Responses of microbial community to different concentration of perchlorate in the Qingyi River

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Abstract

Perchlorate is a refractory and mobile contaminant that is widely distributed in surface water, and due to its tremendous inhibitory effect on mammalian thyroid function, it has gained much attention in recent years. Numerous studies have focused on environmental detection of perchlorate, especially in water. However, less attention has been paid to the effects of perchlorate on the composition of the microbial community in rivers. Upstream of the Qingyi River, an important source of drinking water for local residents, there are two perchlorate manufacturers. In this study, we selected eight study sites from upstream to downstream of the Qingyi River, including sites located upstream and downstream of the perchlorate manufacturers. Our results indicated that perchlorate was detected in all sites except for QYR2, QYR3, and QYR10. The concentration of perchlorate in the five study sites was much higher than the reference dose proposed by the National Academy of Science, and ranged from 187 to 9647.00 µg/L. We utilized 16S rDNA high throughput sequencing to analyze changes in the composition of the microbial community, based on the Illumina 2×250 MiSeq platform. The results showed that, when microbial communities were exposed to high concentration of perchlorate, there was an increase in the ratio of *Betaproteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Saccharibacteria* in the microbial community along with a decrease in the ratio of *Chloroflexi*, *Verrucomicrobia*, and *Gammaproteobacteria*. Our study has provided a theoretical basis for the alteration of the microbial community caused by the perchlorate pollution, which may have a truly important impact on the quality of groundwater.

Keywords Perchlorate · Microbial community · Qingyi river · Biodiversity

Introduction

Perchlorate is a relatively new environmental pollutant that has attracted increasing attention recently. Due to its industrial release and the historic use of chlorine-containing

fertilizers, perchlorate is found widely in soil and groundwater. Because of its chemical stability, it has become a persistent and extremely refractory contaminant (Kang et al. 2006; Balaji et al. 2007; Rajagopalan et al. 2009). Understandably, perchlorate pollution poses serious risk as a hazard for animal and human life (Bulaeva et al. 2015; Flood and Langlois 2014; Opitz et al. 2009). In humans, ingestion of perchlorate may cause adverse health effects as it could inhibit absorption of iodide (Massimo et al. 2004; Leung et al. 2010).

Perchlorate can be detected in rainwater, snow, and groundwater (Tomassini 2010). It is highly mobile and soluble in water, and chemically stable under near-surface toxic environmental conditions (Poghosyan et al. 2014). It is well known that hundreds of tons of perchlorate are produced and sold throughout China, and that there are several perchlorate manufacturers located in the upper reaches of the Qingyi River. Unfortunately, chlorine-containing sewage is discharged directly into the Qingyi River without being treated, a potentially dangerous situation as the Qingyi River

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is the most important source of drinking water for the city of Ya'an.

Despite these concerns, there are few studies that have focused on the relationship between perchlorate contamination and microbial diversity. In this work, we used the Qingyi River as a research object to investigate perchlorate contamination and the resulting changes in microbial diversity. Our study is an attempt to provide a fundamental analysis for changes in the microbial community caused by perchlorate pollution and further elaborate its impact on the quality of groundwater.

Methods

Study area and sample collection

This study was conducted in Qingyi River, which is located in the Sichuan Province in the southwest of China. The Qingyi River originates from the Shuxiying Mountain with an altitude of 4930 m. It is the convergence of the Baoxin River, the Yingjing River, the Tianquan River, and the Sijing River. The samples collected in this paper were from some tributaries of the Qingyi River, such as the Xingjing Creek, the Tianquan Creek, and the Sijing Creek. The eight sample sites in this paper were selected out from national waters as follows: the monitoring point of the Qingyi River, the

confluence of the Sijing Creek and the Tianquan Creek (QYR2), upstream of Sijing Chang Town (QYR3), downstream of Sijing Chang Town (QYR4), the confluence of Tianquan Creek and Yingjing Creek (QYR6), downstream of the Miaogang Bridge (QYR9), upstream of the Miaogang Bridge (QYR10), the Pig's Mouth Section (QYR12), and the Guidufu Section (QYR13) (Fig. 1). Additionally, two perchlorate manufacturers operate along the Qingyi River. Perchlorate manufacturer A was located between QYR3 and QYR4, and perchlorate manufacturer B was located between QYR9 and QYR10. The red solid circle in Fig. 1 represents the location of the perchlorate manufacturers.

