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Boron mine ponds: metagenomic insight to bacterial diversity

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Abstract

Since it can be a source of new microorganisms with biological potential, it is important to identify the microorganisms found in environments with high boron content in terms of ecological and biotechnological application potentials. In this context, deposit waters in environments where boron mining activities perform are important habitats for boronophilic/boronotolerant microorganisms.

In this study, bacterial community in the waste mining ponds of Balıkesir-Bigadiç and Eskişehir-Kırka Boron Mining Operations were investigated by using 16S rDNA gene-targeted Illumina MiSeq sequencing. The greater parts of high-throughput sequences were related to Proteobacteria, Planctomycetes, Bacteriodetes, Verrucomicrobia and Actinobacteria phyla. Cyanobacteria and Parcubacteria were other notable groups with low abundance. Genera belonged to *Blastopirellula*, *Luteolibacter*, *Porhyrobacter* and *Hydrogenophaga* were the most abundant taxa for all the samples. *Sandarakinorhabdus*, *Pseudoanabena, Roseinatronobacter* and *Pontimonas* genera-affiliated reads were also detected at in the October samples. Striking seasonal variations were detected between samples in terms of the type and number of microbial populations.

Keywords: boron, high-throughput sequencing, microbial diversity

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Bor maden göletleri: bakteriyel çeşitliliğe metagenomik bakış

Özet

Biyolojik potansiyeli olan yeni mikroorganizmaların kaynağı olabileceği için, bor içeriği yüksek ortamlarda bulunan mikroorganizmaların belirlenmesi ekolojik ve biyoteknolojik uygulama potansiyelleri açısından önemlidir. Bu bağlamda, bor madenciliği faaliyetlerinin gerçekleştirildiği çevrelerdeki birikinti suları boronofilik / boronotolerant mikroorganizmalar için önemli habitatlardır.

Bu çalışmada, Balıkesir-Bigadiç ve Eskişehir-Kırka Bor Maden İşletmeleri atık madenciliği havuzlarındaki bakteri topluluğu, 16S rDNA gen hedefli Illumina MiSeq dizilimi kullanılarak incelenmiştir. Yüksek verimli dizilerin büyük kısımları Proteobacteria, Planctomycetes, Bacteriodetes, Verrucomicrobia ve Actinobacteria filumları ile ilişkili bulunmuştur. Cyanobacteria ve Parcubacteria, düşük miktara sahip diğer önemli gruplar arasındadır. *Blastopirellula*, *Luteolibacter*, *Porhyrobacter* ve *Hydrogenophaga*'ya ait cinsler tüm örneklerde en çok bulunan taksonlardır. Ekim örneklerinde *Sandarakinorhabdus*, *Pseudoanabena, Roseinatronobacter* ve *Pontimona*s cinsine bağlı okumalar da tespit edilmiştir. Mikrobiyal popülasyonların türü ve sayısı açısından örnekler arasında çarpıcı mevsimsel farklılıklar tespit edilmiştir.

Anahtar kelimeler: bor, mikrobiyal çeşitlilik, yüksek verimli dizileme

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1. Introduction

3.1. Chemical components of sampling area

The high concentrations of metals in the boron mine drainage with two seasons in two different sampling areas were determined as B and Li and they are shown in Table 1. Especially, the concentrations of boron are higher in PA_K1 and PA_K2 at the first season. However, the boron concentration in 2B1 and 2B2 was very high at the second season. The sampling areas show the difference about boron content according to season. The region in Kırka was rich in term of boron in autumn and other region (Bigadic) was rich in term of boron in spring.

Table 1**.** Element contents of sampling areas (K1, K2, B1 and B2; Kırka 1, Kırka 2, Bigadiç 1 and Bigadiç 2, respectively)

Element	K1		K2		B1		B2	
	1st season	**2nd season**	**1st season**	**2nd season**	**1st season**	**2nd season**	**1st season**	**2nd season**
B (5 ppb)	4330569	659014	2400865	159111	790555	2894267	258714	2417816
Ba (0.05 ppb)	670	<80	293	51.32	<80	390	51.04	225
Ca (0.05 ppm)	10	196.70	20.7	44.46	212.40	13.40	55.54	17
K (0.05 ppm)	189	9	107	7.34	12	182	8.05	124
Li (0.1 ppb)	258.449	1.071	146.492	1058.1	1.277	17.929	788.2	12.519
Mg (0.05 ppm)	36	54.00	37	31.56	59	34.00	27	36.00
Na (0.05 ppm)	7.018	468.00	4.561	248.81	476	6.246	275.69	5.025
S (1 ppm)	552	260	450	138	286	506	116	453
Sr (0.01 ppb)	2.904	24.032	4.539	11587.32	27.673	2.708	18834.48	3.815

