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Spatial distribution of vanadium and microbial community responses in surface soil of Panzhihua mining and smelting area, China

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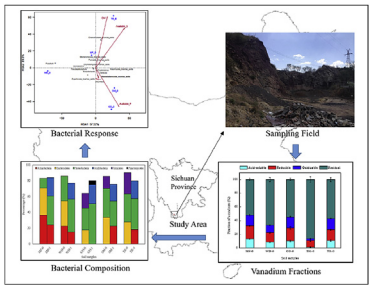
HIGHLIGHTS

- Spatial distribution of vanadium in surface soils in Panzhihua, China, is studied.
- Responses of microbial communities including bacteria and fungi are investigated.
- Organic matter, available P and S have great influences on bacterial structure.
- Bacterial communities converge to similar structure after long-term cultivation.
- Fungal diversities decrease after cultivation with the same most abundant phyla.

GRAPHICAL ABSTRACT



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ABSTRACT

Spatial distribution of vanadium in surface soils from different processing stages of vanadium-bearing titanomagnetite in Panzhihua mining and smelting area (China) as well as responses of microbial communities including bacteria and fungi to vanadium were investigated by fieldwork and laboratory incubation experiment. The vanadium contents in this region ranged from 149.3 to 4793.6 mg kg^{-1}, exceeding the soil background value of vanadium in China (82 mg kg^{-1}) largely. High-throughput DNA sequencing results showed bacterial communities from different manufacturing locations were quite diverse, but *Bacteroidetes* and *Proteobacteria* were abundant in all samples. The contents of organic matter, available P, available S and vanadium had great influences on the structures of bacterial communities in soils. Bacterial communities converged to similar structure after long-term (240 d) cultivation with vanadium containing medium, dominating by bacteria which can tolerate or reduce toxicities of heavy metals. Fungal diversities decreased after cultivation, but *Ascomycota* and *Ciliophora* were still the most abundant phyla as in the original soil samples. Results in this study emphasize the urgency of investigating vanadium contaminations in soils and provide valuable information on how vanadium contamination influences bacterial and fungal communities.

1. Introduction

As the fundamental component of terrestrial ecosystem, soil plays vital roles in providing habitats and nutrient resources for

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organisms (Schadt et al., 2003). However, modern agriculture and industry result in trace metal accumulations in soils, which are threatening terrestrial ecosystems (Nriagu and Pacyna, 1988). As one of trace metals, vanadium is widely distributed in the Earth's crust with an average concentration of around 150 μg g^{-1} (Rehder, 1991). The main sources of vanadium in soils are from vanadium-bearing titanomagnetite, stone coal, uranium-bearing minerals and petroleum-accompanying ore (Hu et al., 2012). The oxidation states of vanadium present in natural environment are +3, +4 and + 5 (Zhang et al., 2012). V(V) is the most common used in industry and the most toxic valence (Luo et al., 2017). The releases of vanadium into environment are associated with geologic and anthropogenic activities, e.g. the combustion of fossil fuel and the manufacture of vanadium products (Burke et al., 2013). Lately, the increasing demand of vanadium in industry leads to serious vanadium pollution in soils (Teng et al., 2011; Yang et al., 2016). Therefore, many studies have been conducted on the carcinogenic and fatal effects of vanadium on animals and plants (Crans et al., 2004; Tian et al., 2014). For instance, Larsson et al. (2013) investigated vanadium toxicity to plants and nitrifying bacteria and found out that soil sorption determined the bioavailability of vanadium. Yang et al. (2014) performed a series of soil column leaching experiments to investigate the leaching behavior in soils and mine tailings, and assessed the potential risk of vanadium pollution. Notably, it has been ascertained that the elevated vanadium in soil leads to chromic kidney disease in Sri Lanka (Jayawardana et al., 2014). Compared with macroorganisms, microorganisms are far more sensitive to environmental stress (Giller et al., 2009; Chen et al., 2017). However, little is known about the influence of vanadium on soil microorganisms including bacteria and fungi, although they have vital functions in soil ecosystems (van der Heijden et al., 1998; Chen et al., 2016).

To fill the knowledge gaps, a study on the spatial distribution of vanadium in surface soil as well as its influence on microbial communities was performed in Panzhihua region, Southwestern China. Panzhihua region concentrates abundant vanadium-bearing titanomagnetite resources and accounts for 64% vanadium supply in China (Teng et al., 2011). It was reported that vanadium distributed abundantly in air, water and soil in Panzhihua region due to the intensive mining and smelting activities, implying soils in this area were polluted by vanadium (Teng et al., 2011).

