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Simultaneous bioremediation of Cr(VI) and lindane in soil by actinobacteria

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ABSTRACT

Environments co-contaminated with metals and organic compounds are difficult to remediate. Actinobacteria is an important group of microorganisms found in soils, with high metabolic versatility and potential for bioremediation. In this paper, actinobacteria were used to remediate soil co-contaminated with Cr(VI) and lindane. Five actinobacteria, tolerant to Cr(VI) and lindane mixture were selected: *Streptomyces* spp. A5, A11, M7, and MC1, and *Amycolatopsis tucumanensis* DSM 45259. Sterilized soil samples were inoculated with actinobacteria strains, either individually or as a consortium, and contaminated with Cr(VI) and lindane, either immediately or after 7 days of growth, and incubated at 30 °C during 14 days. All actinobacteria were able to grow and remove both contaminants, the consortium formed by *Streptomyces* spp. A5, M7, MC1, and *A. tucumanensis* showed the highest Cr(VI) removal, while *Streptomyces* sp. M7 produced the maximum lindane removal. In non-sterile soil samples, *Streptomyces* sp. M7 and the consortium removed more than 40% of the lindane, while *Streptomyces* sp. M7 demonstrated the greatest Cr(VI) removal. The most appropriate strategy for bioremediation of Cr(VI) and lindane co-contaminated soils would be the inoculation with *Streptomyces* sp. M7.

1. Introduction

The great expansion of human activity caused by industrial growth has resulted in an increase in scenarios of serious and complex environmental contamination by both organic compounds (herbicides, plastics, tannins, polyphenols, pesticides, etc.) and inorganic compounds (As, Cd, Cu, Pb, Cr, Hg, etc.) (Volke Sepúlveda and Velasco Trejo, 2002). Mixed pollution caused by simultaneous contamination by organic and inorganic compounds is a widespread global problem that tends to be concentrated in certain types of locations such as industrial zones, oil storage areas, waste dumps, waste recycling sites, and soils and sediments near roads and railways (Volke Sepúlveda and Velasco Trejo, 2002). Co-pollution is a very important issue because more than one third of contaminated sites are found to have more than one type of pollutant (Tang et al., 2010; Mansour, 2012). Environments co-contaminated with metals and organic compounds are considered difficult to remediate because of the mixed nature of these pollutants.

Cr(VI) is a harmful pollutant characterized by its chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity and immunotoxicity (Bagchi et al., 2002), and Cr(VI) compounds are approximately 1000 times more toxic and mutagenic than Cr(III) compounds (USEPA, 1998; Dana Devi et al., 2001). However, Cr(VI) compounds have several uses in industry (Polti et al., 2007; Bhadra and Mahananda, 2013) and chromium contamination by these compounds in soil and water has been detected in and around a wide variety of industrial sites (Benimeli et al., 2003; Nie et al., 2010; Srinivasa Gowd et al., 2010).

The systematic use of pesticides has led to great improvements in terms of agricultural production levels. However, massive and indiscriminate application of pesticide products has also led to adverse effects on human health, the environment, and even the effectiveness of the products themselves (Johri et al., 2000; Phillips et al., 2005). The gamma isomer of hexachlorocyclohexane (γ-HCH), commercially known as lindane, is a highly chlorinated, recalcitrant organochlorine pesticide (OP). Lindane residues persist in the environment and have been reported in soils, water, air, plants, agricultural products, animals, foods, and microbial

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environments, as well as in the human body. Since the toxicity of γ-HCH is well established, it is now imperative to develop methods to remove lindane from the contaminated environments (Fuentes et al., 2011).

In recent years, the intense search for a solution to co-contamination has led to the development of remediation technologies that can address treatment of not just a single compound, but which can simultaneously deal with multiple contaminants (Srivastava et al., 2007; Ma et al., 2010; Wasi et al., 2011). However, studies involving methodologies for simultaneous decontamination of organic and inorganic contaminants remain rare, and have generally been focused on increasing organic biodegradation by reducing the toxicity of metals through their sequestration and precipitation. Such strategies are primarily concerned with degrading the organic contaminants without consideration of metal extraction, and consequently metal remediation is restricted to deactivation. It still remains imperative to develop low-cost removal techniques that can degrade organic compounds while also extracting metals or stabilizing them in a non-toxic or less toxic form (Puzon et al., 2002).

In the last ten years a stronger emphasis has come to be placed on the study of the physiological, biochemical, and molecular approaches to microbial bioremediation of environments co-contaminated with heavy metals and pesticides. Soils with long-term exposure to mixed contamination with organic compounds and heavy metals have been shown to have structural and functional microbial communities with the ability to adapt and grow under these conditions. This suggests that bioremediation based on microorganisms is feasible for recovery of such sites by microbial transformation of both organic compounds and heavy metals into non-toxic products. These strategies depend mainly upon the catabolic biological activities of the microorganisms, and therefore their ability to utilize the contaminants as nutrients and energy sources (Atlas and Unterman, 1999; Boopathy, 2000).

