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Key Points:

- Vanadium distributions in soil, water, and sediment were systematically investigated.
- Microbial communities were distinct in specific matrices.
- Bioremediation by indigenous microorganisms is feasible with long-term cultivation.

Abstract

Vanadium mining activities can cause contamination of the surrounding geological environment. Vanadium may exist in multiple matrices due to its migration and transformation, forming interactive relationships; however the connection between vanadium distributions in multiple matrices and microbial community responses remains largely unknown. Vanadium is a redox-sensitive metal that can be microbiologically reduced and immobilized. To date, bioremediation of vanadium-contaminated environments by indigenous microorganisms has rarely been evaluated. This paper reports a systematic investigation into vanadium distributions and microbial communities in soils, water, and sediment from Panzhihua, China. Large vanadium contents of 1130.1 ± 9.8 mg kg^{-1} and 0.13 ± 0.02 mg L^{-1} were found in surface soil and groundwater. Vanadium in surface water tended to precipitate. Microbial communities isolated from similar environments were alike due to similarity in matrix chemistry, whereas communities were distinct when compared to different matrices, with relatively lower richness and diversity in groundwater. *Proteobacteria* was distributed widely and dominated microbial communities within groundwater. Redundancy analysis shows that vanadium and nutrients significantly affected metal-tolerant bacteria. Long-term cultivation (240 d) suggests the possibility of vanadium bioremediation by indigenous microorganisms, within acid-soluble fraction. This active fraction can potentially release mobile vanadium with shifted redox conditions. Vanadium (V) was bio-reduced to less toxic, mobile vanadium (IV) primarily by enriched *Bacillus* and *Thauera*. This study reveals the biogeochemical fate of vanadium in regional geological environments, and suggests a bioremediation pathway via native vanadium-reducing microbes.

1 Introduction

Heavy metal pollution of the geological environment is a serious environmental issue (Hochella et al., 2005; Gough et al., 2008; Ran et al., 2015), vanadium has gained increasing attention as an emerging contaminant (Yelton et al., 2013; Zhang et al., 2015a). Vanadium, an essential trace element, plays an important role in human health (Rowell et al., 1998; Crans et al., 2004). However, high concentrations of vanadium are harmful (Jayawardana et al., 2014). The U.S. American Conference of Governmental Industrial Hygienists has adopted an exposure limit of 0.05 mg m^{-3} for vanadium as inhalable particulate matter (Assem & Levy, 2009). Phosphate metabolism can be adversely affected by vanadium (Zhang et al., 2014). Ingestion of vanadium can lead to serious diseases, including pulmonary tumors (Chen & Liu, 2017). Additional exposure ailments include: allergic reactions such as asthma, conjunctivitis, and rhinitis. Lung disease is common among workers engaged in vanadium production (Teng et al., 2006). Vanadium widely exists in the Earth's crust alongside minerals, crude oil, and coal (Huang et al., 2015). Global and European vanadium concentrations in soil typically range between 90 and 60 mg kg^{-1} (Yang et al., 2014b). In China, the soil background levels of vanadium are estimated at 82 mg kg^{-1} (Teng et al., 2011). Vanadium enters water naturally through geological weathering of vanadium-containing minerals (Cole et al., 2017). Anthropogenic activities, especially intensive mining of vanadium-bearing minerals, result in excessive amounts of vanadium being released into regional geological environments, causing severe public health issues (Zhang et al., 2012; Chen & Liu, 2017).

Vanadium contamination of the geological environment is widespread globally. Table S1 (Supporting Information (SI)) lists vanadium distributions in solid matrices including soil and

sediment. Surface water in Panzhihua region, Southwestern China also contains high concentrations of vanadium, ranging from 0.076 to 0.285 mg L^{-1} in Jinsha River near a tailing impoundment, exceeding the recommended limit of 0.05 mg L^{-1} for drinking water sources based on standards of China (Wang et al., 2009). In Rifle, Colorado, USA, vanadium concentration in groundwater reached 0.77 mg L^{-1} near a tailing impoundment (Ortiz-Bernad et al., 2004). The Rifle site comprises a former vanadium and uranium ore processing facility. Remediation efforts, such as pilot-scale *in situ* biosequestration and acetate-amended field biostimulation, have been found effective at controlling vanadium (Yelton et al., 2013; Xu et al., 2017). To date, most studies have focused on vanadium within a specific environmental matrix, little is presently known about the connection of vanadium distributions in multiple matrices in regional geological environments.

Microorganisms perform vital functions in environmental ecosystems and are sensitive to environmental stress (Batson et al., 2015; Moore et al., 2018). Accumulated vanadium in such matrices can substantially alter microbial communities (Yang et al., 2014a). Decreased microbial richness and diversity in soil, and reduced soil basal respiration have been found to correlate with vanadium pollution (Cao et al., 2017; Xiao et al., 2017). Nevertheless, characteristics of microbial communities with vanadium present in other environmental matrices, such as water and sediment, have rarely been investigated. For remediation, vanadium has to be removed from these matrices to avoid negative ecological impacts. Conventional adsorption is often employed, but can only transfer vanadium in aqueous phase to solid medium, which may lead to secondary pollution (Gao et al., 2017). In addition, there are three oxidation states of vanadium in the natural environment: vanadium (III), vanadium (IV), and vanadium (V) (Carpentier et al., 2003). V(V) is the most toxic, whereas V(IV) is less toxic and can precipitate in near-neutral conditions; thus reduction of V(V) to V(IV) is recognized as a promising remediation strategy (Imtiaz et al., 2015). Although traditional chemical/electrochemical reductions can achieve this transformation (Wilson and Weber, 1979; Zhang et al., 2009), their cost-effectiveness is questionable and limits field application.

