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Versatility of *Streptomyces* sp. M7 to bioremediate soils co-contaminated with Cr(VI) and lindane

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ABSTRACT

The aim of this work was to study the impact of environmental factors on the bioremediation of Cr(VI) and lindane contaminated soil, by an actinobacterium, *Streptomyces* sp. M7, in order to optimize the process.

Soil samples were contaminated with 25 μg kg^{-1} of lindane and 50 mg kg^{-1} of Cr(VI) and inoculated with *Streptomyces* sp. M7. The lowest inoculum concentration which simultaneously produced highest removal of Cr(VI) and lindane was 1 g kg^{-1}. The influence of physical and chemical parameters was assessed using a full factorial design. The factors and levels tested were: Temperature: 25, 30, 35 °C; Humidity: 10%, 20%, 30%; Initial Cr(VI) concentration: 20, 50, 80 mg kg^{-1}; Initial lindane concentration: 10, 25, 40 μg kg^{-1}.

Streptomyces sp. M7 exhibited strong versatility, showing the ability to bioremediate co-contaminated soil samples at several physicochemical conditions. *Streptomyces* sp. M7 inoculum size was optimized. Also, it was fitted a model to study this process, and it was possible to predict the system performance, knowing the initial conditions. Moreover, optimum temperature and humidity conditions for the bioremediation of soil with different concentrations of Cr(VI) and lindane were determined. Lettuce seedlings were a suitable biomarker to evaluate the contaminants mixture toxicity. *Streptomyces* sp. M7 carried out a successful bioremediation, which was demonstrated through ecotoxicity test with *Lactuca sativa*.

1. Introduction

Industrialization and urbanization have led to serious problems of soil contamination, by both organic (polyphenols, pesticides, etc.) and inorganic compounds (Cd, Cu, Cr, etc.). Mixed pollution is a global problem, hence it affects more than one third of contaminated sites (Tang et al., 2010; Mansour, 2012).

Chromium contamination in soil and water has been detected in and around industrial sites (Benimeli et al., 2003; Nie et al., 2010; Srinivasa Gowd et al., 2010). Cr(VI) is a harmful pollutant, neurotoxic, dermatotoxic, genotoxic, carcinogenic and immunotoxic (Bagchi et al., 2002). On the other hand, residues of the gamma isomer of hexachlorocyclohexane (γ-HCH), commercially known as lindane, have been reported in soils, water, air, plants and animals, because of its indiscriminate use, principally in agriculture practices (Fuentes et al., 2011). Lindane is highly recalcitrant, and produces several health effects, such as neurological problems and cancer (Saez et al., 2012). Moreover, mixed pollution by chromium and lindane has been detected around the world in water, sediment and soil, at concentrations up to 140 mg kg^{-1} and 400 μg kg^{-1}, for chromium and lindane respectively (Benimeli et al., 2003; Maggi et al., 2012; Arienzo et al., 2013; Coatu et al., 2013).

The treatment of co-contaminated soils is complex, as the chemical processes and remediation technologies are different for each group of pollutants (Puzon et al., 2002; Dong et al., 2013). Bioremediation is a low cost technology, which simultaneously allows the degradation of organic compounds and the removal or stabilization of metals into non-toxic or less toxic forms (Owabor

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et al., 2013).

The composition of the microbial population dictates the overall microbial degradation process (Owabor et al., 2013). However, the bioremediation effectiveness is subject to several factors which interact in complex ways, depending on the matrix and contaminant characteristics, among others. When a system is affected by a large number of independent factors, experimental design methods are commonly used to systematically determine the effective factors and their interactions, as well as to model and optimize the whole system. Through full factorial design maximum information regarding the factors is obtained. It is possible to identify the interactions between separate experimental factors and to predict the effect that such interactions could have on the experimental response (Antony, 2003; Mason et al., 2003). Thus, biological treatments could be improved using this approach. Actinobacteria represent an important component of the microbial population in soils. They have demonstrated bioremediation ability (Benimeli et al., 2008; Polti et al., 2009; Albarracín et al., 2010; Alvarez et al., 2012). In particular, *Streptomyces* sp. M7 was able to bioremediate simultaneously Cr(VI) and lindane from non-sterilized soils (Polti et al., 2014). However, to assess whether bioremediation processes are acceptable, it is mandatory to investigate toxic effects of microbial metabolites produced during the pollutant removal (Repetto et al., 2001). Bioindicators change their response in front of changes in environmental pollution. *Lactuca sativa* is a recommended specie for this purpose (Charles et al., 2011), since it allows evaluating lethal and sublethal effects and it can be used in samples with high turbidity, reducing pretreatment interference. Furthermore, it has high sensitivity, so it requires reduced exposure time, it has low cost and does not require sophisticated equipment (Sobrero et al., 2004).