It is important to consider that when allochthonous microorganisms are incorporated into a soil, they usually cannot fully participate in the community activity in a meaningful way. This is why the use of indigenous microorganisms in bioremediation processes is so important. The actinobacteria are a group of bacteria that is found in high concentrations in soils. They play an important ecological role in recycling substances in the natural world, using humic acids for their growth as well as organic matter, which is difficult to degrade (Kieser et al., 2000). The physiological diversity of actinobacteria allows the production of a large number of metabolites with biotechnological importance included antibiotics, which are synthesized and excreted into a medium (Goodfellow et al., 1988; Ensign, 1990; Genilloud et al., 2011). The important role played by actinobacteria in the environment is also demonstrated by their ability to remove oil, rubber, plastics, pesticides, and heavy metals, among other substances (Goodfellow et al., 1988; Vobis, 1997; Benimeli et al., 2003, 2006, 2007a; Albarracín et al., 2005, 2010b; Polti et al., 2009, 2011).

There have been previous studies focused on biotransformation of OPs by actinobacteria, particularly in relation to lindane degradation (Benimeli et al., 2006, 2007a; Fuentes et al., 2011; Saez et al., 2012). *Streptomyces* spp. M7, A2, A5, and A11, isolated from sediments and soils contaminated with OPs, were found to be able to degrade lindane, as revealed by the release of chloride ions when the microorganisms were grown on media containing this pesticide as a sole carbon source (Benimeli et al., 2003, 2006; Cuozzo et al., 2009; Fuentes et al., 2010). Biotransformation of heavy metals [Cu (II), Cd (II), and Cr (VI)] by actinobacteria, particularly in terms of uptake and/or reduction to less toxic forms, has also been studied (Polti et al., 2007; Albarracín et al., 2008a; Siñeriz et al., 2009). *Streptomyces* sp. MC1, isolated from contaminated sugar cane, has shown the ability to reduce Cr(VI) to Cr(III) in both liquid and solid culture media (Polti et al., 2009, 2010). *Amycolatopsis tucumanensis* DSM 45259, isolated from sediments contaminated with heavy metals has also shown resistance to copper and chromium under a variety of culture conditions (Albarracín et al., 2005, 2008b, 2010a).

Table 1
Actinobacteria strains used in this study.

Strain	GenBank accession number	Reference
Streptomyces sp. M7	AY459531	Benimeli et al., 2003
Streptomyces sp. MC1	AY741287	Polti et al., 2007
Amycolatopsis tucumanensis DSM 45259	DQ886938	Albarracín et al., 2005
Streptomyces sp. A2	GU085103	Fuentes et al., 2010
Streptomyces sp. A5	GQ867050	Fuentes et al., 2010
Streptomyces sp. A11	GQ867055	Fuentes et al., 2010

The use of a single population involves many metabolic limitations, which could be avoided by using a mixed community of microorganisms. In nature, microorganisms exist as elements of microbial consortia, made up of multiple populations that coexist and carry out complex chemical processes and physiological functions in order to enable survival of the community. Microbial consortia can combine the catalytic specialties of different species to metabolize new substrates, including pesticides (Dejonghe et al., 2003; Smith et al., 2005; Yang et al., 2010; Fuentes et al., 2011; Shong et al., 2012). A microbial consortium formed by resistant actinobacteria could thus enhance the potential to simultaneously remove Cr(VI) and lindane. In the present work, authors therefore experiment with the bioremediation of soil contaminated with Cr(VI) and lindane using actinobacteria previously identified and characterized.

The effect of metals on biodegradation is still a matter requiring further study, in order to develop cost-effective remediation processes for sites co-contaminated with metals and organic pollutants. The impacts that metals have on biodegradation are complex and are influenced by the matrix structure, which determines the bioavailable metal concentrations. Metals inhibit biodegradation using different mechanisms and patterns, which depend upon the biological and physico-chemical characteristics of each system. A variety of approaches to bioremediation of co-contaminated sites are under development, and they include addition of metal-resistant microorganisms as well as additives that reduce metal bioavailability (Sandrin and Hoffman, 2007).

Several authors have evaluated bioremediation in media co-contaminated with metals and persistent organic compounds. Olaniran et al. (2009) investigated the impact of lead and mercury on biodegradation of 1,2-dichloroethane in soils, and they concluded that heavy metals have a negative impact on this bioprocess. These authors also found that biostimulation can have a positive influence on 1,2-dichloroethane degradation.

