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Review

Response of soil microbial activities and microbial community structure to vanadium stress

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ABSTRACT

High levels of vanadium (V) have long-term, hazardous impacts on soil ecosystems and biological processes. In the present study, the effects of V on soil enzymatic activities, basal respiration (BR), microbial biomass carbon (MBC), and the microbial community structure were investigated through 12-week greenhouse incubation experiments. The results showed that V content affected soil dehydrogenase activity (DHA), BR, and MBC, while urease activity (UA) was less sensitive to V stress. The average median effective concentration (EC_{50}) thresholds of V were predicted using a log-logistic dose-response model, and they were 362 mg V/kg soil for BR and 417 mg V/kg soil for DHA. BR and DHA were more sensitive to V addition and could be used as biological indicators for soil V pollution. According to a polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis, the structural diversity of the microbial community decreased for soil V contents ranged between 254 and 1104 mg/kg after 1 week of incubation. As the incubation time increased, the diversity of the soil microbial community structure increased for V contents ranged between 354 and 1104 mg/kg, indicating that some new V-tolerant bacterial species might have replicated under these conditions.

1. Introduction

Vanadium (V) is a ubiquitous trace element, and the average V content in soil worldwide is 90 mg/kg (Reimann and Caritat, 1998). However, high V content can accumulate in soil because of anthropogenic sources, such as fossil fuel combustion, tailing leakage, and mining and smelting activities (Nriagu, 1998). For example, soil V contents near a V mine in South Africa varied from 1570 to 3600 mg/kg (Panichev et al., 2006), and the V contents in soil ranged from 1268 to 1538 mg/kg around a V-containing stone coal smelting area in China (Xiao et al., 2015).

Much research has reported the potential risk of carcinogenic and other toxic effects due to excessive V exposure (McCrindle et al., 2001; Assem and Levy, 2009). At the end of the 1980, the United Nations Environment Programme listed V as a priority environmental risk element (Hindy et al., 1990). Elevated levels of V in agricultural soils have been of worldwide concern for the last few decades owing to its toxicological effects on humans, animals, and plants (Nriagu, 1998; Assem and Levy, 2009; Xiao et al., 2012). Stone coal, an important source of V has accounted for the majority of the domestic V reserves in China (Jiang et al., 2010). In particular, in western Hunan Province, the intensive extraction of V from stone coal, which resulted in severe soil contamination around smelting areas, has attracted much attention (Xiao et al., 2015).

Soil biological and biochemical properties have been used largely to assess the quality of soils because of their sensitivity to environmental stress (Hinojosa et al., 2004; Ma et al., 2015). The activities of enzymes, microbial biomass, and basal respiration (BR) play major roles in the decomposition of organic matter (OM) in soil, carbon sequestration, and the cycling and use of nutrients; thus, they are important indicators of microbial and biochemical processes and functions (Caldwell, 2005). In addition, these microbial parameters are easy to measure (Gülser and Erdoğan, 2008; Zhang et al., 2010). However, it is necessary to study the microbial community structure using advanced molecular biological techniques because of some limitations of soil enzyme assays and microbial biomass in assessing soil quality (Trasarcepeda et al., 2000; Broos et al., 2007). In recent decades, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has been used to assess changes in the structure of the soil microbial community at a molecular level in response to environmental stress (Zhu et al., 2013; Wang et al., 2016).

Numerous reports have demonstrated that heavy metals, such as Cd, As, Pb, and Cr, exhibit adverse effects on soil microbial and biochemical processes (Gülser and Erdoğan, 2008; Zhang et al., 2010; Liu et al., 2017). Some investigations regarding the influence of V toxicity on soil biota have also been reported. Larsson et al. (2013) studied the inhibitory effect of V on the soil potential nitrification rate and

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substrate-induced respiration over 28 days. Soil enzymatic activity and microbial BR were adversely affected by high V levels in soil (Xiao et al., 2015). Yang et al. (2014) reported that the soil sulfatase activity decreased with increasing V contents, and the structure of bacterial and fungal communities changed significantly at different V levels. However, to date, knowledge concerning the impact of V on soil biology is still limited (Larsson et al., 2013; Yang et al., 2014), especially about the dose relationship between V and soil microbial activity during long-term incubations. Furthermore, there have been few studies regarding the effect of V on the microbial community at a molecular level using PCR-DGGE. Here we conducted greenhouse experiments using traditional soil microbial assays and the advanced molecular PCR-DGGE technique. The aims of the study were: 1) to evaluate the response of soil microbial properties to V stress; 2) to examine the potential of soil microbial properties for assessing V contamination; and 3) to quantify the relationship between V stress and the bacterial community structure.

2. Materials and methods

2.1. Soil sampling and characteristics

The soil sampling site is located in Yuanling County, western Hunan Province, China, where there are intensive, industrial-scale V extraction activities from stone coal. A surface paddy soil sample was collected 20 km from the stone coal smelting area (latitude, 28°21′44.13″, longitude, 110°23′29.91″). After transportation to the laboratory, the soil was air dried and sieved through a 2-mm mesh. The selected physiochemical properties of the soil were as follows: pH, 7.76; available nitrogen (N) content, 151 mg/kg; available phosphorous (P) content, 59.5 mg/kg; and available potassium (K) content, 174 mg/kg. The OM content and cation-exchange capacity (CEC) were 0.15 g/kg and 5.27 cmol/kg, respectively. The total V content in the soil was 104 mg/kg.

