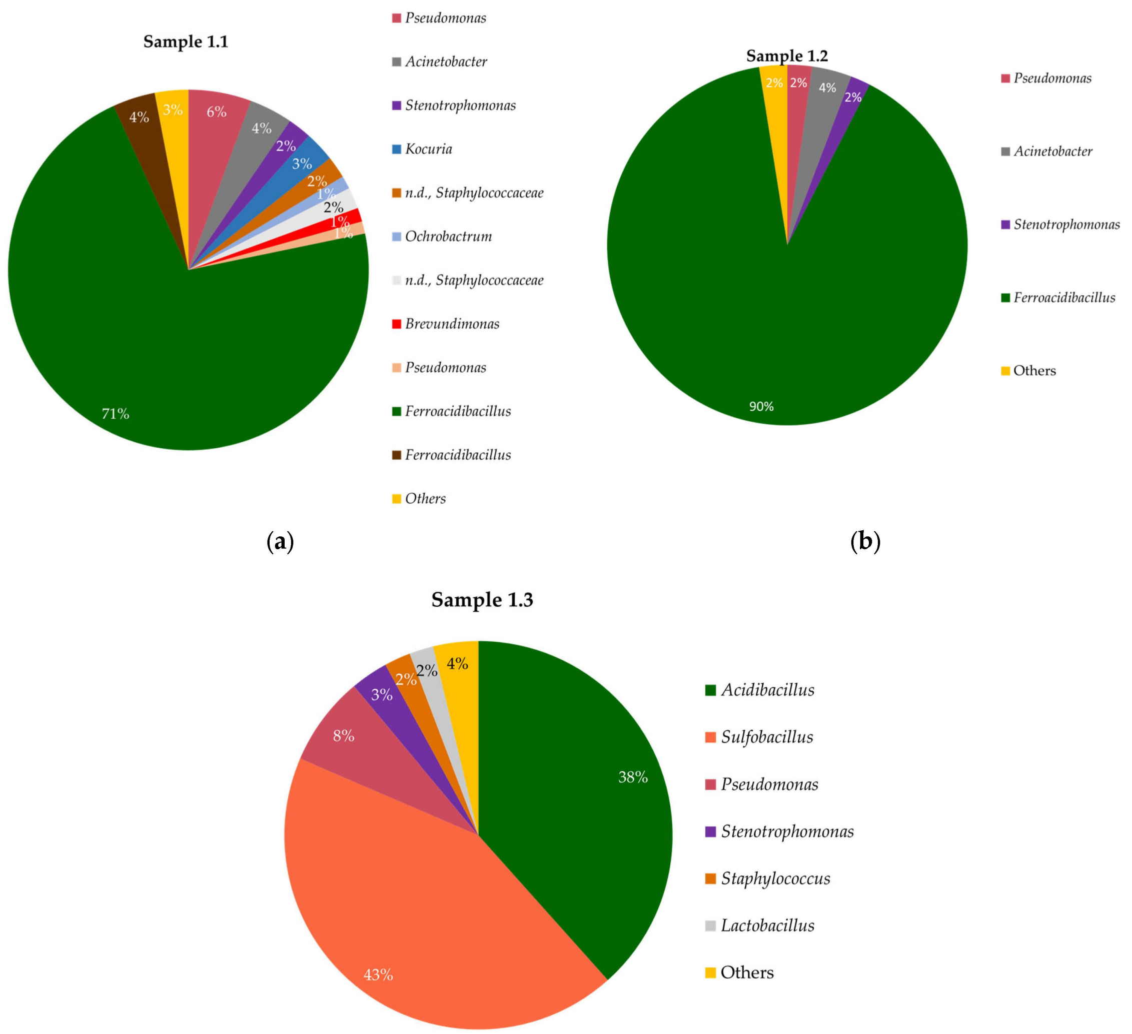
To estimate the leaching/oxidation of the flotation ore tailings and the contribution of microorganisms to these processes, the samples collected from different depths (0.5–1.5 m) in the Sibay tailing dump zones (Figure 2) were analyzed for the presence of microorganisms. The depth of sampling (from the surface up to ~1.5 m) was justified by the ocher color of upper layers, presumably, resulting from the oxidation of sulfide minerals. Analysis of the structure of microbial communities in the studied zones of the tailing dump was carried out by the metabarcoding analysis of hypervariable regions of the 16S rRNA genes. As previously shown [20,25], some species of acidophiles can be successfully predicted by metabarcoding due to the high variability of the V3–V4 regions within the corresponding genera, although the differences in the V3–V4 region are usually not sufficient to differentiate closely related microbial species. In this research, acidophilic bacteria and archaea were also successfully assigned to the known species.