Specifically, surface soil samples from different processing stages of vanadium-bearing titanomagnetite in Panzhihua mining and smelting area were collected to analyze the distribution and morphology of vanadium. More importantly, bacterial and fungal diversities and compositions in the soil samples were detected to investigate the influence of vanadium contamination on microbial communities. Furthermore, soil microorganisms were cultivated with vanadate and nutrients in lab for ~240 d to explore the microbial responses. Lately, the high-throughput DNA sequencing has facilitated major advances in studies of microbial communities by providing more detailed and clear insights into diversities and compositions. Therefore, bacterial and fungal communities in the original and cultivated soil samples were profiled by this advanced technique. Information from this study will be conducive in understanding how bacterial and fungal communities respond to vanadium contamination in soils and other environments.

2. Methods and materials

2.1. Study area and soil sampling

The study sites are located in the Panzhihua region (26°05′-27°21′N, 101°08′-102°15′E), Sichuan Province, China. Surface (upper 20 cm layer) soil samples were collected from five different vanadium manufacturing locations, including mining plant (MP), waste dump (WD), concentrator (CO), smelter (SM) and tailing reservoir (TR) in April 2015 (Fig. 1). Every soil sample (approximately 1.5 kg) consisted of five homogenized subsamples, which were collected at a spatial interval of 50–80 m. The samples were preserved in polyethylene bags and then delivered to the laboratory. The soils were stored at 4 °C in the laboratory after removing stones and plant residue.

2.2. Incubation test

250 mL Jars sealed with rubber plug were employed as experimental reactors. Each jar was inoculated with 100 g collected soil samples and 150 mL simulated groundwater with vanadate and nutrients, which contained the following components (per liter): $C_6H_{12}O_6$ (0.7500 g); NH_4Cl (0.1557 g); KH_2PO_4 (0.0299 g); KCl (0.0283 g); $CaCl_2$ (0.2464 g); $MgCl_2 \cdot 6H_2O$ (1.0572 g); $NaHCO_3$ (0.8082 g) and $NaVO_3$ (0.1795 g). The medium was refreshed every 3 d and the total incubation period was approximately 240 d. All the incubation experiments were carried out at room temperature (22 ± 2 °C).

2.3. Chemical analysis

The soil samples were air-dried at room temperature and sieved through 2 mm mesh before further analysis. Chemical properties of soil samples such as pH, organic matter (OM), total nitrogen (TN), available phosphorus and available sulfur were determined based on previous methods (Ran et al., 2015). Specifically, pH was measured in dissolved soil with a ratio of soil to deionized water of 1:2.5 (w/v), the content of OM was quantified by $K_2Cr_2O_7$-volumetry and TN was determined by semi-micro kjeldahl method. Extracted available phosphorus and available sulfur were measured by molybdenum antimony colorimetry and barium sulfate turbidimetry, respectively. Total vanadium was monitored by inductively coupled plasma mass spectroscopy. V(V) and V(IV) in the incubation experiment were measured by spectrophotometric methods (Hao et al., 2015).

The modified three-step Community Bureau of Reference (BCR) sequential extraction method was used to assess the fraction of vanadium in original soil samples, including acid-soluble phase, reducible phase and oxidizable phase (Yang et al., 2013). Additionally, the residual phase and the total vanadium content in soil samples were determined by microwave digestion (MARS 6, CEM Corp., USA) with aqua regia. The concentrations of vanadium in the extracted and digested samples were finally measured by inductively coupled plasma-optical emission spectrometer (Prodigy XP, Leeman Labs, Inc., USA).

2.4. Microbiological analysis

Molecular biology techniques were employed to detect the structures of microbial communities in the original (MP-0, WD-0, CO-0, SM-0 and TR-0) and cultivated (MP-1, WD-1, CO-1, SM-1 and TR-1) soil samples. Total genomic DNA from 10 soil samples was extracted by FastDNA Spin Kit for Soil (Qiagen, CA, USA) according to the manufacturer's instructions. Bacterial 16S rRNA genes were amplified with PCR primer 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). Fungal 18S rRNA genes were amplified with PCR primer 0817F (5′-TTAGCATGGAATAATRRAATAGGA-3′) and 1196R (5′-TCTGGACCTGGTGAGTTTCC-3′). 25 μL PCR amplification solutions were prepared for each sample in triplicate. After being purified, the PCR products of all samples were quantified by QuantiFluor™-ST microfluorometer (Promega, USA). Then triplicate PCR products were mixed with equal DNA content for