Distribution of *Acetothermia*-dominated microbial communities in alkaline hot springs of Baikal Rift Zone.

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Alkaline hot springs are unique extreme habitats resemble the early Earth and present a valuable resource for the discovery of procaryotic community diversity and isolation of the novel thermophilic *Bacteria* and *Archaea*. One of the model for the possible origin of biochemistry in alkaline hot springs revealed the acetyl-CoA pathway of CO_2 fixation might be the most ancient form of carbon metabolism. Recent phylogenetic studies have suggested that the phylum *Acetothermia* is one of the deep branches of the *Bacteria* domain. Firstly *Acetothermia* (Candidate division OP1) was characterized in a culture independent molecular phylogenetic survey based on the 16S rRNA gene of the sulfide-rich hot spring, Obsidian Pool, a 75 to 95°C hot spring. Two nearly complete genomes of *Acetothermia* were established based on genome-resolved metagenomic analysis and its capability of implementing acetogenesis through the ancient reductive acetyl-CoA pathway by utilizing CO_2 and H_2 was revealed. Although genomic, proteomic and metagenomic approaches investigate basic metabolism and potentional energy conservation of uncultivated candidate phyla but ecological roles of these bacteria and general patterns of diversity and community structure stay unclear. General hydrochemical and geological characterization of alkaline thermal springs of the Baikal Rift zone with high silica concentrations and a nitrogen dominated gas phase is provided. Previous microbiogical studies based on culture-dependent methods recovered a large number of bacterial strains from thermal springs located in Baikal Rift zone. We combined microbial communities analysis by using high-throughput 16S rRNA gene sequencing, biogeochemical measurements, sediment mineralogy and physicochemical characteristics to investigate ecosystems of alkaline hot springs located in the Baikal Rift zone. Uncultivated bacteria belonging to the phylum *Acetothermia*, along with members of the phyla *Firmicutes* and *Proteobacteria*, were identified as the dominant group in hydrothermal sediments communities in the alkaline hot springs of Baikal Rift zone. In

bottom sediments of the Alla hot spring, about 57% of all classified sequences represent this phylum. Geochemistry of fluids and sample type were strongly correlated with microbial community composition. The *Acetothermia* exhibited the highest relative abundance in sediment microbial community associated with alkaline thermal fluids enriched in Fe, Zn, Ni, Al and Cr.

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2 **Baikal Rift Zone**

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Abstract

Alkaline hot springs are unique extreme habitats resemble the early Earth and present a valuable resource for the discovery of procaryotic community diversity and isolation of the novel thermophilic *Bacteria* and *Archaea.* One of the model for the possible origin of biochemistry in alkaline hot springs revealed the acetyl-CoA pathway of CO_2 fixation might be the most ancient form of carbon metabolism. Recent phylogenetic studies have suggested that the phylum *Acetothermia* is one of the deep branches of the *Bacteria* domain. Firstly *Acetothermia* (Candidate division OP1) was characterized in a culture independent molecular phylogenetic survey based on the 16S rRNA gene of the sulfide-rich hot spring, Obsidian Pool, a 75 to 95°C hot spring. Two nearly complete genomes of *Acetothermia* were established based on genome-resolved metagenomic analysis and its capability of implementing acetogenesis through the ancient reductive acetyl-CoA pathway by utilizing CO_2 and H_2 was revealed. Although genomic, proteomic and metagenomic approaches investigate basic metabolism and potential energy conservation of uncultivated candidate phyla but ecological roles of these bacteria and general patterns of diversity and community structure stay unclear.

General hydrochemical and geological characterization of alkaline thermal springs of the Baikal rift zone with high silica concentrations and a nitrogen dominated gas phase is provided. Previous microbiogical studies based on culture-dependent methods recovered a large number of bacterial strains from thermal springs located in Baikal Rift zone.

We combined microbial communities analysis by using high-throughput 16S rRNA gene sequencing, biogeochemical measurements, sediment mineralogy and physicochemical characteristics to investigate ecosystems of alkaline hot springs located in the Baikal Rift zone. Uncultivated bacteria belonging to the phylum *Acetothermia*, along with members of the phyla *Firmicutes* and *Proteobacteria*, were identified as the dominant group in hydrothermal sediments communities in the alkaline hot springs of Baikal Rift zone. In bottom sediments of the Alla hot spring, about 57% of all classified sequences represent this phylum. Geochemistry of fluids and sample type were strongly correlated with microbial community composition. The *Acetothermia* exhibited the highest relative abundance in sediment microbial community associated with alkaline thermal fluids enriched in Fe, Zn, Ni, Al and Cr.

61 *Keywords*: alkaline hot springs, microbial diversity, *Acetothermia*, 16S rRNA

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63 **Introduction**

64 Alkaline hot springs are unique extreme habitats resemble the early Earth and present a valuable

65 resource for the discovery of procaryotic community diversity and isolation of the novel

66 thermophilic *Bacteria* and *Archaea*. One of the model for the possible origin of biochemistry in

67 alkaline hot springs reveal the acetyl-CoA pathway of CO_2 fixation might be the most ancient

68 form of carbon metabolism (Martin & Russel, 2007). Recent phylogenetic studies have

69 suggested that the phylum *Acetothermia* is one of the deep branches of the *Bacteria* domain and

70 can be potentially important organisms in the origin of life (Takami et al., 2012). Firstly

71 *Acetothermia* (Candidate division OP1) was characterized in a culture independent molecular

72 phylogenetic survey based on the 16S rRNA gene of the sulfide-rich hot spring, Obsidian Pool, a

73 75 to 95°C hot spring (Hugenholtz et al., 1998). Single clones of *Acetothermia* have been found

74 in clone-libraries of geochemically diverse environments including the microbial mats at deep-

75 sea hydrothermal fields (Kato et al., 2009), alkaline hot spring (Tobler and Benning, 2011),

76 hydrothermally active sediments of the Guaymas Basin (Teske et al., 2002) and in the

77 northwestern Great Basin (Costa et al. 2009). In addition the phylum *Acetothermia* potentially

78 plays an important metabolic role in the ecosystems of hypersaline Laguna Tebenquiche, Salarde