Microorganisms can reduce V(V) to V(IV) at limited expense (Liu et al., 2017). Microbial V(V) reduction takes place via two pathways: respiration of V(V) through electron transfer, and reduction by microbes for detoxification purposes with vanadium binding to reductases of other electron acceptors (Liu et al., 2016). Pure cultures of *Geobacter metallireducens*, *Shewanella oneidensis* and methanogens appear to function well in terms of this transformation (Zhang et al., 2014). Mixed anaerobic cultures from wastewater treatment plant also detoxify V(V) efficiently due to their greater microbial diversity, adaption, and self-evolution (Zhang et al., 2015a). Comparative evaluation of the performance of dissolved organic carbon sources shows that acetate supports highly-efficient V(V) bio-reduction (Liu et al., 2016). Oxidation of gaseous and solid electron donors, such as hydrogen and elemental sulfur (Jiang et al., 2018; Zhang et al., 2018), can also be coupled to V(V) bio-reduction without residual organics. Furthermore, coexisting common electron acceptors in groundwater, such as nitrate and sulfate, can decrease the efficiency of V(V) bio-reduction since they are more efficient electron acceptors (Liu et al., 2017). However, most existing studies were carried out in engineered systems, with cultures from wastewater treatment facilities. Although bioremediation of vanadium-contaminated environments by indigenous microorganisms is of particular importance, few studies have been carried out.

The present study reports a systematic investigation into vanadium distributions and responses of microbial communities in multiple environmental matrices, including surface soil, vertical soil profile, surface water, sediment, and groundwater. Microorganisms in surface soils and waters were experimentally cultivated with V(V) stress in a laboratory setting to assess the bioremediation potentials of native microorganisms and to discover functional species. Our results provide a detailed understanding of biogeochemical processes related to vanadium which will promote development of effective future strategies for the bioremediation of vanadium-polluted environments.

2 Materials and Methods

2.1 Site Description and Sample Collection

Sampling sites were located in the Panzhihua region (26°05′-27°21′ N, 101°08′-102°15′ E), Sichuan Province, China, at elevation about 1200 m above sea level. Average temperature ranged from 19.7 to 20.5 °C and average annual rainfall was 860 mm. Vanadium mining and smelting activities have been conducted in the region since the 1960s. Samples were collected from four different processing stages, including mining plant (MP), concentrator (CO), smelter (SM), and tailing reservoir (TR) (Fig. S1 SI) in April 2016. Four surface soil (0-10 cm layer) samples (MP-SL0, CO-SL0, SM-SL0, TR-SL0) and four corresponding groundwater samples (MP-GW0, CO-GW0, SM-GW0, TR-GW0) were collected. “SL” and “GW” stand for soil and groundwater, respectively. The number “0” indicates the original samples from the collection. Vertical soil profile samples at depths of 0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm (TR-S1-6) were extracted in the TR area, within 500 m of TR-SL0. Two surface water samples were obtained; one from the TR area (TR-SW0), the other from Jinsha River (JS-SW0). Two sediment samples at TR-SD0 and JS-SD0 were also collected. One tailing sample (TR-TL0) was also collected from TR for reference. “SW”, “SD” and “TL” refer to surface water, sediment and tailing, accordingly. All samples consisted of five subsamples, mixed thoroughly (Cao et al., 2017). Approximately 1.5 kg soil/sediment was selected for each solid sample and packaged within polyethylene bags, and 20 L water was collected for each aqueous sample and stored in polyethylene buckets. All samples were stored at -20 °C within 48 h of collection.

2.2 Physicochemical Analysis

Soil samples were air-dried at ambient temperature (~ 20 °C) and sieved through a 2 mm mesh. pH (soil:water = 1:2.5) was assessed using HI 3221 pH/ORP meter (Hanna Instruments Inc., USA). Organic matter (OM), total nitrogen (TN), available phosphorus (AP), and available sulfur (AS) were measured after sieving through a 0.149 mm mesh (Cao et al., 2017). Briefly, OM was determined by wet oxidation using $K_2Cr_2O_7$. TN was measured by the Kjeldahl method after digesting with H_2SO_4, AP was analyzed using molybdenum antimony spectrophotometry, and AS was monitored by the turbidimetric procedure. Samples were pretreated by microwave digestion (MARS 6, CEM Corp., USA) with aqua regia for measurement of total vanadium and other metal ions. The modified three-step Community Bureau of Reference (BCR) sequential extraction method was employed to evaluate vanadium fractions for solid samples with acid-soluble, reducible, oxidizable, and residual phases (Ure et al., 1993). Contents of vanadium and other metal ions in the digested and extracted samples were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent Technologies 7700 Series, USA).