Another emerging approach is bioaugmentation. Sprocati et al. (2012) used this strategy to remediate soils co-contaminated with diesel oil and heavy metals. The bioaugmentation was performed by introducing a consortium composed of 12 allochthonous bacterial strains, previously isolated from a site with long-term pollution. This strategy showed high efficiency in the bioremediation process.

The aim of this work was to evaluate the use of actinobacteria, as pure or mixed culture, to remediate soil co-contaminated with lindane and Cr(VI).

2. Materials and methods

2.1. Bacterial strains, culture media, and chemicals

Lindane (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 (Benimeli et al., 2003, 2007b). Cr(VI) was added as $K_2Cr_2O_7$ (Polti et al., 2007).

Six previously isolated actinobacteria were assayed: three isolated from environments contaminated with pesticides and heavy metals (Table 1). For the Cr(VI) and lindane resistance tests, minimal medium agar (MM) was used, containing (in g L^{-1}): glucose, 10.0; L-asparagine, 0.5; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.20; $FeSO_4.7H_2O$, 0.01, agar, 15.0 (Amoroso et al., 1998).

The cultures for soil sample (SS) inoculation were obtained by first inoculating the strains in Tryptic Soy Broth (TSB containing (in g L^{-1}): tryptone, 15; soy peptone, 3; NaCl, 5; K_2HPO_4, 2.5; and glucose, 2.5. The cultures were incubated for 3 days at 30 °C (200 rpm) in Erlenmeyer flasks containing 20 mL of medium (Polti et al., 2009).

2.2. Tolerance and antagonism assays

In order to evaluate the select actinobacteria with tolerance to Cr(VI) and lindane, a qualitative assay was performed using MM agar plates (Fig. 1). Rectangular troughs were cut in the center of the plate and then filled with 500 mg L^{-1} of Cr(VI) and/or 250 μg L^{-1} of lindane. The actinobacteria strains were inoculated by streaking perpendicular to the troughs, and the Petri dishes were incubated at 30 °C for 7 days. Microbial growth was used as a qualitative parameter of toxicity tolerance. Control samples for growth were also created using a medium without the addition of toxics (Fuentes et al., 2013). The technique described by Fuentes et al. (2011) was used to determine the presence of potential antagonistic effects among the isolated strains, Petri dishes with solid MM were sown as follows: one of the strains was spread in the center of the plate and faced transversely with the other microorganisms to be assayed. It was considered a strain to be antagonistic to the other evaluated strains if a growth inhibition was observed. In this way, the presence of antagonism among the strains studied was assessed by considering all possible combinations.

2.3. Soil samples: preparation and inoculation

Non-polluted soil samples (SS) were taken from an experimental site near the city of Tucumán in northwest Argentina. The samples were collected from near the surface (5–15 cm deep) and stored in the dark at 10–15 °C until being utilized. Glass pots were filled with 200 g of soil and kept at 20% humidity using distilled water. The SS were steam-sterilized (three successive sterilizations at 24 h intervals, at 100 °C for 1 h each) (Polti et al., 2009). These sterilized soil samples (SSS) were each inoculated with either an individually selected actinobacterium or with the mixed culture (the four actinobacteria selected after the resistance assay) to a final inoculum concentration of 2 g kg^{-1} of soil (dry weight).

The inoculated SSS were then contaminated with 25 μg kg^{-1} of lindane and 50 mg kg^{-1} of Cr(VI), either immediately or after 7 days of incubation, essays were labeled as d0 and d7, indicating that samples were contaminated at day 0 and day 7, respectively. The flasks were then incubated at 30 °C for a total time period of 14 days (14 days for the d0 samples and 7 more days for the d7 samples). Also, inoculated SSS without toxics and non-inoculated SSS with both toxics were used as controls.

The actinobacteria showing better performance in the sterilized SS were selected to carry out studies in non-sterilized SS (NSSS), in order to evaluate the influence of the native microbial flora on their ability to remove Cr(VI) and lindane. Non-sterilized SS were inoculated with the selected actinobacterium or actinobacteria, then contaminated with 25 μg kg^{-1} of lindane and 50 mg kg^{-1} of Cr(VI), either at d0 or d7. These flasks were also incubated at 30 °C for a total time period of 14 days. Similarly, inoculated NSSS without toxics and non-inoculated NSSS with both toxics were used as controls.