In all samples, cell numbers were determined within 10^7 cells/g sample, except for the samples from zone no. 2 (samples 2.1, 2.2, and 2.3) and sample 3.3 collected from zone no. 3: 10^6 cells/g sample.

In the southern zone (no. 1) of the mine tailing dump (Figure 4a–c), *Ferroacidibacillus* spp. were shown to dominate the communities from all three samples (depth, 0.5–1.5 m). Nucleotide sequences of the 16S rRNA gene fragments showed 100% similarity to the species *Ferroacidibacillus (Fr.) organovorans* (former "*Acidibacillus ferrooxidans*") within the family *Alicyclobacillaceae*, phylum *Firmicutes* [26,27]. Bacteria of the genus *Ferroacidibacillus* have been classified as chemolitho-heterotrophs that require both inorganic (iron) and organic compounds for their efficient growth. The optimum temperature and pH for *Fr. organovorans* are 30 °C and 2.9 (1.9 in the case of the type strain), respectively. This acidophilic species was isolated from mine waste regolithic material (pH 2.9) subjected to accelerated weathering in humidity cells at 25 °C. Other isolates have been obtained from diverse environments impacted by metal mining, such as solid wastes, as well as from geothermal areas. The mesophilic species *Fr. organovorans* has been reported to carry out oxidation of ferrous iron and, therefore, indirect oxidative dissolution of sulfidic minerals, such as pyrite [26–28].

The layers of this zone (no. 1) were defined as jarosite crust: an oxidized zone characterized by ocher color, in which jarosite content varied within 1–10%. The proportion of nucleotide sequences assigned to *Ferroacidibacillus* sp. ranged from 71 (or 74%, taking into account another *Ferroacidibacillus* sp.—99.5% similarity) to 90%, decreasing 2.4 times at a depth of 1.5 m (sample 1.3; Figure 4c). In this layer, other acidophiles also prevailed, probably, contributing to the oxidation of sulfides: *Sulfobacillus* spp. (three different 16S rRNA gene sequences), with a total share of 43% in the microbial community. The highest proportion of the nucleotide sequences of the 16S rRNA gene fragment showed 99.5% similarity to one of the validated *Sulfobacillus* species: *Sb. thermotolerans* Kr1^T [29,30]. The closest (99.8%) similarity was indicated to the uncultured *Sulfobacillus* sp. from a low-grade copper sulfide run-of-mine test heap [31]. Two other prevailing *Sulfobacillus* spp. identified in the acidophilic community of zone no. 1 at 1.5 m showed a 100% identity to *Sulfobacillus* sp. Y0017 [32] and uncultured *Sulfobacillus* sp. (99.8% similarity) from the acidic region (pH 2.7) of an abandoned semiarid lead-zinc mine tailing site [33]. The strain Y0017 is a highly efficient pyrite-oxidizing isolate at 45 °C, with an upper temperature limit of 50 °C [32].

Bacteria of the genus *Sulfobacillus* are commonly identified in bioleaching processes and mine wastes, as well as sulfide ore deposits containing precious and nonferrous metals, acid mine drainage, and thermal springs [3,34]. Compared to *Fr. organovorans* identified in the same microbial community in this research, mixotrophic *Sulfobacillus* bacteria possess wider capabilities of oxidizing inorganic substrates: ferrous iron, reduced inorganic sulfur compounds (RISCs), elemental sulfur, and sulfide minerals in the presence of small amounts of organic substances [35], using both inorganic and organic substrates as energy sources [36]. Optimum growth of *Sulfobacillus* bacteria occurs at ≥39 °C, while a lower temperature limit is <20 °C [34].



(**c**)

Figure 4. Proportions of the 16S rRNA gene sequences (%) in microbial communities from the southern zone (no. 1) of the Sibay tailing dump. Microbial communities were analyzed in samples (**a**) 1.1, (**b**) 1.2, and (**c**) 1.3 collected at a depth of 0.5, 1.0, and 1.5 m, respectively. Community structure was determined by metabarcoding analysis of the V3–V4 regions of the 16S rRNA gene. Others, each microorganism represented by <1% of total sequences in the community; n.d., the genus was not determined.

The share of other bacteria (based on the proportion of the 16S rRNA gene sequences) identified in the structure of three communities from the southern zone did not exceed 1–8%, with that of *Pseudomonas* spp. varying from 2 to 8% (Figure 4a–c). Although extremely low pH values are outside the growth optimum range for these minor bacterial components of the communities, they have been identified in several acidic man-made environments, including abandoned mine areas [2,15,37,38]. The data obtained in this research and previous studies by different authors, as well as low amounts of these bacteria (compared to the predominant members) in microbial communities, may indicate their ability to survive under such conditions without active proliferation and functioning.