Aqueous samples were filtrated through 0.22 μm cellulose acetate syringe filters. pH, total vanadium, and other metal ions were measured by the same instruments as used for the solid samples. Total organic carbon (TOC) was determined by a Total Carbon Analyzer (TOC-5000, Shimadzu, Japan) and Cl^-, Br^-, NO_3^-, SO_4^{2-} were quantified by ion chromatography (ICS-900, DIONEX, USA). The obtained data were statistically analyzed with a one-way ANOVA using the software program PAST (Hammer et al., 2001).

2.3 Cultivation of Microorganisms

Bioremediation of V(V)-contaminated environment by indigenous microorganisms in representative matrices through nutrients amendment was assessed. Four 250 mL wild-mouth bottles (Beijing Daxiang Glass Factory, China) equipped with rubber plugs were inoculated with 100 g surface soil samples (MP-SL0, CO-SL0, SM-SL0, TR-SL0). Incubated samples were amended with mineral salt solution containing 750 mg L^{-1} $C_6H_{12}O_6$ and 75 mg L^{-1} V(V) in the form of $NaVO_3$ (Liu et al., 2016). Five 100 mL serum bottles equipped with rubber plugs were inoculated with concentrated microbes from 8 L water samples (MP-GW0, CO-GW0, SM-GW0, TR-GW0, TR-SW0). Similar mineral salt solutions containing 750 mg L^{-1} acetate and 10 mg L^{-1} V(V) were added. Different salt solutions were employed to form different nutrient supplements and pollutant loadings (Zhang et al., 2014). All bottles were placed on a shaker at 22 ± 2 °C. Supplements were added every three days, and the incubation study run for a total of 240 days to achieve reproducible performance with stable microbial communities (Cao et al., 2017). At the end of the incubation, V(V) removals were monitored in three consecutive cycles, a typical cycle comprising 72 h for the soil-inoculated system and 12 h for the water-inoculated system. V(V) was quantified by spectrophotometry (Hao et al., 2015). At the end of the cultivation period, microbial communities in all bioreactors were collected and stored at -80 °C prior to genomic analysis. The incubated samples were marked by the number "1" to distinguish from the inocula.

2.4 Microbial Community Studies

Genomic DNA from all field samples and incubated samples was extracted using the FastDNA SPIN Kit for Soil (Oiagen, CA, USA) according to manufacturer's instructions. The V4-V5 hypervariable regions of total bacterial 16S rRNA genes were amplified from solid samples (Cao et al., 2017) and the V3-V4 hypervariable regions of total bacterial 16S rRNA genes were amplified from aqueous samples (Liu et al., 2017). DNA sequencing was performed on the Illumina MiSeq platform by Shanghai Majorbio Technology (Shanghai, China). Raw gene sequences obtained from this study were deposited at the NCBI Sequence Read Archive with accession numbers SRP073713, SRP121440, SRP120206, and SRP129751.

Sequences were clustered into operational taxonomic units (OTUs) setting a 0.03 distance limit (equivalent to 97% similarity) using the MOTHUR program (version v.1.30.1). Rarefaction curves and Alpha-diversity were also generated using MOTHUR (Amato et al., 2013). Bacterial communities were further presented by non-metric multidimensional scaling (NMDS) analysis using the software program PAST. NMDS plots were generated based on Bray-Curtis similarities calculated between different samples using the OTU table with OTU clustering at the genus level. Redundancy analysis (RDA) was performed to identify relationships between environmental parameters and bacterial groups using the R software package (vegan 2.4.4).

3 Results

3.1 Vanadium Distributions and Speciation

3.1.1 Vanadium Distributions in Multiple Matrices

The vanadium content in surface soil samples from MP, CO, SM, and TR areas varied significantly (Fig. 1a), 1.26, 1.59, 13.8, and 1.63 times that of the soil background value of vanadium in China (82 mg kg^{-1}, $p < 0.05$). Highest vanadium concentration was observed from SM-SL0, confirming previous findings by Cao et al. (2017). Other metals were also measured and higher contents of Zn and Cr were observed (Table S2 SI); all Zn results from the four sites were above the soil background value (74.2 mg kg^{-1}, $p < 0.01$) recommended for China (Li et al., 2011).

Vanadium in the vertical soil profile from TR initially increased and then decreased as the sample depth increased, peaking at TR-S2 (Fig. 1b). Table S2 (SI) also lists values of the other physicochemical indicators, indicating pH was positively related to depth; potentially due to oxidation of ammonium to nitrate with the production of H^+ in upper layers (Li et al., 2017).

Increased concentrations of vanadium were detected in aqueous samples (Fig. 1c), most of which exceeded the limit of 0.05 mg L^{-1} for drinking water sources based on standards of China, except JS-SW0 (Wang et al., 2009). Vanadium concentrations in surface water in the TR area exceeded 0.61 ± 0.03 mg L^{-1} ($p < 0.01$), significantly greater than previous studies (Table S3, SI). Greater vanadium contents were also found in sediment (Fig. 1a). Other physicochemical indicators were also measured (Table S4 SI). Compared to surface water, groundwater showed oligotrophic characteristics with less TOC. Higher chromium concentrations were detected, especially in TR-GW0 and SM-GW0.

