

Cr(VI) and lindane removal by *Streptomyces* M7 is improved by maize root exudates

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Environmental mixed pollution by both organic and inorganic compounds are detected worldwide. Phytoremediation techniques have been proposed as ecofriendly methods for cleaning up polluted sites. Several studies have demonstrated enhanced dissipation of contaminants at the root-soil interface through an increase in microbial activity caused by the release of plant root exudates (REs). The aim of this study was to evaluate the effectiveness for Cr(VI) and lindane removal by *Streptomyces* M7 cultured in a co-contaminated system in presence of maize REs. Our results showed when REs were added to the contaminated minimal medium (MM) as the only carbon source, microbial removal of Cr(VI) and lindane increased significantly in comparison to contaminant removal obtained in MM with glucose 1 g L⁻¹. The maximum removal of 91% of lindane and 49.5% of Cr(VI) were obtained in the co-contaminated system. Moreover, *Streptomyces* M7 showed plant growth promoting traits which could improve plant performance in contaminated soils. The results presented in this study provide evidence that maize REs improved growth of *Streptomyces* M7 when REs were used as a carbon source in comparison to glucose. Consequently, lindane and Cr(VI) removal was considerably enhanced making evident the phytoremediation potential of the actinobacteria-plant partnership.

KEYWORDS

bio/phytoremediation, co-contamination, root exudates

1 | INTRODUCTION

In the last few years, there has been a growing interest in the influence of microorganisms on plant growth and contaminant bioavailability and degradation. Consequently, more and more studies are focusing on the role of plant-associated microorganisms in improving phytoremediation efficiency [1,2]. Phytoremediation techniques, based on the interactions between plants and microorganisms, have been proposed as cost-effective and ecofriendly methods for cleaning up polluted sites [3].

Microbe amended phytoremediation appears to be especially effective for organic contaminants, including organochlorine pesticides (OPs) [4]. Several studies have demonstrated enhanced dissipation of OPs at the root-soil interface [1,4,5]. The rhizosphere effect is attributed to an increase in microbial activity caused by the release of plant root exudates (REs) containing enzymes, amino acids, carbohydrates, low-molecular-mass carboxylic acids, and phenolic compounds [6]. Plant-microbe interactions in relation to heavy metals and trace elements have been also mostly approached within the context of phytoremediation. It has been suggested that soil microorganisms might improve

metal mobilization and uptake by plants [7]. This is the case of several actinobacteria since members of this phylum are heterogeneously distributed in the rhizosphere where secondary metabolite producers are enhanced [8]. This feature is generally attributed to the production of plant growth promoting factors, like indoleacetic acid, or the production of metal binding or chelating compounds such as siderophores [9].

Chromium is a heavy metal widely used in a variety of industrial processes, mainly as Cr(VI) [10]. Industrial effluents containing Cr(VI) are released into water courses, resulting in anthropogenic contamination [11]. Cr(VI) shows a high toxicity, which makes it hazardous even at very low concentration [12].

The OP lindane has been widely used for both agriculture and medical purposes before its use has been restricted in most countries [13]. This pesticide is highly recalcitrant and produces several health effects [14]. Since its persistence, lindane residues has been reported in soils, water, air, plants, agricultural products, and animals, as well as in human body [15,16].

Mixed pollution by chromium and lindane has been detected around the world. For example, in sediments of the Berre Coastal Lagoon, in the Southeast France [17] (maximum: Cr = 119 mg kg⁻¹; lindane = 380 µg kg⁻¹), and in the Romanian Black Sea coast [18] (maximum: Cr = 140 mg kg⁻¹; lindane = 390 µg kg⁻¹).

Bio/phytoremediation of pesticides and heavy metals has been shown to be successful, although at the moment each process has generally been performed individually. Certainly, a multifunctional biological process is needed for bioremediation of co-contaminated sites, since co-contamination represents the real current worldwide concern [19]. Currently, members of the phylum Actinobacteria are being studied because of their ability to bioremediate co-polluted soils. In particular, *Streptomyces* strains are able to bioremediate Cr(VI) and lindane from non-sterilized co-contaminated soils [20,21]. In turn, lindane-degrading *Streptomyces* strains were shown to be able to remove lindane in the presence of maize plants and its REs [22,23]. In this context, the main objective of the present work has been to evaluate the effectiveness for Cr(VI) and lindane removal by *Streptomyces* M7 cultured in a co-contaminated system in presence of maize REs.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Lindane (99% pure) was purchased from Sigma-Aldrich Co. (St. Louis, USA). The solvents used throughout this study, such as acetone and hexane, were pesticide grade. All other chemicals were analytical grade, purchased from standard manufacturers. Cr(VI) was added as K₂Cr₂O₇ [21].

2.2 | Microorganisms and culture media

Streptomyces M7 was previously isolated from wastewater sediments contaminated with pesticides and heavy metals [21,24]. Taxonomic identification of this strain has been confirmed by amplification and partial sequencing of their 16S rDNA genes (GenBank IDs: AY45953) [25].