3.2. The general view of microbial diversity

Species richness estimates (Chao1 and ACE) and diversity indices (Shannon and Simpson) for the four sampling timepoints are presented in Table 2. Reads were repossessed and comprised 2167, 1303, 1387, 1152, 783, 1577, 1044, and 1838 OTUs, with Shannon diversity entropy of 5.13, 4.34, 4.76, 4.18, 3.59, 4.99, 3.80 and 4.47 for 2K_2, 2B_2, 2K_1, 2B_1, PA_B1, PA_K1, PA_B2, PA_K2 respectively, after the quality filter.

Table 2. Diversity estimates of the samples based on high-throughput sequencing data

Set Name	Sample Name	ACE	CHAO	Jackknife	OTUs	NPShannon	Shannon	Simpson	Phylogenetic Diversity	Good's coverage of library(%)
March_2019_Sampling	2K_2	3466.61	3003.44	3164.97	2167	5.32	5.13	0.034	943	95.11
March_2019_Sampling	2B_2	2138.66	1874.56	2180.07	1303	4.47	4.34	0.047	736	97.05
March_2019_Sampling	2K_1	1754.76	1589.52	1797.00	1387	4.88	4.76	0.039	664	97.95
March_2019_Sampling	2B_1	1814.01	1634.13	1901.99	1152	4.29	4.18	0.046	991	97.47
October_2018_sampling	PA_B1	1428.22	1254.60	1420.89	783	3.69	3.59	0.067	743	98.02
October_2018_sampling	PA_K1	2301.05	2110.67	2230.63	1577	5.12	4.99	0.028	903	96.83
October_2018_sampling	PA_B2	2872.30	1749.62	1975.36	1044	3.92	3.80	0.053	1329	97.18
October_2018_sampling	PA_K2	3019.26	2677.00	3103.58	1838	4.65	4.47	0.108	1252	95.80

We identified 45 bacterial phyla in the boron containing samples by 16S rRNA gene sequencing. The most highly represented phyla throughout the samples were Proteobacteria, Planctomycetes Bacteroidetes, Verrucomicrobia and Actinobacteria. The top 10 most abundant phyla in each sample are shown in Figure 2.

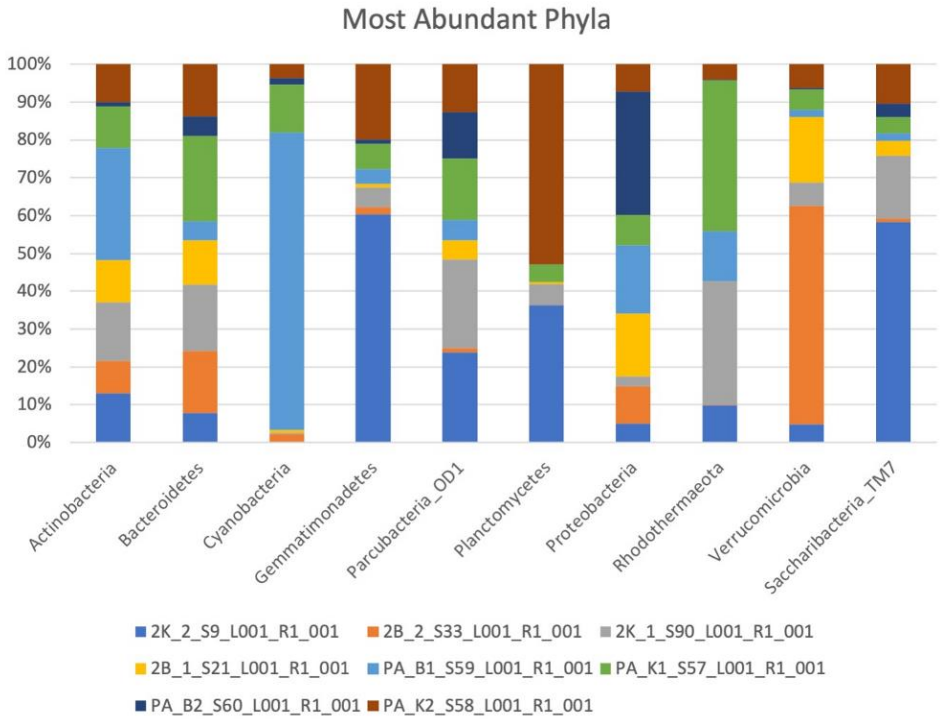

Figure 2. The top 10 most abundant phyla in each sample

At the phylum level, microbial community composition showed seasonal and regional variations, with the phylum Proteobacteria detected at a high level in Bigadiç samples [samples: (PA_B2) 89.10%, (PA_B1) 46.60% vs (PA_K1) 28.69% and (PA_K2) 22.96%)], and more abundant during autumn (41.65%) than during spring (29.10%) samples (Figure 3).