3.1.2 Vanadium Speciation in Solid Matrices

Fractions of vanadium in solid samples were further studied to assess bioavailability. The residual fraction accounted for the highest concentration occurring in surface soils and sediments (Table 1), suggesting that vanadium in solid matrices in this mining region was not bioavailable. In particular, SM-SL0 and TR-SL0 possessed the highest ratios of vanadium in the sum of acid-soluble, oxidizable, and reducible fractions (45.5 ± 2.6% and 43.1 ± 1.3%, $p < 0.05$). The residual fraction had the largest concentration, followed by oxidizable reducible, and acid-soluble fractions in the vertical soil profile, implying that vanadium speciation remains nearly constant with increasing soil depth.

3.2 Microbial Community in Multiple Matrices

3.2.1 Richness, Diversity and Structure

Microbial richness, diversity, and structure of surface soils were investigated. Detected OTUs ranged from 597 to 867 ($p < 0.05$, Table S5 SI). SM-SL0 maintained the greatest richness and diversity as reflected by rarefaction curves (Fig. S2a SI), and Shannon and Simpson indexes. Similar community compositions at phylum level were observed, dominated by *Bacteroidetes* (20.6%-37.9%), *Proteobacteria* (9.6%-37.8%), and *Firmicutes* (6.9%-37.4%) ($p < 0.05$) (Fig. S3a SI). Microbes responsible for metal bio-transformations were present at genus level (Fig. 2a). *Geobacter*, *Pseudomonas* and *Comamonas* existed in all surface soil samples. Significant

accumulation of other genera occurred in specific samples, for instance, *Zoogloea* in MP-SL0 (15.7%) and *Trichococcus* in CO-SL0 (17.1%).

Microbial richness in the vertical soil profile initially increased slightly and then decreased with increasing depth, peaking in TR-S3 (Fig. S2b, Table S5 SI). The Shannon index suggested decreased microbial diversity as depth increased. Samples of vertical soil profile exhibited similar community compositions at phylum level (Fig. S3b SI). Apart from the dominant *Proteobacteria* (11.7%-22.6%) in surface soils, *Actinobacteria* (22.2%-39.0%) and *Acidobacteria* (15.2%-27.4%) also predominated ($p < 0.05$). Microbes related to heavy metals were also revealed at genus level (Fig. 2b). *Actinobacteria* species were abundant in all vertical soil profile samples, whereas more *Streptomyces* was observed in deeper soil samples, especially in TR-S5.

Relatively lower microbial abundances were observed in groundwater compared to soils (Fig. S2c, Table S5 SI), due to the oligotrophic characteristics of the aquifers, especially for MP-GW0, with TOC of 0.94 ± 0.05 mg L^{-1} and NO_3^- of 8.54 ± 0.11 mg L^{-1}. Microbial diversity showed similar tendencies with microbial richness, with highest Shannon index for TR-GW0 and lowest for MP-GW0. *Proteobacteria* (58.0%-89.1%) dominated all groundwater samples at phylum level ($p < 0.05$) (Fig. S3c SI), consistent with accumulation of *Proteobacteria* in soil. *Bacteroidetes* also accounted for a large portion (29.1%) of microbes in SM-GW0, consistent with its appearance in SM-SL0. Microbes active in metal biogeochemical processes were found at genus level (Fig. 2c), including *Novosphingobium* in all groundwater samples, and most abundant in MP-GW0 (74.9%). *Flavobacterium* and *Acinetobacter* were dominant in SM-GW0. *Sphingobium* was abundant in TR-GW0 (21.9%).

Fewer OTUs were found in surface water samples compared to sediments (Fig. S2d, Table S5 SI), suggesting decreased microbial richness in the aqueous matrix. TR-SW0 had lower microbial richness than TR-GW0 possibly due to direct contact with tailings. The Shannon index suggested microbes were more diverse in sediments than in surface water. *Proteobacteria* and *Actinobacteria* appeared in all samples at phylum level (Fig. S3d SI), at relatively larger abundance in surface water than sediments. Metal-related microbes were also observed (Fig. 2d). *Exiguobacterium* was abundant in JS-SD0 (11.5%), but decreased to 0.02% in JS-SW0. *Synechococcus* was only detected in TR-SW0, whereas *Cyanobacteria* was found in TR-SD0.

3.2.2 Similarities and Influences of Environmental Parameters

Similarities between bacterial communities were visualized by NMDS (Fig. 3a). Interestingly, bacterial communities were distinct among different matrices in vanadium-mining geological environments, however, there were similarities within the same matrix. Samples from surface soils, vertical soil profile, and groundwater clustered separately. There were also similarities in samples from the same site, for example, JS-SW0 and JS-SD0, were taken from the same location in Jinsha River.

RDA was performed to reveal influences of environmental parameters on bacteria involved in metal tolerance and biotransformation. In solid matrices, nutrients including OM, TN, and available S were identified to have important influences (Fig. 3b). The first two axes of the RDA explained 48.1% and 23.0% of variations in microbial data. Vanadium was positively associated with *Trichococcus* and also affected abundance of *Geobacter* and *Comamonas*. In aqueous matrices, pH and nutrients, such as TOC and NO_3^-, had significant influences on the

presence of metal-related bacteria (Fig. 3c). Vanadium had a major impact on the abundance of *Synechococcus*.

