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Geographical Distribution and Risk Assessment of Heavy Metals in Nearby River of Heap Bioleaching Plant: A Case Study At the Zijin Copper Mine, China

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Abstract. The Zijin heap bioleaching plant was operated at the end of 2005. Concerns about the potential risk of environmental pollution from heap bioleaching plant arise due to the proximity to the Ting River. In this study, a physicochemical analysis, a geo-accumulation index and a high throughput sequencing technology were applied to determine heavy metals, assess the extent of heavy metal pollution, and research the effect of the heap bioleaching plant on the microbes, respectively. Results showed that the heap bioleaching plant had significant influence on the distribution of S, Pb and Cu and no significant influence on the distribution of As, Fe and Cr. Most of the water samples reached the third class standard of the People's Republic of China for surface water and individual water samples were above the fifth class standard of the People's Republic of China for surface water (GB3838-2002) because of As. The heap bioleaching plant had some effect on the microbial biomass, diversity and the microbial composition. However, the effect on the microbial biomass and diversity were not significant.

1. Introduction

For over half a century, bioleaching has been employed to economically extract metal from certain sulfide minerals [1]. In China, the first commercial heap bioleaching plant, with a capacity of 10,000 t.Cu/a, began operation at the Zijin Copper Mine at the end of 2005 [2].

The ZiJin Copper Mine is located in Fujian province in the southeast of China, which has richer rainfall and higher temperature than the rest of China. Due to the particular geographic location of the Zijin Copper Mine, heavy metals could be more easily to spread from the bioleaching heap to the nearby river with rainwater. In order to research and track of the safety of Zijin Copper Mine heap bioleaching plant, the periphery of the heap bioleaching plant was routine to test and risk assess from 2007.

To identify pollution problems, the anthropogenic contributions should be distinguished from the natural sources. Geochemical approaches, such as the geo-accumulation index (Igeo) [3, 4] is often used to distinguish anthropogenic contributions from the natural sources. Microorganisms are sensitive to heavy metals, and heavy metals have a significant influence on bacterial community structure, microbial biomass and microbial diversity [5], microbial ecology is an important indicator of heavy metal pollution. The application of molecular biology techniques, especially 16S ribosomal RNA high throughput sequencing technology, has progressed significantly in the field of microbial ecology [6].

In this paper, to research and track of the safety of Zijin Copper Mine heap bioleaching plant, the physical and chemical indexes of the water and sludge samples were also determined using ICP-OES, the extent and risks of heavy metal contamination were assessed by the Igeo method. Furthermore, the microbial community structure of samples from different environments were analyzed and compared using the 16S ribosomal RNA high throughput sequencing technology.

2. Materials and Methods

2.1. Sampling sites information

Ting River near Zijin Mining heap bioleaching plant (116°21′48.5″E; 25°12′13.2″N) located in Fujian province, belongs to subtropical maritime monsoon climate, the average temperature is 18.7~21.0 degrees, and the average rainfall is 1031~1369 mm.

2.2. Sample preparation and classification

19 sludge samples (S1-S19) and 11 water samples (W1-W11) were collected using sterile plastic bottles in October of 2015 year. All the samples were stored at 4℃ before determination. The spatial distribution of the sampling sites was shown in Figure 1.

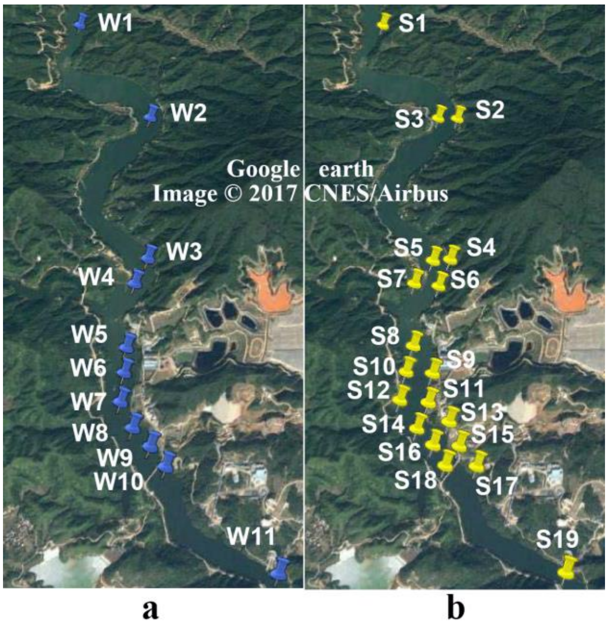

Figure 1. Sample collecting sites. Water samples collecting sites (a), Sludge samples collecting sites (b).

