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Removal of a mixture of pesticides by a *Streptomyces* consortium: Influence of different soil systems

María S. Fuentes [a, b], Enzo E. Raimondo [a], María J. Amoroso [a, b, c], Claudia S. Benimeli [a, b, *]

[a] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, 4000, Tucumán, Argentina
[b] Universidad del Norte Santo Tomás de Aquino, 9 de Julio 165, 4000, Tucumán, Argentina
[c] Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000, Tucumán, Argentina

HIGHLIGHTS

- Removal of three pesticides was simultaneously assayed in liquid and soil systems.
- The pesticides contaminated systems were inoculated with a *Streptomyces* consortium.
- Pesticides removal by the consortium was dependent on the system evaluated.
- Soil textures were determining in the removal of each pesticide in soil microcosms.
- Highest percentages of pesticide removal were obtained in clay silty loam soil.

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ABSTRACT

Although the use of organochlorine pesticides (OPs) is restricted or banned in most countries, they continue posing environmental and health concerns, so it is imperative to develop methods for removing them from the environment. This work is aimed to investigate the simultaneous removal of three OPs (lindane, chlordane and methoxychlor) from diverse types of systems by employing a native *Streptomyces* consortium. In liquid systems, a satisfactory microbial growth was observed accompanied by removal of lindane (40.4%), methoxychlor (99.5%) and chlordane (99.8%). In sterile soil microcosms, the consortium was able to grow without significant differences in the different textured soils (clay silty loam, sandy and loam), both contaminated or not contaminated with the OPs-mixture. The *Streptomyces* consortium was able to remove all the OPs in sterile soil microcosm (removal order: clay silty loam > loam > sandy). So, clay silty loam soil (CSLS) was selected for next assays. In non-sterile CSLS microcosms, chlordane removal was only about 5%, nonetheless, higher rates was observed for lindane (11%) and methoxychlor (20%). In CSLS slurries, the consortium exhibited similar growth levels, in the presence of or in the absence of the OPs-mixture. Not all pesticides were removed in the same way; the order of pesticide dissipation was: methoxychlor (26%)>lindane (12.5%)>chlordane (10%). The outlines of microbial growth and pesticides removal provide information about using actinobacteria consortium as strategies for bioremediation of OPs-mixture in diverse soil systems. Texture of soils and assay conditions (sterility, slurry formulation) were determining factors influencing the removal of each pesticide of the mixture.

1. Introduction

The agricultural practices, necessary to meet the world food demand, have involved pesticides use in simultaneous application or one after another. Thus, these xenobiotic compounds may be simultaneously present in the environment, constituting an important problem, mainly if the pesticides used were organochlorines (OPs), due their high toxicity and persistence (Swarcewicz and Gregorczyk, 2012). Among them, lindane, chlordane and methoxychlor have been widely used in the world for crop protection, against insects and other pests (Basavarajappa et al., 2011; Xiao et al., 2011; Girish and Kunhi, 2013). However, despite the fact that most countries have restricted or banned their use, currently they are detected in sites with and without a history

* Corresponding author. Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, 4000, Tucumán, Argentina.
E-mail address: cbenimeli@yahoo.com.ar (C.S. Benimeli).

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of pesticide application, including the environment and diverse organisms (Ondarza et al., 2014; Agarwal et al., 2015; Arienzo et al., 2015). Also, it is important to highlight the ability of these OPs to be transported for long distances (Sheng et al., 2013). This phenomena is evidenced by the pesticide accumulation in both pristine environments and their wildlife, for example in the Arctic and Antartic (Dietz et al., 2004; Zhang et al., 2015). In the northwest of Argentina the extensive use of OPs has left associated residues in the environment. Thus, Chaile et al. (1999) detected chlordane, lindane and methoxychlor in the Salí River, the main hydrographical system of Tucumán province, in concentrations higher than allowed. It is important to consider that these compounds can be detected simultaneously in different matrices and organisms, despite not having been applied together. In this sense, Ondarza et al. (2014) detected the presence of lindane and chlordanes among others toxic compounds, in edible fish from Negro River basin in the Argentinean Patagonia. For his part, Lupi et al. (2016) studied the behavior of OPs such as DDTs, endosulfans, HCHs, heptachlors, drins and chlordanes, in the Quequén Grande River agricultural watershed, including different environmental matrices, and they found these pesticides in all of them.

Among the different matrices contaminated, the impacted soils with OPs represent serious environmental problems, for that their remediation is urgently required. There are chemical and environmental factors that influence the soil remediation process: solubility and concentration of the pesticide, temperature, pH, oxygen availability, soil texture, organic matter (OM), moisture, nitrogen and phosphorus concentration, micronutrient content as well as bioavailability of the contaminant and the ability of microorganisms to use it (Wilkinson et al., 2002; Rama Krishna and Philip, 2011). Generally, in soil relationship, when the OM content and the pesticide hydrophobicity are high, the adsorption and retention of the pesticide in the solid matrix is greater (Alexander, 1995). However, an increase in the clay content could produce higher pesticide adsorption, in soils with low OM contents (Đurović et al., 2009). Sandy soils are usually very permeable to air and water, but have low water holding and nutrient storage capacity. Their high porosity, due to the enormous size of the particles, is responsible for soil aeration and potential infiltration of contaminant (Rucks et al., 2004). Clay soils have high water holding capacity, but aeration is usually poor. This kind of soil has a larger surface area and therefore more contact contaminant-microorganism, which improves contaminant biodegradation. However, OPs may be adsorbed in the particles of clay soils, limiting their bioavailability and their biodegradation (Đurović et al., 2009). On the contrary, loam and silty loam soils retain adequate amounts of water and they have no aeration difficulties.

The bioremediation of environments contaminated with OPs, by using microorganisms as actinobacteria, represents one cost-effective alternative against the chemical and physical processes used. These microorganisms represent a special group of Gram-positive bacteria with ability to degrade pesticides (Briceño et al., 2012; De Paolis et al., 2013; Bourguignon et al., 2014; Mesquini et al., 2015). Among them, *Streptomyces* genus may be useful for OPs treatment because they have a mycelial growth, relatively rapid growth rates, ability for colonization of semi-selective substrates, susceptibility to genetic manipulation (Shelton et al., 1996) and they are able to produce surfactants, which may facilitate the bioavailability of the toxic compounds (Colin et al., 2016). In this way, previous studies have demonstrated that different *Streptomyces* strains were able to remove OPs (Benimeli et al., 2003; Fuentes et al., 2010; Saez et al., 2012). Besides, Fuentes et al. (2011, 2013, 2014, 2016) reported that *Streptomyces* consortia enhance the efficiency of OPs biodegradation. For this reason, they are promising tools for bioremediation of different matrices contaminated with OPs, however, there are no reports regarding simultaneous bioremediation of lindane, methoxychlor and chlordane by them. Therefore, the main objective of the present work was to investigate the potential removal of an OPs mixture in different soil systems by an indigenous *Streptomyces* defined consortium.

2. Materials and methods

2.1. Chemicals

Pesticides used in this study were lindane (LIN) (99% pure), methoxychlor (MTX) (99.8% pure) and technical-grade chlordane (98.4% pure); in the case of chlordane only the isomer gamma was evaluated (named as CLD). Pesticides were purchased from Sigma-Aldrich, and their properties are given in Table 1, according to the Pesticide Properties Database (PPDB, 2012). All other chemicals used throughout the study were of analytical grade and were purchased from standard manufacturers.