Figure 3. Taxonomic profiles of samples at the phylum level (A), order level (B) and genus level (C) revealed by 16S rRNA gene-targeted Illumina MiSeq sequencing (All samples, first and second season).

3.2.1. Sampling region B1

Proteobacteria was the predominant group for both October and March samples, 46.60% and 56.38% respectively (Fig. 4). OTUs were distributed mainly within *Sandarakinorhabdus* (17.98%), *Porhyrobacter* (14.86%), *Luteolibacter* (12.36%), *Pontimonas* (11.65%), *Limnothrix* (10.45%), *Pseudoanabena* (9.44%), *Hyphomonas* (5.21%) (Proteobacteria) in this region for both sampling. OTUs of *Algoriphagus* and *Polymorphobacter* genera were detected in low abundance. *Sandarakinorhabdus* and *Pontimonas* related OTUs were found to be more represented in PA_B1 than in other sampling areas.

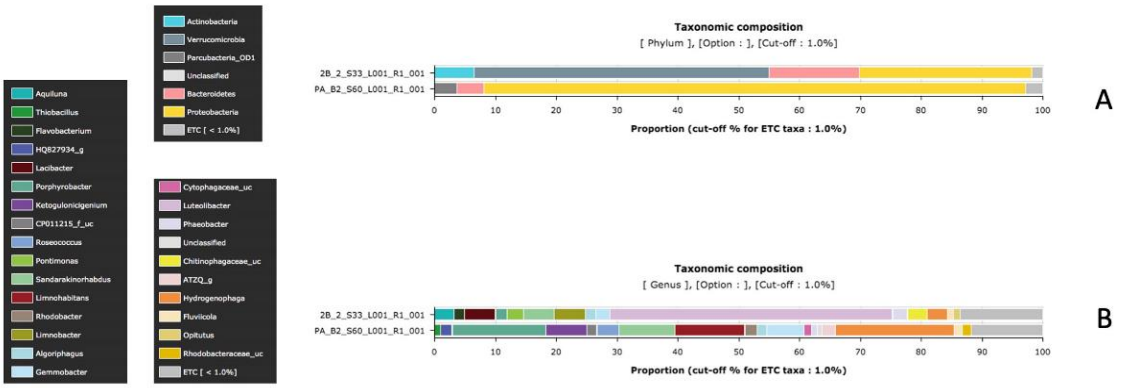

Figure 4. Taxonomic profiles of samples at the phylum level (A) and genus level (B) revealed by 16S rRNA gene-targeted Illumina MiSeq sequencing (B1 sampling, first and second season)

3.2.2. Sampling region B2

Sample 2B_2 was different from the others by harboring 48.7% Verrucomicrobia. Proteobacteria related sequences was 28.32% in October sampling, while it increased to 89.02% in March (Fig. 5). OTUs belonging to *Hydrogenophaga* (19.48%), *Porphyrobacter* (15.42%), *Limnohabitans* (11.46%) and S*andarakinorhabdus* (9.27%) were found to be quite abundant at the genus level in sample PA_B2. OTUs belonging to *Luteolibacter* was 46.44% in sample 2B_2 and this genus not detected in October. Low abundance OTUs were associated with the genera *Gemmobacter*, *Rhodobacter* and *Ketogulonicigenium*. OTUs of *Sandarakinorhabdus*, *Hydrogenophaga*, *Porphyrobacter*, *Limnohabitans* and *Roseococcus* in this region were not detected in Kırka (PA_K1 and PA_K2) samples.