To convenient the analysis and research the effect of heap bioleaching plant on the nearby river, samples were divided into 3 groups according to their spatial distribution, and the details were shown in Table 1.

Table 1. Sample grouping information

Groups	Sludge samples	Water samples
Upstream	S1, S2, S3, S4, S5, S6, S7	W1, W2, W3, W4
Nearby	S8, S9, S10, S11, S12, S13, S14, S15, S16	W5, W6, W7, W8, W9
Downstream	S17, S18, S19	W10, W11

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2.3. Physicochemical analysis of the samples

Sludge was filtered and dried thoroughly in the oven, grinded into powder. About 0.5g sludge powder was weighed, added 15mL nitric acid, 5mL hydrochloric acid and 2mL hydrochloric acid successively, heated until the liquid was evaporated completely and white smoke fumed, added 5mL deionized water and heated to boiling, added 5mL hydrochloric acid and heated to boiling, then cooled and added deionized water to 10mL[1,2]. The pre-treated sludge samples were filtered with super membrane filters (0.2 μm pore size, Sigma-Aldrich) and determined using ICP-OES[5]. The river water samples were determined using ICP-OES directly after filtration with super membrane filters (0.2 μm pore size, Sigma-Aldrich) [5].

2.4. Statistical analysis

The pH, Eh and heavy metals were analysed with separately one-way ANOVA (analyses of variance). All these statistical analysis were performed using SPSS 19.0 for Windows[7].

2.5. Geo-accumulation index analysis

The geo-accumulation index (*I*geo) was used to assess the extent of heavy metal pollution in sediments. This index was originally introduced by Müller as follows [3, 8]:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5\times B_n}\right) \tag{1}$$

Where C_n is the measured metal concentration of n, and B_n is the geochemical background concentration of metal n. In this study, the background value of soils in Fujian Province was used as the Bn (with Cr, Cu, Fe and Pb concentrations equalling 14 $mg\cdot kg^{-1}$, 22.8 $mg\cdot kg^{-1}$, 4.24 $mg\cdot kg^{-1}$, 41.3$mg\cdot kg^{-1}$, respectively)[9]. A value of 1.5 was used as the background matrix-correction factor to account for the lithogeny effect. The class and pollution status of samples were assessed according to table 2.

Table 2. Class and pollution status of samples by geo-accumulation indexes (Igeo)

Igeo	Class	Pollution Status	Risk
<0	1	Unpolluted	No risk
0-1	2	Unpolluted to moderate	Low risk
1-2	3	Moderate	Moderate risk
2-3	4	Moderate to heavy	Moderate risk to high risk
3-4	5	Heavy	High risk
4-5	6	Heavy to extreme	High risk to very high risk
>5	7	Extreme	Very high risk

2.6. DNA extraction, miseq sequencing and data processing

Whole DNA of samples was extracted using the E.Z.N.A. bacterial DNA kit (OMEGA, D3350-01) according to the manufacturer's instruction. 16S rRNA genes were sequenced with the 340F/805R primers [10]. Sequencing was conducted on an Illumina miSeq high throughput sequencing technology platform [11].

Paired-end reads of the original DNA fragments from high throughput sequencing were merged using FLASH[12] and assigned to each sample according to the unique barcodes. The 16S rRNA genes were processed using an open-source software QIIME [13]. Chimera Slayer tool was used for chimera detection[14], then CD-HIT package[15] and QIIME script "pick_de_novo_otus.py"[16] were used to pick operational taxonomic units (OTUs) by making OTU table, sequences with ≥ 97% similarity were assigned to the same OTUs[17]. Representative sequences for each OTU were picked and the RDP classifier was used to annotate taxonomic information for each representative sequence[18].