79 Atacama, Chile (Fernandes et al. 2016). In the site at a salinity of 62 g/L and a pH of 7.4 these

80 bacteria dominated (25% of the total sequences). The thermophilic chemolithoautotrophic

81 microorganisms of this phylum are predicted to be one of the earliest evolutionary branches of

82 the Bacteria domain. Two nearly complete genomes of *Acetothermia* were established based on

83 genome-resolved metagenomic analysis and its capability of implementing acetogenesis through

84 the ancient reductive acetyl-CoA pathway by utilizing CO_2 and H_2 was revealed (Takami et al.,

85 2012; Rinke et al. 2013). Besides that significant differences in metabolic capacities and favor of

86 heterotrophy with consequences on the biogeochemical transformations occurring in the oil

87 reservoir were suggested (Hu et al., 2016). They carry out an almost complete folate-dependent

88 acetyl-coenzyme pathway for carbon fixation and acetogenesis (Takami et al. 2012). Moreover,

89 these bacteria contain both aerobic and anaerobic pairs of genes for the metabolism of pyruvate

90 (Badhai, Ghosh & Das, 2015). Although genomic, proteomic and metagenomic approaches

91 reveal basic metabolism and potential energy conservation of uncultivated candidate phyla

92   (Takami et al., 2012; Farag et al., 2014; Nobu et al., 2016) but ecological roles of these bacteria
93   and general patterns of diversity and community structure stay unclear.
94   General hydrochemical and geological characterization of alkaline thermal springs of the Baikal
95   rift zone with high silica concentrations and a nitrogen dominated gas phase is provided
96   (Lasareva et al. 2010; 2011; Shvartsev et al. 2015). Previous microbiogical studies based on
97   culture-dependent methods recovered a large number of bacterial strains from thermal springs
98   located in Baikal Rift zone. Isolates included alkaliphilic and alkalitolerant thermophilic bacteria
99   (Zaitseva et al. 2004; Kublanov et al. 2009; Lavrentieva et al. 2009; Namsaraev et al. 2010;
100 Rosanov et al. 2014a; 2014b), anoxygenic phototrophic bacteria (Kompantseva & Gorlenko,
101 1988; Gorlenko et al. 2014; Kuznetsov & Gorlenko 2014; Gaisin et al., 2015; Nuyanzina-
102 Boldareva et al., 2016). Our previous study focused on the microbial activities and included
103 results of the determination of the microbial processes rates in microbial mats and sediments in
104 some studied hot springs (Namsaraev et al. 2003; Bryanskaya et al. 2006; Zaitseva et al. 2007)
105 and microbial taxonomic diversity (Radnagurueva et al. 2016; Lavrentieva et al. 2018).
106 This study aimed to characterize the hydrotherms microbial communities by focusing on the
107 deep-branching thermophilic phylum *Acetothermia* distribution and reveal the main
108 environmental factors affect the microbial diversity.
109 **METHODS**
110 **Sample collection and chemical measurements**
111   Four alkaline hot springs located in the Baikal Rift Zone were sampled (Fig. 1). Sampling
112 for Alla thermal field was done from six hot spring sources on the left bank of Alla river at GPS
113 location $N54^0 41^/ 735^{//}$ $E110^0 44^/ 710^{//}$, Garga hot spring at $N54^0 19^/ 203^{//}$ $E110^0 59^/ 646^{//}$, Kuchiger
114 at $N54^0 52^/ 934^{//}$ $E111^0 00^/ 050^{//}$ and Umkhey at $N54^0 59^/ 253^{//}$ $E111^0 07^/ 152^{//}$.
115   The samples of water, bottom sediments, and microbial mats were collected in June-July
116 of 2014 and 2015. Temperature at the sampling sites was measured using a Long E905050
117 electronic digital thermometer (Prima, Portugal); pH was measured using a pHep2 portable pH
118 meter (HANNA, Portugal). The redox potential (Eh) was measured using a portable oxidation
119 reduction potential (ORP) meter (HM Digital, Portugal). Water mineralization was measured
120 using a TDS-4 conductometer (HM Digital, Singapore). Concentrations of Na^+ and K^+ were
121 measured in a PinAAcle 900F (PerkinElmer Inc., USA) atomic absorbance spectrometer.

122   The concentration of calcium ions was determined by the titrimetric method of
123   determining the hardness, and magnesium content was accounted as the difference in hardness
124   and calcium. The concentration of nitrate ions was determined by the photometric method with
125   salicylic acid. The concentration of nitrite ions was determined by a photometric method with
126   the Griss reagent. The concentration of sulfate ions was measured by the turbidimetric method.
127   Chloride content was determined by Moor's argentometric titrimetric method for determination
128   of carbonate and bicarbonate ions; iron was assayed by a photometric method.

129   Water samples were taken in polyethylene tubes (50 ml), which had been conditioned in
130   15% and 5% solutions of HNO_3 for 10 minutes, heated in the microwave system up to 100 °C
131   and then washed with deionized water; the nitric acid being prepared in a two-stage distillation
132   using SubPUR Milestone quartz bowling and DST-100 fluoroplastic distillation system. In
133   addition, water samples were if necessary filtered through sterile syringe nozzles of Cromafil
134   Xtra PVDF-45/25 type and then acidified with nitric acid of Ultrapure grade (to pH 1-2).

135   The microelement analysis was made by magnetic secretory mass spectrometry with
136   inductive coupled plasma (SF-ICP-MS Element XR). Mass signals were fixed in low, medium
137   and high resolutions versus spectra independence from interference overlays. Diluted multi-
138   element (ICP-MS-68A) solutions A and B (High-Purity Standards) and mono-element solutions
139   B, P, Na, Mg, Ca, K and Al (Inorganic Ventures) were used as dilution solutions. Corrections
140   for the instrument drift, matrix effect, and mass fractionation were carried out by the addition of
141   In (Inorganic Ventures) having the final concentration in the sample equal to 1 ppb. When
142   making solutions, deionized water (18.2 MΩ/cm) prepared by the Purelab Maxima Elga system
143   was used. The limits of detection for Be, Rb, Y, Zr, Nb, Mo, Ag, Sn, Sb, Cs, Hf, Tl, Bi, Th, U,
144   Sc, Ti, V, Cr, Co, Ga, Cd, Ba, Ta, W, Ge, As, Se varied from 0.1 to 0.01 ppb. Those for Li, P,
145   Mn, Cu, Ni, Zn, Pb were from 1 to 0.1 ppb and for B, Na, Mg, Ca, Fe, Al, K from 0.1 to 0.001
146   ppb; respectively.