To prepare the inoculum for evaluating ACC deaminase activity, *Streptomyces* M7 was cultured on Tryptic Soy Broth (TSB), containing in g L⁻¹: tryptone, 15; soy peptone, 3; NaCl, 5; K₂HPO₄, 2.5; and glucose, 2.5. The pH was adjusted to 7.3. Flasks were incubated at 30 °C for 3 days.

Minimal medium (MM) containing in g L⁻¹: glucose, 10; NH₄SO₄, 2; K₂HPO₄, 0.5; MgSO₄ · 7H₂O, 0.2; FeSO₄ · 7H₂O, 0.01 was used for several assays. The pH was adjusted to 7.

To prepare spore suspensions (inoculum for the bioremediation assay) and for CFU counts, *Streptomyces* M7 was cultured in Starch Casein medium (SC-agar), containing in g L⁻¹: starch, 10; casein, 1; K₂HPO₄, 0.5; agar, 15. The pH was adjusted to 7. Petri dishes were incubated at 30 °C for 7 days.

REs were obtained from maize plants cultured in nutrient solution, containing: KNO₃ 1.5 mM; Ca(NO₃)₂ 1 mM; MgSO₄ 0.5 mM; NH₄H₂PO₄ 0.25 mM; EDTA-Fe 11.9 µM; H₃BO₃ 11.5 µM; MnSO₄ 1.25 µM; ZnSO₄ 0.2 µM; CuSO₄ 0.075 µM; (NH₄)₆Mo₇O₂₄ 0.025 µM. The pH was adjusted to 5.8 [26].

All the media were sterilized by autoclaving at 121 °C for 15 min.

2.3 | Plant growth promoting characteristics

The ability of *Streptomyces* M7 to solubilize inorganic phosphate was assessed in modified MM (without K₂HPO₄) supplied with (g L⁻¹): Ca₃(PO₄)₂, 5 and agar, 15. Plates were incubated at 30 °C for 7 days. A clear halo around the bacterial colony indicated solubilization of mineral phosphate.

Siderophore production was detected in modified MM (without FeSO₄) using the Chrome Azurol S (CAS) method described by Schwyn and Neilands [27].

The ability of *Streptomyces* M7 to produce IAA were evaluated in modified MM (without NH₄SO₄) supplemented with 2 mg ml⁻¹ of tryptophan, pH 7. After 5 days of incubation at 30 °C, cultures were centrifuged and the presence of IAA was evaluated after incubating 0.5 ml of the supernatant with 0.5 ml of Salkowski's reagent (1 g L⁻¹ FeCl₃; 28.5 ml water; 22 ml H₂SO₄ [28]) for 30 min, at 25 °C, in darkness. The production of IAA was recognized by the presence of red coloring and evaluated spectrophotometrically (540 nm) using pure IAA as a standard calibration curve.

ACC deaminase activity was checked according to the method of Glick [29] with modifications. *Streptomyces* M7 was first cultured in TSB medium. A solution of ACC (0.5 M) (Sigma Chemical Co., USA) was filter sterilized (0.22 µm Millipore) and

frozen at -20°C . The microorganism was streaked onto modified MM (without NH_4SO_4) supplemented with 3.0 mM ACC as a nitrogen source. Plates were incubated at 30°C for 7 days.

2.4 | Collection and analysis of root exudates

Endophyte-free maize (*Zea mays*) seeds not treated with fungicide were first surface sterilized using 2% mercuric chloride [30] and then germinated. Groups of twenty maize seedlings were aseptically transferred to flasks where they were grown in 200 ml of a nutrient solution under sterile conditions, in a climate controlled room. The nutrient solution in the culture flasks was replaced every 2 days for 2 weeks, with the exiting solution being collected and stored at 4°C . The solution collected from each flask was used as the source of REs (adapted from Luo et al. [26]). These exudates in solution were lyophilized, re-dissolved in an appropriate volume of sterile distilled water, and then filter sterilized ($0.22\ \mu\text{m}$ Millipore) to obtain a dissolved organic carbon concentration (DOC). The DOC of the final solution was measured with an organic carbon analyzer (TOC-L CPN, Shimadzu).

The protein concentration was determined according to the method described by Bradford [31]. Quantitative analysis of carbohydrates was carried out using High-Pressure Liquid Chromatography (Gilson) with a Rezex RPM-Monosaccharide column ($300 \times 7.8\ \text{mm}$, Phenomenex) and refraction index detector (Gilson 132). Sample was eluted with ultrapure water at a flow rate of $0.6\ \text{ml}\ \text{min}^{-1}$, at 85°C . Sugars of REs were identified by comparison of the retention times of peaks from the REs with those of standard compounds.