3. Results and Discussion

3.1. Physiochemical properties and spatial distribution of heavy metals in the water samples

Spatial distribution patterns and physiochemical properties of heavy metals in the river were shown in Table 3-4. The Eh and pH of water samples were in the range of 313-324 mV and 6.43-7.54, respectively. The Eh of the heap bioleaching plant nearby and downstream was obviously higher ($p<0.05$) than the upstream. The pH of the heap bioleaching plant nearby was slightly higher ($p>0.05$) than the upstream and downstream respectively. This was opposite to the results of 2009 [19] and consistent with the data of Longjiang river dosing sites [5]. This may be related to the use of a large amount of soda lime for remediation in 2012 pollution incidents.

Previous reports showed that S, Cu, Fe, Pb, As and Cr are the main elements of Zijin copper mine [20]. Thus, these six elements were determined to verify heap bioleaching plant pollution and influence on the nearby river. Soluble S concentration significantly decreased in the water samples followed the river direction, the spatial distribution difference of Pb in water was not significant and Cu could hardly be detected in water samples. However, the S, Pb and Cu content in downstream sludge notably increased compared with upstream sludge ($p<0.05$). The distribution of S, Pb and Cu illustrated there was no S, Pb and Cu discharged into the river now, some S, Pb and Cu migrated into the river from the heap bioleaching plant and was precipitated into the river sludge in the past time. The spatial distribution difference of As in water and the spatial distribution differences of Fe, Cr in sludge were not notable ($p>0.05$) along the river. Furthermore, As in sludge and Fe, Cr in water could hardly be detected. These results showed that heap bioleaching plant had no significant influence on the distribution of As, Fe and Cr.

The heavy metals concentration of water samples were compared with the current national standard of the People's Republic of China for surface water (GB3838-2002). Results showed that most of the water samples reached the third class standard of the People's Republic of China for surface water (GB3838-2002), and a small amount of water samples was above the fifth class standard of the People's Republic of China for surface water (GB3838-2002) because the high concentration of As [21].

Table 3. Physiochemical properties and distribution of heavy metals in water (mg/L)

Groups	pH	Eh	As	Pb	Soluble S
Upstream	6.92±0.44	315.5±3.11	0.1±0.02	0.03±0.01	30.28±5.94
Nearby	7.04±0.44	320±2.24	0.09±0.02	0.03±0.01	24.87±7.55
Downstream	6.46±0.04	321.5±3.54	0.09±0.00	0.03±0.00	6.67±2.07

Table 4. Spatial distribution of heavy metals in sludge (mg/kg)

Groups	Cr	Cu	Fe	Pb	S
Upstream	90.9±14.6	14.9±3.5	46685.7±4697.0	20.7±14.3	197.1±98.4
Nearby	93.3±23.1	87.3±102.9	45022.2±6635.7	34.2±22.3	390.2±576.3
Downstream	132±95.2	79.3±43.9	50633.3±22171.5	70.7±53.4	680±281.6

3.2. Geo-accumulation index analysis and pollution assessment of sludge samples

The geo-accumulation index was developed by Muller based on his study on heavy metals in fluvial stream sediments [8]. It has been widely used because it takes into account the effects of the natural elements and human activities on pollution [4]. The geo-accumulation indexes of Cr, Cu, Fe and Pb were calculated in this study (Fig. 2 and Table 5). Fe had the largest geo-accumulation index，Cr came the second, and the geo-accumulation indexes of Fe and Cr had no notable changes in all the sludge samples ($p>0.05$). These results indicated that the heap bioleaching plant had no obvious effect on the Fe and Cr accumulation in sludge, the high Igeo index of Fe and Cr result from the natural sources rather than the anthropogenic contributions from heap bioleaching plant. The geo-accumulation index of Cu differed significantly ($p<0.05$), which in the heap bioleaching plant nearby and downstream samples increased notably than in upstream samples. Most of the geo-accumulation indexes of Pb were less than 0, only a few of samples from the heap bioleaching plant nearby and downstream were more than 0 and less than 1. These results implied that the anthropogenic contributions from heap bioleaching plant increased the Cu and Pb pollution of the sludge samples. However, the effect of heap bioleaching plant on Pb was subtle.