147   **2.2 Mineralogy**

148   Sediments collected from all four lakes were analyzed for mineral and chemical
149   composition. X-ray diffraction (XRD) was used to characterize the mineralogy of sediments
150   collected from each lake. Oven-dried sediments were pulverized with a mortar and pestle and
151   analyzed by powder X-ray diffraction methods using the Bruker Phaser 2D (Cu Kα₁ –

152   radiation). The analysis of phase composition of ground adjournment was executed with bank

153   ICCD PDF Relase 2012.

154   **2.3 Molecular analyses**

155   The samples of microbial mats and bottom sediments were collected in sterile vessels. Prior to

156   analysis, the samples were stored at –20°C. For the molecular biological analysis, the samples

157   were fixed with 70% ethanol. Total DNA of the samples was extracted using the Marmur method

158   (Marmur, 1961) and purified using the phenol method.

159   The V3-V4 region of the 16S rRNA genes was amplified with the primer pair 343F and 806R

160   combined with Illumina adapter sequences as described in early (Martemyanov, 2016). The 16S

161   libraries were sequenced on MiSeq (Illumina) at the SB RAS Genomics Core Facility (ICBFM

162   SB RAS, Novosibirsk, Russia).

163   **2.4 Data analysis**

164   Raw sequences were analyzed with UPARSE pipeline (Edgar, 2013) using Usearch v8.1. The

165   UPARSE pipeline included merging of paired reads; read quality filtering; length trimming;

166   merging of identical reads (dereplication); discarding singleton reads; removing chimeras and

167   OTU clustering using the UPARSE-OTU algorithm. The OTU sequences were assigned a

168   taxonomy using the RDP classifier 2.11 (Wang, 2007).

169   Principal Component Analysis (PCA) was performed using MatLab11 software (The MatWorks,

170   Inc.). A constrained ordination was carried out by a Canonical Correspondence Analysis (CCA)

171   to correlate environmental variables with microbial phyla and samples. CCA was performed with

172   XLSTAT (Addinsoft, France).

173   **RESULTS**

174   The temperature at the sampling points varied from 35.6 to 75°C, the hottest fluids were

175   observed in Garga and Alla hydrotherms (Table 1). The pH of the source water changed from 8.3

176   to 10, the oxidation-reduction potential varied from -36 to -447 mV. All hydrothermal

177   environments were characterized by low mineralization (from 0.18 to 0.73 g/L) and hydrogen

178   sulfide contents (from 0.02 to 13.25 mg/L).

179   An inventory of major and trace elements in the thermal springs revealed significant

180   differences across sample sites. A comparison of the geochemistry and predominant solid phases

181   assesses the similarity of the five Alla sample sites in terms of high concentrations of Mg (0.2-

182   0.6% of total cations), Ca (2.6-13% of total cations), Cr (up to 208 μg/L), Fe (up to 1.3 mg/L), Ni

183   (up to 467 µg/L), Zn (344-1234 µg/L) and Al (up to 445 µg/L). The concentration of other

184   analyzed trace metals in the Alla hydrotherm water ranges as follows: Sn (3-6 µg/L), Sb (0.3-0.7

185   µg/L), Th (0.1-0.4 µg/L), U (0.1-1.4 µg/L), Ti (16-57 µg/L), Co (up to 1.4 µg/L), Cu (16-68

186   µg/L), Pb (66-328.5 µg/L), As (0.37-1.2 µg/L) and Se (0.13-0.45 µg/L). The mean values of

187   analyzed trace metals in Alla hydrotherm water are higher than the reported allowable values for

188   fresh water (Market, 1994) and compared with other studied hot springs (Table 2). These

189   elements are mainly from natural sources, anthropogenic pollution wasn't detected in Alla hot

190   spring.

191   Principal component analysis of temperature, pH and geochemistry indicate the presence

192   of at least four hydrotherms clusters in the Baikal Rift zone that differ in temperature, pH and

193   concentrations of Na^+, Ca^{2+}, Mg^{2+}, Fe, trace metals and hydrogen sulfide (Fig. 2). PCA of the

194   data collected on alkaline hot springs showed that temperature, trace metals and hydrogen sulfide

195   determined the distribution of samples on the plot (Fig. 2). Equal contributions to PC1 that

196   determined 27% of the variations were made by numerous trace elements, especially Ga, Nb, Y,

197   Sn, Th, Sc and Dy. Temperature and hydrogen sulfide content contributed the most to the second

198   principal component, PC2, explaining 23% of the variations. pH and Mn content had a main

199   contribution in PC3, explained 14% of the variations.

200   Geochemistry of fluids and sample type strongly correlated with microbial community

201   composition. Microbial mats exhibit low diversity and taxa related to the *Chloroflexus* (up to

202   56% of all sequences at the 58°C) or *Proteobacteria* (up to 70% of total sequences in the low-

203   temperature zone) were dominated. Microbial mat from 41°C harbor characterized more divers

204   community and contain abundant microbial taxa affiliated with *Atribacteria*, *Nitrospira*,

205   *Chloroflexi* and *Proteobacteria* (Fig.3).

206   The sequences retrieved from water samples indicated that *Cyanobacteria* dominated the

207   microbial community (55.7-70.8% of all retrieved sequences) in Alla hydrotherm. Both

208   Proteobacteria and Deinococcus-Thermus affiliated sequences occurred water in high-

209   temperature zone (60-74 °C) and at 55°C, respectively.