In the southwestern zone (no. 2) of the mine tailing dump, the community was represented by two predominant genera: *Pseudomonas* (share, 48%–56% of all sequences) and *Stenotrophomonas* (21%–23%) (Figure 5a–c). Other bacteria were minor components of the communities. Moreover, no acidophiles were identified in the community structure. This zone of the tailing dump was characterized by the high content of pyrite (36%–56%) and the absence of jarosite (Table 3), indicating negligible bio-oxidation of ferrous iron and, therefore, sulfides by acidophilic chemolithotrophs.

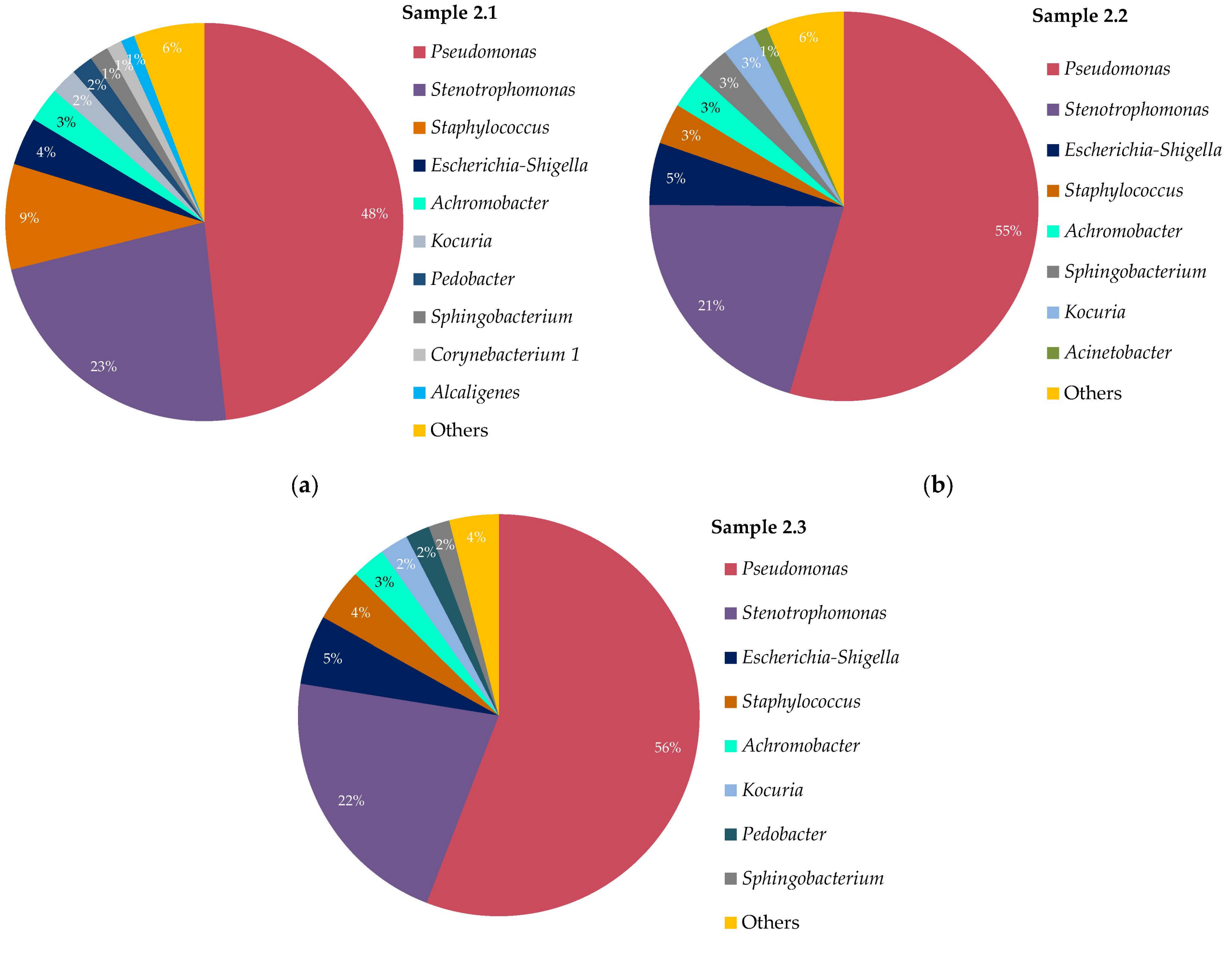


Figure 5. Proportions of the 16S rRNA gene sequences (%) in microbial communities from the southwestern zone (no. 2) of the Sibay tailing dump. Microbial communities were analyzed in samples (**a**) 2.1, (**b**) 2.2, and (**c**) 2.3 collected at a depth of 0.5, 1.0, and 1.5 m, respectively. Community structure was determined by metabarcoding analysis of the V3–V4 regions of the 16S rRNA gene. Others, each microorganism represented by <1% of total sequences in the community.