2.2. Microorganisms and culture media

The actinobacteria consortium comprised of *Streptomyces* sp. A2, A5, A11, and M7 was used in this study. These strains were isolated from OPs-contaminated soil and sediment samples (Benimeli et al., 2003; Fuentes et al., 2010) and the consortium was previously selected based on its proven ability to remove lindane and dechlorinate this pesticide (Fuentes et al., 2011).

Minimal medium (MM), which was used for growth of the microorganisms and pesticides removal assays, consisted of (g L^{-1}): L-asparagine, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.01. The pH was adjusted to 7.0 prior to sterilization (Hopwood, 1967).

Tryptic Soy Broth (TSB) was purchased from Sigma-Aldrich and used for inocula preparation; this medium consisted of (g L^{-1}): trypticase, 17.0; soy peptone, 3.0; NaCl, 5.0; K_2HPO_4, 2.5; glucose, 2.5. The pH was adjusted to 7.3 ± 0.2 prior to sterilization.

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

2.3. Soil samples

Three soil samples of different textures were taken from diverse experimental sites of the province of Tucumán (Argentina), from soil surface (5–15 cm depth). Table 2 lists the physico-chemical characteristics of these soils. They were conditioned following the methodology described by Benimeli et al. (2008).

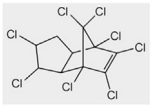
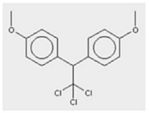
2.4. Inoculum formulation

In order to obtain appropriate inocula, the strains were individually pre-cultured in Erlenmeyer flasks containing 30 mL of TSB, at 30 °C on a rotary shaker (200 rpm) for 72 h. Then the cultures were centrifuged at 8500 ×g for 10 min at 4 °C. Supernatants were eliminated and the biomass of each strain was inoculated together as a defined consortium in MM, soil microcosms or slurries systems, as applicable.

2.5. Biodegradation of pesticides mixture in liquid medium

The actinobacteria consortium, obtained as described above, was inoculated (2.0 g L^{-1}) in Erlenmeyer flasks of 250 mL containing 100 mL of MM simultaneously contaminated with LIN, CLD and MTX (1.66 mg L^{-1} each one). The cultures were incubated at 30 °C under constant agitation (200 rpm), for 16 days and the procedure was carried out in triplicate with the respective biotic

Table 1
Properties of the used pesticides (from the Pesticide Properties Database (PPDB, 2012)).

Pesticide	IUPAC name	CAS number	Chemical group	Uses	Molecular mass (g mol^{-1})	Solubility in water (mg L^{-1})	Log (K_{ow})[a]	K_{oc}[b] (cm^3 g^{-1})	Degradation half-life[c] (days)	Chemical structure
Chlordane (CLD)	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	57-74-9	Organochlorine	Insecticide	409.78	0.1	2.78	20000	365	
Lindane (LIN)	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane	58-89-9	Organochlorine	Insecticide, acaricide, veterinary substance	290.82	8.52	3.50	1270	980	
Methoxychlor (MTX)	1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane	72-43-5	Organochlorine	Insecticide, veterinary substance	345.65	0.1	5.83	80000	120	

[a] Octanol–water partition coefficient.
[b] Organic carbon sorption distribution coefficient.
[c] Average value of field and laboratory studies.

(MM inoculated with the consortium) and abiotic (pesticides contaminated MM without inoculum) controls. Samples were taken for determining microbial growth by enumeration of bacteria (1 mL), residual pesticides concentration (15 mL) and release chloride ions (1 mL).

2.6. Biodegradation of pesticides mixture in sterile and non-sterile soil microcosms

Glass pots with conditioned soil were filled with 100 g of soil of each texture (CSLS, SS and LS) at 20% moisture (dry weight base), and kept for 36 h at 25 °C so that water equilibrated in the soil. For sterile soil microcosms, the pots were stem sterilized (three successive sterilizations every 24 h, at 100 °C for 1 h each) and the soil humidity was adjusted at the end of this process (Benimeli et al., 2008).

Soil microcosms (sterile or non-sterile) were contaminated with appropriate stock solutions of LIN, CLD and MTX (final concentration: 1.66 mg kg^{-1} soil, each one). Then, the microcosms were inoculated with the actinobacteria consortium (2.0 g kg^{-1}) obtained as described above and incubated at 30 °C for 16 days. The procedure was carried out in triplicate with the respective biotic and abiotic controls. Soil samples were taken for determining microbial growth (1 g) and residual pesticides concentration (5 g).

Table 2
Physico-chemical characterization of assayed soil samples.

Parameters	Soil #1	Soil #2	Soil #3
pH	7.3	6.2	7.6
Salinity (dSm^{-1})	0.8	0.8	1.0
Carbonate $CaCO_3$ (%)	0.3	ND	0.2
Oxidizable organic matter (%)	0.5	0.4	0.5
Available phosphorus (ppm)	17.2	24.7	6.9
Total Nitrogen (%)	0.04	0.03	0.05
Clay (%)	30.9	0.9	8.4
Silt (%)	56.4	1.4	41.4
Sand (%)	12.7	97.7	50.2
Texture	Clay silty loam (CSLS)	Sandy (SS)	Loam (LS)

ND: not detected.

2.7. Biodegradation of pesticides mixture in sterile soil slurries

The experiments were carried out in 250 mL Erlenmeyer flasks containing 40 g of sterile SCLS and 60 mL of sterile distilled water. The resulting slurries were again autoclaved following the previously described methodology (Fuentes et al., 2014). The loss of liquid due to the sterilization process was calculated by difference in weight between the bottles before and after sterilization, and was adjusted by adding sterile distilled water. Slurries were contaminated with LIN, CLD and MTX (1.66 mg L^{-1} each one, final concentration) and were incubated at 30 °C under constant stirring (200 rpm), for 16 days. This procedure was carried out in triplicate with the respective biotic and abiotic controls. Samples were taken for determining residual pesticides concentration (5 mL) and microbial growth (1 mL).

2.8. Analytical procedures

The microbial growth of actinobacteria consortium in liquid cultures, sterile soils and slurries was measured by enumeration of bacteria (CFU mL^{-1} or CFU g^{-1}), carrying out serial 10-fold and plating them in triplicate on SC (Jézéquel et al., 2005).

For determining residual OPs concentration from the cell free supernatants of liquid culture, solid phase extraction (SPE) using a C18 column (Varian, Lake Forest, USA) was carried out, and the extracts obtained were analysed by GC-μECD.

For determining residual OPs concentration from soil and slurry systems an extraction technique was performed according to Quintero et al. (2005). 5 g of soil or 5 mL of slurry samples were transferred to centrifuge tubes and mixed with water, methanol, and *n*-hexane, in a relationship of 4:1:5 mL respectively. The tubes were shaken for 10 min. Then, they were centrifuged (8500 ×g, 10 min, 4 °C). The organic phase results were taken and evaporated to dryness under reduced pressure. The residues were resuspended in hexane and analyzed by GC-μECD.

The equipment used was a Gas Chromatograph Agilent 7890A equipped with a HP5 capillary column (30 m × 0.320 mm × 0.25 μm) and ^{63}Ni-μECD detector, a split/splitless Agilent 7693B injector and Agilent Chem-Station software, following the

chromatographic conditions described previously (Saez et al., 2014). Quantitative sample analysis was performed using appropriate calibration standards (AccuStandard).

Chloride ions released were measured using a technique described by Bidlan and Manonmani (2002), with minimal modifications. Briefly, 50 μL cell-free supernatants were mixed with 50 μL each of 0.15 N HNO_3 and 0.1 N $AgNO_3$. The reaction mixture was incubated 20 min at room temperature and the turbidity was determined immediately at 600 nm. A calibration curve was performed using appropriate concentrations of NaCl solution.