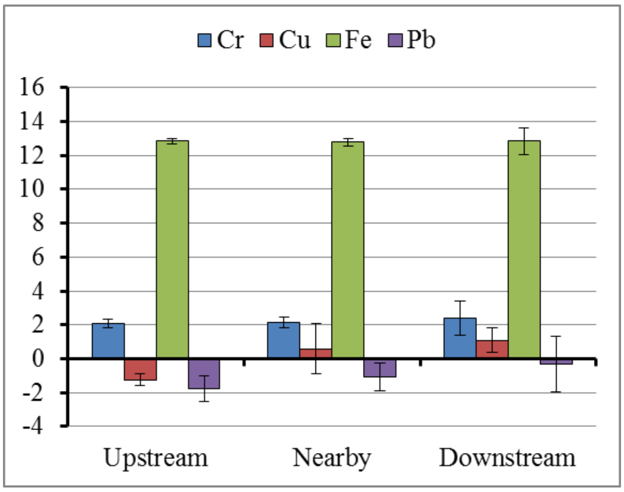

Figure 2. Geo-accumulation indexes (Igeo) of heavy metals in sludge samples

Table 5. Pollution status statistical table of various heavy metals

Class	Pollution Status	Number of samples				Percentage			
		Cr	Cu	Fe	Pb	Cr	Cu	Fe	Pb
1	Unpolluted	0	11	0	15	0.0	57.9	0.0	78.9
2	Unpolluted to moderate	0	5	0	4	0.0	26.3	0.0	21.1
3	Moderate	6	1	0	0	31.6	5.3	0.0	0.0
4	Moderate to heavy	12	1	0	0	63.2	5.3	0.0	0.0
5	Heavy	1	1	0	0	5.3	5.3	0.0	0.0
6	Heavy to extreme	0	0	0	0	0.0	0.0	0.0	0.0
7	Extreme	0	0	19	0	0.0	0.0	100.0	0.0

3.3. Microbial diversity and composition of the samples

Microbial biomass (richness) and diversity (Shannon index) results of the samples were shown in Fig.3. In the sludge samples, the microbial biomass and diversity in the downstream samples lowered than the other two groups. In the water samples, the microbial biomass and diversity decreased followed the river. However, the difference was not notable ($p>0.05$). These results indicated that the heap bioleaching plant had some effect on the microbial biomass and diversity. However, the effect of heap bioleaching plant on microbial biomass and diversity was not significant.

Microbial community structures results of different samples were shown in Fig. 4. There were some *Leptospirillum* existed in some of the upstream samples, this is consistent with the previous reports [19]. These results indicated the *Leptospirillum* is the inherent microbe of this river. More *Leptospirillum* and *Thiobacillus* existed in the the heap bioleaching plant nearby and downstream samples than in the upstream samples implied the heap bioleaching plant influenced on the microbial community structures. There are notable microbial species differences between the water and sludge samples. *Sulfuricurvum* could hardly be detected in the sludge samples, *Sulfuricurvum* was the dominant microbe in all the river water samples, and the difference in all the water samples was not notable. These results indicated *Sulfuricurvum* mainly existed in the water, and *Sulfuricurvum* was also the inherent microbe of the river water.