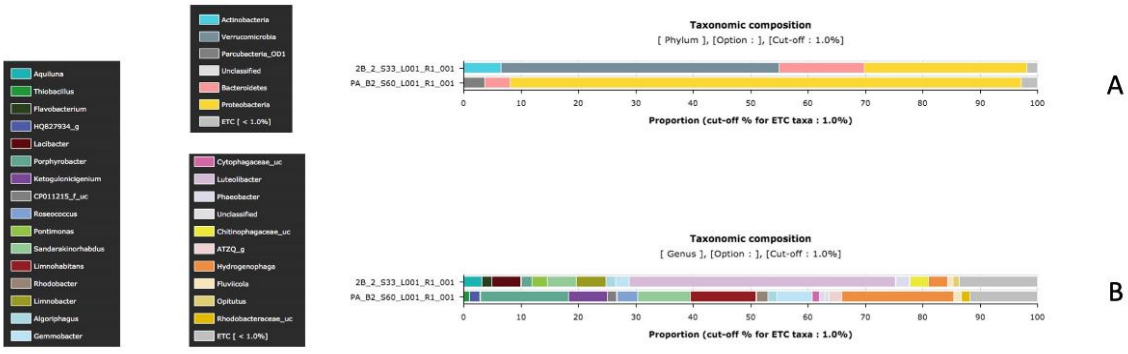

Figure 5. Taxonomic profiles of samples at the phylum level (A) and genus level (B) revealed by 16S rRNA gene-targeted Illumina MiSeq sequencing (B2 sampling, first and second season)

3.2.3. Sampling region K1

Proteobacteria, Bacteriodetes, Actinobacteria, Parcubacteria and Rhodothermoaota were the main groups (Fig. 6) in Kırka samples (PA_K1 and PA_K2). OTUs were mostly associated with the orders Rhodobacterales,

Planctomycetales, Saprospirales and Nitrilirupturales. *Blastopirellula*, *Roseinatrobacter* and *Brumimicrobium* were the most represented genera. Other low abundance OTUs belonged to the genera *Planktosalinus*. OTUs of *Blastopirellula* were more abundant in Kırka region and not detected in Bigadiç samples.

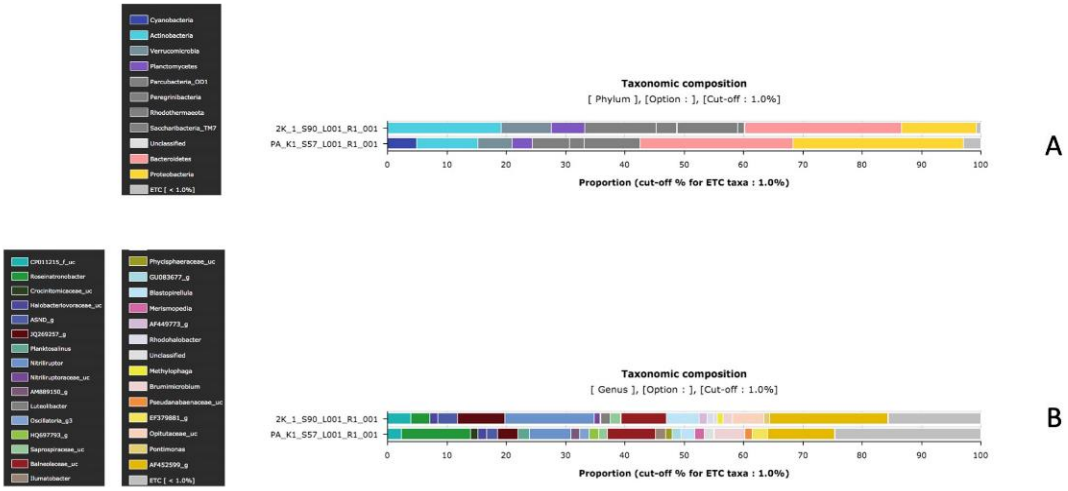

Figure 6. Taxonomic profiles of samples at the phylum level (A) revealed by 16S rRNA gene-targeted Illumina MiSeq sequencing (K1 sampling, first and second season)

3.2.4. Sampling region K2

Sampling region K2 was different from the others by harboring up to 35.24% Planctomycetes. Planctomycetes, Proteobacteria, Bacteriodetes, Actinobacteria and Parcubacteria were the main groups in this region. *Blastopirellula* was the most represented genera both October and March samplings (Figure 7).