In all cases, samples were taken at the end of each assay to determine the lindane and chromium concentrations, although microbial growth was evaluated only in the sterilized SS assay. The extraction and determination procedure for γ-HCH residues in soil was performed according to Fuentes et al. (2011), while, potentially bioavailable chromium in the soil was measured by a physical method: 100 g of soil were centrifuged at 5050 g during 60 min, to reproduce the maximal plant suction (soil water potential: −1500 kPa, conventional wilting point) (Csillag et al., 1999). After centrifugation, the supernatant was recovered, filtered at 0.45 mm and analyzed by AAS for Cr content (A.P.H.A, 1989). Microbial growth was determined as CFU g^{-1} by transferring 1 g of soil from each pot into a sterile flask, containing 9 ml of a sterile sodium hexametaphosphate solution (1.66 g l^{-1}, pH 7). Samples were then vortexed during 10 min and 10-fold serial dilutions were made in NaH_2PO_4 (0.05 M, pH 7) and plated out onto solid MM in triplicate. Plates were incubated at 30 °C for 72 h (Polti et al., 2009).

2.4. Statistical analysis

All assays were carried out in triplicate. Statistical analyses were conducted using the Microcal Origin Working Model Version 6.0.

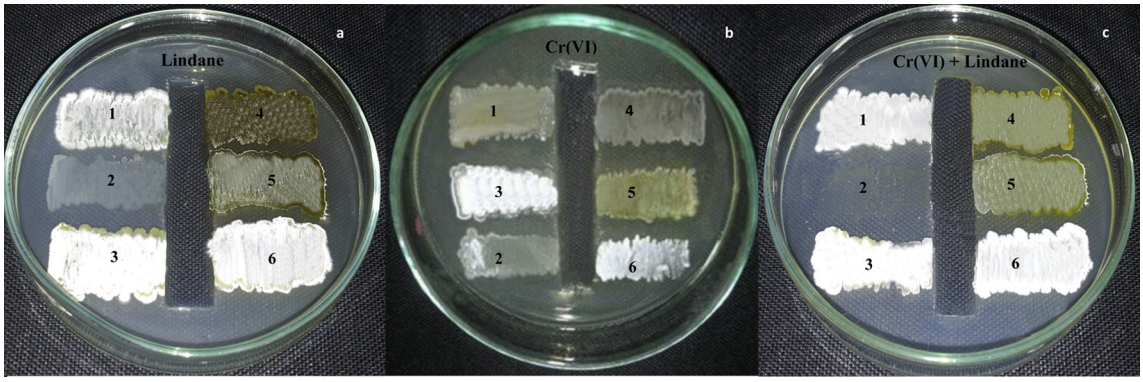

Fig. 1. Qualitative tolerance test. a) Cr(VI) 500 mg L^{-1}; b) Lindane; 250 μg L^{-1};.c) Cr(VI) 500 mg L^{-1} + lindane 250 μg L^{-1}. 1: *Streptomyces* sp. A5; 2: *Streptomyces* sp. A2; 3: *Streptomyces* sp. A11; 4: *Streptomyces* sp. M7; 5: *Streptomyces* sp. MC1; 6: *Amycolatopsis tucumanensis*.

Paired *t*-test analysis and one-way ANOVA were used with a probability level of $p < 0.05$.

3. Results and discussion

3.1. Selection of actinobacteria tolerant to Cr(VI) and lindane

Studies of tolerance to Cr(VI) and lindane were performed on MM agar because the toxic elements do not interact with the medium components and they therefore remain bioavailable to the actinobacteria (Amoroso et al., 2001, 2002; Rathnayake et al., 2013). Qualitative growth evaluation in the presence of 500 mg L^{-1} of Cr(VI) and/or 250 μg L^{-1} of lindane was carried out by comparing growth in the presence or absence of the two contaminants, both individually and in combination. When the individual toxic elements were assayed, *Streptomyces* spp. A5, A11, M7, MC1, and *Amycolatopsis tucumanensis* all showed similar growth to that observed in the uncontaminated control, while *Streptomyces* sp. A2 showed little growth and was thus considered to have low tolerance to Cr(VI) and lindane (Fig. 1a and b). Previously, Polti et al. (2007) used Cr(VI) 260 mg L^{-1}, and Benimeli et al. (2003) used 10 μg L^{-1} of lindane to select Cr(VI) or lindane tolerant actinobacteria, respectively. Therefore, toxic concentrations used in the present work ensure selection of bacteria with high tolerance to such compounds.

Degradation of organic contaminants by microorganisms generally corresponds to an inducible system. However, in co-contaminated environments the presence of heavy metals inhibits the degrading metabolism, so it is necessary to evaluate the toxicity of both pollutants in combination in order to select the most suitable microorganisms for bioremediation processes (Alisi et al., 2009; Thavamani et al., 2012; Moreira et al., 2013). Therefore, as described above the two types of contaminants were mixed to evaluate their combined effect on the six actinobacteria under study.

