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Evaluation of bacterial biosensors to determine chromate bioavailability and to assess ecotoxicity of soils

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HIGHLIGHTS

- Bacterial biosensors pCHRGFP1 and pCHRGFP2 are able to measure chromate in soils.
- Biosensors are alternative methods to EPA 7199 and DPC for chromate measurement.
- Soil properties influence the rates of water-extractable chromate decrease.
- Springtails grazers of bacteria influence the chromate fate in soil.
- Reproduction of springtails correlates with bioavailable chromate in soil.

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ABSTRACT

Chromate can be considered a potent environmental contaminant and consequently, an understanding of chromate availability and toxicity to soil biology is essential for effective ecological assessment of metal impact in soils. This study shows the response of two bacterial bioreporters, pCHRGFP1 *Escherichia coli* and pCHRGFP2 *Ochrobactrum tritici*, to increasing concentrations of chromate in two different soils. The bioreporters, carrying the regulatory gene *chrB* transcriptionally fused to the *gfp* reporter system, exhibited different features. In both, the fluorescence signal and the chromate concentration could be linearly correlated but *E. coli* biosensor functioned within the range of 0.5–2 μM and *O. tritici* biosensor within 2–10 μM chromate. The bioreporters were validated through comparative measurements using the chemical chromate methods of diphenylcarbazide and ionic chromatography. The bacterial sensors were used for the estimation of bioavailable fraction of chromate in a natural soil and OECD artificial soil, both spiked with chromate in increasing concentrations of 0–120 mg Cr(VI) kg^{-1} of soil. OECD soil showed a faster chromate decrease comparing to the natural soil. The toxicity of soils amended with chromate was also evaluated by ecotoxicological tests through collembolan reproduction tests using *Folsomia candida* as test organism. Significant correlations were found between collembolans reproduction and chromate concentration in soil (lower at high chromate concentrations) measured by biosensors. Data obtained showed that the biosensors tested are sensitive to chromate presence in soil and may constitute a rapid and efficient method to measure chromate availability in soils.

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1. Introduction

The rapid development of the industry related with paper production, fertilizers, pesticides and others has conducted to the discharge of large amounts of metal-contaminated residues into the environment, resulting in a serious problem of environmental contamination. Unlike organic contaminants, metals are not biodegradable and tend to accumulate in living organisms, becoming toxic and carcinogenic. For instance, chromium is a metal with different oxidation states, although only hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)] are stable in the environment (Krishna and Philip, 2005). The hexavalent chromium compounds exist mainly as chromate and dichromate and have high solubility, bioavailability and mobility. These compounds are associated with several diseases such as allergic reactions, contact dermatitis and cancer of the lung (Ramírez-Díaz et al., 2008). The Agency for Toxic Substances & Disease Registry (ATSDR) from USA included the Cr(VI) in hazardous substances list

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(http://www.atsdr.cdc.gov/spl/index.html) since 2011. Chromium is commonly used in metal finishing and tanning industries and, therefore, soil may be contaminated with chromium through wastewaters and land disposal of sewage sludges (Zheng et al., 2007).

The soil is a very complex and heterogeneous matrix whose biodiversity supports the provision of several ecosystem services (nutrient cycling and soil formation) of most importance for food production and maintaining socioeconomic activities (Bronick and Lal, 2005). The preservation of soils depends on monitoring soil contamination in order to prevent the dispersion of pollution and avoid drastic consequences. Therefore, it is important to map the concentration of toxic compounds present in soils. The development of cost effective methods to measure soil contamination, namely regarding the contaminant fraction most available for soil organisms, is needed. According to the literature, metal toxicity and particularly, chromate toxicity in soil depends on soil properties such as organic matter content, the concentration of metal ions (iron and manganese) that influence the oxidation–reduction cycle of chromium (Kotaś and Stasicka, 2000), soil texture (percentage of clay, silt and sand) and pH (Dube et al., 2001; Banks et al., 2006).

There are various classical methods for metal detection. These include atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), and inductively coupled plasma mass spectrometry (ICP-MS). These methodologies involve expensive instrumentation, require additional chemical compounds, which are pollutants and are unable to detect metal bioavailable concentrations. Nevertheless, the measurement of the bioavailable fraction of metals is a parameter of high interest since it determines the toxicity of metals to the organisms (Bontidean et al., 2004).