2.5 | Bioremediation assay

Streptomyces M7 was grown in SC-agar at 30°C until sporulation (approximately 7 days). Spores were then scraped from the surface of the plates and washed twice with sterile distilled water. Spores were suspended in an equal volume of sterile distilled water to prepare the inoculum [32]. In order to evaluate the performance of *Streptomyces* M7 on lindane and chromium removal, flasks with 30 ml of modified MM with $1\ \text{g}\ \text{L}^{-1}$ glucose were artificially contaminated with $2\ \text{mg}\ \text{L}^{-1}$ lindane and/or $25\ \text{mg}\ \text{L}^{-1}$ of Cr(VI), and inoculated with *Streptomyces* M7 (about $2 \times 10^5\ \text{CFU}\ \text{ml}^{-1}$). Then, in order to evaluate the effect of maize REs on lindane and chromium removal by *Streptomyces* M7, flasks with 30 ml of modified MM (without glucose) were supplemented with maize REs. Thus, REs were added to the flasks to a final concentration of $0.4\ \text{g}\ \text{C}\ \text{L}^{-1}$ (the same amount of carbon present in $1\ \text{g}\ \text{L}^{-1}$ glucose). Then, MM was artificially contaminated with lindane ($2\ \text{mg}\ \text{L}^{-1}$) and/or $25\ \text{mg}\ \text{L}^{-1}$ of Cr(VI) and inoculated with *Streptomyces* M7 (about $2 \times 10^5\ \text{CFU}\ \text{ml}^{-1}$). Flasks were incubated at 30°C , 150 rpm during 5 days. Non-contaminated MM and non-inoculated MM were used as

controls. Samples were taken at 3 and 5 days of incubation for determining microbial growth and contaminants concentrations.

2.6 | Analytical determinations of lindane and Cr(VI)

Total Cr content was determined by Atomic Absorption Spectrometry, using a Perkin Elmer Analyst 400 (AAS). Cr(VI) concentration was determined using the Cr(VI) specific colorimetric reagent 1,5-diphenylcarbazide, dissolved in acetone to a final concentration of 0.05% [21]. The absorbance was measured at 540 nm. The Cr(VI) concentration was estimated using a calibration curve (0–1 mM).

Lindane extraction from aqueous systems was carried out by ultrasonication. For this purpose, 3 ml of n-hexane were added to 1 ml of sample and sonicated for 30 min. Then an appropriate volume of the organic phase was taken and dehydrated with Na_2SO_4 . Finally, 1 ml of the extract obtained was put in a sealable vial for subsequent injection into the gas chromatograph with microelectron capture detector (GC/ μECD). Lindane concentration in extracts was quantified in a gas chromatograph (Agilent 7890A) equipped with ^{63}Ni microelectron capture detector, HP5 capillary column ($30\ \text{m} \times 0.53\ \text{mm} \times 0.35\ \text{m}$), a split/splitless Agilent 7693B injector and Agilent ChemStation software. The chromatographic conditions were as follows: inlet temperature: 250°C , carrier gas (nitrogen) flow rate: $25\ \text{cm}\ \text{s}^{-1}$, initial oven temperature: 180°C increasing to 250°C at $40^{\circ}\text{C}\ \text{min}^{-1}$ and increasing to 280°C at $10^{\circ}\text{C}\ \text{min}^{-1}$. The detector temperature was 320°C and the injection volume was 1 ml. Quantification of the pesticide was performed using a calibration curve with appropriated dilutions of lindane calibration standards (AccuStandard, New Haven, USA).

2.7 | Statistical analyses

Three replicates were conducted in all assays and the results are the average of them. One-way analysis of variance (ANOVA) was used to test the significant differences. When significant differences were found, Tukey test was used to separate the effects. Tests were considered significantly different at $p < 0.05$. Statistical analyses were performed using a professional version of Infostat statistical software.

3 | RESULTS

3.1 | Physico-chemical characteristics of maize root exudates and plant growth promoting traits of *Streptomyces* M7

Lyophilized REs from approximately 150 maize plants grown in hydroponics were re-dissolved in water. The pH of the

solution was 7.3. Total proteins content of REs was 1.1 mg g^{-1} , and carbohydrates such as sucrose 11.8 g L^{-1} and glucose 1.8 g L^{-1} were detected. DOC was 34.1 mgC g^{-1} which allowed preparing a stock solution with a known amount of carbon.

Streptomyces M7 was able to solubilize inorganic phosphate and secrete siderophores, since a clear and reddish hydrolysis halos around the colonies were observed, respectively. Positive ACC deaminase activity was also detected since the microorganism was capable of growth using ACC as the only source of N. Finally, IAA production was $13.2 \pm 1.6 \mu\text{g ml}^{-1}$.