2.2. Experimental setup and laboratory incubation

Air-dried soil samples (800 g) were placed in individual plastic pots (10 cm in height and 5 cm in diameter), which were adjusted to 70% of the field water-holding capacity and pre-incubated at 25 °C for 2 weeks. External V in the form of ammonium metavanadate (NH_4VO_3) was added to the potting soil at six levels (0, 50, 150, 250, 500, and 1000 mg V/kg soil). Thus, the V levels in the treated soil samples were 104 (background content), 154, 254, 354, 604, and 1104 mg V/kg soil. Each treatment had triplicates. The incubation experiments were conducted in a thermostatic incubator at 25 °C with a relative humidity of 80%. During the incubation, deionized water was sprayed evenly to maintain the soil moisture at 70% of the field water-holding capacity. Soil samples from each tube were collected at 1, 3, 5, 8 and 12 weeks to analyze urease activity (UA), dehydrogenase activity (DHA), BR, microbial biomass carbon (MBC), and the available V content. The structural diversity of the soil microbial communities in the samples was determined using PCR-DGGE after 1- and 12-week incubation periods.

2.3. Sample analysis

2.3.1. Soil physicochemical properties

The physicochemical properties of the soil samples were analyzed according to the methods described by Lu (1999). Soil pH was determined using a pH meter at a soil weight to deionized water volume ratio of 1:2.5 (w/v). The OM content was determined using a volumetric $K_2Cr_2O_7$-heating method, and the CEC was determined by extraction using a 1.0 mol/L ammonium acetate solution (pH of 7.0). The soil available N content was measured using the alkaline hydrolysis method. Soil available P was extracted by sodium bicarbonate and determined using the molybdenum blue method. Available K in the soil was extracted by ammonium acetate (NH_4AC) and determined by flame photometry. The soil samples were digested using a mixture of HNO_3-H_2O_2 to determine the total V content (USEPA, 1996). The bioavailable V content was evaluated by extraction using 0.05 mol/L ethylenediaminetetraacetic acid (EDTA) (Teng et al., 2011).

2.3.2. Soil microbial activity

The UA and DHA for each soil sample were determined by the method of Tabatabai (1994) and expressed as mg NH_4^+-N/kg soil and mg triphenyl formazan (TPF)/kg soil in 24 h, respectively. The substrates used for determining the activity of particular enzymes were 10% urea for urease and 2,3,5-triphenylterazolium chloride (TTC) for dehydrogenase, and the incubation period was 24 h at 37 °C. The NH_4^+ released by the urease-mediated enzymatic hydrolysis of urea was determined colorimetrically at 578 nm. The released triphenyl formazan was extracted with methanol and assayed at 485 nm. BR (CO_2 evolution without added substrate) was determined using the alkali-trap method described by Page (1982), i.e., it was determined by incubating 20-g (oven-dry basis) aliquots of moist soil samples for 24 h in gas-tight vessels, and it was expressed as mg CO_2/(24 h kg) dry soil. The MBC content was determined by the chloroform fumigation-extraction method, as modified by Gregorkh et al. (1990). Carbon in the extracts was determined by a TOC-VCPH analyzer (TOC-VCPH, Shimadzu, Japan). An extraction efficiency coefficient of 0.45 was used to convert the difference in soluble C between the fumigated and the unfumigated soils to MBC.

2.3.3. DNA extraction and PCR-DGGE amplification

DNA was extracted from 0.5-g soil samples fractions using the Fast DNA Spin Kit (Qbiogene, Montreal, QC, Canada). Bacterial 16 S rRNA genes were PCR-amplified using the universal forward primer F341GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC-GGG AGG CAG CAG-3′) and the reverse primer R518 (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer et al., 1993). PCR amplification was performed on an iCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA) in a 50-μL reaction volume. The following cycling programs were used: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min. The PCR products were extended at 72 °C for 10 min, and then they were stored at 4 °C for the subsequent DGGE analysis.

DGGE was performed using a Bio-Rad Dcode™ Universal Mutation Detection System (Gel Doc™ XR+, Bio-Rad). The extended PCR products were loaded on a 0.8-mm thick polyacrylamide gel (10% w/v, acrylamide: bisacrylamide of 37.5:1) using a denaturing gradient from 40% to 60% (100% comprised 7 mol/L urea and 40% (v/v) deionized formamide). The electrophoresis was performed in 1× Tris-acetate-EDTA (TAE) buffer under a constant voltage of 55 V for 12 h and a constant temperature of 60 °C. After the run, the gels were stained for 20 min in 1× TAE containing 0.5 mg/mL ethidium bromide, rinsed with distilled water, and then photographed. DGGE bands of the microbial community were detected and digitized following the subtraction of the average background across the entire lane (Quantity One 4.62, Bio-Rad).

2.4. Statistical analysis

2.4.1. Median effective concentration threshold for microbial properties

The median effective concentration (EC_{50}) values of the soil V content (X, in mg V per kg), at which there was a 50% reduction based on the response (Y) of microbial properties, were determined with a log-logistic dose-response model (Larsson et al., 2013), given by:

$$Y = \frac{c}{1 + \exp\left(b \times \ln\frac{X}{EC_{50}}\right)}$$