All water samples (≈ 2.5 L) were collected in triplicate and stored directly at 4 °C in September of 2016. The water samples were approximately 500 mL and were filtrated using vacuum filtration through a 0.45 μm . The filtrate was then used for further analysis. Other water samples were concentrated by vacuum filtration through a 0.22 μm nitrocellulose filter for environmental DNA extraction.

Physicochemical analysis of water samples

Conductivity, chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), Sulfide (S), Sulfate (SO_4^{2-}), and ammonium nitrogen (N-NH_4) were measured based on the standard methods previously described (Huang 2000). Temperature was determined, the conductivity was

Fig. 1 Location of 8 sampling sites along the Qingyi River in Ya'an City. The tributary, upstream, downstream sites along the Qingyi River are abbreviated as QYR2, QYR3, QYR4, QYR6, QYR9, QYR10, QYR12, QYR13. The red solid circle represented the perchlorate manufacturer, and the A represented the perchlorate manufacturer located between QYR3 and QYR4, B represented the perchlorate manufacturer located between QYR9 and QYR10

measured with a conductivity tester (Leici DDS-307A, Shang Hai, China), and the pH was determined with a pH meter (Leici PHS-3C, Shang Hai, China). Next, we obtained measurements of the ClO_4^- and Cl^- ions in the water samples using an ion chromatography (IC) system (Dionex ICS-1000), as previously described (Zhang et al. 2015). All data were performed in triplicate.

DNA extraction

Total environmental genomic DNA passed through a 0.22 μm filter was isolated using a Power Water DNA Isolation kit (MoBio Laboratories, Inc. USA), according to the manufacturer's instructions. The quality and concentration of the isolated environmental DNA was determined with a NanoDrop Spectrophotometer (Thermo Fisher Scientific Inc., USA). DNA samples were stored at -20°C for 16S rRNA gene sequencing.

Illumina MiSeq sequencing of environmental samples

The sequencing library was generated and Illumina MiSeq sequencing was performed at Novo ge, Ltd. (Peking, China). Sequencing of the V3–V4 region amplicons was performed following the manufacturer's instructions (Illumina, San Diego, CA, USA) (Caporaso et al. 2010, 2012). Briefly, the V3–V4 hypervariable region was amplified with the barcoded primers 343F/804R (343F: TACGGRAGGCAG CAG, 804R: CTACCRGGGTATCTAATCC) (Zeng et al. 2015; Gunajit et al. 2017; Liu et al. 2017). All PCR reactions were performed using a T100™ Thermal Cycler (Bio-Rad, USA). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), following the manufacturer's recommendations, with index codes added. The quality of the library was assessed on a Qubit® 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina 2 × 250 MiSeq platform.

Data analysis

All raw data were processed through the QIIME 1.80 platform (Caporaso et al. 2010, 2012), and paired-end reads were merged with a combination of the FLASH V1.2.7 and the PANDASEQ 2.9 (Magoc and Salzberg 2011). Assembled sequences were high-quality filtered and analyzed with QIIME (Bokulich et al. 2013; Caporaso et al. 2010, 2012). The chimera sequences were detected and removed, according to the UCHIME algorithm 4.2.40 (Edgar et al. 2011). The operational taxonomic units (OTUs) were carried out using UPARSE 7.0, with $\geq 97\%$ similarity (Edgar 2013). For each representative sequence, the Unite Database was used

based on the Blast algorithm that was calculated with the QIIME software (Version 1.7.0) to annotate the taxonomic information. The phylogenetic relationship of different OTUs was conducted using the MUSCLE software 3.8.31 (Edgar. 2004). OTUs abundance information was normalized using a standard of the sequence number corresponding to the sample with the least sequences and alpha diversity and beta diversity were conducted with the normalized data. Alpha diversity was calculated with QIIME (Version 1.7.0) and displayed using R software (Version 2.15.3). Beta diversity, such as principal component analysis (PCA), principal coordinate analysis (PCoA), and non-metric multidimensional scaling (NMDS), was conducted by QIIME and R package vegan (Zhang et al. 2018).