The northeastern zone (no. 3) was characterized by a qualitative structure different from that of the communities from other zones, with *Ferroplasma* (*Fm.*) spp. (100% similarity to *Fm. acidiphilum*) prevailing at depths $\leq$1.0 m (Figure 6a,b). These acidophilic archaea oxidize ferrous iron at low pH values in the presence of small amounts of organic material. They are commonly reported as members of acidophilic communities in sulfide ore deposits, processes of bioleaching of sulfide raw materials, and AMD [34,39,40]. The proportion of *Pseudomonas* spp. was within 2–8% at depths $\leq$1.0 m and increased 2–9 times at 1.5 m

(Figure 6c). In this layer, a pronounced succession of community members was noted. At 0.5–1.0 m, *Ferroacidibacillus* spp. prevailed, while at 1.5 m, no iron or sulfur oxidizers were identified.

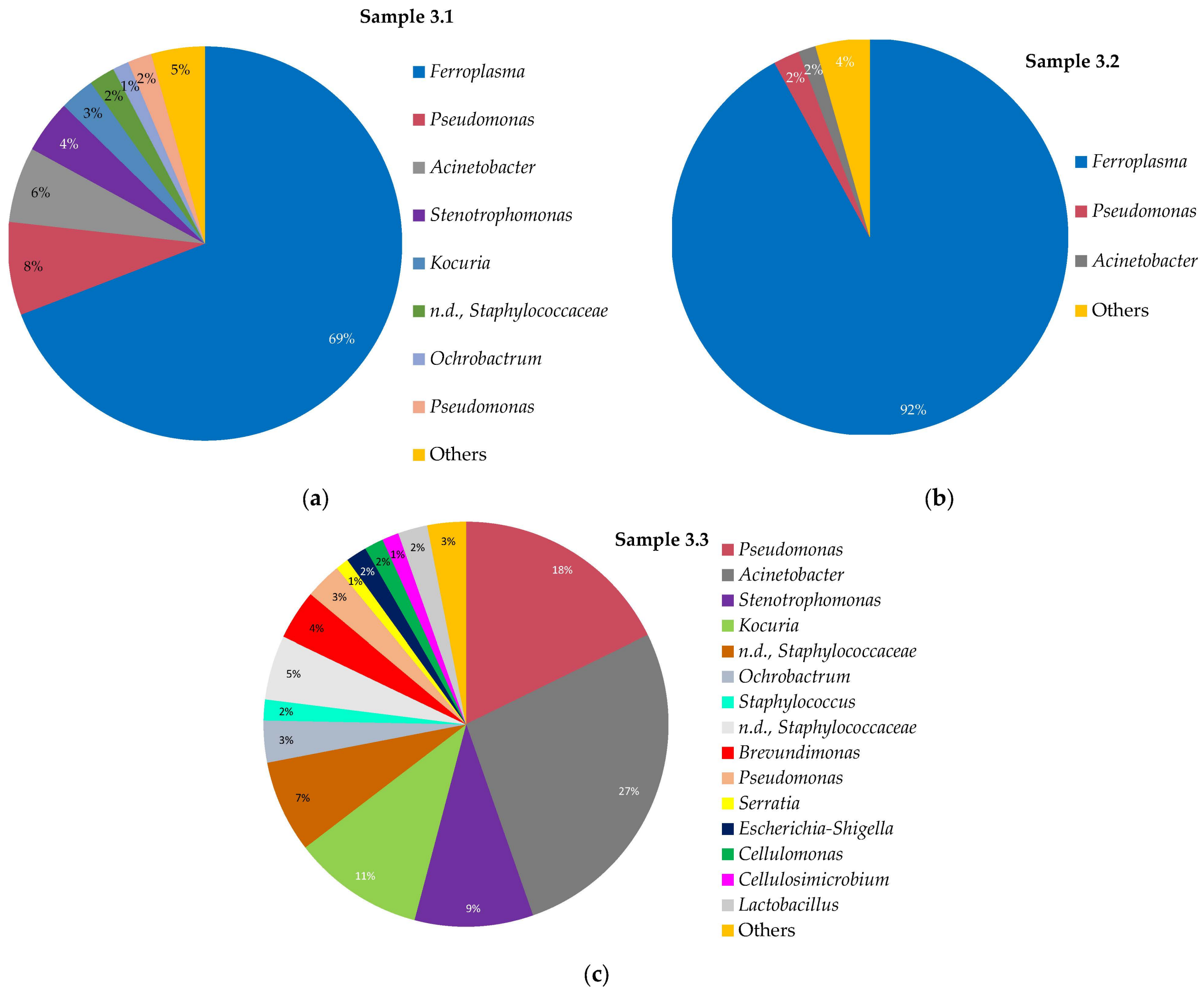


Figure 6. Proportions of the 16S rRNA gene sequences (%) in microbial communities from the northeastern zone (no. 3) of the Sibay tailing dump. Microbial communities were analyzed in samples (**a**) 3.1, (**b**) 3.2, and (**c**) 3.3 collected at a depth of 0.5, 1.0, and 1.5 m, respectively. Community structure was determined by metabarcoding analysis of the V3–V4 regions of the 16S rRNA gene. Others, each microorganism represented by <1% of total sequences in the community; n.d., the genus was not determined.

Finally, in the northwestern zone (no. 4), which was characterized by 3% of jarosite in the composition of the sample, two main genera probably contributed to the oxidation processes of the layer at a depth of 1.5 m: *Sulfoacidibacillus* (*Sf.*) and *Sulfobacillus* (Figure 7). The genus *Sulfobacillus* was represented by the same species as in the southern zone (no. 1) (nucleotide sequences of the variable regions of the 16S rRNA gene showed 100 and 99.8% similarity to that of the strain Y0017) (sample 1.3; Figure 4c). *Sulfoacidibacillus* spp. (100 and 99.5% similarity to *Sf. thermotolerans*) is a former genus "*Acidibacillus*" belonging to the family *Alicyclobacillaceae* (phylum *Firmicutes*). These