The aims of this work were to statistically optimize environmental factors for bioremediation of lindane and Cr(VI) by *Streptomyces* sp. M7 in soil, and further, to prove the efficiency of this bioprocess by using *L. sativa* as bioindicator.

2. Materials and methods

2.1. Bacterial strain, culture medium and chemicals

Lindane (γ-HCH) (99% pure) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used during the study were analytical grade and purchased from standard manufacturers. Cr(VI) was added as $K_2Cr_2O_7$ (Benimeli et al., 2003, 2007; Polti et al., 2007).

The actinobacterium used was *Streptomyces* sp. M7, previously isolated from sediments contaminated with organochlorine pesticides and heavy metals, and then characterized by Benimeli et al. (2003). The *Streptomyces* sp. M7 inoculum was obtained by cultivating the strain in Tryptic Soy Broth, containing (in g L^{-1}): tryptone, 15; soy peptone, 3; NaCl, 5; K_2HPO_4, 2.5; and glucose, 2.5, during 3 days at 30 °C (200 rpm) (Polti et al., 2009).

2.2. Soil samples: preparation and inoculation

Non-polluted soil samples (SS) were collected from an experimental site near the city of Tucumán, in northwest Argentina. The samples were taken from near the surface (5–15 cm deep) and stored in darkness at 10–15 °C until being used. 200 g of soil were put into each glass pot, and humidity content was fixed using distilled water.

The SS were inoculated with *Streptomyces* sp. M7 (0.5, 1, 2, or 4 g kg^{-1}) and contaminated with lindane and Cr(VI). The glass pots were then incubated during 14 days. Also, inoculated SS without toxics and non-inoculated SS with both toxics were used as controls. In all cases, samples were taken at the end of each assay to determine both lindane and bioavailable chromium residual concentrations.

2.3. Analytical determinations

The extraction procedure for γ-HCH in soil was performed as follow: 5 g of soil were transferred to centrifuge tubes and mixed with 10 mL of a 4:1:5 water-methanol-hexane solution. The tubes were hermetically sealed and shaken during 10 min in order to allow the extraction of lindane from soil to the organic phase, and then centrifuged (2500*g* during 10 min) for separation of the organic and aqueous phases. Organic phase was evaporated to dryness. The residues were suspended in hexane and analysed by Gas Chromatography. Extracts were quantified in a Gas Chromatograph Agilent 7890 A equipped with a HP5 capillary column (30 m × 0.53 mm × 0.35 m) and ^{63}Ni μECD detector, a split/splitless Agilent 7693B injector and Agilent Chem Station software. Quantitative sample analysis was performed using appropriate calibration standards (AccuStandard) (Fuentes et al., 2011).

Potentially bioavailable chromium in the soil was extracted by a physical method: 100 g of soil were centrifuged at 5050*g* during 60 min, in order to reproduce the maximum plant suction (soil water potential: 1500 kPa, conventional wilting point) (Csillag et al., 1999). Supernatant was recovered, filtered at 0.45 nm and analysed by Atomic Absorption Spectrometry, using a Perkin Elmer Analyst 400 (AAS) for Cr content (Polti et al., 2011).

2.4. Experimental design and statistical analysis

Experimental design and analysis were performed using MINITAB 17(PA, USA) statistical software. Statistical significance values for the means were evaluated using one-way analysis of variance. Differences were accepted as significant when $p < 0.05$. In order to identify the main effects of the selected factors and the interactions among them, a 2^3 full factorial design was applied. Three extra replicates were included as centre points. The experimental variables evaluated are presented in Table S1 (see Supplementary Table S1 in EES Online), which shows the two alternative options tested for each factor. All assays were performed in triplicate and the results are presented as the mean value ± standard deviation. Associations between variables were assessed by using Pearson's correlation coefficient.

In each sample two responses were evaluated, including residual Cr(VI) and lindane. The results of the experimental design were studied and interpreted using MINITAB 17 (PA, USA) statistical software to estimate the response of the dependent variable (Martorell et al., 2012).

2.5. Phytotoxicity test

To assess the bioremediation success, three parameters were assessed on lettuce seedlings (*Lactuca sativa*): germination, root elongation and hypocotyl elongation. Thirty seeds were placed into sterile Petri plates containing 15 g of soil sample bioremediated by *Streptomyces* sp. M7. Biotic and abiotic soil samples were used as controls. Petri plates were sealed and incubated at 22 ± 2 °C in darkness, during 5 days. At the end of the incubation period, the number of germinated seeds was registered. The length of roots and hypocotyl was measured by using a millimetre scale. Vigour index ((mean root length + mean hypocotyl length) × (percent germination/10)) was also calculated (Bidlan et al., 2004; Fuentes et al., 2013; Saez et al., 2014).