2.9. Statistical analyses

All assays were conducted in triplicate and the results are the average of them. One-way analysis of variance (ANOVA) was used to test the significant differences between treatments in liquid systems, soil microcosms and slurries. When significant differences were found, Tukey post-test was used to separate the effects among treatments. Tests were considered significantly different at $P < 0.05$. These statistical analyses were performed using professional versions of Infostat and Statistica 6.0 software.

3. Results and discussion

3.1. Biodegradation of mixed pesticides in liquid medium

When the actinobacteria consortium (*Streptomyces* sp. A2-A5-A11-M7) was cultured in MM supplemented with LIN, MTX and CLD as the only carbon source, microbial growth was observed, reaching a final biomass of $(9.63 \pm 0.04)x10^4$ UFC mL^{-1} (Table 3). On the contrary, no microbial growth was observed on MM without pesticides. This result could mean that the pesticides are not toxic to microorganisms in the assayed concentration, that the pesticides are used to sustain microbial growth since another carbon source was not added to MM, and that toxic metabolites with inhibitory effect on growth were not accumulated. In addition, Fuentes et al. (2011, 2014, 2016) found that all the actinobacteria strains of the consortium showed the ability to grow in MM in the presence of each one of the OPs used, without the addition of other carbon sources.

The decrease in concentration of the three organochlorine pesticides in inoculated MM compared to that not inoculated was considered as microbial degradation. The GC analysis confirmed a substantial dissipation of the three pesticides (Table 3). At the end of incubation, almost total removal of MTX and CLD was observed (99.5 and 99.8, respectively) and the removal of LIN was 40%. There was no degradation observed of the three pesticides in uninoculated MM controls ($P > 0.05$), so removal of OPs could be due to microbial activity and not to physicochemical factors. These results are not surprising, since in a previous work Fuentes et al. (2010) demonstrated that all the actinobacteria of the assayed consortia was able to remove 100% CLD, when these strains were individually cultured in MM supplemented with 1.66 mg L^{-1} CLD. In the same study, it was observed that individual cultures of *Streptomyces* sp. A2 and A5 in MM contaminated with MTX, in absence of another carbon source, could remove almost 100% of the pesticide, while *Streptomyces* sp. A11 and M7 showed lower percentages of removal (46 and 73%, respectively) (Fuentes et al., 2010). This finding is very important, since it demonstrates that in the presence of a complex and toxic mixture of pesticides, the actinobacteria consortium exhibited the highest removal ability for MTX and CLD, which had already been observed for some of the individual actinobacteria cultures. On the contrary, removal of LIN was affected by the presence of the other two compounds because Fuentes et al. (2011) have observed that the same consortium was able to remove 61.7% LIN when it was cultured in MM supplemented only with this pesticide.

In the same way, Sineli et al. (2016) demonstrated that the strain *Streptomyces* sp. M7, one of the strains belonging to the assayed consortium, was able to grow in the presence of a mixture of α-HCH, β-HCH and γ-HCH and remove them efficiently from MM. These authors detected a removal of 45% for the gamma isomer (LIN) in the mixture; this percentage was similar to that observed in this study.

The different percentages of pesticide degradation in a mixture were also demonstrated by other authors. For instance, Rama Krishna and Philip (2011) observed great differences in the removal efficiency of three toxic compounds in a submerged soil system contaminated with a mixture of pesticides (carbofuran, lindane, and methyl parathion at a final concentration of 2 mg g^{-1} of soil), where carbofuran degradation was maximum whereas minimum degradation was observed for lindane in the liquid phase. They also found that in the mixture of these pesticides, the degradation efficiency was less than the efficiency detected in systems contaminated with one pesticide at a time.

Additionally, Fuentes et al. (2013) observed that the same actinobacteria consortium used in the present work was able to remove a mixture of chlorpyrifos and pentachlorophenol to different extents (40.2 and 5.2%, respectively) when these microorganisms were cultured in MM without the addition of a supplementary carbon source.

It is important to note that the different percentages and velocities of removal obtained in this assay could be due to the physico-chemical characteristics of the pesticides such as solubility in water, degradation half-life, chemical structure, among others (Table 1) and/or the different concentrations of each pesticide used in the assay. Commercially available technical-grade chlordane added to culture medium is a mixture of over 140 different but related compounds; the two most common components are α-chlordane and γ-chlordane (Dearth and Hites, 1990). These compounds constitute 60–75% of commercial chlordane (Yamada et al., 2008). MM were contaminated with 1.66 mg L^{-1} of LIN, MTX and CLD, but in the case of technical-grade chlordane, only the isomer gamma was determined by GC (named as CLD). For this reason, the concentration of γ-chlordane in the mixture was lower than the concentration of lindane and methoxychlor. The greater and faster

Table 3
Microbial growth, removal of the pesticides mixture (lindane, methoxychlor and chlordane, 1.66 mg L^{-1} each one) and chloride ion release by the consortium *Streptomyces* sp. A2-A5-A11-M7 inoculated in MM.

Incubation time (days)	Microbial growth (UFC mL^{-1}) × 10^4	LIN removal (%)	MTX removal (%)	CLD removal (%)	Chloride ion concentration (mg L^{-1})[a]
0	0.48 ± 0.02	ND	ND	ND	26.01 ± 2.09
8	3.55 ± 0.07	14.8 ± 6.4	42.3 ± 0.2	99.5 ± 0.1	47.32 ± 1.99
16	9.63 ± 0.04	40.4 ± 2.0	99.5 ± 0.1	99.8 ± 0.2	33.21 ± 3.39

ND: not detected.
LIN: lindane; CLD: chlordane; MTX: methoxychlor.
[a] Cl^- concentration of control without pesticides was 25.46 ± 2.09 mg L^{-1} (mean during the assay).

removal of chlordane could be due to its lower concentration in the medium. Moreno-Medina et al. (2014), during pesticides mixture degradation experiments in a mineral salt medium, revealed that the removal of coumaphos was more complete than methyl parathion, attributing this result to the different characteristics and concentrations of the evaluated pesticides. Hirano et al. (2007) found that the degradation rate of γ-CLD was higher than that of α-CLD in river sediments, concluding that even though isomers may have the same molecular formula, structural differences can also result in different biodegradation rates.

This ability of the actinobacteria to remove mixed pesticides without any nutrient sources is not always observed in all microorganisms. For example, Kulshrestha and Kumar (2010) found that a *Fusarium* strain was not able to degrade DDT and chlorpyrifos simultaneously in a liquid medium deficient in normal carbon and nitrogen sources; however, liquid medium with mannitol (1%) and sodium nitrate (0.1%) enhanced the degradation efficiency of both compounds. Also, Zuo et al. (2015) informed that the recombinant *Pseudomona putida* KT2440 was able to degrade a mixture of six pesticides in a liquid medium when it was supplemented with 0.2% glucose.

It is well known, that the elimination of halogens from halogenated xenobiotics is a key step in their degradation because the carbon-halogen bond is relatively stable (Fetzner and Lingens, 1994). Because dehalogenation plays a central role in biodegradation of many chlorinated compounds, the release of chloride ions into the culture medium to assess the OPs-degrading ability of the actinobacteria consortium was studied. Release of chloride ions was measured at different time intervals and the maximum value was observed at eight days of incubation, in concordance with the total removal of CLD (Table 3). The abiotic MM control, without inoculated microorganisms, showed no variations within 16 days of incubation. No linear correlation between chloride ions and pesticides removal was found, which could be explained by the complexity of the pesticides mixture tested. The increase in cell biomass, the dissipation of OPs in the medium and the release of chloride ions to the culture medium, clearly show that the majority of the pesticides mixture degradation occurred through microbial activity.