3.3 Behavior of Cultivated Indigenous Microorganisms

3.3.1 V(V) Removals and Reduction Products

Gradual decreases in V(V) with time were observed in three consecutive cycles for all cultivated systems (Fig. 4a, Fig. 4b). Removal efficiencies of V(V) ranged from 65.2 ± 1.9% to 98.7 ± 3.6% ($p < 0.05$) in a typical cycle (72 h) in soil-inoculated bioreactors, with removal rates from 0.68 ± 0.07 to 0.97 ± 0.09 mg $L^{-1} \cdot h^{-1}$ ($p < 0.05$). 78.0 ± 3.5% to 88.3 ± 3.7% of V(V) ($p < 0.05$) was removed in a typical cycle (12 h) by microbes in water, with removal rates from 0.59 ± 0.02 to 0.74 ± 0.03 mg $L^{-1} \cdot h^{-1}$ ($p < 0.05$). After microbially mediated V(V) reduction in soil-inoculated reactors, the percentage of acid-soluble fraction for vanadium in cultivated soil increased (Fig. 4c) owing to reduction products.

When V(V) was reduced by microbes from the inoculated water samples, blue precipitates appeared concomitantly over the incubation period. Fig. 4d shows the high-resolution spectrum of V 2p where a sub-band is located at about 516.3 eV corresponding to V(IV) (Zhang et al., 2018; Cai et al., 2017), the main component of mineral sincosite [$CaV_2(PO_4)_2(OH)_4 \cdot 3H_2O$] in bioreactors (Qiu et al., 2017).

3.3.2 Evolution of Microbial Community

The microbial community changed significantly after cultivation. More abundant microbes were found in all soil-inoculated bioreactors compared with the corresponding original samples, as reflected by rarefaction curves in Fig. S4a (SI) and richness indexes in Table S6 (SI). The Shannon index also indicated that microbes after cultivation became more diverse. In comparison, the richness and diversity of microbes in water samples further decreased after cultivation (Fig. S4b, Table S6 SI). NMDS results indicated that bacterial communities became similar after long-term cultivation with vanadium and nutrients (Fig. S4c, Fig. S4d SI).

Functional species related to detoxifying V(V) increased in abundance during cultivation. For soil-inoculated bioreactors (Fig. 5a), previously detected V(V) reducers such as *Geobacter* were found to decrease with enriched *Bacillus*, especially in MP-SL1 (2.8%). Previously identified *Streptomyces* in the vertical soil profile accumulated significantly, especially in CO-SL1. Other heavy-metal tolerant microbes were also detected, such as *Lysobacter*, *Microvirga* and *Ramlibacter*.

Functional species accumulated significantly in water samples with larger relative abundance of microbes after cultivation; however, we observed a decrease in previously detected metal-tolerant bacteria such as *Acinetobacter* due to the high concentrations of V(V) (Fig. 5b). Newly observed *Longilinea* and enriched *Pseudomonas* were detected with large abundance after cultivation, especially in SM-GW1 and in TR-SW1, respectively. Notably, the abundance of the Fe(III) reducing microbe *Thauera* increased considerably in all cultivated systems, particularly in CO-GW1, *Rhodococcus* exhibited higher relative abundance after cultivation, especially in TR-GW1, and *Brevundimonas* also increased in MP-GW1.

4 Discussion

4.1 Connection of Vanadium Distributions in Geological Environments

Intensive anthropogenic activities have directly contributed to the occurrences and spread of vanadium in mining geological environments. Dusts containing residual vanadium are produced concomitantly during mining, smelting, and disposal processes and enter the soil through natural weathering processes such as drying and rewetting (Vicars & Sickman, 2011; Yang et al., 2014b). Ash identified in TR (TR-TL0) also possesses abundant vanadium (6932.1 ± 15.3 mg kg^{-1}) and could be a potential source of vanadium release into the environment. This suggests that severely contaminated surface soil is the primary sink of vanadium in Panzhihua, especially in SM, implying that remediation of smelting areas should be a priority. Given that polymetallic ores are mined in Panzhihua region, it is most likely that the detected Zn and Cr originated from mining activities. In this case, combined pollution characteristics should be considered when remediation is implemented. Higher contents of vanadium in surface soil layers in the vertical soil profile provided evidence that vanadium originated from atmospheric dry deposition and precipitation (Yang et al., 2017). Although the strong adsorption proprieties of soil could reduce infiltration to a certain extent (Qu et al., 2016), concentration of vanadium at depths of 80-100 cm still exceeded the soil background value of vanadium in China (82 mg kg^{-1}), implying that strong migration took place when vanadium entered surface soil. Moreover, soil could be a source of vanadium in other matrices due to migration of vanadium. Such movement of vanadium could pose significant difficulties to remediation engineering and could be a potential threat to groundwater quality.