3.2 | *Streptomyces* M7 growth and lindane and Cr(VI) removal

Growth and simultaneous removal of Cr(VI) and lindane by *Streptomyces* M7 was evaluated in artificially co-contaminated liquid system. At 3 days of the assay, the highest microbial growth was obtained in the uncontaminated medium ($1.69 \times 10^7 \pm 1.93 \times 10^6 \text{ CFU ml}^{-1}$) while the lowest value was registered in the co-contaminated medium ($2.46 \times 10^5 \pm 1.28 \times 10^4 \text{ CFU ml}^{-1}$) (Figure 1A). These differences were significant at a statistical level ($p < 0.05$). Cr(VI) removal of 10.3 and 14.3% were registered in the co-contaminated system and in the presence of Cr(VI) alone, respectively. Lindane removal of 21.4% was registered in the co-contaminated system while 23.7% of removal was registered in the treatment with lindane. No removal of contaminants was detected in the abiotic control (Figure 1A). At 5 days of the assay, microbial growth and contaminants removals were increased. Once again, the highest microbial growth was obtained in the uncontaminated medium ($1.51 \times 10^8 \pm 9.95 \times 10^6 \text{ CFU ml}^{-1}$), while the lowest value was registered in the co-contaminated medium ($2.00 \times 10^6 \pm 7.25 \times 10^4 \text{ CFU ml}^{-1}$). These differences were significant at a statistical level ($p < 0.05$) (Figure 1B).

Cr(VI) removal of 15.9 and 15.6% were registered in the co-contaminated system and in the presence of Cr(VI) alone, respectively. Lindane removal of 28.0% was registered in the co-contaminated treatment, while 40.8% of pesticide removal was registered in the system with lindane alone. These differences were significant at a statistical level ($p < 0.05$). No removal of contaminants was detected in the abiotic control (Figure 1B).

Once the positive performance of *Streptomyces* M7 on liquid co-contaminated medium was evaluated, glucose was replaced by maize REs on the MM.

Streptomyces M7 grew in the presence of REs as the only carbon source (Figure 2) reaching a greater biomass than the biomass obtained with glucose in the uncontaminated medium, both at 3 and 5 days of assays (Figure 1). These

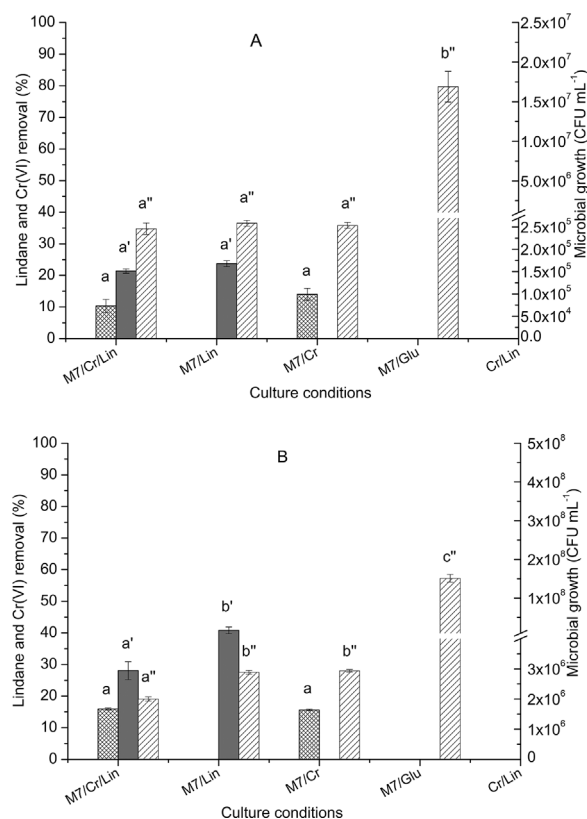


FIGURE 1 Microbial growth and lindane and Cr(VI) removal by *Streptomyces* M7 cultured in minimal medium at 3 (A) and 5 (B) days. Lin: lindane; Cr: Cr(VI). Bars showing different letters indicate they were significantly different ($p < 0.05$, Tukey's post-test)

differences were significant at a statistical level ($p < 0.05$). Likewise, the highest microbial growth was obtained in the uncontaminated medium, while the lowest value was recorded in the co-contaminated medium, both at 3 and 5 days of assays. However, microbial growth was notably higher in the co-contaminated system supplemented with REs ($2.63 \times 10^7 \pm 6.69 \times 10^5 \text{ CFU ml}^{-1}$) (Figure 2B) in comparison to the co-contaminated system with glucose ($2.00 \times 10^6 \pm 7.25 \times 10^4 \text{ CFU ml}^{-1}$), at 5 days of the assay (Figure 1B). These differences were significant at a statistical level ($p < 0.05$).

Cr(VI) and lindane removal by *Streptomyces* M7 increased significantly in the presence of REs in comparison to glucose assays, both at 3 and 5 days. These differences were significant at a statistical level ($p < 0.05$). It was highlighted the maximum removal of 91.2% of lindane and 49.5% of Cr(VI) in the co-contaminated system at 5 days of the assay (Figure 2B). Similar to these values were lindane and chromium removal obtained when one or other contaminant were in the MM. In fact, no statistically significant differences were found in contaminants removal between co-contaminated and single-contaminated treatments ($p > 0.05$). No removal of contaminants was detected in the abiotic control (Figure 2B).