bacteria are similar to *Ferroacidibacillus* spp. determined in the samples from other zones of the tailing dump and oxidize not only ferrous iron but also RISCs, together with the utilization of organic compounds (organic substances are preferred to inorganic substrates). Moderately thermophilic and extremely acidophilic bacteria of this genus are characterized by a temperature growth optimum of 43 °C and pH optimum of 1.8 [26,27]. *Ferroplasma* spp. were also identified in this community (100% similarity to *Fm. acidiphilum*).

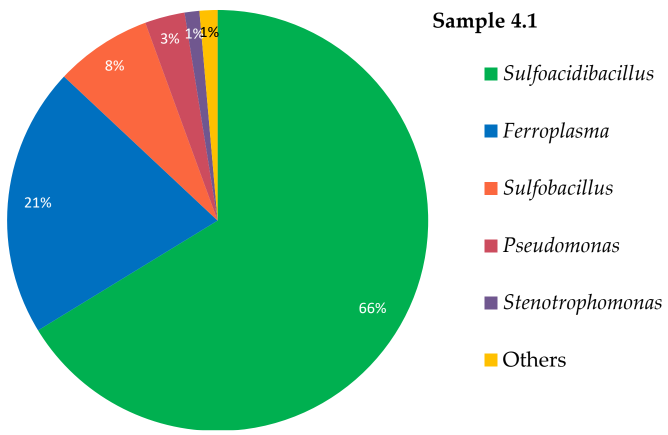


Figure 7. Proportions of the 16S rRNA gene sequences (%) in microbial communities from the northwestern zone (no. 4) of the Sibay tailing dump. Microbial communities were analyzed in sample 4.1 collected at a depth of 1.5 m. Community structure was determined by metabarcoding analysis of the V3–V4 regions of the 16S rRNA gene. Others, each microorganism represented by <1% of total sequences in the community.

Thus, the qualitative structure of the microbial communities differed depending on the zone of the tailing dump and the depth of the sample collection (Figures 4–7). At least one indicator of the activity of extremely acidophilic iron- and/or sulfur-oxidizing microorganisms in the surface layers of the tailing dump (compared to deeper layers) was found: a higher content of jarosite formed due to the precipitation of microbially oxidized iron—Fe^{3+} (Table 3). With depth, the jarosite content tended to change, and a moderate (Figure 4) or, less commonly, pronounced succession of microorganisms in the communities was noted (Figure 6). Overall, sulfur- and iron-oxidizing acidophiles were represented by a few genera: *Ferroacidibacillus* and *Sulfoacidibacillus* (former "*Acidibacillus*"), *Sulfobacillus* (phylogenetically close to *Sb. thermotolerans*), and *Ferroplasma*. Their presence and proportion in the community depended both on the zone of the tailing dump and the depth of sampling. Among other genera, *Pseudomonas* prevailed in the layers characterized by weak oxidation of sulfide minerals.