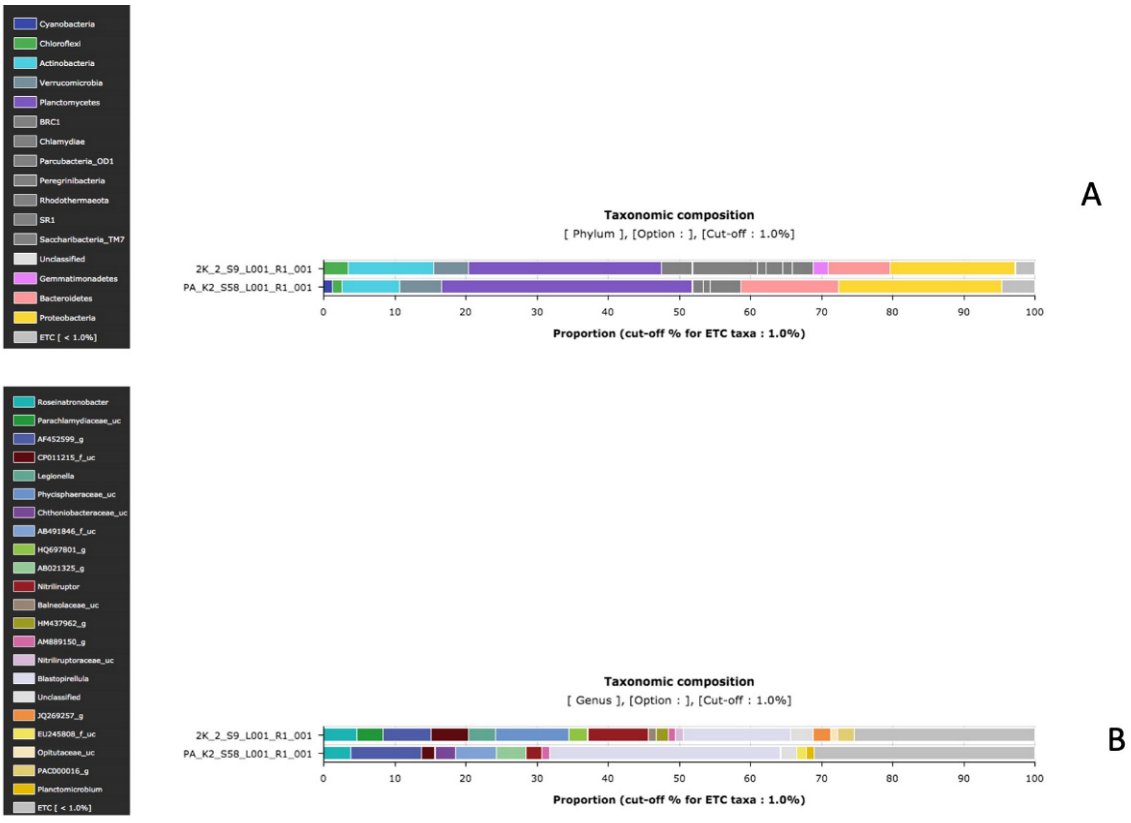

Figure 7. Taxonomic profiles of samples at the phylum level (A) revealed by 16S rRNA gene-targeted Illumina

3.3. Investigation of Dominant Taxa

Aquatic bacteria belonging to the deep-branching phylum Planctomycetes play a major role in global carbon and nitrogen cycles. Anammox bacteria branch deeply within the Planctomycetes phylum and they were subsequently characterized in natural environments, including marine, estuary, freshwater, and terrestrial habitats [24].

Cyanobacteria play an important role in changeable the habitat. After artificially degrading (including abundant minerals) they settle in inanimate substrates. These habitats are characterized by high concentrations of heavy metals, lack of water, high levels of isolation and deficient nutrient contents [25]. They have different great adaptability traits that allow cyanobacteria to grow, reproduce and dominate in many other habitats where other life forms are absolutely unknown and may even be difficult to survive. However, in our study we have found that Cyanobacteria did not appear as one of the dominant groups except for the PA_B1 sample according to MiSeq reads data. *Gloeobacter* is also belonged to Cyanobacteria phylum was also abundant in B1 sample.

The bacteria of Proteobacteria were dominant in various environments. This phylum has gram negative bacteria and large metabolic diversity [26, 27] in widespread environments. In this study, the phylum was analyzed in four sampling at the two seasons.

It is obvious that subtle changes in the environment, such as varying metal concentrations, nutrient availability and the presence of natural aquifers, will affect microbial communities near and within mineral-rich areas. However, all the efforts for characterizing the microbiome in mineral-rich samples is helping us understand the distinct difference between microbial communities between different types of ore..

4. Conclusions and discussion

Extreme environments have been attracted attention and studied in various conditions by culture dependent and culture independent techniques due to the widely spread capacities. Since no systematic study on the microbial ecology aspects of Boron mines has so far been reported, results of this work will be useful to determine and to characterize the indigenous microbiota of the Boron mines in Turkey.

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