Results and discussion

The Qingyi River has long served as a source of drinking and irrigation water for nearby residents. Perchlorate is a known competitive inhibitor of iodide, and can interfere with the normal functioning of the thyroid, thus altering the functioning of the thyroid itself (Fisher and McLanahan 2008, Srinivasan and George 2009; York et al. 2005; Goleman et al. 2006; Opitz et al. 2009). Long-term exposure to perchlorate may result in widespread thyroid disease or fetal teratogenicity in the area. Physicochemical analysis of the water in the samples examined herein has revealed a high concentration of perchlorate, except in the areas QYR2, QYR3, and QYR10, which were located upstream of the perchlorate manufacturer (Table 1; Fig. 1). The concentration of perchlorate in areas QYR4 and QYR9 was especially high, reaching 3285.00 $\mu\text{g/L}$ and 9647.00 $\mu\text{g/L}$, respectively. The areas QYR4 and QYR9 were located near the two perchlorate manufacturers (Fig. 1). Our findings indicated that industrial wastewater from perchlorate manufacturing has caused tremendous perchlorate contamination in the Qingyi River. The concentration of perchlorate exhibited an obvious declining trend from upstream to downstream, yet the concentration of perchlorate in area QYR13 maintained the high concentration of 187.00 $\mu\text{g/L}$, which was nearly 7 times more than the reference dose proposed by the National Academy of Science (Rajagopalan et al. 2009, National Academy of Sciences 2005).

We examined the microbial communities in the Qingyi River at different levels, such as the class, family, genus, order, and phylum levels, based on the OTUs data. As described in Fig. 1, the areas QYR2, QYR3, QYR4, QYR6, QYR9, QYR10, QYR12, and QYR13 were selected as sites for this study. For each of the study sites, the ratio of each microbial community was averaged and the final ratio of every microbial group was obtained. To visually investigate the effect of perchlorate on the

Table 1 Physicochemical characteristics of the water samples

Study sites	pH	Temperature	Conductivity (us/cm)	N (mg/L)	P (mg/L)	CODcr (mg/L)	Sulfide (mg/L)	NH ₄ ⁺ (mg/L)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	Perchlorate (μg/L)
QYR2	7.66	8.8	250	0.824	0.158	8	0.064	0.16	2.5141	42.2393	0.00
QYR3	7.72	9.3	267	2.170	0.105	6.6	0.015	0.08	1.2027	30.9067	0.00
QYR4	7.81	9.3	384	2.200	0.100	7.2	0.005	0.32	16.0668	33.8613	3285.00
QYR6	7.04	9.6	270	1.000	0.158	8.9	0.038	0.13	5.5975	42.7382	647.00
QYR9	7.76	10.2	374	1.160	0.211	16.6	0.056	0.04	53.7889	18.8637	9647.00
QYR10	7.81	10.8	207	1.230	0.168	0.3	0.041	0.04	2.1836	19.7702	0.00
QYR12	7.25	9.5	293	0.910	0.710	0.6	0.010	0.08	3.8254	40.6691	253.00
QYR13	7.28	9.7	310	1.060	0.790	7.6	0.013	0.12	3.7383	47.4500	187.00

environmental microbial community, the typical sites QYR3, QYR4, QYR9, and QYR10 were chosen for further analysis based on their location upstream and downstream of the perchlorate manufacturer (location details see Fig. 1).

Based on the OTUs data, the relative abundance of the microbial community in phylum, class, order, family, and genus was analyzed (Fig. 2). A total of ten bacterial phyla, including one unknown taxonomic population, were detected in the eight study sites. Among all eight study sites, the microbial community was dominated by *Proteobacteria*, as previously reported (Archilla et al. 2001). The ratio of *Proteobacteria* in the different sites was 73.9%, 74.0%, 49.1%, 73.4%, 68.8%, 66.7%, 41.5%, and 46.9% in QYR2, QYR3, QYR4, QYR6, QYR9, QYR10, QYR12, and QYR13, respectively. Interestingly, there were obvious differences in the microbial communities: some genera increased significantly, while some genera decreased significantly.

At the phylum level (Fig. 2e), the ratio of *Saccharibacteria*, *Bacteroidetes* was higher in areas QYR4 and QYR9 compared with QYR3 and QYR10, yet, the ratio of *Chloroflexi*, *Verrucomicrobia* was lower in areas QYR4 and QYR9 compared with QYR3 and QYR10. At the class level (Fig. 2a), the ratio of *Betaproteobacteria*, *Thermoleophilia*, *Sphingobacteriia*, and *Flavobacteriia* was higher in QYR4 and QYR9 compared with QYR3 and QYR10. At the order level (Fig. 2d), the ratio of *Burkholderiales*, *Solirubrobacterales*, *Sphingobacteriales*, and *Flavobacteriales* was higher in QYR4 and QYR9 compared with QYR3 and QYR10. At family level (Fig. 2b), the ratio of *Comamonadaceae*, *Chitinophagaceae*, and *Flavobacteriaceae* was higher in QYR4 and QYR9 compared with QYR3 and QYR10, while the ratio of *Pseudomonadaceae* was lower in QYR4 and QYR9 compared with QYR3 and QYR10. At genus level (Fig. 2c), the ratio of *Burkholderiales*, *Solirubrobacterales*, *Sphingobacteriales*, and *Flavobacteriales* was higher in QYR4 and QYR9 compared with QYR3 and QYR10. As shown in Fig. 2, the composition of microbial communities was quite different among the eight study sites, and the dominant genera of microbial communities at these sites were quite different. We think this may be related to the physicochemical properties of rivers.