There are results obtained in previous studies conducted in our laboratory that reinforce these hypotheses. Fuentes et al. (2011) have observed that certain soil microorganisms like *Streptomyces*, have enzymes with dehalogenase activity. Also, Cuozzo et al. (2009) have demonstrated that the synthesis of dechlorinase enzyme was observed when *Streptomyces* sp. M7 was cultured in the presence of lindane. Finally, Cuozzo et al. (2012) observed that all the actinobacteria employed in the preset work released chloride ions into the culture medium when they were cultured in MM supplemented with CLD as the only carbon source.

3.2. Biodegradation of pesticides mixture in sterile soil microcosms

Studies of pesticides mixture removal were conducted inoculating the actinobacteria consortium on sterile contaminated soil microcosms (1.66 mg kg^{-1} of soil, each pesticide), for a period of 16 days. These studies have been carried out with three different soils: SCLS, SS, LS (Table 2). This kind of study can provide a closer insight about microorganisms and their growth requirements, before any *in situ* intervention for decontamination is carried out.

As shown in Fig. 1, the actinobacteria consortium showed similar growth profiles in the three soil microcosms with different assayed textures. In all cases, the cell population increased rapidly in four days and it was followed by a stationary phase from the fourth to the sixteenth day; moreover, no significant differences in the bacterial enumeration were observed in the soils with different textures ($P > 0.05$). In addition, there were no differences in the microbial growth between the contaminated soils and the controls without pesticides, during the 16 days of incubation (Fig. 1a, b and c). These results reinforce the hypothesis that actinobacteria are well adapted to proliferate in natural soils contaminated with xenobiotics and that they are able to colonize these matrices. Previously, Benimeli et al. (2008) found that the strain *Streptomyces* sp. M7 was able to grow on sterile soils with and without LIN. Besides, another actinobacteria consortium (*Streptomyces* sp. A6, A12, A14 y M7) showed no growth inhibition when it was inoculated on sterile soils contaminated with MTX (Fuentes et al., 2014).

Influence of different textures of soil on simultaneous pesticides removal was evaluated by determining residual concentration of LIN, CLD and MTX in the soil samples. In the SCLS, the three OPs were not removed in the same percentage (Fig. 1a). MTX dissipation was the most important and it was observed from the beginning of incubation, showing 39% of removal at the 16 days of incubation. For LIN, the decrease in its concentration was observed from the eighth day, reaching a percentage of 30% removal at the end of assay. Finally, the removal of CLD was 23%.

For the three pesticides, the abiotic controls (contaminated soil with the OPs mixture, in the absence of the microbial consortium) had final removal percentages of 18, 10 and 5% (for MTX, LIN and CLD, respectively), which would be due to chemical reactivity of pesticides (Cao et al., 2013). However, it is important to notice that at the end of the incubation period, statistically significant differences ($P < 0.05$) between controls and their corresponding tests were detected. So, discounting the abiotic removal of pesticides, a 20% average removal for each pesticide could be attributed to the microbial consortium in sterile SCLS system (MTX: 21%; LIN: 20%; CLD: 18%). This result clearly shows that the majority of the degradation occurred through microbial activity.

In sterile SS microcosms the following removal percentages were observed: LIN 48%, MTX 27% and CLD 23%, after 16 days of incubation (Fig. 1b). However, removal percentages of three pesticides in contaminated soils with inoculation were similar to those of non-inoculated, without significant differences between them ($P > 0.05$). Thus, in the case of sandy soils the elimination of pesticides would not be provided by the activity of the microbial consortium, but probably due to the chemical reaction between the pesticides and the soil particles.

In the microcosms formulated with LS, the following removal percentages were observed: LIN 12% and MTX 4%; for CLD removal was not observed (Fig. 1c). As in SS microcosms, there was no significant differences between actinobacteria inoculated microcosms and abiotic controls, for the three assayed pesticides ($P > 0.05$).

Soil acts like an active filter, where pesticides are degraded by chemical, physical and biological means. The degradation of pesticides in the soil is a function of their availability and the ability of microorganisms to utilize them (Rama Krishna and Philip. 2011). The availability and degradability of a pesticide in the soil varies a lot from one pesticide to another and depends upon the soil type. The physicochemical properties of soil could certainly have a profound influence on the efficiency of pesticides biodegradation. These parameters affect both their equilibrium concentration in the soil system, as well as the possible adsorption in soil components (Bhatia et al., 2011; Kogbara et al., 2015).

In the present work, the order of removal of pesticides in soils was SCLS > SS > LS. It is important to notice that SCLS was characterized by a predominance of fine-grained particles. The high content of clay and silt particles could provide more sites for bacteria to attach, leading to a higher utilization of OPs as a carbon and energy source. In this way, several studies demonstrated that higher specific surface area of silts and clays in the fine soil textures probably enhanced availability of the contaminant sorbed in the