210   In the hydrothermal samples we detected numerous sequences of *Acetothermia*,

211   *Atribacteria* and *Aminicenantes*, respectively, representing up to 57.9, 28.4 and 15.1% of total

212   sequences. Members of *Acetothermia* have been shown to occur across a temperature range

213   including high temperature hydrothermal field Alla (64-68°C) to low-temperature sediments of

Umkhey hot spring (35-37°C). Generally, the sequences affiliated with candidate phylum represent ups 7.1, 33.7 and 78.7% of all classified sequences in water, microbial mats and sediments, respectively. We also studied the effect of environmental conditions (temperature, pH, major ion and trace elements contents) on *Acetothermia* community structure in different types of samples. Members of *Acetothermia* were still detectable but to a much lesser extent (i.e., 5.9% of all sequences) in Umkhey sediments (Table 3).

Geochemical Context of Alkaline Geothermal Habitats

To reveal major differences across sites we analyzed macro- and minor elements along with solid phases of sediments. Although the five sites with higher *Acetothermia* abundance at Alla hydrotherms are all sulfidic and sub-oxic, they are geochemically distinct from one another and yield significantly different microbial communities (Fig. 2 and 4). The anoxic, submerged sediments sampled at Al1 (64.4 °C) are dominated by solid phases of quartz (SiO_2), albite (Na, Ca Al_3O_8), carlinite (Tl_2S) and characterized by high Fe, Cr and Al contents. Microbial community has been shown to be dominated by *Firmicutes* (38% of all classified sequences), *Proteobacteria* (27%) and *Acetothermia* (18% of all classified sequences). *Acetothermia*-dominated communities were sampled at Al2 and Al3 with Ca- and Mg-rich water. Solid phases at Al2 site were represent only calcite ($CaCO_3$), but at Al3 site albite, calcian and lopezit were found in addition to calcite. The aqueous phase of Al2 contains high concentrations of Ca (12.6 mg/L), Cr (up to 208 µ/L) and Fe (up to 1294 µ/L). (Tables 1 and 2).

Canonical correspondence analysis predicted that the trace element content in the springs had a strong influence over the microbial community composition. It was showed that the abundance of the phyla *Acetothermia* and *Atribacteria* was positively correlated with the aqueous concentration of Fe, Zn, Ni, Al and Cr (Fig. 4). Similarly, the abundance of the phylum *Firmicutes* was strongly correlated with the aqueous concentration of lithium and strontium, whereas the phyla *Proteobacteria* and *Deinococcus-Thermus* were positively correlated with the potassium.

The sequences of the 16S rRNA gene affiliated with candidate genera were numerous in the *Acetothermia*-dominated microbial communities (Table 4). The members of Atribacteria_genera_incertae_sedis were found in all sedimentary microbial communities and varied from 0.2 to 14.8% of total abundance. The members of Aminicenantes_genera_incertae_sedis were highly numerous in Al2 and Al3 sampling sites.

245   Figure 5 show Venn diagram with the number of unique and shared genera between
246 *Acetothermia*-dominated sediment microbial community. Microbial communities were
247 characterized by a high level of similarity, the number of unique genera for each community was
248 less than the number of shared genera.

249 **DISCUSSION**

250   Early-branching lineages of *Bacteria* and *Archaea* and their metabolic possibilities might
251 be cricial in understanding the origin and evolution of life on Earth (Colman et al., 2016; Farag
252 et al., 2014; Nobu et al., 2016). Uncultivated bacteria belonging to the candidate phylum
253 *Acetothermia*, along with members of the phyla *Firmicutes* and *Proteobacteria*, were identified
254 as the dominant group in sedimentary microbial communities in the alkaline hydrothermal
255 springs of Baikal Rift zone. Geochemistry of fluids and sample type were strongly correlated
256 with microbial community composition.

257   A unique feature of the Alla hot springs was the high abundance of members of the
258 phylum *Acetothermia* in the bottom sediments community.

259   Similarly, Badhai et al. showed that more than 24% of the classified sequences were
260 represent the phylum *Acetothermia* in the hot spring of Tarabalo (Odisha, India) (Badhai et al.
261 2015). The main environmental variables affecting the diversity and microbial composition of
262 the community were nitrate-nitrogen, fluoride, bicarbonate (HCO_3^{-}), lead, cadmium and zinc
263 concentrations.

264   Another example of *Acetothermia*-dominated microbial community is the Prony Bay
265 Hydrothermal Field (PHF) (Pisapia et al., 2017). In this active serpentinization site high-pH
266 fluids rich in H_2 and CH_4 discharged from carbonate chimneys at the seafloor, but in a shallower
267 lagoon environment. It was showed that a central role of uncultivated bacteria belonging to the
268 *Firmicutes, Acetothermia* and *Omnitrophica* in the ecology of the PHF. Mineralogical studies
269 showed a predominance of brucite mixed with Mg-carbonates and aragonite, with an increasing
270 number of Ca-carbonates closer to the outer surface of the chimney that was deposited when sea
271 water began to mix with hyperalcalic hydrothermal fluids (Pisapia et al., 2017). Despite
272 *Acetothermia* relative proportions and ubiquity the metabolic capacities of this phylum remain
273 entirely unknown at PHF.

274   In the study of Rempfert et al., 2017, a phylogenetic variety of subsurface microbial
275 communities of Samail Ophiolite (Oman) water from gabbro and peridotite aquifers was

276 investigated. It was shown that the geochemical composition of water controls the composition
277 of the microbial community; similar microbial assemblages group according to fluid type
278 (Rempfert et al. 2017). Nevertheless, the phylotypes associated with the candidates phyla OD1,
279 GAL15 and *Acetothermia*, as well as the *Betaperobacteria* were abundant in all the samples
280 studied. Based on geochemical and microbiological results, the authors suggested that several
281 metabolisms in groundwater may occur, including methanogenesis, acetogenesis and
282 fermentation, as well as oxidation of methane, hydrogen and small organic acids using nitrate
283 and sulfate as electron acceptors.

284 **CONCLUSION**

285 In general, the analysis of the previous studies showed that the distribution of
286 *Acetothermia* in hydrothermal environment is associated with the geochemical / mineralogical
287 properties of the environment that affect the composition of the microbial community and their
288 abundance. Nonetheless, the distribution of *Acetothermia* in modern extreme ecosystems could
289 be conceivably evolved independently in the fluctuating environments between anaerobic
290 organics-depleted and aerobic-enriched states with maintaining a part of primordial genes for
291 basic energy and carbon metabolism in the limited microhabitat similar to the ancient
292 hydrothermal environments.