4. Conclusions

An integral approach used in this research combined chemical–mineralogical, physical–mechanical, microbiological, and molecular–biological methods. The data obtained allowed a thorough characterization of the oxidized surface soil layers from four zones of the Sibay tailing dump. The main conclusions of this research are as follows:

- The surface layers of the tailing dump were formed by two main soil types: brown and gray soils characterized by low pH values. Brown soils were associated with oxidized upper layers dominated by secondary sulfates of the jarosite group formed via (bio)oxidation of pyrite. Gray soils included secondary sulfates of the melanterite group characteristic of the intermediate step of sulfide oxidation;
- The study of the microbial communities isolated from different zones of the tailing dump indicated that their structure depended on the zone of the tailing dump and the depth of sampling. Moderate or, less commonly, pronounced succession of microorganisms in the communities was shown;
- Sulfur- and iron-oxidizing acidophiles contributed to the oxidation of sulfide minerals and the formation of jarosite in the upper soil layers of the tailing dump. They were represented by the following genera: *Ferroacidibacillus*, *Sulfoacidibacillus*, *Sulfobacillus*,

and *Ferroplasma*. In the layers characterized by the weak oxidation of sulfide minerals, *Pseudomonas* spp. prevailed;

- Processes of (bio)oxidation and secondary mineral formation in the tailing dump may change the physical properties of soils. Based on the particle size distribution, as well as the physical and mechanical characteristics of the tailing samples, soils from the southwestern and northwestern zones were characterized by high stability of slopes and, therefore, the possibility of further development of the tailing dump: mining for metal extraction or land reclamation.

Data on the granulometric, physical, chemical, and mechanical features of the tailing dump soils, as well as acidophilic sulfur and iron oxidizers, can be used to predict negative environmental impacts in the mining region, including further AMD formation. The results obtained in this study are of importance to the technologies for the subsequent treatment of ore flotation wastes deposited in the Sibay tailing dump, as well as land reclamation activities in this area.

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References

1. Patrick, V.B.; Fleuren-Kemilä, A.K. Influence of pH on the toxic effects of zinc, cadmium, and pentachlorophenol on pure cultures of soil microorganisms. *Environ. Toxicol. Chem.* **1997**, *16*, 146–153.
2. Cho, M.-J.; Park, S.-Y.; Kim, C.-G. Acidic neutralization by indigenous bacteria isolated from abandoned mine areas. *Appl. Sci.* **2022**, *12*, 3324. [CrossRef]
3. Bond, P.L.; Druschel, G.K.; Banfield, J.F. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Appl. Environ. Microbiol.* **2000**, *66*, 4962–4971. [CrossRef] [PubMed]
4. Plokhov, A.S.; Pashkevich, M.A.; Isakov, A.E.; Chukaeva, M.A. Study of the ways to utilize ore dressing tailings for obtaining a useful component. In *Topical Issues of Rational Use of Natural Resources, Proceeding of the International Forum-Contest of Young Researchers, Saint Petersburg, Russia, 18–20 April 2018*; Routledge: London, UK, 2019; pp. 379–386.
5. Korneeva, T.; Yurkevich, N.; Kurcher, D.; Saeva, O. A Geochemical and geophysical characterization of acid mine drainage and sulfide tailings at Karabash mine site (South Ural, Russia). *Int. J. Adv. Sci. Eng. Technol.* **2016**, *4*, 114–120.
6. Opekunova, M.G.; Opekunov, A.J.; Papyan, E.E.; Somov, V.V. Phytoindicational properties of the vegetation in landscape transformation studies on Sibay chalcopyrite deposits (Southern Urals). *Contemp. Probl. Ecol.* **2017**, *10*, 301–314. [CrossRef]
7. Kharko, P.A.; Plokhov, A.S. Evaluation of the impact of copper-sulphide deposits on the mineral composition of the bottom sediments of small rivers. *Eurasian Sci. J.* **2019**, *6*, 1–9. (In Russian)
8. Figueiredo, J.; Vila, M.C.; Góis, J.; Biju, B.P.; Futuro, A.; Martins, D.; Dinis, M.L.; Fiúza, A. Bi-level depth assessment of an abandoned tailings dam aiming its reprocessing for recovery of valuable metals. *Miner. Eng.* **2019**, *133*, 1–9. [CrossRef]
9. Muravyov, M. Bioprocessing of mine waste: Effects of process conditions. *Chem. Pap.* **2019**, *73*, 3075–3083. [CrossRef]
10. Elghali, A.; Benzaazoua, M.; Taha, Y.; Amar, H.; Ait-khouia, Y.; Bouzahzah, H.; Hakkou, R. Prediction of acid mine drainage: Where we are. *Earth-Sci. Rev.* **2023**, *241*, 104421. [CrossRef]
11. Makarov, A.B. Main types of technogenous-mineral deposits of the Urals: Formation conditions, peculiarities of composition and directions of usage. *News Ural State Min. Univ.* **2007**, *22*, 61–68. (In Russian)