No inhibition of growth was seen in *Streptomyces* spp. A5, A11, M7, MC1, or *Amycolatopsis tucumanensis*. However, little growth was seen in *Streptomyces* sp. A2 and it was thus considered to be a strain with low tolerance to this mixture of contaminants, probably because the combination of Cr(VI) and lindane enhanced their inhibitory activity against this strain (Fig. 1c). This effect has in fact already been observed by other authors studying co-contaminated systems (Argentina, 2003, 2005; Sandrin and Maier, 2003; Sandrin and Hoffman, 2007; Alisi et al., 2009). However, these results indicate that the contaminant concentrations used were not inhibitory for the growth of five of the six actinobacteria under the experimental testing conditions. The contaminant concentrations tested were selected based upon previous studies, while also taking international standards for permissible levels in soils into consideration (9 mg kg^{-1} for Cr(VI) and 10 μg kg^{-1} for lindane), in order to ensure that microorganisms with high toxicity resistance could be obtained (Argentina, 2003, 2005; Benimeli et al., 2006). The concentrations used are also consistent with those observed by a variety of authors in co-contaminated environments (Roane et al., 2001; El Deeb and Altalhi, 2009; Olaniran et al., 2009; Orton et al., 2013; Shi et al., 2013), who have reported lindane and Cr(VI) concentrations in the order of μg L^{-1} and mg L^{-1}, respectively, in different environmental compartments such as soil, groundwater, rainwater, etc. Such contamination levels are sufficient to produce acute toxicity in animals (Srivastava et al., 2007; Harris et al., 2011). Based upon their tolerance to the individual toxic elements and the mixture, the strains *Streptomyces* spp. A5, A11, M7, MC1, and *Amycolatopsis tucumanensis* were initially selected for use in the further studies discussed below. However, when these individual strains confronted each other on solid MM, it was observed that *Streptomyces* sp. A11 had an inhibitory effect on the growth of *Streptomyces* sp. MC1 and *Streptomyces* sp. M7. These results suggested that it would be best to develop a consortium with *Streptomyces* spp. A5, MC1, M7, and *Amycolatopsis tucumanensis* for removal of lindane and Cr(VI), and these four strains were therefore the ones selected for use in further assays.

3.2. Bioremediation of sterilized soil samples co-contaminated with Cr(VI) and lindane

This study was conducted in order to determine the ability of the selected actinobacteria to grow and to remove Cr(VI) and lindane in sterilized SS. To carry out these assays, 50 mg kg^{-1} of Cr(VI) was used, which corresponds to 5.5 times the permissible level for soils as cited above (9 mg kg^{-1}). However, because of its high level of toxicity, lindane was used at a concentration of 25 μg kg^{-1}, which corresponds to 2.5 times its acceptable level in soils (10 μg kg^{-1}) (Argentina, 2003, 2005).

In control flasks, bioavailable chromium was reduced from 50 to 12 mg kg^{-1}. This result agree with previously reported by other authors (Kotas and Stasicka, 2000; Stewart et al., 2003; Mandiwana et al., 2007; Polti et al., 2011), where a fraction of chromium was adsorbed by soil compounds. Over time, this concentration was kept constant. Bioavailable chromium concentration detected in control flasks (12 mg kg^{-1}) was considered as 100% to further calculations. The changes in lindane concentration in controls were also evaluated. No variations of lindane concentrations in both control series were observed (data not shown), so, there was no evidence of noticeable contribution of abiotic processes to the pesticide removal.

3.2.1. Contamination at d0: assay performance evaluation

After 14 days of incubation at 30 °C, the SSS contaminated at d0 were analyzed. The growth of the individual strains *Streptomyces* spp. A5, M7, and *Amycolatopsis tucumanensis* as well as the growth of the consortium was significantly inhibited by the contaminants ($p < 0.05$). However, *Streptomyces* sp. MC1 showed similar growth levels in the presence or absence of both contaminants (Table 2). This result agrees with those of previous studies carried out in sterilized SS contaminated with 50 mg kg^{-1} of Cr(VI) and inoculated with *Streptomyces* sp. MC1 (Polti et al., 2009). On the other hand, for *Streptomyces* sp. M7 Benimeli et al. (2008) found no growth inhibition in SS contaminated with 100 μg kg^{-1} of lindane, and it would thus appear that the combined presence of the two types of contaminants probably caused the observed growth inhibition in this strain.

After 14 days of incubation, bioavailable chromium levels were determined. *Streptomyces* spp. MC1, M7, A5, and the consortium were able to completely remove the bioavailable chromium, while

Table 2
Microbial growth in SSS after 14 days at 30 °C.

Strain	Non-contaminated soil samples	Soil samples contaminated at d0	Soil samples contaminated at d7
A. tucumanensis	2×10^8 (a)[A]	2×10^7 (b)	4×10^7 (ab)
Streptomyces sp. MC1	2×10^8 (a)	6×10^7 (a)	5×10^7 (a)
Streptomyces sp. M7	2×10^8 (a)	2×10^7 (b)	4×10^8 (a)
Streptomyces sp. A5	2×10^8 (a)	4×10^7 (b)	2×10^8 (a)
Consortium	1×10^9 (c)	8×10^6 (d)	2×10^8 (c)

A: means with different letters are significantly different ($p < 0.05$). Expressed in CFU L^{-1}.

