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ScienceDirect
Applied Soil Ecology 34 (2006) 125–139
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Response of microbial communities to different doses of chromate in soil microcosms

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Received 26 August 2005; received in revised form 10 January 2006; accepted 22 March 2006

Abstract

The toxic effect of chromate on soil microbial communities is not well documented, although microorganisms control biogeochemical cycling, contribute to formation of soil structure, regulate the fate of organic matter applied to soil. In this study the effects of short- and middle-term chromate on the soil microbial community were investigated. The shifts in the size and in the diversity of culturable heterotrophic bacterial community, the resistance to Cr(VI) of heterotrophic bacteria, the presence of cyanobacteria, the activity of 19 enzymes, and the ATP content were monitored over time (120 days) in soil microcosms artificially contaminated with three concentrations of chromate (50, 250 and 1000 mg kg^{-1} soil). The chromate contamination affected the structure and the diversity of the soil bacterial community. Bacterial strains isolated from the microcosm contaminated with the highest concentration of chromate were identified by 16S rDNA gene sequencing. All isolates belonged to the genus *Pseudomonas*, were able to reduce Cr(VI), and showed a high resistance to chromate. To our knowledge, this is the first report that shows *Pseudomonas* strains having the capability to resist up to 40 mM of Cr(VI) on minimal medium. The cyanobacterial group was more sensitive to chromate contamination than culturable heterotrophic bacteria. No cyanobacterial growth was detected in enrichment cultures from the soil polluted with the highest chromate concentration. Some enzymes were inhibited by high concentrations of chromate, whereas others were stimulated. The ATP content in microcosms was strongly affected by chromate. We conclude that the soil microbial community responds to chromate pollution through changes in community structure, in metabolic activity, and in selection for Cr(VI)-resistance.

Keywords: Microbial community; Chromate; Culturable bacteria; Cyanobacteria; *Pseudomonas*

1. Introduction

Chromium world production is on the order of 10^7 tonnes per year (Cervantes et al., 2001). Chromium can exist in several oxidation states, of which the most stable and common forms in the environment are trivalent [Cr(III)] and hexavalent [Cr(VI)] species. These two chromium species display quite different chemical properties and affect organisms in different ways. In fact, in contrast to other metals, the hazard of chromium is dependent on its oxidation state. Hexavalent chromium is water-soluble, mutagenic, oncogenic and highly toxic to all organisms; trivalent chromium is relatively water-insoluble and 100 times less toxic than Cr(VI) (Francisco et al., 2002). Chromate, which is the most prevalent form of Cr(VI) present in several industrial solid/liquid waste products, is considered one of the principal pollutants in the United States by the Environment Protection Agency (EPA) (Kamaludeen et al., 2003).

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0929-1393/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2006.03.003

In the soil Cr(III) and Cr(VI) can inter-convert, with reduction of Cr(VI) to Cr(III) being generally favored in most environmental conditions (Kimbrough et al., 1999). In presence of organic matter Cr(VI) is reduced to Cr(III), but high concentrations of Cr(VI) may exceed the reducing capability of the environmental conditions (Cervantes et al., 2001). Soils which have been seriously contaminated by chromium coming from chromate and dichromate-producing factories and chromium plating facilities may contain up to 12,000 mg of Cr(VI) per kg of soil (Bader et al., 1999; Li, 2004; Jeyasingh and Philip, 2005). Moreover, a part of Cr(III) can be transformed in Cr(VI) in Bartlett positive soils (Bartlett and James, 1979; Viti and Giovannetti, 2001).

Several studies have reported isolation and characterization of Cr(VI)-resistant bacteria from contaminated soils (Badar et al., 2000; McLean et al., 2000; Camargo et al., 2003, 2004; Megharaj et al., 2003; Viti et al., 2003), but the toxic effect of chromate on soil microbial communities is not well documented despite its importance as an environmental pollutant (Viti and Giovannetti, 2001; Shi et al., 2002). In previous studies it has been difficult to assess the chromium effects on soil microbial community structure because in addition to chromium other heavy metals were present in the same soil or because comparable unpolluted soil was not available (Viti and Giovannetti, 2001; Shi et al., 2002). Therefore, the main goal of this research was to investigate the responses of the soil microbial community to chromate exposure alone by using soil microcosms artificially polluted with three concentrations of chromate. The use of soil microcosms is specially considered a useful tool for providing information on changes that occur over time in a microbial community stressed by heavy metals (Kelly et al., 1999; Ranjard et al., 2000; Rasmussen and Sørensen, 2001).

In this study we monitored the effects of chromate on the number, resistance to Cr(VI) and diversity of culturable heterotrophic bacteria. In addition, the activity of several extracellular enzymes and microcosm ATP content were quantified in order to compare results from culture-dependent and culture-independent methods. Finally, the impact of chromate on cyanobacteria, a sensitive group to environmental stresses, was tested.

2. Materials and methods

2.1. Microcosms

The soil used for the microcosms was collected near Cerbaia (Florence, Italy). It was chosen because it had no previous exposure to heavy metal contamination. Samples were collected at a depth of 2–20 cm. Immediately after collection the soil was sifted with a sterilized sieve (diameter 2 mm) to remove gravel and plant residues, and then stored at 4 °C. The soil characteristics determined as described in Viti and Giovannetti (2001) were: organic carbon 20.35 g kg^{-1} soil, nitrogen Kjeldahl 1.05 g kg^{-1} soil, available phosphorus 0.044 g kg^{-1} soil, total chromium 0.037 g kg^{-1} soil, pH 7.5. The soil matrix was comprised of 35% clay, 52% silt and 13% sand (silt–clay–loam).

The soil was divided into four parts, each of 3.5 kg. One was used for the control soil microcosms, and the other three were amended with 50 mg Cr(VI) kg^{-1} soil or 250 mg Cr(VI) kg^{-1} soil or 1000 mg Cr(VI) kg^{-1} soil. Cr(VI) was added as K_2CrO_4. In order to set-up control and chromate treated microcosms, the four soil samples were treated, using a spray bottle, with a same volume of sterilized deionized water (control microcosm) or K_2CrO_4 solutions (polluted microcosms). Both control and artificially polluted soils were vigorously mixed in order to uniformly distribute the added chromate and distributed into glass containers (50 for each microcosm type) of approximately 125 mL volume. Unpolluted microcosms were nominated control, microcosms polluted with 50 mg Cr(VI) kg^{-1} soil were nominated 50, microcosms polluted with 250 mg Cr(VI) kg^{-1} soil were nominated 250 and microcosms polluted with 1000 mg Cr(VI) kg^{-1} soil were nominated 1000. All values in this study were reported on a dry-weight soil basis.

At the beginning of the incubation soil water content was adjusted to 45% water-holding capacity and subsequently maintained at that level by weighing the microcosms, every 2 days, and adding sterile distilled water to compensate for any weigh loss. Unpolluted and polluted soil microcosms were incubated at 22 °C.

Cr(VI) level in soil microcosms was analyzed using EPA Method 3060A (USEPA, 1995) and EPA Method 7196A (USEPA, 1996). At the end of experiments the concentrations of Cr(VI) in 50, 250 and 1000 were about 10, 16 and 22% of entered, respectively.

2.2. Sampling of microcosms

Samples were taken after 7, 30 and 120 days. At each sampling time nine containers from each microcosm type were randomly chosen, and subsequently subdivided in three groups. Then the three containers from each group were mixed in order to obtain a bulk sample to be analyzed. Three bulk samples for each microcosm type were analyzed separately.