12. Quispe, D.; Pérez-López, R.; Acero, P.; Ayora, C.; Nieto, J.M. The role of mineralogy on element mobility in two sulfide mine tailings from the Iberian Pyrite Belt (SW Spain). *Chem. Geol.* **2013**, *345*, 119–129. [CrossRef]
13. Zhang, Q.; Wei, P.; Banda, J.F.; Ma, L.; Mao, W.; Li, H.; Hao, C.; Dong, H. Succession of microbial communities in waste soils of an iron mine in Eastern China. *Microorganisms* **2021**, *9*, 2463. [CrossRef] [PubMed]
14. Chung, A.P.; Coimbra, C.; Farias, P.; Francisco, R.; Branco, R.; Simão, F.S.; Gomes, E.; Pereira, A.; Vila, M.C.; Fiúza, A.; et al. Tailings microbial community profile and prediction of its functionality in basins of tungsten mine. *Sci. Rep.* **2019**, *9*, 19596. [CrossRef] [PubMed]
15. Sazykin, I.; Khmelevtsova, L.; Azhogina, T.; Sazykina, M. Heavy metals influence on the bacterial community of soils: A review. *Agriculture* **2023**, *13*, 653. [CrossRef]
16. Fashola, M.O.; Ngole-Jeme, V.M.; Babalola, O.O. Physicochemical properties, heavy metals, and metal-tolerant bacteria profiles of abandoned gold mine tailings in Krugersdorp, South Africa. *Can. J. Soil Sci.* **2020**, *100*, 217–233. [CrossRef]
17. El Baz, S.; Baz, M.; Barakate, M.; Hassani, L.; El Gharmali, A.; Imziln, B. Resistance to and accumulation of heavy metals by actinobacteria isolated from abandoned mining areas. *Sci. World J.* **2015**, *2015*, 761834. [CrossRef]
18. Ndeddy Aka, R.J.; Babalola, O.O. Identification and characterization of Cr-, Cd-, and Ni-tolerant bacteria isolated from mine tailings. *Bioremediation* **2017**, *21*, 1–19. [CrossRef]
19. Silverman, M.P.; Lundgren, D.G. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. *J. Bacteriol.* **1959**, *77*, 642–647. [CrossRef]
20. Panyushkina, A.; Fomchenko, N.; Babenko, V.; Muravyov, M. Effect of temperature on biobeneficiation of bulk copper–nickel concentrate with thermoacidophilic microbial communities. *Metals* **2021**, *11*, 1969. [CrossRef]
21. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pẽa, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]
22. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef] [PubMed]
23. Yakhontova, L.K.; Zvereva, V.P. *Fundamentals of the Mineralogy of Hypergenesis*; Dalnauka: Vladivostok, Russia, 2000. (In Russian)
24. Bratanov, N.S. Formation of technogenic minerals on the territory of degtyarsky copper-pyrite deposit. In *Ecological and Technosphere Safety of Mining Regions, Proceeding of VII International Scientific and Practical Conference, Ekaterinburg, Russia, 9 April 2019*; Semyachkov, A.I., Ed.; Ural State Mining University: Ekaterinburg, Russia, 2019; pp. 46–49. (In Russian)
25. Muravyov, M.; Panyushkina, A. Distinct roles of acidophiles in complete oxidation of high-sulfur ferric leach product of zinc sulfide concentrate. *Microorganisms* **2020**, *8*, 386. [CrossRef] [PubMed]
26. Holanda, R.; Hedrich, S.; Ňancucheo, I.; Oliveira, G.; Grail, B.M.; Johnson, D.B. Isolation and characterisation of mineral-oxidising "*Acidibacillus*" spp. from mine sites and geothermal environments in different global locations. *Res. Microbiol.* **2016**, *167*, 613–623. [CrossRef] [PubMed]
27. Johnson, D.B.; Holmes, D.S.; Vergara, E.; Holanda, R.; Pakostova, E. *Sulfoacidibacillus ferrooxidans*, gen. nov., sp. nov., *Sulfoacidibacillus thermotolerans*, gen. nov., sp. nov., and *Ferroacidibacillus organovorans*, gen. nov., sp. nov.: Extremely acidophilic chemolitho-heterotrophic Firmicutes. *Res. Microbiol.* **2023**, *174*, 104008. [CrossRef] [PubMed]