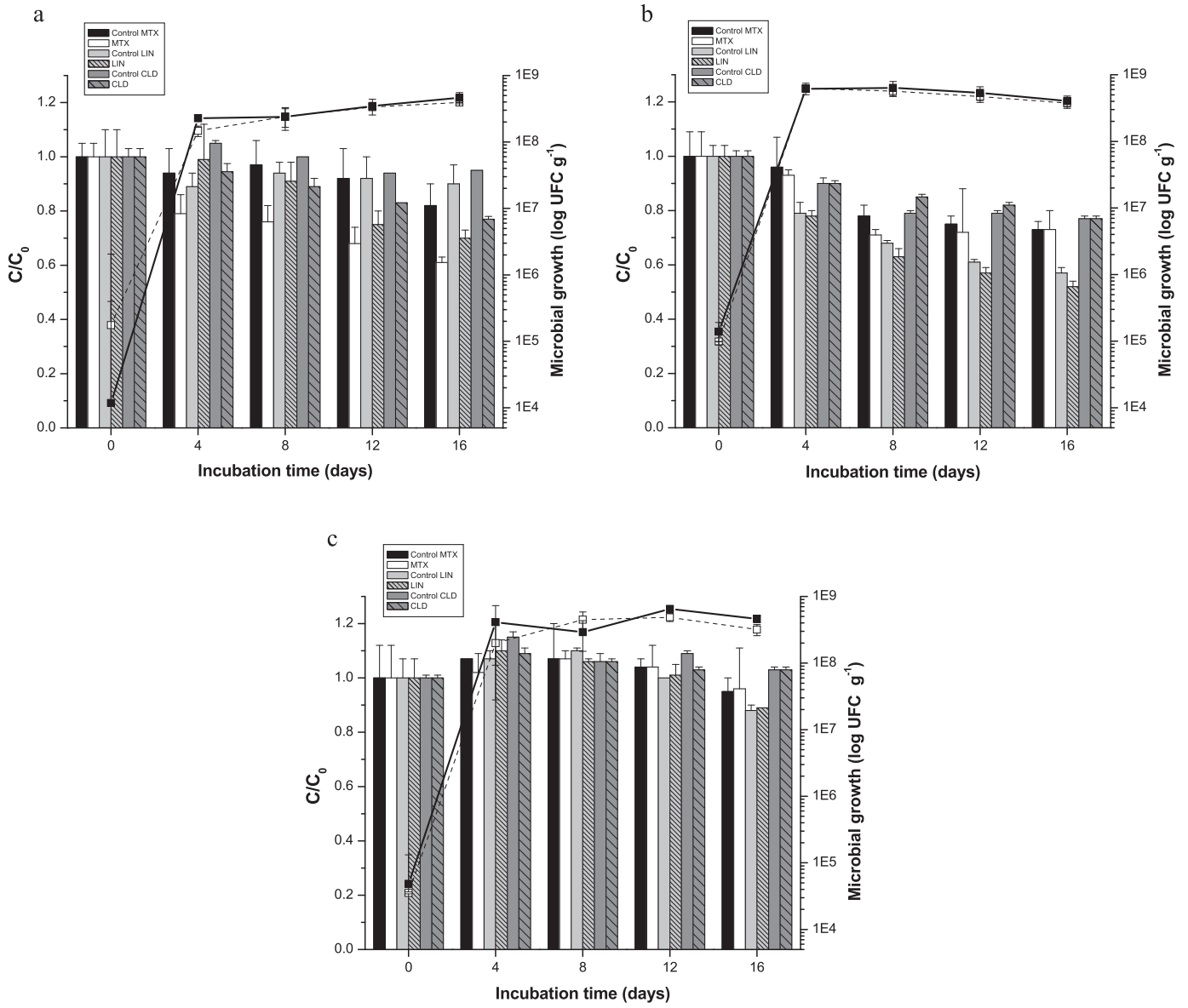


Fig. 1. Microbial growth and removal of a mixture of lindane, chlordane and methoxyclor by the consortium *Streptomyces* sp. A2-A5-A11-M7 inoculated in sterile soil microcosms of different textures. a) clay silty loam soil (CSLS); b) sandy soil (SS); c) loam soil (LS). ■: microbial growth in non-contaminated soil (biotic control); □: microbial growth in contaminated soil. C/C_0: relationship between final and initial pesticide concentration.

soil particles to microbes (Cui et al., 2011).

Furthermore, in SCLS the maximum removal for MTX followed by LIN and CLD was observed. These results confirm that in a soil contaminated with a mixture of pesticides, removal does not occur at the same rate for each compound. Similar results were observed by Rama Krishna and Philip (2011). These authors studied the degradation of a mixture of LIN, methyl parathion and carbofuran in submerged soils by a microbial consortium, showing maximum degradation for methyl paration and minimum for LIN. The variation in OPs degradation in this SCLS was probably due to the difference in the bioavailability of pesticides for microbes. Water solubility of LIN is greater than the other two pesticides studied, however MTX has a shorter half-life and CLD has the lowest octanol water partition coefficient (Table 1). These properties could be responsible for the different bioavailability and consequent removal of pesticides in silty clay loam soils. Singh (2002) found that adsorption of pesticides is positively correlated with octanol water partition coefficient and negatively correlated with their water solubility.

The better aeration of the coarse textured soils would provide aerated microhabitats for bacterial activity during bioremediation processes, since oxygen is generally a limiting nutrient in soils. Nevertheless, soils rich in large particles such as SS might also easily allow the contaminant loss by volatilization and deep percolation, as has already been reported in the literature for other pesticides. In the present study, no significant differences were found in the bioremediation process between inoculated SS and controls without inoculum ($P > 0.05$). This result could be due to well-drained sandy soil, with low organic matter content and large size of their pores helped the percolation of pesticides through it. In the same way, Rama Krishna and Philip (2008) desmostrated that sandy soils with small amounts of organic matter retain carbofuran, methyl parathion and LIN less than soils with high clay and/or organic content.

In the case of LS, the low percentages of LIN and MTX removal obtained could be due to the insufficient incubation time for the adaptation of the consortium in soil with different composition and texture. In this sense, Cuozzo et al. (2012) studied the CLD removal in this type of soil using *Streptomyces* sp. A5 and they did not observe dissipation of this compound during the first 15 days of assay, however, after this time the organism was able to remove it. This finding could support the hypothesis that the incubation time was insufficient for this kind of soil.

This study seeks to understand the efficiencies of the pesticides mixture biodegradation in different soil textural classes, but the relationship between degradation rates of pesticides and soil properties is still not clearly defined and more investigations are necessary.

3.3. Biodegradation of pesticides mixture in non-sterile silty clay loam soil microcosms

Since the highest removal percentages for all of the assayed pesticides were obtained in SCLS and also the removal ability of the consortium was evident, this type of soil was selected for testing pesticides mixture biodegradation in non-sterile microcosms (Fig. 2). No significant CLD removal was observed in both control without inoculum and in the contaminated and inoculated samples (0 and 5%, respectively). In contrast, the consortium was able to remove LIN, detecting a 25% dissipation at the end of the assay, while in the non-inoculated control a LIN removal of 14% was observed. This result indicates an actual LIN removal of 11%, which could be attributable to the actinobacteria consortium. In this case, it could be assumed that the native microflora of soil was involved in removing the pesticide. However, in the assay with the same type of soil in sterile conditions, controls without inoculum showed a similar LIN removal, so the depletion of the pesticide in this kind of soil could also be related to the reactivity of pesticides on clay particles. The dissipation of MTX was evident from the fourth day of incubation, showing a maximum removal of 21% at the end of the incubation time; in this case, the abiotic removal of the pesticide

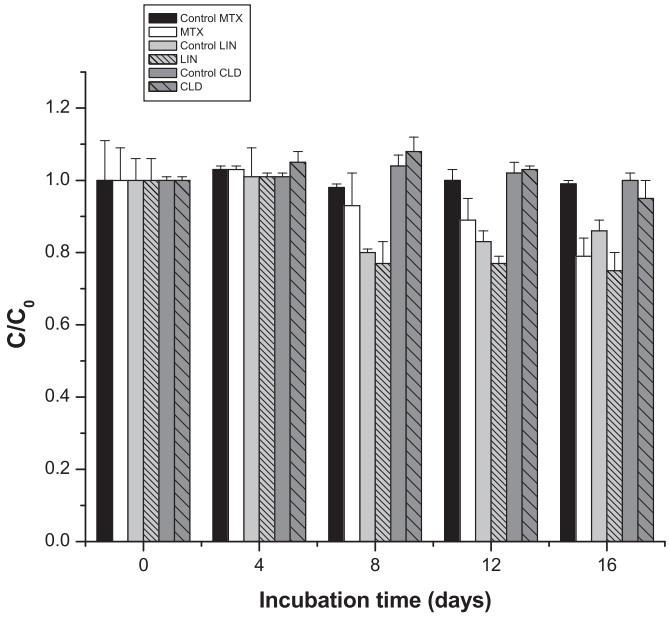


Fig. 2. Removal of a mixture of lindane, chlordane and methoxyclor by the consortium *Streptomyces* sp. A2-A5-A11-M7 inoculated in non-sterile SCLS microcosms. C/C_0: relationship between final and initial pesticide concentration.

was only about 1%. Therefore, in soil microcosms without inoculation with the actinobacteria consortium the concentration of MTX remained almost constant over time, so the native microflora of these soils could not be involved in removing this pesticide.

According to the literature, the participation of native soil microflora in processes of bioremediation of pesticides is variable. For example, Bidlan et al. (2004) found that during the bioremediation of soils contaminated with technical hexachlorocyclohexane by a bacterial consortium, the degradation of the different HCH isomers by the indigenous microflora was limited. In the same way, Cycoń et al. (2013) evaluated the biodegradation of an organophosphorus pesticides mixture in soils of different textures by *Serratia marcescens*, and they observed that the indigenous microflora of each kind of soil was able to degrade the pesticides under study.

The depletion of pesticides observed in the current work, demonstrates that actinobacteria was able to remove them even in the presence of native microflora, although its effectiveness could be reduced in non-sterile systems, in view of the lower removal percentages obtained. In the same way, Mandal et al. (2014) found that *Bacillus alkalinitrilicus* was able to remove efficiently the insecticide imidacloprid in sterile and non-sterile clay loam soil microcosms, with a small insecticide percentage removal under unsterile conditions.

