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°C 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).](image)

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.

| 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          |
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[12]. 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 (Igeo) was used to assess the extent of heavy metal pollution in sediments. This index was originally introduced by Müller as follows [3, 8]:

\[ I_{geo} = \log_2 \left( \frac{C_n}{1.5 \times B_n} \right) \]  

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·kg\(^{-1}\), 22.8 mg·kg\(^{-1}\), 4.24 mg·kg\(^{-1}\), 41.3mg·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| 90.92±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](image)

**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.

5. References
[1] Brierley C and Brierley J 2013 Progress in bioleaching: part B: applications of microbial processes by the minerals industries Applied Microbiology and Biotechnology 97 7543
[2] Ruan R, Wen J and Chen J 2006 Bacterial Heap-Leaching: Practice in Zijinshan Copper Mine Hydrometallurgy 83 77

[3] Zhang L, Liao Q, Shao S, Zhang N, Shen Q and Liu C 2015 Heavy Metal Pollution, Fractionation, and Potential Ecological Risks in Sediments from Lake Chaohu (Eastern China) and the Surrounding Rivers Int J Environ Res Public Health 12 14115

[4] Song J, Yang X, Zhang J, Long Y, Zhang Y and Zhang T 2015 Assessing the Variability of Heavy Metal Concentrations in Liquid-Solid Two-Phase and Related Environmental Risks in the Weihe River of Shaanxi Province, China Int J Environ Res Public Health 12 8243

[5] Zhang M, Huang F, Wang G, Liu X, Wen J, Zhang X, Huang Y and Xia Y 2017 Geographic distribution of cadmium and its interaction with the microbial community in the Longjiang River: risk evaluation after a shocking pollution accident Sci Rep 7 227

[6] Amann R I, Ludwig W and Schleifer K H 1995 Phylogenetic identification and in situ detection of individual microbial cells without cultivation Microbiol. Rev. 59 143

[7] Li J, Zheng Y, Yan J, Li H, Wang X, He J and Ding G 2013 Effects of different regeneration scenarios and fertilizer treatments on soil microbial ecology in reclaimed opencast mining areas on the Loess Plateau, China PLoS One 8 e63275

[8] Muller G 1969 Index of geoaccumulation in sediments of the Rhine River Geojournal 2 108

[9] Wei F, Chen J and Wu Y. Background Element Values in Soils of China. 1990, Beijing(china): China Environmental Science Press. 329.

[10] Sinclair L, Osman O A, Bertilsson S and Eiler A 2015 Microbial community composition and diversity via 16S rRNA gene amplicons: evaluating the illumina platform PLoS One 10 e0116955

[11] Unno T 2015 Bioinformatic Suggestions on MiSeq-based Microbial Community Analysis J Microbiol Biotechnol

[12] Magoc T and Salzberg S L 2011 FLASH: fast length adjustment of short reads to improve genome assemblies Bioinformatics 27 2957

[13] Kuczynski J, Stombaugh J, Walters W A, Gonzalez A, Caporaso J G and Knight R 2012 Using QIIME to analyze 16S rRNA gene sequences from microbial communities Curr Protoc Microbiol Chapter 1 Unit 1E 5

[14] Haas B J, Gevers D, Earl A M, Feldgarden M, Ward D V, Giannoukos G, Ciulla D, Tabbaa D, Highlander S K, Sodergren E, Methe B, DeSantis T Z, Human Microbiome C, Petrosino J F, Knight R and Birren B W 2011 Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons Genome Res 21 494

[15] Li W, Fu L, Niu B, Wu S and Wooley J 2012 Ultrafast clustering algorithms for metagenomic sequence analysis Brief Bioinform 13 656

[16] Caporaso J G, Kuczynski J, Stombaugh J, Bittinger K, Bushman F D, Costello E K, Fierer N, Pena A G, Goodrich J K, Gordon J I, Huttenhower G A, Kelley S T, Knights D, Koenig J E, Ley R E, Lozupone C A, McDonald D, Muegge B D, Pirrung M, Reeder J, Sevinsky J R, Turnbaugh P J, Walters W A, Widmann J, Yatsunenko T, Zaneveld J and Knight R 2010 QIIME allows analysis of high-throughput community sequencing data Nat Methods 7 335

[17] Li W and Godzik A 2006 Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences Bioinformatics 22 1658

[18] Wang Q, Garrity G M, Tiedje J M and Cole J R 2007 Naïve bayesian classifier for rapid assignment of rna sequences into the new bacterial taxonomy Applied and environmental microbiology 73 5261

[19] Liu X, Chen B, Chen J, Zou L, Zhang M, Wen J and Liu W 2016 Biogeographical distribution of acidophiles and their effects around the Zijinshan heap bioleaching plant Chemistry and Ecology 32 419

[20] Liu X, Chen B, Chen J, Mingjiang Z, Jiankang W, Dianzuo W and Renman R 2016 Spatial variation of microbial community structure in the Zijinshan commercial copper heap bioleaching plant Minerals Engineering 94 76

[21] Administration C S E P. (2002) standard of the People's Republic of China for surface water Beijing.
Acknowledgments
This work was supported by the National Natural Science Foundation of China under grant numbers U1402234, 41573074; the nation high-level youth talents special support plan; the Guangxi scientific research and technology development plan under grants GuikeAB16380287 and GuikeAB17129025; the public welfare fund of the Ministry of Environmental Protection of People's Republic of China under grant number 201509049; Program of International S & T Cooperation 2016YFE0130700; and the fund of General Research Institute for Nonferrous metals under grant numbers 53321 and 53348.