28. Ňancucheo, I.; Oliveira, R.; Dall'Agnol, H.; Johnson, D.B.; Grail, B.; Holanda, R.; Nunes, G.L.; Cuadros-Orellana, S.; Oliveira, G. Draft genome sequence of a novel acidophilic iron-oxidizing *Firmicutes* species, "*Acidibacillus ferrooxidans*" (SLC66^{T}). *Genome Announc.* **2016**, *4*, 53–54. [CrossRef] [PubMed]
29. Bogdanova, T.I.; Tsaplina, I.A.; Kondrat'eva, T.F.; Duda, V.I.; Suzina, N.E.; Melamud, V.S.; Tourova, T.P.; Karavaiko, G.I. *Sulfobacillus thermotolerans* sp. nov., a thermotolerant, chemolithotrophic bacterium. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 1039–1042. [CrossRef]
30. Panyushkina, A.E.; Babenko, V.V.; Nikitina, A.S.; Selezneva, O.V.; Tsaplina, I.A.; Letarova, M.A.; Kostryukova, E.S.; Letarov, A.V. *Sulfobacillus thermotolerans*: New insights into resistance and metabolic capacities of acidophilic chemolithotrophs. *Sci. Rep.* **2019**, *9*, 15069. [CrossRef]
31. Demergasso, C.S.; Galleguillos, P.A.P.; Escudero, L.V.G.; Zepeda, V.J.A.; Castillo, D.; Casamayor, E.O. Molecular characterization of microbial populations in a low-grade copper ore bioleaching test heap. *Hydrometallurgy* **2005**, *80*, 241–253. [CrossRef]
32. Johnson, D.B.; Okibe, N.; Roberto, F.F. Novel thermo-acidophilic bacteria isolated from geothermal sites in Yellowstone National Park: Physiological and phylogenetic characteristics. *Arch. Microbiol.* **2003**, *180*, 60–68. [CrossRef]
33. Mendez, M.O.; Neilson, J.W.; Maier, R.M. Characterization of a bacterial community in an abandoned semiarid lead-zinc mine tailing site. *Appl. Environ. Microbiol.* **2008**, *74*, 3899–3907. [CrossRef]
34. Kondrat'eva, T.F.; Pivovarova, T.A.; Tsaplina, I.A.; Fomchenko, N.V.; Zhuravleva, A.E.; Murav'ev, M.I.; Melamud, V.S.; Bulayev, A.G. Diversity of the communities of acidophilic chemolithotrophic microorganisms in natural and technogenic ecosystems. *Microbiology* **2012**, *81*, 1–24. [CrossRef]
35. Karavaiko, G.I.; Krasil'nikova, E.N.; Tsaplina, I.A.; Bogdanova, T.I.; Zakharchuk, L.M. Growth and carbohydrate metabolism of sulfobacilli. *Microbiology* **2001**, *70*, 245–250. [CrossRef]
36. Zhuravleva, A.E.; Ismailov, A.D.; Tsaplina, I.A. Electron donors at oxidative phosphorylation in bacteria of the genus *Sulfobacillus*. *Microbiology* **2009**, *78*, 811–814. [CrossRef]

37. Teng, W.; Kuang, J.; Luo, Z.; Shu, W. Microbial diversity and community assembly across environmental gradients in acid mine drainage. *Minerals* **2017**, *7*, 106. [CrossRef]
38. Selvi, A.; AlSalhi, M.S.; Devanesa, S.; Maruthamuthu, M.K.; Mani, P.; Rajasekar, A. Characterization of biospheric bacterial community on reduction and removal of chromium from tannery contaminated soil using an integrated approach of bio-enhanced electrokinetic remediation. *J. Environ. Chem. Eng.* **2021**, *9*, 106602. [CrossRef]
39. Panyushkina, A.; Muravyov, M.; Fomchenko, N. A Case of Predominance of *Alicyclobacillus tolerans* in microbial community during bioleaching of pentlandite-chalcopyrite concentrate. *Minerals* **2022**, *12*, 396. [CrossRef]
40. Norris, P.R. Acidophile diversity in mineral sulfide oxidation. In *Biomining*; Rawlings, D.E., Johnson, D.B., Eds.; Springer: Berlin, Germany, 2007; pp. 199–216.