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Figure 1(on next page)

Location of sampling sites.
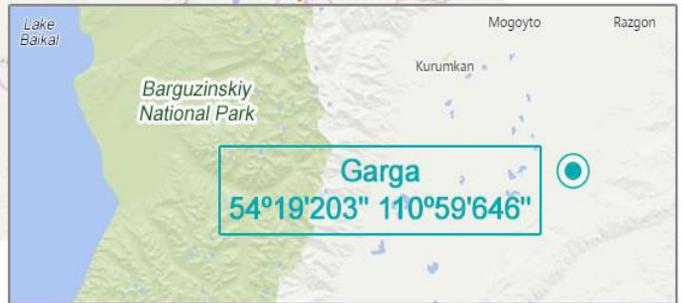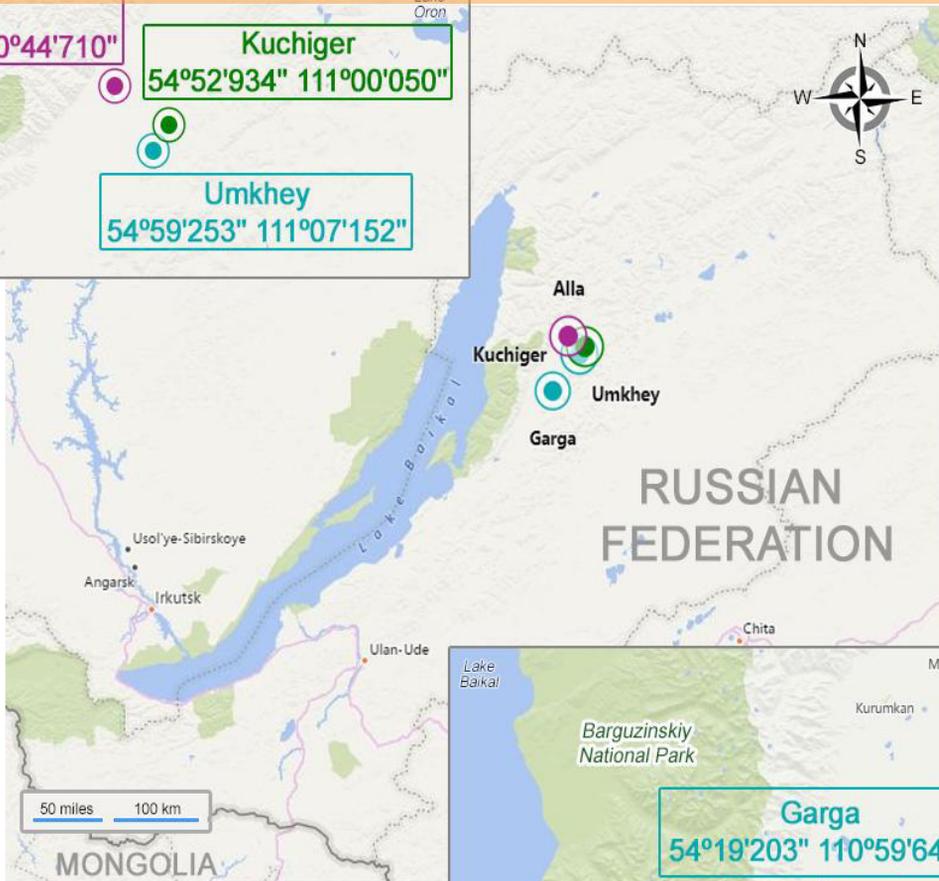
Figure 2(on next page)

Principal component analysis (PCA) of environmental parameters in studied hot springs.

PC2, 23%

▲ Al-1

Ku-1 ▲

Um-1 ▲

Ga

H

Nb,

Y, Pr, Al,

Ti, V,

▲ Al-4 **PC1, 27%**

-15

-10

-5

SO$_4^{2-}$

Cr, Sn, Sb,

Co, Ni, Zn,

As Pb, Hf

Ga-1 ▲

Ga-2 ▲

▲ Al-3

U, Se,

Temperature

▲ Al-2

▲ Al-5

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10

5

0

-5

-10

-15

0

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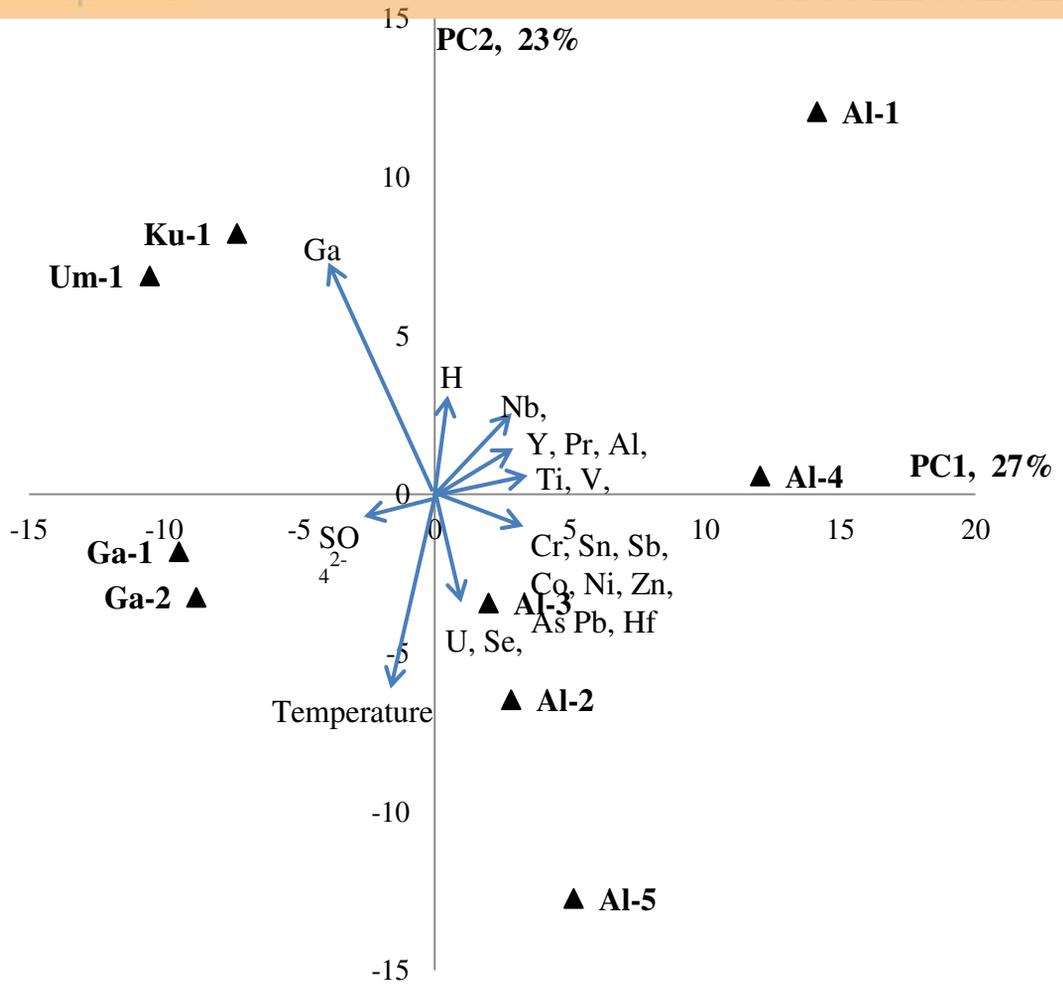
Figure 3(on next page)