Assays using organisms have been developed to evaluate the toxicity of contaminants in the environment, which is directly related to their bioavailable fraction (Girotti et al., 2008). Assays using microorganisms have been seen as an excellent methodology since they have short life cycle, can be easily maintained in laboratory cultures at low cost and are highly sensitive and selective to specific analytes (Tibazarwa et al., 2001). In this way, the development of metal-specific biosensor tools functioning on the basis of a reporter system has been acquiring increasing attention. The use of microbial cells as the biological recognition element may be an important tool in environmental studies to evaluate the extent of contaminated areas and to monitor bioremediation processes. Two examples are the use of MC1061 (pzntRluc) and AE104 (pchrBluc) biosensors to detect the bioavailable fraction of cadmium, zinc, mercury and chromium in soil (Ivask et al., 2002). More recently, the use of biosensors constructed through the fusion of the regulatory gene, *chrB* of the *chr* resistance determinant of *Ochrobactrum tritici* 5bvl1 (Branco et al., 2008) with the reporter gene, green fluorescence protein (*gfp*), has been reported as an efficient and sensible approach to detect chromate in environmental waters spiked with chromate (Branco et al., 2013). These biosensors, pCHRGFP1 *Escherichia coli* and pCHRGFP2 *O. tritici*, have revealed distinct sensitivity to chromate concentrations. In the present work, these two biosensors were used with the aim to detect and monitor Cr(VI) in different chromate contaminated soils showing the usefulness of the biosensors as an alternative tool for monitoring chromate. Collembolan reproduction tests with *Folsomia candida*, following standard procedures (usually performed to evaluate the habitat function of contaminated soils), were also performed as a way to relate the quantification of Cr(VI) with the toxicity toward this invertebrate species. Additionally, measurements of Cr(VI) in aqueous solutions through biosensors were compared with measurements performed by classic chemical methods (using diphenylcarbazide and ionic chromatography methods) in the same solutions to further validate de usefulness of the biosensors for chromate measurements.

2. Material and methods

2.1. Test soils

In this study two different soil types were tested: (1) a natural soil collected in the campus of Coimbra Agronomic School and (2) an artificial standard soil from the Organization for Economic Cooperation and Development (OECD). The natural soil is characterized by 62.4% of sand, 21.2% of silt, 16.4% of clay (sandy loam texture; LNEC, 1970), pH 6.9, cation exchange capacity of 0.025 cmol g^{-1} (ISO, 1994), organic matter content of 3.3 ± 0.1% (loss on ignition at 500 °C for 6 h) and water-holding capacity of 36.2% (ISO, 1999). The OECD artificial soil was composed by 69.5% of sand, 10% of *Sphagnum* peat (air dried and sieved at 2 mm), 20% of clay and 0.5% of calcium carbonate to adjust the pH to 6 ± 0.5. Its water-holding capacity was 65.1% (ISO, 1999).

Contamination gradients of sodium chromate were prepared, in natural and OECD artificial soil, immediately before the beginning of the experiments. Portions of 300 g (dry weight equivalent; DW) of the natural and the OECD artificial soils were weighted and different amounts of a sodium chromate stock solution (1 M) were added to each soil to obtain the final gradient of contamination of 0, 10, 20, 40, 60, 80 and 120 mg Cr(VI) kg^{-1} soil (Table 1).

2.2. Ecotoxicological tests

Collembola reproduction tests were performed using the springtails *F. candida* and following the procedures described in the ISO 11267 (ISO, 1999). The test organisms were taken from the laboratory cultures of the University of Coimbra. Synchronized cultures were prepared following the procedures described by Natal-da-Luz et al. (2009) and only organisms 10–12 d old were used in the reproduction tests. Springtails were exposed to a concentration gradient of Cr(VI) in the natural soil and OECD artificial soil (procedures for soils contamination are described in Section 2.1). Soil moisture was adjusted to 50% of the water-holding capacity before being used in the tests. At the beginning of the experiment the soil moisture and the pH were measured in each test treatment. For each concentration five replicates were prepared. The replicates consisted of glass flasks (4 cm of diameter, 7 cm of height) with 30 g of fresh soil and ten springtails. Two milligrams of granulated dry yeast were added as food at the beginning and after 14 d of test. The test containers were covered with a lid during the test and opened weekly for a few seconds to allow aeration. The experiment was conducted at a constant temperature of 20 ± 2 °C, and under a photoperiod of 16 h light and 8 h dark. At the day 14, the water loss was reestablished by compensating the weigh losses of the test containers with distilled water when the weight loss was higher than 2%. After 28 d of exposure, each test container was emptied into a small vessel, which subsequently was filled with water. After the addition of few drops of blue ink and gentle stirring, the animals floating on the water surface were

Table 1
Natural and OCDE soil treatments used in this study. Soils were spiked with the different concentrations of chromate.

Soil treatments	Final concentration of chromate in soils (mg kg^{-1})	μM chromate g^{-1} of soil
C0	0	0
C1	10	19.2
C2	20	38.5
C3	40	79.9
C4	60	115.4
C5	80	153.9
C6	120	230.8

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