It's well known that the diversity of aquatic microbial communities is considered a key component of a functioning aquatic ecosystem. Microbial taxonomic turnover could mediate ecosystem-scale responses to changing environmental conditions, including the physicochemical factors of rivers (Zeglin 2015; Nold and Zwart 1998; Cotner and Biddanda 2002; Gessner et al. 2010). There were two perchlorate manufacturers located near the Qingyi River (location details see Fig. 1) and perchlorate was detected in sites QYR3 and QYR9 with the higher concentrations of 3285.00 μg/L and 9647.00 μg/L, respectively. We believe

Fig. 2 Relative abundance of microbial groups in every study site at class (a), family (b), genus (c), order (d), and phylum (e) levels

that the high concentration of perchlorate in the Qingyi River resulted in the changing of microbial communities. Thus, the component of microbial communities was quite different between sites QYR3 and QYR4. The microbial communities of other sites located upstream also changed.

In aquatic ecosystems, microbial activity plays an important role in ecosystem function, and the components of the microbial community may change to keep with changes in the environment (Zeglin 2015; Nold and Zwart 1998; Cotner and Biddanda 2002; Gessner et al. 2010). It is obvious that, when the microbial communities were exposed to a high concentration of perchlorate, the ratio of *Saccharibacteria*, *Bacteroidetes*, *Betaproteobacteria*, *Thermoleophila*, *Sphingobacteriia*, *Flavobacteriia*, *Burkholderiales*,

Solirubrobacterales, *Sphingobacteriales*, *Flavobacteriales*, *Comamonadaceae*, *Chitinophagaceae*, *Flavobacteriaceae*, *Burkholderiales*, *Solirubrobacterales*, *Sphingobacteriales*, and *Flavobacteriales* increased and that the rate of *Chloroflexi*, *Verrucomicrobia*, and *Pseudomonadaceae* declined. *Burkholderiales*, *Comamonadaceae*, and *Burkholderiales* were classified into *Betaproteobacteria*, while *Bacteroidetes*, *Sphingobacteriia*, *Sphingobacteriales*, *Chitinophagaceae*, *Flavobacteriaceae*, *Flavobacteriales*, and *Flavobacteriia* were classified into *Bacteroidetes*. *Solirubrobacterales*, *Solirubrobacterales*, and *Thermoleophila* were classified into *Actinobacteria*, and *Pseudomonadaceae* was classified into *Gammaproteobacteria*. Thus, when microbial communities were exposed to a high concentration of perchlorate, the

ratio of *Betaproteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Saccharibacteria* rose and the ratio of *Chloroflexi*, *Verrucomicrobia*, and *Gammaproteobacteria* declined. As previously reported, many perchlorate-reducing strains are classified as *Betaproteobacteria*, *Dechlorospirillum*, *Azospira*, and *Dechlorospirillum* (Achenbach et al. 2001; Zhang et al. 2002; Coates et al. 1999; Coates and Achenbach et al. 2004; Ye et al. 2012). Therefore, we could infer that the existence of perchlorate resulted in the increasing of perchlorate-reducing strains, such as *Betaproteobacteria*, and decline of some other genera, such as *Chloroflexi*, *Verrucomicrobia*, and *Gammaproteobacteria*. Although the ratio of *Bacteroidetes*, *Actinobacteria*, and *Saccharibacteria* increased, their role in the microbial community remains unknown.