Relative abundance of the dominant phyla in samples of different type.

(A) – sediments, (B) – water, (C) – microbial mat

Legend:

- Proteobacteria
- Acetothermia
- Cyanobacteria/Chloroplast
- Atribacteria
- Euryarchaeota
- Chloroflexi
- Deinococcus-Thermus
- Firmicutes
- Aminicenantes
- Nitrospirae
- Bacteroidetes
- Aquificae
- Crenarchaeota
- Acidobacteria
- Others
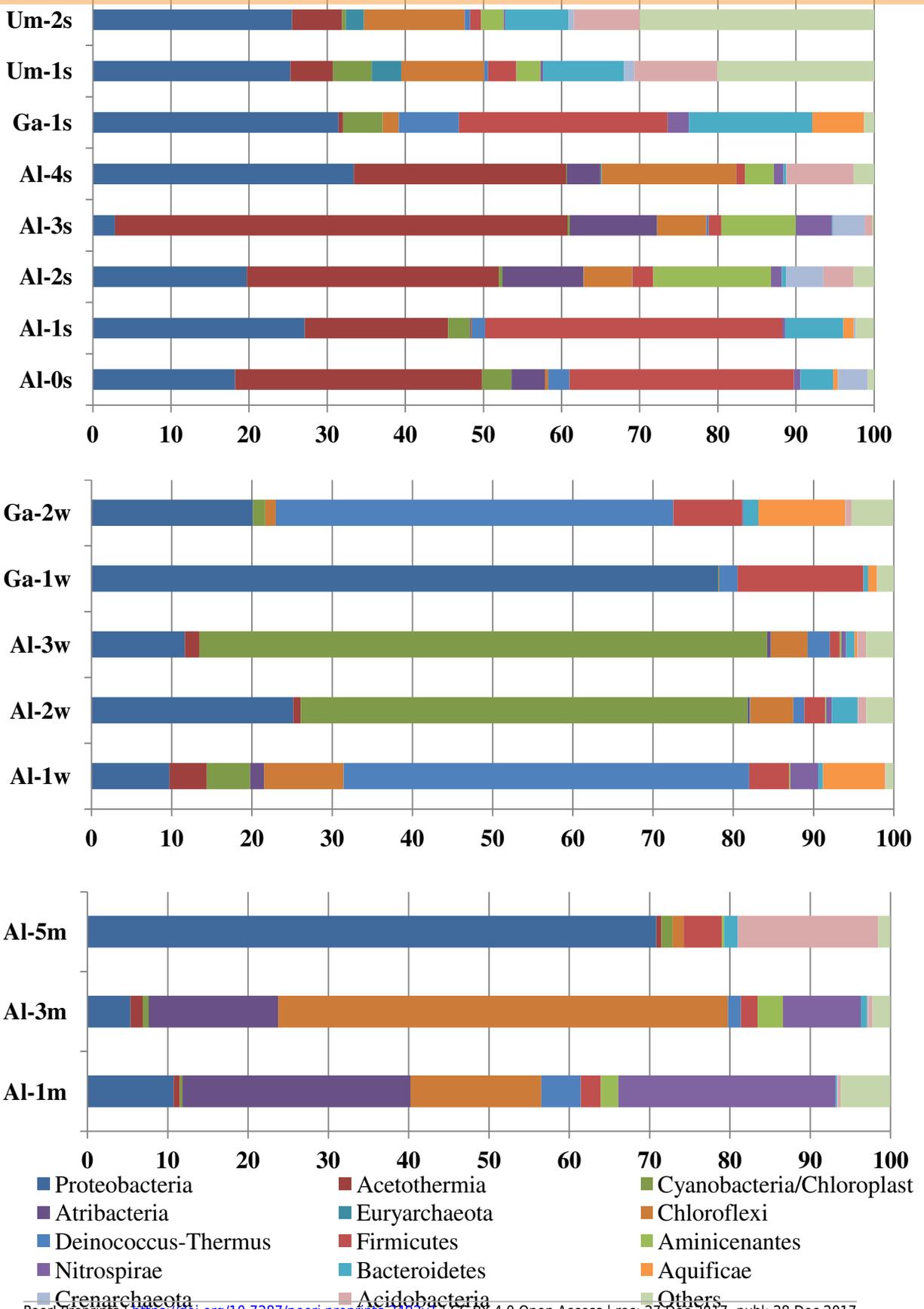
Figure 4 (on next page)

Canonical correspondence analysis (CCA) of dominant phyla, samples and trace elements contents.