Although bioavailability of vanadium in solid matrices had been previously observed in Panzhihua (Cao et al., 2017), local mining activities have increased the bioavailability of vanadium compared to that of vanadium-bearing titanomagnetite. Acid-soluble fraction increased in all surface soil and in TR-TL0; this fraction is the most active and readily dissolves during rain events (Wang et al., 2009; Jayawardana et al., 2014). The incidence of acid rain events in China is currently increasing third in the world (Yu et al., 2017) and dissolution of vanadium from surface soils is of primary concern. Notably, vanadium in surface soils of CO and TR had greater bioavailability; active fractions have potential geochemical activity that can be transformed into bioavailable vanadium. For instance, reduction of Fe (hydr)oxides under low E_H have led to release of associated vanadium (Shaheen et al., 2014; 2016). Nevertheless, geochemical processes taking place in our study have reduced bioavailable potentials, for example: decreased ratios of acid-soluble, oxidizable, and reducible fractions observed in sediment samples. In the vertical soil profile, the bioavailability of vanadium decreased with depth, potentially due to reduced vanadium fixation by exchangeable bound fractions through surface sorption or surface precipitation (Tokalıoğlu & Kartal, 2005) lowering groundwater contamination.

Surface water could be a sink of vanadium, thus affecting distributions of vanadium in other matrices. The high concentrations of vanadium in surface water in the TR could have derived from tailings leaching (Yang et al., 2014b). Infiltration of vanadium in TR-SW0 to groundwater could be due to the observed higher concentration of vanadium in TR. Vanadium concentrations in the Jinsha River decreased from a maximum of 0.285 mg L^{-1} (Wang et al., 2009) to 0.013 ± 0.002 mg L^{-1} (present study, $p < 0.01$), implying sedimentation because V(V) is precipitated through microbial metabolism under anoxic conditions and adsorbed onto suspended

particles (Zhang et al., 2015b). As the redox potentials fluctuate, vanadium may desorb and re-mobilize (Shaheen et al., 2016). Apart from anthropogenic activities, excessive vanadium in groundwater could result from geological weathering of vanadium-containing minerals, noting that the Panzhihua region possesses rich reserves of vanadium-bearing titanomagnetite where vanadium can be released into groundwater through water-rock interaction (Wright & Belitz, 2010).

Our results have revealed the connectivity of vanadium distributions in the Panzhihua region; however, gaps in our knowledge of these relationships still exist for other vanadium-contaminated sites. For example, research at the Rifle site, Colorado, USA, focused on vanadium in groundwater, and did not assess other matrices such as soil (Yelton et al., 2013). Vanadium in European soils (i.e. Germany and Italy) was researched (Shaheen et al., 2014; Guagliardi et al., 2018), however, surrounding water contamination was not investigated. The present study emphasizes the importance of comprehensive investigations into vanadium distributions in geological matrices due to inherent interactions driven by geological activities. Such investigations could then facilitate successful bioremediation.

4.2 Microbial Community Responses

Metal-tolerant microbial species increased in the presence of vanadium. *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* that dominate surface soils are frequently involved in reduction of V(V) (Zhang et al., 2015a). Identified *Geobacter* and *Pseudomonas* immobilized V(V) through bio-reduction (Ortiz-Bernad et al., 2004; Mirazimi et al., 2015). *Comamonas* can transfer Se(VI) to selenium nanoparticles (Zheng et al., 2014). *Zoogloea* and *Trichococcus* are capable of reducing Cr(VI) to Cr(III) (Solisio et al., 1998; Liu et al., 2017). These members of the microbial community accumulated in our study and were also found in the vanadium-contaminated environment (Cao et al., 2017). They potentially participate in V(V) reduction because vanadium is a redox-sensitive metal similar to selenium and chromium (Jiang et al., 2018). In the vertical soil profile, *Acidobacteria* that accounted for a large proportion of the microbial population can adapt to vanadium-polluted environments (Cao et al., 2017). The abilities of *Actinobacteria* species to reduce Cr(VI) and *Streptomyces* to remove chromium have been demonstrated previously (Polti et al., 2014). These two species also appeared with large relative abundances in our subsurface soil samples from the Panzhihua region. These organisms could reduce V(V) because most reported Cr(VI) reducers could bio-reduce V(V) with less toxicity (Wang et al., 2018). Regarding groundwater, *Novosphingobium* which appeared in all groundwater samples is known to metabolize cobalt (Raghu et al., 2008). *Novosphingobium* potentially removes V(V) through dissimilatory metal-reduction. *Flavobacterium* and *Acinetobacter* can reduce heavy metals (Kumar et al., 2011), *Sphingobium* can tolerate toxic metals (Wang et al., 2013). These three species also appeared abundantly in our groundwater samples and could contribute to vanadium removal through similar biosorption. There were common microbes in samples from the same site; for example, *Bacteroidetes* appeared in abundance in both JS-SW0 and JS-SD0. However, these commonalities weakened at genus level, with respective metal-related microbes. Previous research suggests that *Synechococcus* tolerates copper (Stuart et al., 2013), *Exiguobacterium* and *Cyanobacteria* reduce Cr(VI) and Fe(III) (Mohapatra et al., 2017; Xu et al., 2016). These bacteria were also present in our surface water and sediment samples. Vanadium is moderately toxic, with toxicity less than chromium and copper, metal-tolerant bacteria could exhibit tolerance to high concentrations of vanadium

(Aihemaiti et al., 2018). Our results suggest the metal-tolerant microbial communities associated with our samples have the ability to tolerate, reduce, and immobilize V(V).