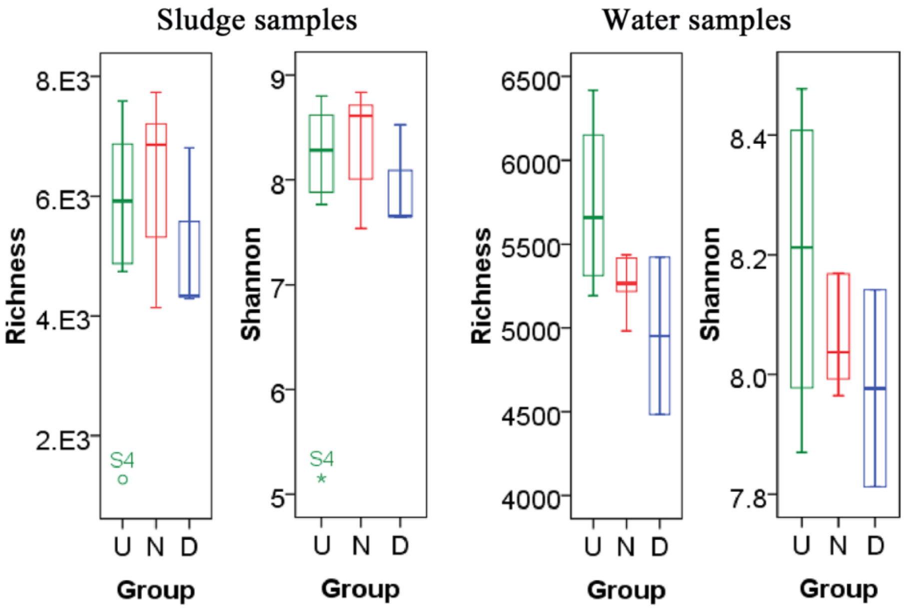

Figure 3. Microbial biomass and diversity of different samples (Upstream-U; Nearby-N; Downstream-D)

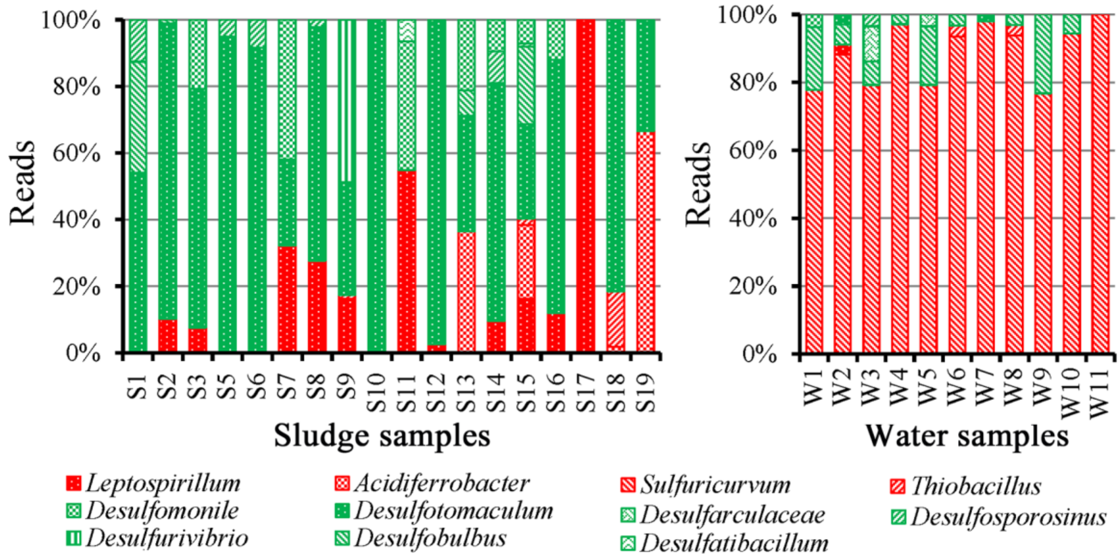

Figure 4. microbial community structures of different samples

4. Conclusions

The heap bioleaching plant had significant influence on the distribution of S, Pb and Cu and no significant influence on the distribution of As, Fe and Cr. Most of the water samples reached the third class standard of the People's Republic of China for surface water and individual water samples were above the fifth class standard of the People's Republic of China for surface water (GB3838-2002) because of As. The heap bioleaching plant had some effect on the microbial biomass, diversity and the microbial composition, however, the effect on the microbial biomass and diversity were not significant.

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