Arrows indicate the direction and magnitude of trace elements content associated with phyla (open triangles) and samples studied (black circles).

Acetothermia

Alla-1

Alla-4

Atribacteria

Fe

Alla-2

Deinococcus-Thermus

Zn

Chloroflexi

Ni

Al, Cr *Proteobacteria*

K

Alla-5

0

Alla-3

Garga-2

Li

Sr Garga-1

Cyanobacteria

Firmicutes

Bacteroidetes

-0,5

Kuchiger

Umhey

-1

CCA2 14.9%

CCA1 43.2%

-1   -0,5   0   0,5   1

0,5
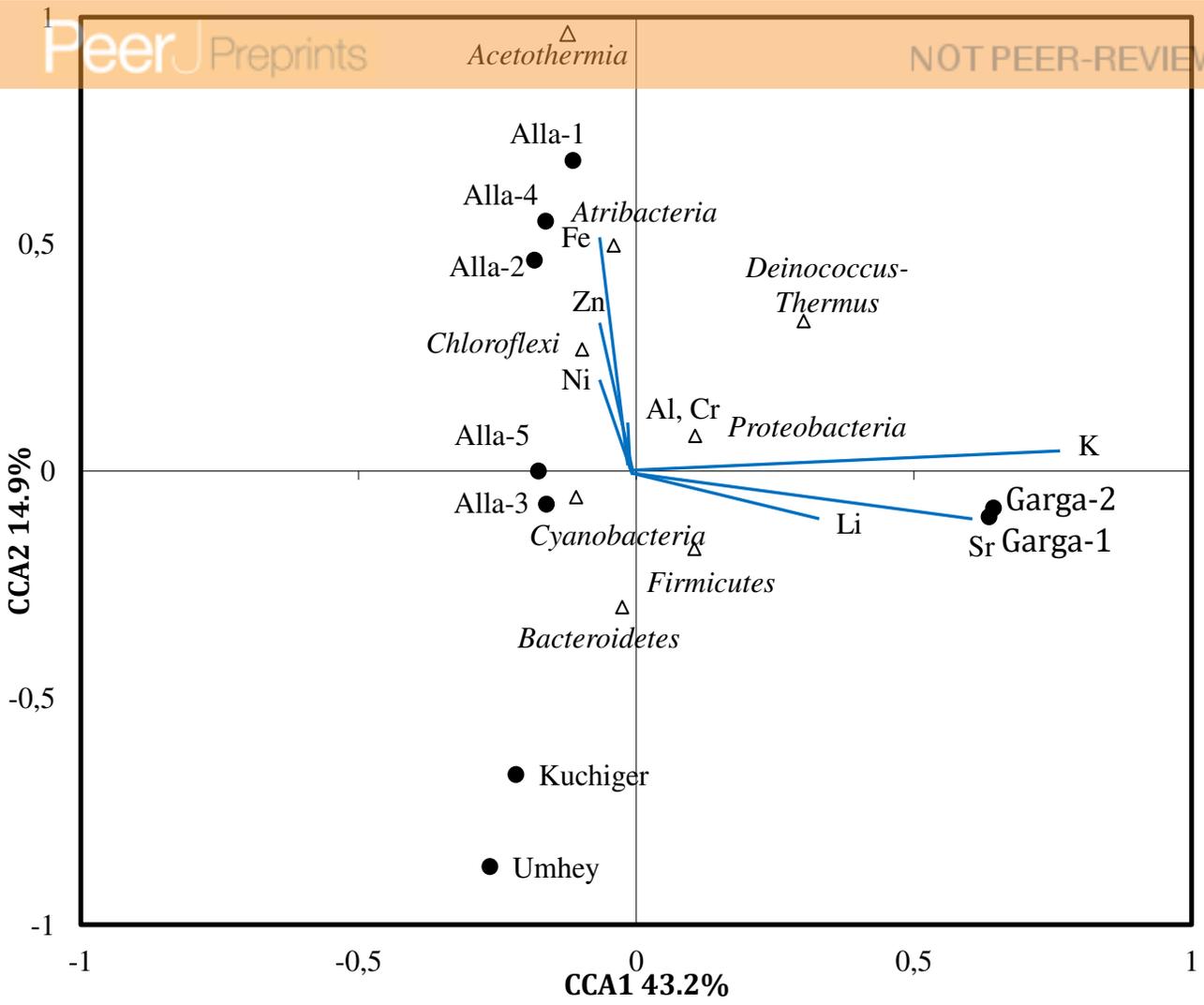
Figure 5 (on next page)