Amycolatopsis tucumanensis removed only 5% (Fig. 2). Previous studies have demonstrated that the bioavailable chromium fraction is exclusively formed by Cr(VI) (Polti et al., 2011), and it can therefore be inferred that the reduction of bioavailable chromium is due to either Cr(VI) reduction to Cr(III) or to bioaccumulation of chromium by *Streptomyces* spp. MC1, M7, and A5. However, since *Amycolatopsis tucumanensis* showed little ability to reduce the bioavailable chromium, its Cr(VI) tolerance must reflect a different mechanism, such as metal exclusion by a permeability barrier or active transport of the metal away from the cell (Bruins et al., 2000). In this case, the metal resistance mechanism does not have relevance in terms of bioremediation processes. It can also be mentioned that Albarracín et al. (2008b) demonstrated copper accumulation by *Amycolatopsis tucumanensis*, with electron microscopy studies demonstrating the presence of copper binding-proteins inside the cell. This mechanism would thus seem to be specific to copper, or at least it is not utilized with chromium.

Polti et al. (2009) previously reported that *Streptomyces* sp. MC1 removed more than 90% of bioavailable chromium after 14 days of incubation in SSS contaminated with 50 mg kg^{-1} of Cr(VI). In the present work, this strain maintained this ability despite the presence of a second pollutant.

Streptomyces spp. MC1, M7, A5, *Amycolatopsis tucumanensis* and the consortium were all able to remove significant amounts of lindane ($p < 0.05$). *Streptomyces* sp. MC1 and *Streptomyces* sp. M7 showed the highest removal levels (44% and 41% respectively), while *Streptomyces* sp. A5 and the consortium removed 22% and 21%, respectively. The level of removal by *Amycolatopsis tucumanensis* was 36% (Fig. 3).

3.2.2. Contamination at d7: assay performance evaluation

In order to evaluate the importance of metabolic activation and environmental adaptation of actinobacteria prior to soil contamination, sterilized SS were first inoculated with the actinobacteria under study and incubated for 7 days, after which the SSS were contaminated with Cr(VI) and lindane and incubated for 7 more days. Actinobacteria growth was first evaluated in the presence and absence of Cr(VI) and lindane (Table 2). Growth inhibition was not observed in the contaminated SSS in comparison with the non-contaminated controls. This would indicate that soil colonization prior to contamination improved actinobacteria development, in contrast to the results seen with the samples where contamination took place at d0, where the contaminants adversely affected growth in most cases.

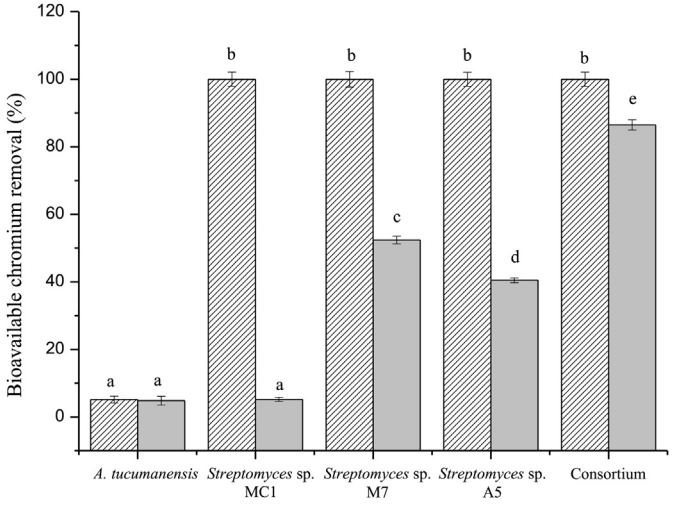

Fig. 2. Bioavailable chromium removal in sterilized SS, after 14 days at 30 °C. Means with different letters are significantly different ($p < 0.05$). Soil samples were contaminated at d0 (▨) or at d7 (▬).

