

Microbial Community Structure Diversity in the Dewatered Sludge from 4 Different Waste Water Treatment Plants used for CSRB in Colder Season

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Abstract. Compacted Sludge Reducing Barrier (CSRB) was tested to be a feasible barrier for Acid Mine Drainage (AMD) from tailings in case the ground water contamination. Because of its double function (encapsulating and reducing), the microbial community structure diversity in the sludge played a key role. In this paper, we researched the correlation between heavy metals and microbial structure diversity in the dewatered sludge from 4 different sewage treatment process plants in Lanzhou city, a developing area of northwest China, in the colder season. The results indicated that the microbial community structure diversity differed and were unique among the different sewage plants; their correlation with heavy metals was also unique independently.

1 Introduction

The contamination of groundwater by tailings and acidic mine drainage (AMD) is an environmental problem that has long puzzled mining and environmental scientists. At the same time, the problem of safe disposal of dewatered sludge from municipal sewage treatment plants has not been solved effectively for a long time. In order to achieve the "win-win" and "treating waste with waste" strategy of solving environmental problems, Professor Zhang, Huyuan from Environmental Geotechnical Engineering Research Group of Lanzhou University, China innovatively proposed a Compacted Sludge Reducing Barrier (CSRB) solution. After years of research, CSRB, a tailing impoundment liner with dual functions of encapsulating by the low permeability coefficient and fixing heavy metals by microbial reduction, is found to be feasible [1-5].

In previous researches, the dewatered sludge from the same sewage treatment plant was used to study the microbial mechanism [6-8], or the sludges in the warmer season were used to study [9]. However, the effects of different municipal wastewater treatment processes on the microbial community structural diversity in dewatered sludge and the correlation of heavy metal factors in the colder season have been rarely reported. In this paper, at the beginning of January 2020, dewatered sludges from 4 municipal sewage treatment plants with different locations and different sewage treatment

processes in Lanzhou city, China were selected to conduct high-throughput sequencing of microbial community structure diversity research and heavy metal determination, and to conduct horizontal comparison and correlation analysis to obtain more abundant MICROBIAL mechanism data of CSRB.

2 Material and method

2.1 Sampling sites and conditions

The sewage sludges were sampled from 4 different waste water treatment plants in Lanzhou city, Gansu province, China, in the beginning of January 2020. They were dewatered sludges from the Dewatering Workshop. Some samples were put in the 15 mL sterile centrifuge tube immediately, put in an ice box and transported back to the laboratory, then stored in the -70 °C refrigerator waiting for the microbial community structure diversity test. Other samples were taken with the plastic bags to the laboratory, air-dried naturally, ground, passed to 0.5 mm sieve, then stored in the dry place waiting for the heavy metal content test. 3 parallel samples were got from each plant individually.

Lanzhou city lies in the upper reaches of Yellow River, in the Northwest China. It is the capital of Gansu province, a developing area with the bottom GDP ranking of the whole country. The population of

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Lanzhou is 3.6 million with the area of 14.1 thousand km². Yellow River goes through the center of the city from the west to the east for 40 km. The 4 different waste water treatment plants from east to west along the River are Lanzhou Yanerwan Waste Water Treatment Plant (YEJA) with an improved A2/O sewage treatment process, Lanzhou Yanchang Waste Water Treatment Plant (YCJA) with an improved SBR and TCBS sewage treatment process, Lanzhou Qilihe Waste Water Treatment Plant (QLJA) with the A2/O sewage treatment process, Lanzhou Xigu Waste Water Treatment Plant (XGJA) with the A2/O sewage treatment process. In December 2019, the highest temperature was 8 °C while the lowest was -13 °C; the best air quality index was 46 while the worst was 148.

2.2 Microbial community structure diversity test

Microbial community structure diversity was detected by Illumina HiSeq 2500 sequencing platform. Paired End sequencing was used to construct a small fragment library. Reads were splitted; OTUs (Operational Taxonomic Units) were clustered; and species annotation and abundance analysis were performed.

2.3 Heavy metals content test

Heavy metals content in sludge dry powder were analyzed by BRUKER S1 TITAN Handheld XRF Analyzer.

2.4 Data processing and analysis

Microbial Species Abundance Cluster Analysis was performed using BMKCloud (www.biocloud.net). Figures were drawn by R 3.5.2 software.

3 Result and discussion

3.1 Heavy metals content in the sludge

As shown in Table 1, the Cr average content in QLJA sludge sample was the highest, 342 ppm, while that in YEJA was the lowest, 202 ppm. The Mn average content in XGJA sludge sample was the highest, 483 ppm, compared with the lowest 254 ppm in YEJA sample. The Fe content in XGJA was the highest, 87333 ppm, with the lowest 19667 ppm in YEJA sample. The highest Cu content was in QLJA sample, 83 ppm. The lowest Zn content was in YCJA sludge sample, 234 ppm.