Venn diagram showing the number of unique and shared genera between Acetothermia-dominated sediment microbial community.
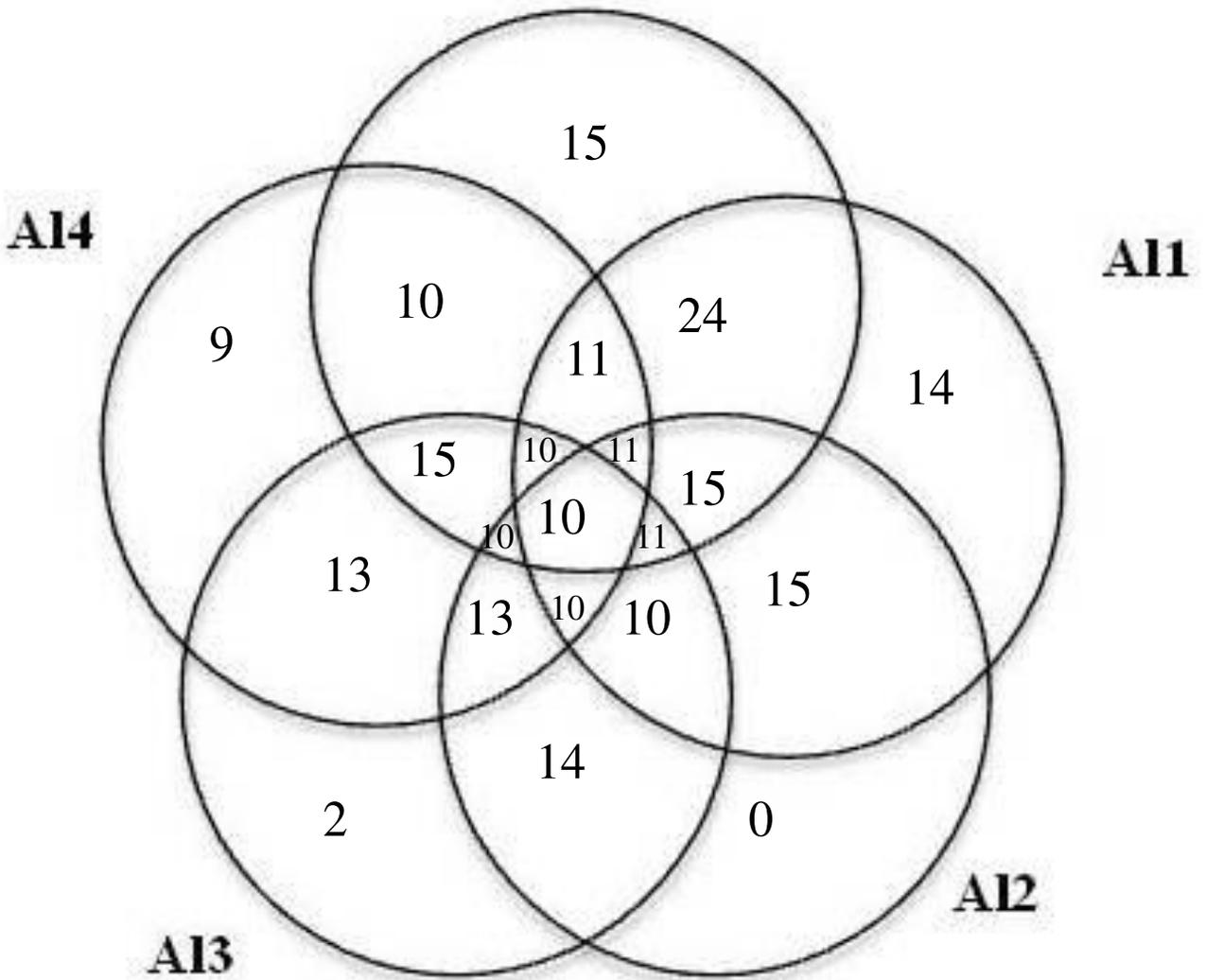
Table 1(on next page)

Physicochemical parameters of the four alkaline hot springs.

Sampling site	Alla hot spring	Umkhey hot spring	Kuchiger hot spring	Garga hot spring
Temperature range (◦C)	34.4-68	35.6-37	35-45	55.2-75
pH	8.9	8.8	9.0	7.9
Sodium, mg/l	89.01-109.54	100.82	94.41	312.04
Calcium, mg/l	2.00-3.01	2.00	1.00	25.05
Magnesium, mg/l	0.61-1.22	1.22	0.61	0
Iron III, mg/l	0.1-1.03	0.38	<0.05	<0.05
Bicarbonate, mg/l	91.53-109.83	51.87	73.22	118.99
Carbonate, mg/l	7.50-22.5	22.5	15.0	0
Nitrate, mg/l	1.33-4.7	5.19	1.65	3.97
Sulfate, mg/l	49.3-53.9	74.8	55.9	515.32
Chloride, mg/l	18.7-21.9	19.86	17.73	50.7

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Table 2(on next page)

Trace metal concentration in water samples from alkaline hot springs of Baikal Rift Zone with reference values for freshwater.

[1] Rempfert et al. 2017; [2] Markert, 1994; [3] Xing & Liu, 2011

Sampling site	Co (µg/l)	Ni (µg/l)	Zn (µg/l)	Pb (µg/l)	Al (µg/l)	Cu (µg/l)	Fe (µg/l)	Mn (µg/l)	Cr (µg/l)	As (µg/l)
Alla hydrotherm	0.48-1.43	282-467	344-1234	66-329	70.6-444.9	16-68	549-1294	8.7-19.8	27.2-207.7	0.37-1.2
Umkhey hydrotherm	0.05	0.16	3.1	0.3	38.5	0.1	15.5	1.4	0.07	0.03
Kuchiger hydrotherm	0.05	0.51	9.5	1.2	188.2	0.1	171.6	2.2	0.41	0.05
Garga hydrotherm	0.05	0.05	3.9-4.3	0.27-0.54	15.3-16.5	0.1-1.6	8.8-10.9	19.7-20.0	0.3-0.6	0.03-0.04
NSHQ3B[1]	0.06	2.11	5.23	-	BDL	0.70	BDL	-	5.82	0.38
WAB55[1]	0.177	0.29-2.41	-	-	0.003-0.008	0.64-2.92	0.112	-	2-10.7	1.2
WAB188[1]	0.177	2.9-3.52	-	9.35	0.003	0.127-2.67	20	-	2.5-4.37	1.2
Freshwater[2]	5	0.3	5.0	3.0		0.003	$\leq 1^3$	1-200		

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Table 3(on next page)

Distribution and relative abundance (%) of Acetothermia in hot springs of Baikal rift zone

n.d. – not determined

Sample type	Alla hot spring	Garga hot spring	Umkhey hot spring	Average abundance (%)	Maximum relative abundance (%)
Microbial mat	0.6-1.5	n.d.	n.d.	0.95	1.5
Water	0.9-4.7	0	n.d	2.47	4.7
Sediments	18.3-57.9	0.6	5.4-6.4	17.39	57.9

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Table 4 (on next page)

Distribution and relative abundance (% of all classified sequences) of candidate genera in Acetothermia-dominated microbial community

	Al-0	Al-1s	Al-2s	Al-3s	Al-4s
Acetothermia_genera_incertae_sedis	37.7	24.9	45.9	62.5	42.3
Aminicenantes_genera_incertae_sedis	0.0	0.0	21.5	10.3	5.8
Atribacteria_genera_incertae_sedis	5.1	0.2	14.8	12.1	6.7
Omnitrophica_genera_incertae_sedis	0.0	0.0	1.3	0.0	0.3

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