Vanadium environmental contamination influenced microbial communities. In surface soils, the greatest vanadium content, and microbial richness and diversity were found in SM-SL0, indicating that the presence of vanadium might stimulate growth and proliferation of specific microbial communities. Moreover, both microbial richness and diversity were positively related to vanadium concentration in groundwater, since vanadium could act as a nutrient (Yang et al., 2014a). RDA suggests that concentrations of vanadium shape the structure of metal tolerant microbes, consistent with results from similar research in the Panzhihua region (Cao et al., 2017). Our findings suggest that the addition of nutrient supplements could enhance functional species for efficient bioremediation (Callister et al., 2010; Yelton et al., 2013). Due to the varying geological conditions, metal tolerant microbial communities differ. The results from the present study offer helpful insights into the biogeochemical fate of vanadium in vanadium-contaminated sites with specific microbial communities.

4.3 Evaluation of Microbial Remediation

V(V) in contamination could be reduced by indigenous microorganisms via the addition of a carbon source, indicating the potential for bioremediation. Moreover, the observed V(V) bioremediation efficiencies (65.2 ± 1.9% to 98.7 ± 3.6% of 75 mg L^{-1} within 72 h in soil-inoculated bioreactors and 78.0 ± 3.5% to 88.3 ± 3.7% of 10 mg L^{-1} within 12 h by microbes in water) exhibited advantages in comparison to results from pure cultures and field tests. For example, 30 days were required to reduce 2 mM V(V) by mesophilic and thermophilic methanogens (Zhang et al., 2014), whereas 99% of 6 mM V(V) in an aquifer were removed in 22 d with acetate bio-stimulation (Yelton et al., 2013). Notably, V(V) was reduced relatively quickly by microbes from matrices that were heavily contaminated, such as SM-SL0 (Fig. 4a) and TR-GW0 (Fig. 4b), because microbial communities in these matrices had adapted to high levels of vanadium. In water samples, less mobile and toxic V(IV) formed from V(V) bio-reduction, alleviating water contamination by indigenous microorganisms. However, reduction products formed an acid-soluble fraction within soil. This active fraction could release mobile vanadium when the redox condition shifts, providing a secondary source of microbial-mediated contamination.

Soil microorganisms were stimulated by nutrient addition, with increased richness and diversity occurring after cultivation (Fig. S4a, Table S6 SI)), despite the presence of vanadium at higher concentrations. Vanadium played an important role in shaping microbial community structure, especially for aquatic microbial communities, as accumulated functional species related to V(V) reduction at genus level after incubation differed from those in the inocula (Fig. 5b). Microbial communities converged after cultivation (Fig. S4c, Fig. S4d SI). Similar microbial communities after cultivation had been observed in vanadium-loaded soils (Cao et al., 2017); it was hypothesized that long-term incubation with the same substrate could lead to aggregation of microbial communities (Yang et al., 2014b).

Metal-tolerant microbes in our samples were susceptible to high concentrations of V(V) and were replaced over the incubation period, suggesting a succession of microbial communities. For soil-inoculated bioreactors, *Bacillus* was significantly enriched after cultivation, and has also been detected at a high-metal content site in Guanajuato, Mexico, where it was found capable of removing V(V) (Rivas-Castillo et al., 2017). Enriched *Bacillus* could therefore play a direct role

of reducing V(V) in our soil-inoculated systems. Other enriched species observed in our study have previously been reported capable of tolerating heavy metals, such as the ctinobacterial strain *Streptomyces* in marine sediments which tolerates Ni (Undabarrena et al., 2017), *Lysobacter* which has genes resistant to Co (Puopolo et al., 2016), *Microvirga* from the arid Taklamakan desert which strongly resists Pb through intracellular precipitations (Luo et al., 2014), and *Ramlibacter* from chromite ore processing residue-contaminated soils which is resistant to Cr (Min et al., 2017). These metal-tolerant species accumulated abundantly after long-term incubation, and were potentially tolerant to vanadium because vanadium is less toxic than the afore-mentioned metals. In water-inoculated bioreactors, *Longilinea* and *Pseudomonas* were newly observed, both of which are capable of V(V) reduction. Similar microorganisms have been identified in groundwater bioremediation efforts as a result of organic carbon source addition (Liu et al., 2017; Zhang et al., 2015a). *Thauera* is able to reduce Fe(III) under anaerobic conditions (Ma et al., 2015). *Rhodococcus* in leachate-contaminated soil can reduce Pb(IV) (Emenike et al., 2016), and *Brevundimonas* in subtropical paddy soils of China is known to participate in Fe(III) reducing processes (Peng et al., 2016). These species were also enriched in our inoculated bioreactors, which could also function in V(V) reduction through a similar electron transport pathway (Hao et al., 2018). In particular, functional species for V(V) reduction were more abundant in, and even dominated microbial communities within water compared to soil, implying that bioremediation could be more efficient for V(V)-contaminated water environments by indigenous microorganisms. Addition of vanadium could result in decreasing microbial richness and diversity in groundwater, as suggested by Yelton, et al., (2013). Enriched functional bacteria varied due to different initial microbial community structure, geological conditions, and applied electron donors. However, the present study indicates the possibility of immobilizing V(V) through *in situ* bio-stimulation of indigenous microorganisms by providing sufficient electron donors. During practical implementation, optimal electron donors could be mixed thoroughly with solid matrices or injected directly into groundwater to reduce V(V) in the geological environment by indigenous microorganisms. Other factors, such as nutrient addition, geological structure, and operating conditions, should also be taken into account.