It is important to notice that the removal percentages of the three pesticides in non-sterile SCLS were lower than those obtained by working with sterile SCLS. This effect could be due to changes caused to the soil properties during the sterilization process, which can affect microbial growth and degradation of pesticides on this matrix. It has been reported that all known sterilization methods commonly cause some secondary effect in soil and they alter its physical and chemical properties. In this sense, Berns et al. (2008) observed two major changes in the structure of the soil after its autoclave sterilization: destruction and reduction of silt particle aggregates occurred, with a consequent increase in the clay fraction and surface area of soil; and increase in the concentration of soluble organic carbon in soil liquid phase took place, due to microbial lysis and organic matter degradation; this soluble organic carbon is readily biodegradable and serves as additional carbon source for microorganisms. Zamani et al. (2015) also observed that other soil parameters, such as optical density, pH, electrical conductivity and percentage of extractable phosphorus, were affected during its sterilization.

In the present work, the changes produced in the soil during sterilization could have affected the pesticides removal, which was favored in sterile soil microcosms due to the increase of soluble organic carbon, which could act as an additional source of carbon for the inoculated microorganisms.

3.4. Biodegradation of pesticides mixture in slurry systems

Slurry bioreactors are useful technologies for bioremediation of soils with high clay and organic matter content, contaminated with recalcitrant toxic substances that exhibit a hysteresis behavior, or when the bioremediation process should be performed in short times. These bioreactors have been used for the degradation of contaminant compounds, such as pesticides, explosives and aromatic hydrocarbons (Robles-González et al., 2012; Saez et al., 2014).

In this context, the ability of the consortium *Streptomyces* sp. A2-A5-A11-M7 to remove simultaneously LIN, CLD and MTX was evaluated in a batch slurry bioreactor, formulated with sterile SCLS. Fig. 3 shows that the actinobacteria consortium exhibited similar growth levels in the presence or absence of the pesticides mixture ($P > 0.05$): microbial growth was exponential until 4 days of incubation and thereafter remained constant in both cases, so the

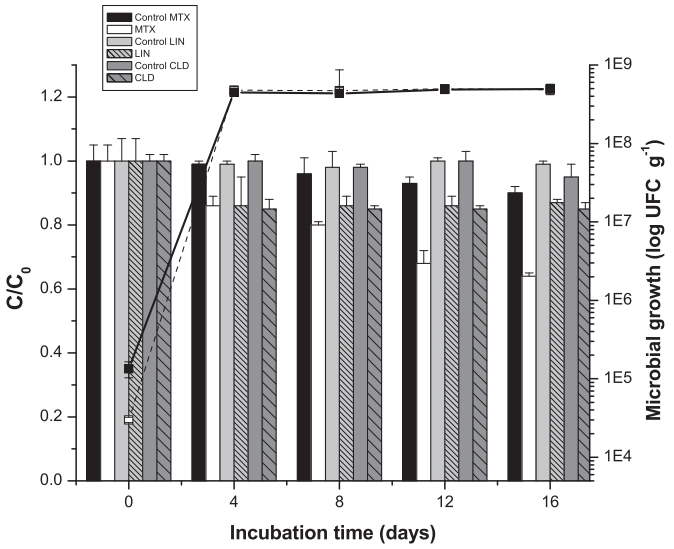


Fig. 3. Microbial growth and removal of a mixture of lindane, chlordane and methoxyclor by the consortium *Streptomyces* sp. A2-A5-A11-M7 inoculated in sterile SCLS slurry. ■: microbial growth in non-contaminated soil (biotic control); □: microbial growth in contaminated soil. C/C_0: relationship between final and initial pesticide concentration.

parent compounds or its metabolites could not exert adverse effects on microbial growth. These results agree with those of studies carried out in sterile microcosmos of SCLS. Similarly, Rajashekara Murthy et al. (2010) demonstrated that a defined bacterial consortium inoculated in a slurry bioreactor contaminated with different concentrations of t-HCH had high survival until the final stage of pesticide degradation.

As in the sterile SCLS microcosms, not all OPs were removed from slurries in the same way. Dissipation of MTX was the most important and it became from the beginning of the assay, showing a removal of 35.8% at the end of the 16 days of incubation; controls without inoculum demonstrated MTX removal of 9.8% (Fig. 3), so the MTX removal attributable to the actinobacteria consortium ability was 26%. LIN showed a decrease on its concentration during the first four days of incubation and then remained constant, reaching a removal of 13.46% at the end of assay; in this case, the change detected in the residual concentration of LIN in the abiotic controls was only about 0.96%, so the removal of the pesticide by actinobacteria consortium was 12.5%. The removal of CLD was only 15% (5% removal in abiotic controls), resulting in a microbial removal of 10% at the end of 16 days incubation.

It is important to notice that the use of SCLS slurry reactors allowed an increase in the percentage of removal of MTX in relation to that obtained in microcosms with this kind of soil. This could be due to the fact that this slurry system allows increase rates of mass transfer and contact contaminants-microorganisms and nutrients-microorganisms (Robles-González et al., 2008). Previously, Fuentes et al. (2014) reported an increase in the removal of MTX using slurry systems, compared with the biodegradation obtained by using soil systems. However, this improvement in the removal was not observed for the other two pesticides.

Different efficiencies in the biodegradation of pesticides mixture were just reported in soil slurries. For example, Monsalvo et al. (2014) observed that 2-methyl-4-chlorophenoxyacetic acid (MCPA) was partially removed in a granular sludge bed reactor when the herbicide was treated as sole carbon source, but when the reactor was fed with a complex mixture of pesticides, the MCPA removal efficiency was less than 10%. The LIN removal percentages observed in soil slurry system at the present work were similar to the LIN removal average that has been reported for slurry bioreactors in the literature (Okeke et al., 2002; Laquitaine et al., 2016). While the removal of MTX was favored using the slurry system in relation to soil microcosms, this was not the case with regard to LIN and CLD. Perhaps, an increase in the LIN and CLD degradation efficiency for the soil slurry system tested in the current work would require longer incubation periods to achieve higher values (Robles-González et al., 2012). Also, by comparing the pesticide degradation between slurry and liquid systems, it was observed a reduced degradation in slurries compared with aqueous medium. Similar results were found by Tiwari and Guha (2014) for chlorpyrifos.

Regarding a comparison of pesticides removal in SCLS systems used (sterile, non-sterile soil microcosms and sterile slurry systems), the type of system has a significant effect on the removal of CLD, LIN and MTX by *Streptomyces* sp. A2-A5-A11-M7. For each pesticide of the mixture, its removal was influenced by the type of assayed system. In the case of LIN and CLD, the maximum removal was observed in sterile SCLS microcosms. In contrast, MTX removal by the *Streptomyces* consortium was improved in SCLS slurry system.

In this study, it was demonstrated that the assayed *Streptomyces* consortium was able to grow and remove the OPs mixture from the different tested systems, thus offering a tool for the bioremediation of soils contaminated with multiple organochlorine pesticides. However, texture of the soils and assays conditions (sterility, slurry formulation) were determining factors that influenced the removal of each pesticide of the mixture. This consortium will be useful for its application in the field-scale bioremediation of OPs-contaminated sites in the future.