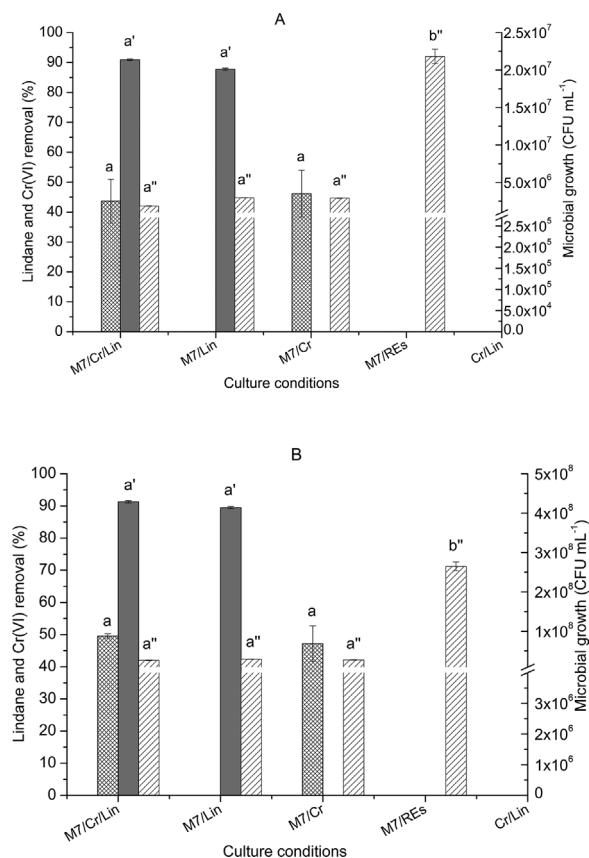


FIGURE 2 Microbial growth and lindane and Cr(VI) removal by *Streptomyces* M7 cultured with maize root exudates (REs) at 3 (A) and 5 (B) days. Lin: lindane; Cr: Cr(VI). Bars showing different letters indicate they were significantly different ($p < 0.05$, Tukey's post-test)

4 | DISCUSSION

Degradation of organic contaminants by microorganisms generally corresponds to an inducible mechanism. 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 microorganism for bioremediation processes [21]. Thus, Cr(VI) (25 mg L^{-1}) and lindane ($250 \text{ } \mu\text{g L}^{-1}$) were mixed to evaluate their combined effect on the actinobacteria *Streptomyces* M7, cultured on artificially contaminated liquid medium. Previous studies have already demonstrated that this strain was tolerant to both contaminants in solid medium and soil [20,21]. However, its performance in a liquid system where contaminants are highly available has never been evaluated. The highest growth of *Streptomyces* M7 was obtained in the uncontaminated medium, while the lowest growth was recorded in the co-contaminated system, indicating the limiting effect of both contaminants on microbial growth. These observations agree with the findings of Cárdenas-González and Acosta-Rodríguez [33] who reported that the

biomass of a strain of *Paecilomyces* sp. decreased significantly when it was exposed to increasing concentrations of Cr(VI). Heavy metals can cause adverse effects on biological systems, producing breaks in DNA by oxidative stress [34]. According to Moreira and Moreira [35], toxic metals can hinder any and all biological activity, causing different types of responses, among them, the decrease of growth. However, the concentration of contaminants used in this work did not inhibit the *Streptomyces* M7 growth. This could be related to the fact that the genome of *Streptomyces* M7 counts with a significant number of genes coding for proteins involved in counteracting the oxidative stress caused by Cr(VI), which is why the microorganism can tolerate the presence of the metal (Davila Costa, personal communication).

Percentages of Cr(VI) and lindane removals were lower in the co-contaminated medium in comparison to percentages of removals in MM contaminated with one or another contaminant. It was highlighted the maximum lindane removal when the bacterium grew only with lindane, at 5 days of the assay. As mentioned before, microbial degradation of organic pollutants generally corresponds to an inducible system. Cuozzo et al. [36] demonstrated the presence of a specific dechlorinase activity in *Streptomyces* M7 whose synthesis was induced when the pesticide was present in the culture medium. However, in co-contaminated environments, the presence of heavy metals in toxic concentrations leads to the inhibition of degradative metabolism, which would explain the less removal of lindane in the presence of Cr(VI). Meanwhile, Polti et al. [37] demonstrated that the bioavailable chromium fraction is exclusively formed by Cr(VI), 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* M7. The latter was recently demonstrated by Davila Costa (personal communication) in preliminary assays in our laboratory.

It is convenient to clarify that the concentrations of Cr(VI) and lindane used in this work were selected based on previous studies by Polti et al. [21] who worked with *Streptomyces* M7 grown in co-contaminated soil with $25 \text{ } \mu\text{g kg}^{-1}$ of lindane (2 mg L^{-1} in the present work) and 50 mg kg^{-1} of Cr(VI) (25 mg kg^{-1} in the present work). The concentrations also correspond to those indicated by different authors in co-contaminated environments, who report concentrations of lindane and Cr(VI) in the order of $\mu\text{g L}^{-1}$ and mg L^{-1} , respectively, in different environmental compartments [38]. These levels of contamination are sufficient to produce acute toxicity in animals [39] thus exceeding the levels allowed by Argentinean legislation ($10 \text{ } \mu\text{g kg}^{-1}$ for lindane and 8 mg kg^{-1} for Cr(VI)) [15,37].