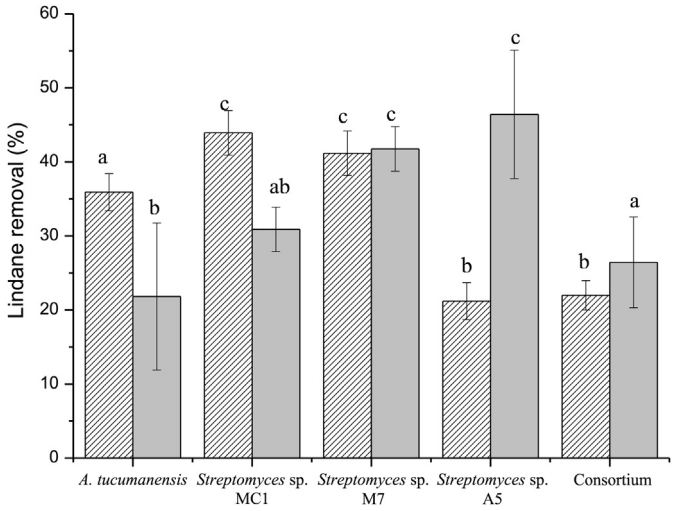

Fig. 3. Lindane removal in sterilized SS, after 14 days at 30 °C. Means with different letters are significantly different ($p < 0.05$). Soil samples were contaminated at d0 (▨) or at d7 (▬).

Bioavailable chromium was determined in the SSS 7 days after their contamination at d7. The consortium of four strains showed the highest level of removal (86%). *Streptomyces* sp. M7 and *Streptomyces* sp. A5 removed 52% and 40%, respectively, while *Streptomyces* sp. MC1 and *Amycolatopsis tucumanensis* removed only 5% of the bioavailable chromium (Fig. 2). It is noteworthy that although in this assay the removal time was reduced in half compared to the samples with immediate contamination (d0), the consortium here maintained its high level of Cr(VI) reducing ability.

Residual lindane was also evaluated 7 days after contamination. *Streptomyces* sp. M7 and *Streptomyces* sp. A5 produced the highest removal levels (42% and 46% respectively). *Amycolatopsis tucumanensis* and the consortium showed the lower levels of lindane removal (22% and 26%, respectively), while *Streptomyces* sp. MC1 removed 31% (Fig. 3).

These results are consistent with previous studies conducted with *Streptomyces* sp. M7, carried out by Benimeli et al. (2008). They determined after 14 days of incubation that this strain was capable of removing 60% of the lindane from SS contaminated with 100 μg kg^{-1} of this pesticide. However, in the present work *Streptomyces* sp. M7 removed 52% of the lindane with an initial concentration of 25 μg kg^{-1} in the presence of Cr(VI), confirming that the metal had a slight negative effect on the biodegradation process.

3.2.3. Selection of the actinobacteria with the most efficient removal results

Different behaviors were observed when comparing the assays performed with immediate (d0) and post-colonization (d7) soil contamination. Growth of *A. tucumanensis* increased in the post-colonization assay, although Cr(VI) and lindane removal was similar under both sets of conditions. Cr(VI) removal by *Streptomyces* sp. MC1, *Streptomyces* sp. M7, and the consortium decreased in the d7 samples, while lindane removal was unchanged. Considering that the exposure time to contaminants was half of that allowed for the immediately contaminated d0 samples, it is possible to infer that these strains first removed lindane and later chromium. Furthermore, *Streptomyces* sp. A5 significantly

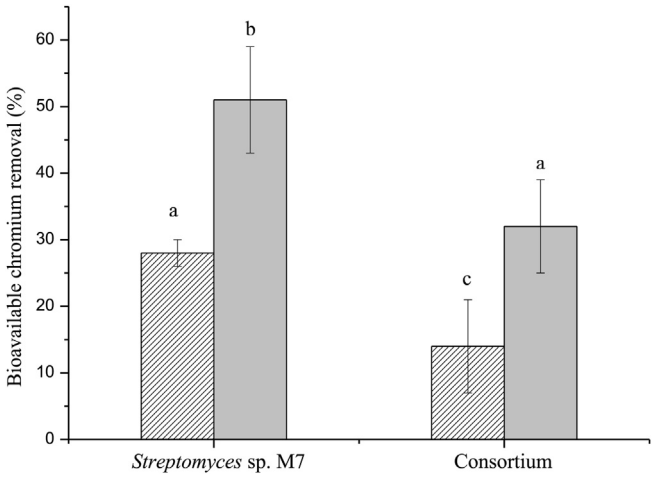

Fig. 4. Bioavailable chromium removal in non sterilized SS, after 14 days at 30 °C. Means with different letters are significantly different ($p < 0.05$). Soil samples were contaminated at d0 (▨) or at d7 (▬).