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References

Agarwal, A., Prajapati, R., Singh, O.P., Raza, S.K., Thakur, L.K., 2015. Pesticide residue in water—a challenging task in India. Environ. Monit. Assess. 187, 54.
Alexander, M., 1995. How toxic are toxic chemicals in soil? Environ. Sci. Technol. 29, 2713–2717.
Arienzo, M., Albanese, S., Lima, A., Cannatelli, C., Aliberti, F., Cicotti, F., Qi, S., De Vivo, B., 2015. Assessment of the concentrations of polycyclic aromatic hydrocarbons and organochlorine pesticides in soils from the Sarno River basin, Italy, and ecotoxicological survey by *Daphnia magna*. Environ. Monit. Assess. 187, 52.
Basavarajappa, M.S., Craig, Z.R., Hernández-Ochoa, I., Paulose, T., Leslie, T.C., Flaws, J.A., 2011. Methoxychlor reduces estradiol levels by altering steroidogenesis and metabolism in mouse antral follicles in vitro. Toxicol. Appl. Pharmacol. 253, 161–169.
Benimeli, C.S., Amoroso, M.J., Chaile, A.P., Castro, G.R., 2003. Isolation of four aquatic streptomycetes strains capable of growth on organochlorine pesticides. Bioresour. Technol. 89, 133–138.
Benimeli, C.S., Castro, G.R., Chaile, A.P., Amoroso, M.J., 2008. Lindane uptake and degradation by aquatic *Streptomyces* sp. strain M7. Int. Biodeterior. Biodegrad. 59, 148–155.
Berns, A.E., Philipp, H., Narres, H.D., Burauel, P., Vereecken, H., Tappe, W., 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur. J. Soil Sci. 59, 540–550.
Bhatia, G., Srivastava, A., Srivastava, P.C., 2011. Degradation of lindane (γ-HCH) in a mollisol as effected by different soil amendments. J. Environ. Prot. 2, 1207–1210.
Bidlan, R., Manonmani, H.K., 2002. Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by *Serratia marcescens* DT-1P. Process Biochem. 38, 49–56.