During plant growth, roots release a range of organic compounds that can potentially enhance the biodegradation of contaminants in a variety of ways, including by

stimulation of bacterial growth. Thus, characterization of REs composition provided insight about the potential of plant species as rhizospheric carbon sources, which in turn is highly relevant considering that root turnover, provides a large source of carbon in rhizoremediation [40]. The content of carbohydrates and proteins detected in this work suggest that maize REs represent a viable carbon, nitrogen, and energy source for microbial growth. Personeni et al. [41] found that glucose was a compound commonly detected in REs from maize plants grown in hydroponics. Alvarez et al. [22,23] detected sugars, proteins, and phenolic compounds in REs of a different variety of maize than the used in the present work. It is known that the chemical characteristics of plant compounds vary not only between different species but also between members of the same species, especially in plants such as maize, from which multiple varieties are available [42]. In view of these results, it may be possible that the studied actinobacteria was competitive at the maize rhizosphere level. In fact, *Streptomyces* M7 grew in presence of maize REs as the only carbon source. When REs was added to the contaminated MM, microbial removal of Cr(VI) and lindane increased significantly with respect to contaminants removal obtained in MM with glucose. Moreover, the maximum removal of lindane and Cr(VI) were obtained in the co-contaminated system. Alvarez et al. [22] found percentages of lindane removal between 55 and 37% when *Streptomyces* strains were cultured with maize REs, significantly lower than those found in this study. These differences could be explained by the fact that REs are a complex mixture of substrates, some of which could repress and/or diminish xenobiotic degrading activities, especially at high concentrations of carbon as was used in that work (100 g C L^{-1}). Similar results were found by Rentz et al. [40] and Louvel et al. [43] who informed that REs from different plant species repressed the degrading phenanthrene activity of *Pseudomonas putida*. Considering that co-metabolism is the mechanism most commonly used by the organic chemical degrading microbiota [44], it is possible that *Streptomyces* M7 first used REs to grow, and then the pesticide as a secondary substrate. In relation to this, Benimeli et al. [25] found that the removal of different OPs by *Streptomyces* M7 was more efficient when other carbon sources were present in the culture medium. However, according to Polti et al. [21], first, there would have been the reduction of chromium and then the lindane degradation. The authors reported that chromium and lindane removal did not occur simultaneously. As mentioned before, the Cr(VI) reduction to Cr(III) is a process which uses NADH from bacterial metabolism and, therefore, any process that affects its production affects the reduction of Cr(VI) [45]. Also, electron acceptors affect significantly lindane degradation. It is a co-metabolic

process, which improves with an additional energy source [30]. REs energy source could be used primarily to obtain NADH for Cr(VI) reduction, and REs as carbon source could be used for lindane removal also.

In general, when *Streptomyces* strains are grown in MM, they arrive to stationary phase between 48 and 72 h of incubation, even in the presence of toxics [25,37]. It was also known that Cr(VI) reduction in *Streptomyces* strains occurs at the end of the exponential phase and beginning of the stationary phase [45]. This could explain why no differences were found between Cr(VI) residual concentration at 3 and 5 days of incubation. This strong biological effect of *Streptomyces* M7 is highly relevant, taking into account the feasibility of its potential application on co-polluted and planted soils: *Streptomyces* strains are well adapted to grow in soil and maize plants are well adapted to acid conditions such as those generated during the degradation of lindane, in addition to its metal tolerance [30,37]. Although the complex interactions existing in the rhizosphere are not detectable in *in vitro* assays, liquid-based experiments, where the pollutant is more bioavailable, seem to be favorable for initial studies related to plant-microbe behavior in polluted systems [46]. Moreover, *Streptomyces* M7 showed several PGP traits: inorganic phosphate solubilization, siderophore production, ACC deaminase activity, and IAA production. Phosphate solubilization and chelating agents as siderophore can influence the metal availability in soils [1]. It is known that IAA and ACC deaminase activity can significantly improve plant growth and biomass production in contaminated soils. In fact, many authors have attributed bacterial-induced increases in plant growth in the presence of metals due to the production of IAA [47,48].