Table 1. Heavy metals content in the different dewatered sludge samples.

Sludge samples	Cr content±SD (ppm)	Mn content±SD (ppm)	Fe content±SD (ppm)	Cu content±SD (ppm)	Zn content±SD (ppm)
YEJA	202±85.2	254±133.5	19667±8082.9	67±7.8	279±25.0
YCJA	248±38.8	417±65.0	40333±13316.7	65±3.1	234±28.3
QLJA	342±49.0	300±37.6	23667±	83±1.5	236±6.1

			3214.6		
XGJA	251±68.7	483±44.0	87333±11590.2	76±1.7	235±2.0

In the 4 different dewatered sludge samples, the Fe content was the highest while the Cu content was the lowest. Cr, Mn and Zn were in the same medium quantity level.

3.2 Microbial community structure diversity in the sludge

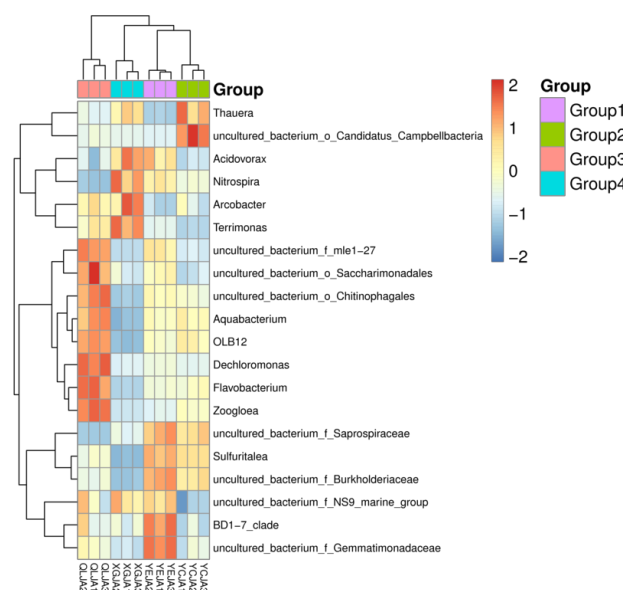


Fig.1. Microbial species abundance clustering heat map at genus level.

As shown in Fig.1, in the heat map clustering results, the color represented the genus abundance; longitudinal clustering showed the similarity of abundance of different genus among samples. The closer the distance between two genus was, the shorter the branch length was, indicating that the abundance of these two genus was more similar among samples. The horizontal clustering showed the similarity of the abundance of each genus in different samples. Just like the vertical clustering, the closer the distance between two samples, the shorter the branch length, indicating that the abundance of each genus in these two samples was more similar. The corresponding value of the heat map was the Z value obtained after the standardization of the relative abundance of genus in each row. The color gradient from blue to red indicated the relative abundance from low to high. In the figure, the first row was samples grouped information, and the color corresponded to the graph column.

3 parallel sludge samples from YEJA had 481, 480, 476 genus with 501, 502, 496 species respectively; samples from YCJA had 413, 473, 473 genus with 431, 493, 494 species respectively; samples from QLJA had 454, 460, 470 genus with 474, 481, 489 species; samples from XGJA had 474, 471, 473 genus with 490, 489, 490 species.

From Fig.1, we could infer that each wastewater treatment plant had its own unique microbial community structure diversity.

As shown in Fig.2, at genus level, different groups, which was 4 different wastewater treatment plants, had significant different microbial community structure in the statistical level. The donated microbial populations were really different.