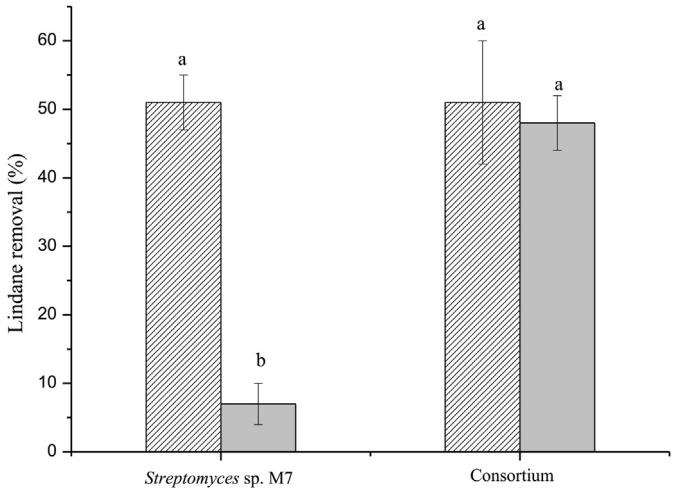

Fig. 5. Lindane removal in non-sterilized SS, after 14 days at 30 °C. Means with different letters are significantly different ($p < 0.05$). Soil samples were contaminated at d0 (▨) or at d7 (▬).

improved its growth with delayed contamination and this was reflected in increased lindane removal levels (from 21% to 46%).

In order to select the actinobacteria most efficient in removing both contaminants, the concentrations of Cr(VI) and lindane removed by each strain and the consortium were analyzed. When contamination was carried out at d0, no difference was observed in terms of Cr(VI) removal by the *Streptomyces* strains. However, in the d7 contamination assay the consortium showed the highest removal percentage, suggesting that the comparative removal rate could be even higher since the time of exposure to the contaminants was reduced to half in comparison with the d0 contamination assay. On the other hand, maximum lindane removal was produced by *Streptomyces* sp. M7 under both conditions (d0 and d7 assays). Based upon these results (Figs. 2 and 3), *Streptomyces* sp. M7 and the consortium formed by the four actinobacteria were selected for a final set of studies using contaminated, non-sterilized soil samples.

3.2.4. Bioremediation of non-sterilized soil samples contaminated with Cr(VI) and lindane

In control flasks, bioavailable chromium was reduced from 50 to 18 mg kg^{-1}. This result agree with previously found (Polti et al., 2011), in non-sterilized soils. The bioavailable chromium fraction was lower in SSS than in NSSS, sterilization process modifies adsorption properties of soil, probably exposing or activating reactive groups of soil, and also the adsorbing surfaces that control the heavy metals solubility (Egli et al., 2006). Similarly to that observed in SSS, over time, this concentration was kept constant. Bioavailable chromium concentration detected in control flasks (18 mg kg^{-1}) was considered as 100% to further calculations. The changes in lindane concentration in controls were also evaluated. No variations of lindane concentrations in both control series were observed, so, there was no evidence of noticeable contribution of autochthonous microflora on the pesticide removal.

In both assays, removal of bioavailable chromium by *Streptomyces* sp. M7 was significantly higher ($p < 0.05$) than by the consortium (Fig. 4). Furthermore, removal of bioavailable chromium by *Streptomyces* sp. M7 in the d7 contamination assay (51%) was similar to that observed in the sterilized SS with this strain, indicating that the presence of the native soil flora did not have a significant impact on the ability of *Streptomyces* sp. M7 to remove Cr(VI).

On the other hand, the bioavailable chromium removal produced by the consortium decreased significantly in the NSSS in comparison with the SSS. The main barrier to the use of communities in bioprocesses is the need for simultaneous control of both individual organisms as the ecosystem as a whole. It is possible that the different consortium members had different behaviors in relation to the native flora, resulting in a decrease in the overall performance of the consortium (Shong et al., 2012).

Lindane removal was higher than 40% for the consortium in both assays (d0 and d7), as well as for *Streptomyces* sp. M7 in the d7 contamination assay (Fig. 5). The low lindane removal level seen for *Streptomyces* sp. M7 in the d7 contamination assay could be due to a decreased level of growth in a non-sterilized SS, whereas when the contaminants were introduced immediately in the d0 contamination assay the lindane may have exerted a selective pressure in favor of this pesticide-resistant actinobacteria, allowing its growth to surpass that of the native flora.

4. Conclusions

Based upon these results, it appears that *Streptomyces* sp. M7 and the consortium make up of the four actinobacterial strains tested could be useful for bioremediation of soils co-contaminated with Cr(VI) and lindane. Also, when considering the process for bioremediation of a co-contaminated site there are two strategies that could be used, either inoculation of a portion of non-sterilized soil with actinobacteria and then using this to inoculate a contaminated environment, or else using actinobacteria grown in a synthetic medium. This latter approach reduces operational time in the laboratory and therefore reduces costs as well.

The differences seen in the results obtained from *Streptomyces* sp. M7 and the consortium are minimal and the use of either would be adequate. However, taking into account the importance of cost/benefit ratios in biotechnological processes and the fact that the use of a consortium is more complex and time consuming and also carries higher risks of contamination, the use of *Streptomyces* sp. M7 alone would seem to be most suitable for these types of processes.

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