In conclusion, the results presented in this study provide evidence that maize REs improved growth of *Streptomyces* M7 when REs were used as a carbon source in comparison to glucose. Consequently, lindane and Cr(VI) removal was considerably enhanced. Otherwise, *Streptomyces* M7 showed plant growth promoting traits which could improve plant performance in contaminated soils, making evident the phytoremediation potential of the actinobacteria-plant partnership.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

- [1] Kidd PS, Barceló J, Bernal MP, Navari-Izzo F. Trace element behaviour at the root-soil interface: implications in phytoremediation. *Environ Exp Bot* 2009;67:243–59.
- [2] Weyens N, van der Lelie D, Taghavi S, Vangronsveld J. Phytoremediation: plant–endophyte partnerships take the challenge. *Curr Opin Biotechnol* 2009;20:248–54.
- [3] San Miguel A, Ravanel P, Raveton M. A comparative study on the uptake and translocation of organochlorines by *Phragmites australis*. *J Hazard Mater* 2013;244–245:60–9.
- [4] Gerhardt KE, Huang XD, Glick BR, Greenberg BM. Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Sci* 2009;176:20–30.
- [5] Becerra Castro C, Prieto-Fernández A, Kidd PS, Weyens N. Improving performance of *Cytisus striatus* on substrates contaminated with hexachlorocyclohexane (HCH) isomers using bacterial inoculants: developing a phytoremediation strategy. *Plant Soil* 2013;362:247–60.
- [6] Curl EA, Truelove B. *The Rhizosphere*. Berlin: Springer-Verlag; 1996.
- [7] Gremion F, Chatzinotas A, Harms H. Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil. *Environ Microbiol* 2003;5:896–907.
- [8] Basil AJ, Strap JL, Knotek-Smith HM, Crawford DL. Studies on the microbial populations of the rhizosphere of big sagebrush (*Artemisia tridentata*). *J Ind Microbiol Biotechnol* 2004;31:278–88.
- [9] Dimkpa CO, Merten D, Svatos A, Büchel G. Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. *J Appl Microbiol* 2009;107:1687–96.
- [10] Bankar AV, Kumar AR, Zinjarde SS. Removal of chromium (VI) ions from aqueous solution by adsorption onto two marine isolates of *Yarrowia lipolytica*. *J Hazard Mater* 2009;170:487–94.
- [11] Cheung KH, Gu JD. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. *Int Biodeterior Biodegrad* 2007;59:8–15.
- [12] Cheung KH, Gu JD. Reduction of chromate (CrO₄²⁻) by an enrichment consortium and an isolate of marine sulfate-reducing bacteria. *Chemosphere* 2003;52:1523–9.
- [13] Vijgen J, Abhilash PC, Li YF, Lal R. Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs—a global perspective on the management of lindane and its waste isomers. *Environ Sci Pollut Res* 2011;18:152–62.
- [14] Saez JM, Benimeli CS, Amoroso MJ. Lindane removal by pure and mixed cultures of immobilized actinobacteria. *Chemosphere* 2012;89:982–7.
- [15] Fuentes MS, Sáez JM, Benimeli CS, Amoroso MJ. Lindane biodegradation by defined consortia of indigenous *Streptomyces* strains. *Water Air Soil Pollut* 2011;222:217–31.
- [16] Ridolfi AS, Alvarez GB, Rodríguez Giraul ME. Organochlorinated contaminants in general population of Argentina and other Latin American Countries. In: Alvarez A, Polti MA, editors. *Bioremediation in Latin America. Current research and perspectives*. New York: Springer; 2014. p. 17–40.
- [17] Arienzo M, Masuccio AA, Ferrara L. Evaluation of sediment contamination by heavy metals, organochlorinated pesticides, and polycyclic aromatic hydrocarbons in the Berre coastal lagoon (Southeast France). *Arch Environ Contam Toxicol* 2013;65:396–406.
- [18] Coatu V, Țigănuș D, Oros A, Lazăr L. Analysis of hazardous substance contamination of the marine ecosystem in the Romanian Black Sea coast, part of the Marine Strategy Framework Directive (2008/56/EEC) implementation. *Rech Mar* 2013;43:144–86.
- [19] Alvarez A, Saez JM, Davila Costa JS, Colin VL. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere* 2017;166:41–62.
- [20] Aparicio JD, Simón Solá MZ, Benimeli CS, Amoroso MJ. Versatility of *Streptomyces* sp. M7 to bioremediate soils co-contaminated with Cr (VI) and lindane. *Ecotoxicol Environ Saf* 2015;116:34–9.
- [21] Polti MA, Aparicio JD, Benimeli CS, Amoroso MJ. Simultaneous bioremediation of Cr(VI) and lindane in soil by actinobacteria. *Int Biodeterior Biodegrad* 2014;88:48–55.
- [22] Alvarez A, Yañez LM, Benimeli CS, Amoroso MJ. Maize plants (*Zea mays*) root exudates enhance lindane removal by native *Streptomyces* strains. *Int Biodeterior Biodegrad* 2012;66:14–8.
- [23] Álvarez A, Benimeli CS, Saez JM, Giuliano A. Lindane removal using *Streptomyces* strains and maize plants: a biological system for reducing pesticides in soils. *Plant Soil* 2015;395:401–13.
- [24] Benimeli CS, Amoroso MJ, Chaile AP, Castro GR. Isolation of four aquatic streptomycetes strains capable of growth on organochlorine pesticides. *Bioresour Technol* 2003;89:133–8.
- [25] Benimeli CS, Castro GR, Chaile AP, Amoroso MJ. Lindane uptake and degradation by aquatic *Streptomyces* sp. strain M7. *Int Biodeterior Biodegrad* 2007;59:148–55.
- [26] Luo L, Zhang S, Shan XQ, Zhu YG. Oxalate and root exudates enhance the desorption of DDT from soils. *Chemosphere* 2006;63:1273–9.
- [27] Schwyn B, Neilands B. Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 1987;160:47–56.
- [28] Szkop M, Sikora P, Orzechowski S. A novel, simple, and sensitive colorimetric method to determine aromatic amino acid aminotransferase activity using the Salkowski reagent. *Folia Microbiol (Praha)* 2012;57:1–4.
- [29] Glick BR. The enhancement of plant growth by freeliving bacteria. *Can J Microbiol* 1995;41:109–17.
- [30] Benimeli CS, Fuentes MS, Abate CM, Amoroso MJ. Bioremediation of lindane contaminated soil by *Streptomyces* sp. M7 and its effects on *Zea mays* growth. *Int Biodeterior Biodegrad* 2008;61:233–9.
- [31] Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–54.
- [32] Amoroso MJ, Castro GR, Carlino FJ, Romero NC. Screening of heavy metal-tolerant actinomycetes isolated from the Salí River. *J Gen Appl Microbiol* 1998;44:129–32.
- [33] Juan F. Cárdenas-González, Ismael Acosta-Rodríguez. Hexavalent chromium removal by a *Paecilomyces* sp. fungal strain isolated from environment. *Bioinorg Chem Appl* 2010;2010. Article ID 676243, 6 pages. <https://doi.org/10.1155/2010/676243>
- [34] Gastaldo J, Viau M, Bencokova Z, Joubert A. Lead contamination results in late and slowly repairable DNA double-strand breaks and impacts upon the ATM-dependent signaling pathways. *Toxicol Lett* 2007;173:201–14.

