



Changes in enzyme activities and microbial biomass after “in situ” remediation of a heavy metal-contaminated soil

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Abstract

Microbial properties such as microbial biomass carbon (MBC), arylsulfatase, β -glucosidase and dehydrogenase activities, and microbial heterotrophic potential, together with several chemical properties such as pH, CaCl_2 soluble heavy metal concentrations, total organic carbon and hydrosoluble carbon were measured to evaluate changes in soil quality, after “in situ” remediation of a heavy metal-contaminated soil from the Aznalcóllar mine accident (Southern Spain, 1998). The experiment was carried out using containers, filled with soil from the affected area. Four organic amendments (a municipal waste compost, a biosolid compost, a leonardite and a litter) and an inorganic amendment (sugarbeet lime) were mixed with the top soil at the rate of 100 Mg ha^{-1} . Unamended soil was used as control. *Agrostis stolonifera* L. was sown in the containers. The soil was sampled twice: one month and six months after amendment application. In general, these amendments improved the soil chemical properties: soil pH, total organic carbon and hydrosoluble carbon increased in the amended soils, while soluble heavy metal concentrations diminished. At the same time, higher MBC, enzyme activities and maximum rate of glucose mineralization values were found in the organically amended soils. Plant cover was also important in restoring the soil chemical and microbial properties in all the soils, but mainly in those that were not amended organically. As a rule, remediation measures improved soil quality in the contaminated soils.

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1. Introduction

Remediation techniques of heavy metal-contaminated soils are based on the extraction or the stabilization of the contaminants. The first ones are generally carried out “ex situ”, and imply soil structure deterioration and high costs, which limits their use on

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vast contaminated areas. Stabilization techniques are carried out “in situ” and are less expensive. Soils can naturally reduce mobility and bioavailability of heavy metals as they are retained in soil by sorption, precipitation and complexation reactions. This natural attenuation process (natural remediation) can be accelerated by the addition of amendments (Bolan and Duraisamy, 2003). Vegetation cover also prevents wind-blow of contaminated particles and reduces water pollution by interception of a substantial proportion of the incident precipitation (Tordoff et al., 2000). Therefore, the application of amendments and the development of a plant cover play an important role in the restoration of the physical, chemical and biological properties of these contaminated soils.

Heavy metals exhibit toxic effects towards soil biota: they can affect key microbial processes (Obbard, 2001) and decrease the number and activity of soil microorganisms (Maliszweska-Kordybach and Smreczak, 2003). Thus, the biological properties of such soils are usually severely affected. Conversely, long-term heavy metal effects can increase bacterial community tolerance Bååth et al. (1998) as well as the tolerance of fungi such as arbuscular mycorrhizal (AM) fungi, which can play an important role in the restoration of contaminated ecosystems (Del Val et al., 1999).

Nevertheless, microorganisms respond quickly to changes and can rapidly adapt to environmental conditions. Changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, providing an early sign of soil improvement or an early warning of soil degradation (Pankhurst et al., 1995).

The use of organic wastes and compost as a source of organic matter and nutrients is a common practice to improve soil physical and chemical properties (Entry et al., 1997), reducing the need for inorganic fertilisers (Madejón et al., 2001). Biochemical soil properties may also be enhanced by organic matter addition (García-Gil et al., 2000; Madejón et al., 2003). Different results have been reported about trace element risks and urban wastes. While Guisquiani et al. (1995) reported an increase in the level of potentially harmful trace elements, Moreno et al. (2002) showed reduction in Cd toxicity after sewage sludge application.

The Aznalcollar mine accident occurred in 1998 in Southern Spain and affected 46,00 ha of the riverbanks

of the Guadiamar and Agrio rivers (Grimalt et al., 1999). Severe trace element pollution was observed in most of the sludge-affected soils (Cabrera et al., 1999). Remediation works started soon after the accident and consisted of: (1) removal of the sludge from the surface together with a layer of soil of around 10 cm; (2) application of amendments; (3) disk harrowing (20 cm) to mix soil within the ploughed depth. After sludge removal and amendment application, the soils still exhibit residual heavy metal pollution. Therefore, further remediation practices are needed to ameliorate the contamination and restore the soils from this vast area. The Regional Government “Junta de Andalucía” decided to give priority to the immobilization of the contaminants, with additional monitoring activities and a perspective of creating a green corridor in the Valley of the Guadiamar river.

The aim of this study was to evaluate the effectiveness of several amendments and a plant cover in restoring soil chemical and biological properties of a heavy metal-contaminated soil from the Guadiamar area in semi-field conditions. Changes in soil quality were determined as changes in microbial biomass carbon (MBC), arylsulfatase and β -glucosidase activities (extracellular enzymes), dehydrogenase activity (intracellular enzyme) and microbial heterotrophic potential.

2. Materials and methods

2.1. Experimental design

The experiment was carried out in 24 containers (70 cm long \times 60 cm wide \times 40 cm deep) placed outdoors. The containers were filled with the first 25 cm of a clay loam soil (1.2 g cm⁻³ bulk density), affected by the Aznalcollar mine accident (Table 1). Four different organic amendments were used in this experiment: two urban composts; a municipal waste compost (MWC) from a the city refuse treatment plant of Villarrasa (Huelva, Southern Spain), and a biosolid compost (BC) made by wastewater sludge mixed with green wastes, and two natural organic amendments: a litter (LIT) from a deciduous forest and leonardite (LEO), a low-rank coal between peat and sub-bituminous rich in humic acids (Table 2). An inorganic amendment, sugarbeet lime, a residual material from

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