5 Conclusions

Vanadium distribution from multiple matrices in Panzhihua region, China, was systematically studied. Vanadium contents in all surface soils and the vertical soil profile invariably exceeded the soil background value of vanadium in China (82 mg kg^{-1}). High vanadium concentrations in groundwater were also detected, whereas vanadium in surface water tended to precipitate onto sediments. Microbial community structures from different environmental matrices were diverse, with relatively lower richness and diversity found in water samples. *Proteobacteria* were widespread, and dominated microbial communities in groundwater. Metal-tolerant bacteria were associated with vanadium content and nutrients. V(V) was successfully bio-reduced to less toxic V(IV) by indigenous microorganisms after 240 d cultivation. The structure of microbial communities changed considerably during cultivation. Enrichments of *Bacillus* and *Thauera* could be responsible for bio-transformations of V(V) to V(IV).

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Table 1. Chemical speciation of vanadium in solid matrices based on modified three-step Community Bureau of Reference (BCR) sequential extraction procedure. MP: mining plant; CO: concentrator; SM: smelter; TR: tailing reservoir; JS: Jinsha River.

	Acid-soluble (%)	**SD**	**Reducible (%)**	**SD**	**Oxidizable (%)**	**SD**	**Residual (%)**	**SD**
MP-SL0	1.97 ± 0.52	a	3.24 ± 1.02	a	5.83 ± 0.59	a	89.0 ± 3.32	a
CO-SL0	1.39 ± 0.59	a	9.54 ± 1.05	b	8.79 ± 0.12	a	80.3 ± 4.21	b
SM-SL0	7.48 ± 0.15	b	18.7 ± 2.02	c	19.3 ± 2.56	b	54.5 ± 1.21	c
TR-SL0	10.9 ± 0.95	c	16.0 ± 0.25	c	16.2 ± 0.56	b	56.9 ± 1.22	d
p	< 0.001		< 0.001		< 0.001		< 0.001	
TR-SD0	7.61 ± 0.23	a	15.6 ± 0.26	a	16.3 ± 0.99	a	60.5 ± 2.25	a
JS-SD0	0.20 ± 0.08	b	5.73 ± 1.21	b	2.42 ± 0.18	b	91.7 ± 3.79	b
TR-TL0	1.67 ± 0.25	c	6.08 ± 0.12	b	10.4 ± 0.21	c	81.8 ± 2.15	c
Ore	0.07 ± 0.01	b	7.95 ± 0.98	c	10.5 ± 1.25	c	81.5 ± 4.15	c
p	< 0.001		< 0.01		< 0.001		< 0.001	
TR-S1	5.58 ± 0.15	a	8.98 ± 0.25	a	25.2 ± 2.56	a	60.2 ± 3.79	a
TR-S2	6.36 ± 0.59	ab	9.05 ± 0.26	a	26.3 ± 0.12	a	58.3 ± 2.15	ab
TR-S3	4.78 ± 0.52	ac	8.54 ± 2.02	a	25.9 ± 0.56	a	60.7 ± 1.22	a
TR-S4	2.91 ± 0.15	b	8.18 ± 1.05	a	26.0 ± 0.99	a	62.9 ± 2.25	a
TR-S5	2.87 ± 0.95	b	9.32 ± 1.02	a	26.2 ± 0.59	a	61.6 ± 3.32	a
TR-S6	0.53 ± 0.23	c	8.15 ± 2.02	a	25.8 ± 2.56	a	65.5 ± 1.21	ac
p	< 0.001		> 0.05		> 0.05		> 0.05	

SD: significant difference

Figure Captions

Fig. 1. Vanadium distributions in multiple matrices in Panzhihua region, China. (a) surface soils and sediments ($p < 0.001$), (b) vertical soil profile ($p < 0.001$), and (c) groundwater and surface water ($p < 0.001$). Error bars indicate standard deviation.

Fig. 2. Microbial community compositions revealed by high-throughput sequencing in multiple matrices at genus level. (a) surface soils; (b) vertical soil profile; (c) groundwater; and (d) surface water and sediments.

Fig. 3. NMDS plots and RDA of microbial communities. (a) NMDS plots for all original samples; RDA for (b) solid samples and (c) aqueous samples.

Fig. 4. Dynamics of V(V) in all cultivated systems with chemical speciation and product examinations. V(V) removals in three consecutive cycles in (a) soil-inoculated system and (b) water-inoculated system; (c) chemical speciation of vanadium in soils after cultivation; and (d) XPS analysis of generated precipitates in water-inoculated system. A typical cycle comprises 72 h for soil-inoculated system and 12 h for water-inoculated system (see Section 2.3). Red arrows indicate the replacement of mineral salt solution at the beginning of each cycle. The chemical speciation and XPS data are collected at the end of the incubation period.

Fig. 5. Accumulated functional species related to detoxifying V(V) at genus level in different cultivated systems. (a) soil-inoculated system; (b) water-inoculated system.