- [35] Moreira FR, Moreira JC. Os efeitos do chumbo sobre o organismo humano e seu significado para a saúde. *Rev Panam Salud Publica* 2004;15:119–29.
- [36] Cuozzo SA, Rollán GG, Abate CM, Amoroso MJ. Specific dechlorinase activity in lindane degradation by *Streptomyces* sp. M7. *World J Microbiol Biotechnol* 2009;25:1539–46.
- [37] Polti MA, Atjian MC, Amoroso MJ, Abate CM. Soil chromium bioremediation: synergic activity of actinobacteria and plants. *Int Biodeterior Biodegradation* 2011;65:1175–81.
- [38] Olaniran AO, Balgobind A, Pillay B. Impacts of heavy metals on 1,2-dichloroethane biodegradation in co-contaminated soil. *J Environ Sci* 2009;21:661–6.
- [39] Harris ESJ, Cao S, Littlefield BA, Craycroft JA. Heavy metal and pesticide content in commonly prescribed individual raw Chinese herbal medicines. *Sci Total Environ* 2011;409:4297–305.
- [40] Rentz J, Alvarez P, Schnoor J. Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. *Environ Microbiol* 2004;6:574–83.
- [41] Personeni E, Nguyen C, Marchal P, Pages L. Experimental evaluation of an efflux-influx model of C exudation by individual apical root segments. *J Exp Bot* 2007;58:2091–9.
- [42] Nardi S, Sessi E, Pizzeghello D, Sturaro A. Biological activity of soil organic matter mobilized by root exudates. *Chemosphere* 2002;46:1075–81.
- [43] Louvel B, Cébron A, Leyval C. Root exudates affect phenanthrene biodegradation, bacterial community and functional gene expression in sand microcosms. *Int Biodeterior Biodegrad* 2011;65:947–53.
- [44] De Schrijver A, De Mot R. Degradation of pesticides by actinomycetes. *Crit Rev Microbiol* 1999;25:85–119.
- [45] Polti MA, Amoroso MJ, Abate CM. Intracellular chromium accumulation by *Streptomyces* sp. MC1. *Water Air Soil Pollut* 2011;214:49–57.
- [46] Schwitzguébel JP, Meyer J, Kidd PS. Pesticides removal using plants: phytodegradation versus phytostimulation. In: Mackova M, Dowling DN, Macek T, editors. *Phytoremediation rhizoremediation*. Berlin: Springer; 2006. p. 179–98.
- [47] Shilev S, Fernández A, Benloch M, Sancho ED. Sunflower growth and tolerance to arsenic is increased by the rhizospheric bacteria *Pseudomonas fluorescens*. In: Morel JL, Echevarria G, Goncharova N, editors. *Phytoemediations of metal contaminated soils*. Springer: Netherlands; 2006. p. 315–8.
- [48] Dell'Amico E, Cavalca L, Andreoni V. Improvement of Brassica napus growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biol Biochem* 2008;40:74–84.

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