Next, we investigated the shared microbial genera that existed between the different samples using a Venn diagram (Fig. 3a). Approximately a quarter (1283 genera, 25.61%) of the total genera detected were common to QYR2, QYR6, QYR12, and QYR13. Meanwhile, an approximate 24.82% of the total genera were shared by QYR9, QYR10, QYR12, and QYR13 (Fig. 3b). Yet, only about 18.96% of the total genera were shared by QYR3, QYR4, QYR12, and QYR13 (Fig. 3c). Moreover, 9.54% of the total genera were shared by QYR9 and QYR10 and only 4.23% of the total genera

were shared by QYR3 and QYR4 (Fig. 3b, c). On the contrary, they shared about 39.02% of the total genera (Fig. 3a). These study sites were clearly close to each other, as can be seen from Fig. 1. However, because the rate of shared genera was different, suggested that the existence of perchlorate in river may have changed the microbial diversity. To illustrate the diversity of bacterial communities, the Shannon and Simpson diversity index was applied and found to be higher in QYR2, QYR6, QYR9, QYR10, QYR12, and QYR13 than in QYR3 and QYR4. Moreover, the value of the diversity index presented similarity among QYR2, QYR6, QYR9, QYR10, QYR12, and QYR13, but higher than QYR3 and QYR4 (Table 2).

PCoA is a type of method that studies the similarities and variances of data (Xiao et al. 2016). We calculated the microbial assemblages of each sample under different levels of perchlorate pollution based on the QIIME software (Version 1.7.0). As shown in Fig. 4, the principal component 2 (PC2) explained 25.5% of the variation and the principal component 1 (PC1) explained 40.48% of the variation. Also evident from Fig. 4, is the similarity of the community structures of different sites present in the four groups. We also investigated the composition of the bacterial community in the different sites using non-metric multidimensional scaling

Fig. 3 Venn diagrams of shared OTUs (number of OTUs) among eight sites. The shared OTUs between (a) QYR2, QYR3, QYR12 and QYR13; (b) QYR9, QYR10, QYR12 and QYR13; QYR3, QYR4, QYR12 and QYR13

Table 2 Alpha diversity among microbial communities of study samples

Sample name	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage	PD whole tree
QYR2	1839	7.894	0.988	2731.948	2852.885	0.972	0.972
QYR3	995.333	4.265	0.674	1877.663	1448.124	0.986	0.986
QYR4	1146.333	6.491	0.953	1616.039	1676.156	0.984	0.984
QYR6	1892	7.598	0.971	2808.879	2970.638	0.971	0.971
QYR9	1788.75	7.526	0.978	2460.915	2628.142	0.975	0.975
QYR10	2096.666	8.347	0.99	2717.242	2850.767	0.974	0.974
QYR12	1651.667	7.022	0.954	2300.689	2394.217	0.977	0.977
QYR13	1923	7.899	0.98	2559.222	2696.994	0.975	0.975

Fig. 4 PCoA (principal co-ordinates analysis) among microbial communities of eight study sites

Fig. 5 NMDS (Nonmetric Multidimensional Scaling) among microbial communities of eight study sites

(NMDS) analysis (Fig. 5). The results, as shown in Fig. 5, reveal that the stress of NMDS was 0.1, suggesting that the result is representative.

A heat map was utilized to analyze the hierarchical clustering, based on the similarities and differences in the microbial community that were conducted among

Fig. 6 Taxa heat map analysis among microbial communities of eight study sites

the eight study sites. The changes in the abundance of microbial genera were visually examined with the color changes in graph. The differences of microbial genera among all study sites were examined at the genus, family, order, class, and phylum levels, results from which are displayed in Fig. 6, and which were consistent with those results found in Fig. 2. The existence of perchlorate results in altering of the composition of the microbial community. As shown in Fig. 6c, *Bacillus*, *Flavobacterium*, *Mycobacterium*, *Aeromicrobium*, and *Devosia* were shared by QYR3 and QYR4. *Pseudomonas* and *Brevundimonas* were solely detected in QYR3, while *Lactococcus* and *Nocardioide*s were detected in QYR4. *Aquabacterium*, *Pseudarcicella*, *Limnhabitans*, *Sediminibacterium*, *Perluclidibaca*, and *Acinetobacter* were solely detected in QYR9 and *Brevundimoas* and *Sphingomonas* were obviously observed in QYR10.

Conclusions

Perchlorate is a ubiquitous environmental contaminant. The widespread presence of perchlorate in water may change the microbial community, especially in river water. However, there are few studies addressing this issue. In the present study, we focused on the distribution of perchlorate in the Qingyi River and the effect of perchlorate on its microbial community. Our findings were that perchlorate was widely detected in the Qingyi River, with concentrations ranging from 0 to 9467 µg/L. The ratio of *Betaproteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Saccharibacteria* had risen, while the ratio of *Chloroflexi*, *Verrucomicrobia*, and *Gammaproteobacteria* declined. These results demonstrated there was a strong relationship between of microbial community alterations and varying concentrations of perchlorate.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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