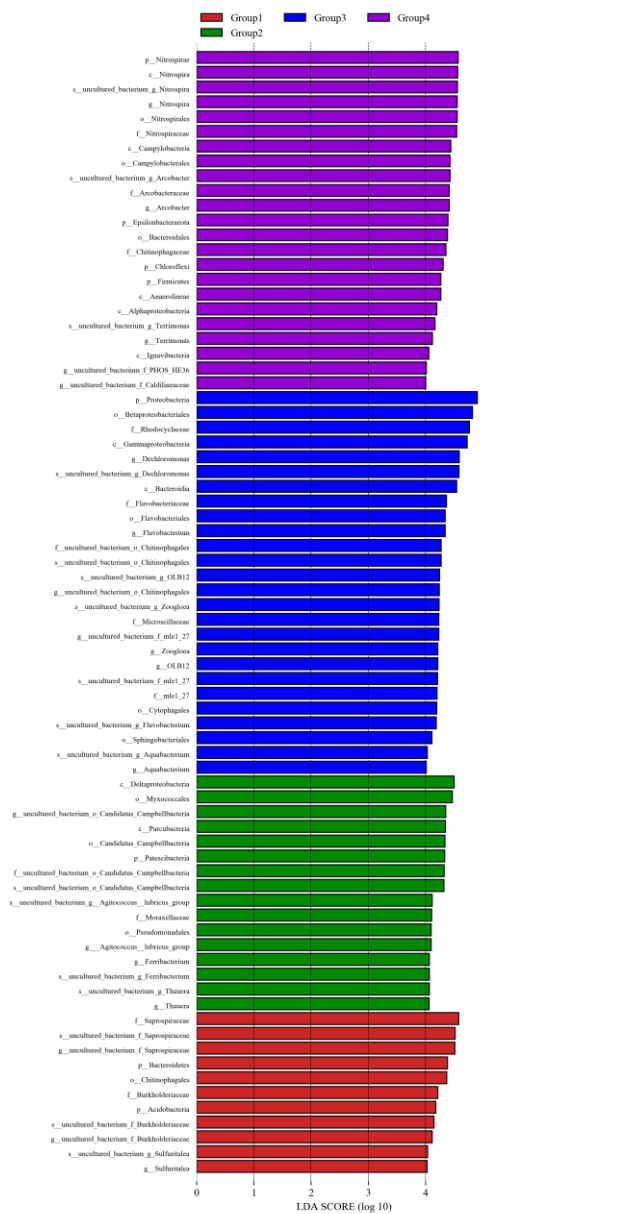


Fig.2. Efsa analysis of significant difference between groups(threshold value of LDA was 4.0).

3.3 Correlations between heavy metals and microbial community structure diversity

As shown in Fig.3, we could infer that Nitrospira and uncultured_bacterium_f_A4b were positively correlative to Cr content; Acidovorax, uncultured_bacterium_f_Saprospiraceae, Nitrospira and uncultured_bacterium_f_A4b were positively correlative to Mn and Fe content; Terrimonas,

uncultured_bacterium_f_NS9_marine_group, Arcobacter, Thauera, uncultured_bacterium_f_Saprospiraceae, Nitrospira, and uncultured_bacterium_f_A4b were positively correlative to Cu content; Terrimonas, uncultured_bacterium_f_NS9_marine_group, Arcobacter, Thauera and Nitrospira were positively correlative to Zn content significantly.

In Fig.3, we also could infer that Aquabacterium, uncultured_bacterium_o_Chitinophagales, Flavobacterium, OLB12, Dechloromonas and uncultured_bacterium_f_mle1-27 were negatively correlative to Cr content significantly; Zoogloea, Aquabacterium, uncultured_bacterium_o_Chitinophagales, Flavobacterium, OLB12 and Dechloromonas were negatively correlative to Mn content significantly; Zoogloea, uncultured_bacterium_o_Chitinophagales, Flavobacterium and OLB12 were negatively correlative to Fe content significantly; Zoogloea, Aquabacterium, uncultured_bacterium_o_Chitinophagales, Flavobacterium, OLB12, Sulfuritalea, uncultured_bacterium_f_Burkholderiaceae, uncultured_bacterium_o_Saccharimonadales, Dechloromonas and uncultured_bacterium_f_mle1-27 were negatively correlative to Cu content significantly; From Fig.3, About Zn content, the significant negative correlation microbial genus were Aquabacterium, uncultured_bacterium_o_Chitinophagales, Flavobacterium, OLB12, Sulfuritalea, uncultured_bacterium_f_Burkholderiaceae and uncultured_bacterium_f_mle1-27.

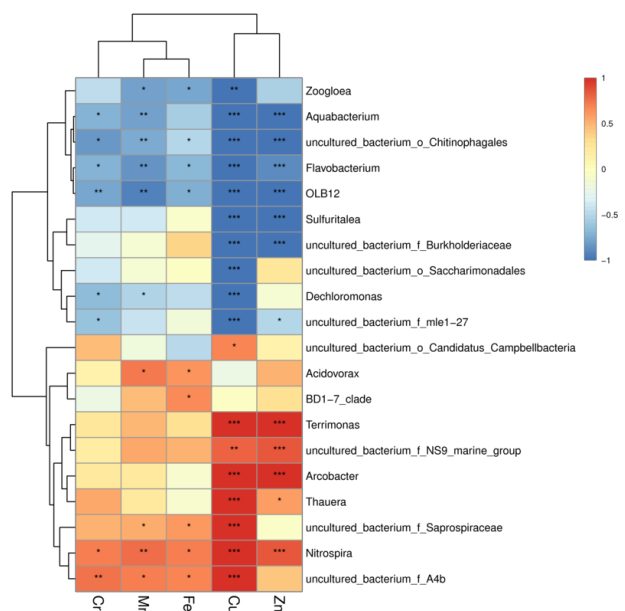


Fig.3. Correlation analysis heat map between heavy metals and microbial community structure diversity (p=0.05, genus abundance was within top 20).

4 Conclusion

The microbial community structure diversity differed and were unique among the different sewage plants.

Their correlation with heavy metals was also unique independently.

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