Bidlan, R., Afsar, M., Manonmani, H.K., 2004. Bioremediation of HCH-contaminated soil: elimination of inhibitory effects of the insecticide on radish and green gram seed germination. Chemosphere 56, 803–811.
Bourguignon, N., Fuentes, M.S., Benimeli, C.S., Cuozzo, S.A., Amoroso, M.J., 2014. Aerobic removal of methoxychlor by a native Streptomyces strain: identification of intermediate metabolites. Int. Biodeterior. Biodegrad. 96, 80–86.
Briceño, G., Fuentes, M.S., Jorquera, M.A., Palma, G., Amoroso, M.J., Diez, M.C., 2012. Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil. Int. Biodeterior. Biodegrad. 73, 1–7.
Cao, X., Yang, C., Liu, R., Li, Q., Zhang, W., Liu, J., Song, C., Qiao, C., Mulchandani, A., 2013. Simultaneous degradation of organophosphate and organochlorine pesticides by *Sphingobium japonicum* UT26 with surface-displayed organophosphorus hydrolase. Biodegradation 24, 295–303.
Chaile, A.P., Romero, N., Amoroso, M.J., Hidalgo, M.V., Apella, M.C., 1999. Organochlorine pesticides in Sali river. Tucumán–Argentina. Rev. Boliv. Ecol. 6, 203–209.
Colin, V.L., Cortes, Á.A., Aparicio, J.D., Amoroso, M.J., 2016. Potential application of a bioemulsifier-producing actinobacterium for treatment of vinasse. Chemosphere 144, 842–847.
Cui, X., Hunter, W., Yang, Y., Chen, Y., Gan, J., 2011. Biodegradation of pyrene in sand, silt and clay fractions of sediment. Biodegradation 22, 297–307.
Cuozzo, S.A., Rollán, G.C., Abate, C.M., Amoroso, M.J., 2009. Specific dechlorinase activity in lindane degradation by *Streptomyces* sp. M7. World J. Microbiol. Biotechnol. 25, 1539–1546.
Cuozzo, S.A., Fuentes, M.S., Bourguignon, N., Benimeli, C.S., Amoroso, M.J., 2012. Chlordane biodegradation under aerobic conditions by indigenous *Streptomyces* strains. Int. Biodeterior. Biodegrad. 66, 19–24.
Cycoń, M., Żmijowska, A., Wójcik, M., Piotrowska-Seget, Z., 2013. Biodegradation and bioremediation potential of diazinon-degrading *Serratia marcescens* to remove other organophosphorus pesticides from soils. J. Environ. Manag. 117, 7–16.
De Paolis, M.R., Lippi, D., Guerriero, E., Polcaro, C.M., Donati, E., 2013. Biodegradation of α-, β-, and γ-hexachlorocyclohexane by *Arthrobacter fluorescens* and *Arthrobacter giacomelloi*. Appl. Biochem. Biotechnol. 170, 514–524.
Dearth, M.A., Hites, R.A., 1990. Highly chlordane dimethanofluorenes in technical chlordane and in human adipose tissue. J. Am. Soc. Mass Spectrom. 1, 99–103.
Dietz, R., Riget, F.F., Sonne, C., Letcher, R., Born, E.W., Muir, D.C., 2004. Seasonal and temporal trends in polychlorinated biphenyls and organochlorine pesticides in East Greenland polar bears (*Ursus maritimus*), 1990–2001. Sci. Total Environ. 331, 107–124.
Fetzner, S., Lingens, F., 1994. Bacterial dehalogenases: biochemistry, genetics and biotechnological applications. Microbiol. Rev. 58, 641.
Fuentes, M.S., Benimeli, C.S., Cuozzo, S.A., Amoroso, M.J., 2010. Isolation of pesticide degrading actinomycetes from a contaminated site: bacterial growth, removal and dechlorination of organochlorine pesticides. Int. Biodeterior. Biodegrad. 64, 434–441.
Fuentes, M.S., Saez, J.M., Benimeli, C.S., Amoroso, M.J., 2011. Lindane biodegradation by defined consortia of indigenous *Streptomyces* strains. Water, Air, Soil Pollut. 222, 217–231.
Fuentes, M.S., Briceño, G.E., Saez, J.M., Benimeli, C.S., Diez, M.C., Amoroso, M.J., 2013. Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized *Streptomyces* strains. BioMed. Res. Int. 2013, 392573.
Fuentes, M.S., Álvarez, A., Saez, J.M., Benimeli, C.S., Amoroso, M.J., 2014. Methoxychlor bioremediation by defined consortium of environmental Streptomyces strains. Int. J. Environ. Sci. Technol. 11, 1147–1156.
Fuentes, M.S., Colin, V.L., Amoroso, M.J., Benimeli, C.S., 2016. Selection of an actinobacteria mixed culture for chlordane remediation: pesticide effects on microbial morphology and bioemulsifier production. J. Basic Microbiol. 56, 127–137.
Girish, K., Kunhi, A.A.M., 2013. Microbial degradation of gamma-hexachlorocyclohexane (lindane). Afr. J. Microbiol. Res. 7, 1635–1643.
Hirano, T., Ishida, T., Oh, K., Sudo, R., 2007. Biodegradation of chlordane and hexachlorobenzenes in river sediment. Chemosphere 67, 428–434.
Hopwood, D.A., 1967. Genetic analysis and genome structure in *Streptomyces coelicolor*. Bacteriol. Rev. 31, 373.
Jézéquel, K., Perrin, J., Lebeau, T., 2005. Bioaugmentation with a *Bacillus* sp. to reduce the phytoavailable Cd of an agricultural soil: comparison of free and immobilized microbial inocula. Chemosphere 59, 1323–1331.
Kogbara, R.B., Ayotamuno, J.M., Worlu, D.C., Fubara-Manuel, I., 2015. A case study of petroleum degradation in different soil textural classes. Recent Pat. Biotechnol. 9, 108–115.
Kulshrestha, G., Kumari, A., 2010. Simultaneous degradation of mixed insecticides by mixed fungal culture isolated from sewage sludge. J. Agric. Food Chem. 58, 11852–11856.
Laquitaine, L., Durimel, A., De Alencastro, L.F., Jean-Marius, C., Gros, O., Gaspard, S., 2016. Biodegradability of HCH in agricultural soils from Guadeloupe (French West Indies): identification of the lin genes involved in the HCH degradation pathway. Environ. Sci. Pollut. Res. 23, 120–127.
Lupi, L., Bedmar, F., Wunderlin, D.A., Miglioranza, K.S.B., 2016. Organochlorine pesticides in agricultural soils and associated biota. Environ. Earth Sci. 75, 519.
Mandal, K., Singh, B., Jariyal, M., Gupta, V.K., 2014. Bioremediation of fipronil by a *Bacillus firmus* isolate from soil. Chemosphere 101, 55–60.
Mesquini, J.A., Sawaya, A.C., López, B.G., Oliveira, V.M., Miyasaka, N.R., 2015. Detoxification of atrazine by endophytic *Streptomyces* sp. isolated from sugarcane and detection of nontoxic metabolite. Bull. Environ. Contam. Toxicol. 95, 803–809.
Monsalvo, V.M., Garcia-Mancha, N., Puyol, D., Mohedano, A.F., Rodríguez, J.J., 2014. Anaerobic biodegradability of mixtures of pesticides in an expanded granular sludge bed reactor. Water Sci. Technol. 69, 532–538.
Moreno-Medina, D.A., Sánchez-Salinas, E., Ortiz-Hernández, M.L., 2014. Removal of methyl parathion and coumaphos pesticides by a bacterial consortium immobilized in Luffa cylindrica. Rev. Int. Contam. Ambient. 30, 51–63.
Okeke, B.C., Siddique, T., Arbestain, M.C., Frankenberger, W.T., 2002. Biodegradation of γ-hexachlorocyclohexane (lindane) and α-hexachlorocyclohexane in water and a soil slurry by a *Pandoraea* species. J. Agric. Food Chem. 50, 2548–2555.
Ondarza, P.M., González, M., Fillmann, G., Miglioranza, K.S.B., 2014. PBDEs, PCBs and organochlorine pesticides distribution in edible fish from Negro River basin, Argentinean Patagonia. Chemosphere 94, 135–142.
PPDB, 2012. Pesticide Properties Database (PPDB) Developed by the Agriculture and Environment Research Unit. University of Hertfordshire, UK (Accessed 05 April 16) from. http://sitem.herts.ac.uk/aeru/footprint/en/index.htm.
Quintero, J.C., Moreira, M.T., Feijoo, G., Lema, J.M., 2005. Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems. Chemosphere 61, 528–536.
Rajashekara Murthy, H.M., Thakur, M.S., Manonmani, H.K., 2010. Degradation of technical grade hexachlorocyclohexane in soil slurry by a defined microbial consortium. Int. J. Environ. Res. 4, 471–478.
Rama Krishna, K., Philip, L., 2008. Adsorption and desorption characteristics of lindane, carbofuran and methyl parathion on various Indian soils. J. Hazard. Mater. 160, 559–567.
Rama Krishna, K., Philip, L., 2011. Bioremediation of single and mixture of pesticide-contaminated soils by mixed pesticide-enriched cultures. Appl. Biochem. Biotechnol. 164, 1257–1277.
Robles-González, I.V., Fava, F., Rinderknecht-Seijas, N., Poggi-Varaldo, H.M., 2008. A review on slurry bioreactors for bioremediation of soils and sediments. Microb. Cell Factories 7, 1.
Robles-González, I.V., Ríos-Leal, E., Sastre-Conde, I., Fava, F., Rinderknecht-Seijas, N., Poggi-Varaldo, H.M., 2012. Slurry bioreactors with simultaneous electron acceptors for bioremediation of an agricultural soil polluted with lindane. Process Biochem. 47, 1640–1648.
Rucks, L., García, F., Kaplán, A., Ponce de León, J., Hill, M., 2004. Propiedades físicas del suelo. Universidad de la República, Montevideo. Uruguay.
Saez, J.M., Benimeli, C.S., Amoroso, M.J., 2012. Lindane removal by pure and mixed cultures of immobilized actinobacteria. Chemosphere 89, 982–987.
Saez, J.M., Álvarez, A., Benimeli, C.S., Amoroso, M.J., 2014. Enhanced lindane removal from soil slurry by immobilized Streptomyces consortium. Int. Biodeterior. Biodegrad. 93, 63–69.
Shelton, D.R., Khader, S., Karns, J.S., Pogell, B.M., 1996. Metabolism of twelve herbicides by *Streptomyces*. Biodegradation 7, 129–136.
Sheng, J., Wang, X., Gong, P., Joswiak, D.R., Tian, L., Yao, T., Jones, K.C., 2013. Monsoon-driven transport of organochlorine pesticides and polychlorinated biphenyls to the Tibetan plateau: three year atmospheric monitoring study. Environ. Sci. Technol. 47, 3199–3208.
Sineli, P.E., Tortella, G., Costa, J.D., Benimeli, C.S., Cuozzo, S.A., 2016. Evidence of α-, β-and γ-HCH mixture aerobic degradation by the native actinobacteria *Streptomyces* sp. M7. World J. Microbiol. Biotechnol. 32, 1–9.
Singh, N., 2002. Sorption behaviour of triazole fungicides in Indian soils and its correlation with soil properties. J. Agric. Food Chem. 50, 6434–6439.
Swarcewicz, M.K., Gregorczyk, A., 2012. The effects of pesticide mixtures on degradation of pendimethalin in soils. Environ. Monit. Assess. 184, 3077–3084.
Tiwari, M.K., Guha, S., 2014. Kinetics of biotransformation of chlorpyrifos in aqueous and soil slurry environments. Water Res. 51, 73–85.
Wilkinson, S., Nicklin, S., Faull, J.L., 2002. Biodegradation of fuel oils and lubricants: soil and water bioremediation options. Prog. Ind. Microbiol. 36, 69–100.
Xiao, P., Mori, T., Kondo, R., 2011. Biotransformation of the organochlorine pesticide trans-chlordane by wood-rot fungi. New Biotechnol. 29, 107–115.
Yamada, S., Naito, Y., Funakawa, M., Nakai, S., Hosomi, M., 2008. Photodegradation fates of cis-chlordane, trans-chlordane, and heptachlor in ethanol. Chemosphere 70, 1669–1675.
Zamani, J., Hajabbasi, M.A., Alaie, E., 2015. The effect of steam sterilization of a petroleum-contaminated soil on pah concentration and maize (*Zea mays* L.) growth. Int. J. Curr. Microbiol. Appl. Sci. 4, 93–104.
Zhang, Q., Chen, Z., Li, Y., Wang, P., Zhu, C., Gao, G., Xiao, K., Sun, H., Zheng, S., Liang, Y., Jiang, G., 2015. Occurrence of organochlorine pesticides in the environmental matrices from King George Island, west Antarctica. Environ. Pollut. 206, 142–149.
Zuo, Z., Gong, T., Che, Y., Liu, R., Xu, P., Jiang, H., Quiao, C., Song, C., Yang, C., 2015. Engineering *Pseudomonas putida* KT2440 for simultaneous degradation of organophosphates and pyrethroids and its application in bioremediation of soil. Biodegradation 26, 223–233.
Đurović, R., Gajić-Umiljendić, J., Đorđević, T., 2009. Effects of organic matter and clay content in soil on pesticide adsorption processes. Pesticides Phytomed. 24, 51–57.