Ecotoxicology and Environmental Safety 190 (2020) 110143

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Enhanced bioremediation of lindane-contaminated soils through microbial bioaugmentation assisted by biostimulation with sugarcane filter cake

Enzo E. Raimondo[a,b], Juan D. Aparicio[a,b], Ana L. Bigliardo[a], María S. Fuentes[a], Claudia S. Benimeli[a,c,*]

[a] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, 4000, Tucumán, Argentina
[b] Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000, Tucumán, Argentina
[c] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, Belgrano 300, 4700, Catamarca, Argentina

ARTICLE INFO

Keywords:
Lindane
Bioaugmentation
Actinobacteria
Biostimulation
Sugarcane filter cake

ABSTRACT

Lindane is a toxic and persistent organochlorine pesticide, whose extensive use generated its accumulation in different environmental matrices. Bioremediation is a promising technology that can be used combining bioaugmentation and biostimulation processes to soil restoration. The aim of the present work was to determine the conditions of maximum lindane removal by bioaugmentation with an actinobacteria consortium and biostimulation with sugarcane filter cake (SCFC). The assays were carried out on lindane-contaminated silty loam (SLS), clayey (CS), and sandy (SS) soils. Through complete factorial designs, the effects of three abiotic factors (moisture content, proportion and size of SCFC particles) were evaluated on lindane removal. In addition, a response optimizer determined the optimal conditions for pesticide removal in bioaugmented and biostimulated soils, in the range of levels studied for each factor. In these conditions, bioaugmentation of biostimulated soils increased the pesticide removal (SLS: 61.4%, CS: 70.8%, SS: 86.3%), heterotrophic microbial counts, and soil enzymatic activities, and decreased lindane $T_{1/2}$, regarding the non-bioaugmented biostimulated controls, after 14 days of assay. The values of these parameters confirmed the efficiency of the bioremediation process. Finally, the viability of the four strains was demonstrated at the end of the assay. The results indicate that the simultaneous application of bioaugmentation with the actinobacteria consortium and biostimulation with SCFC constitutes a promising tool for restoring soils contaminated with lindane, by using the optimal conditions obtained through the factorial designs.

1. Introduction

Lindane, the gamma isomer of hexachlorocyclohexane (γ-HCH), is an organochlorine pesticide that was extensively used in order to minimize economic losses caused by pests, insects, and diseases. It was used worldwide from about 1950 to 1980 in amounts exceeding 600000 tons overall (Vijgen et al., 2011). Due to its non-target toxicity, persistence, and bioaccumulation along the food chain (Vijgen et al., 2011; Saez et al., 2017), lindane was officially listed as persistent organic pollutant in the Stockholm Convention for Protecting Human Health and the Environment from Persistent Organic Pollutants in 2009 (Vijgen et al., 2011). In Argentina, this pesticide was completely banned, both for phytosanitary and medicinal purposes (Ministerio de Salud, 2017). However, its indiscriminate and excessive use in agroecosystems in the past resulted in its release to the environment, causing a global contamination (Rama-Krishna and Philip, 2011;Aparicio et al., 2018a, Grondona et al., 2019).

In particular, the soil contamination occurs at an alarming rate and may alter the microbial activities, life cycles, and/or population sizes, which has a direct or indirect effect on soil fertility and cultivated crops. These changes in soil quality and health, introduced by pesticide contamination, can be studied by evaluating biological parameters such as enzymatic activities (Jastrzębska, 2011; Tejada et al., 2011). Among the most studied soil enzymes are oxidoreductases, which participate in the respiratory chain mediating the energy transformation, as well as in the humic acids synthesis and in soil formation (Achuba and Peretiemo-Clarke, 2008), and hydrolases, which catalyze the conversion of nutrients from unavailable to forms readily assimilable by plants and microorganisms (Renella et al., 2006).

Because biological degradation is recognized as the main mechanism during the pesticide removal (Chishti et al., 2013), several microbial degradation technologies are being developed to ensure a

* 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).

https://doi.org/10.1016/j.ecoenv.2019.110143
Received 7 August 2019; Received in revised form 24 December 2019; Accepted 27 December 2019

harmless, effective, and economic restoration of soils contaminated with lindane residues. In this sense, bioremediation by biostimulation with organic amendments, such as animal manures, sugarcane bagasse, sugarcane filter cake, straw, sawdust, compost, among others, can be highlighted. The application of these organic materials increases the aeration, porosity, and water-holding capacity of soils, as well as the nutrient content, which promotes the microbial activity and improves the microbial diversity (Goss et al., 2013; Ren et al., 2018). Sugarcane filter cake (SCFC) is a residue obtained during the clarification process of sugarcane juice. Its composition includes earthy materials, organic impurities, and a great amount of nutrients such as N, P, K, Ca, and Mg (Fundora-Tellechea et al., 2016). In the literature, it was reported that 30–50 kg of SCFC are obtained for each ton of processed sugarcane. This residue is used in sugarcane fields to improve the physico-chemical properties of soils (Ramos-Anacleto et al., 2017). However, its use is not completely efficient, so SCFC is considered as a source of environmental contamination (García-Torres et al., 2011). Due to its high micro- and macronutrient contents and its biostimulant properties, SCFC represents an interesting alternative to increase the efficiency of bioremediation processes (Fundora-Tellechea et al., 2016).

On the other hand, bioremediation by bioaugmentation has gained great importance in recent decades (Alessandrello et al., 2017). Among the microorganisms used for this purpose, those belonging to the *Streptomyces* genus stand out, which have the ability to degrade different pesticide types, including organochlorines (Benimeli et al., 2007; Alvarez et al., 2017). *Streptomyces* strains represent a source of a wide range of enzymes; therefore, they are capable of performing transformations of organic compounds (Ballav et al., 2012). Moreover, their use as defined microbial consortia is more appropriate for bioremediation processes than their use as pure cultures, since the consortia biodiversity increases the number of catabolic pathways available for the biodegradation, enhancing the contaminant removal efficiency (Fuentes et al., 2011; 2014; 2016).

Currently, bioremediation studies by simultaneous application of bioaugmentation with *Streptomyces* and biostimulation with SCFC are scarce. Thus, it is important to understand the parameters that condition a bioremediation process, for which factorial designs are applied. This experimental methodology allows determining the relevance of the individual and interactive effects exerted by certain variables on the experimental response, modeling the system, and optimizing the process (Aparicio et al., 2018a).

The aim of the present work was to determine the conditions of maximum lindane removal in three soil types by simultaneous application of bioaugmentation with a defined actinobacteria consortium and biostimulation with SCFC, for which complete factorial designs were carried out. The efficiency of the bioremediation process in optimal conditions was also analyzed by monitoring total heterotrophic microorganisms, kinetic parameters of lindane removal, soil enzymatic activities, and survival of the *Streptomyces* strains.

2. Materials and methods

2.1. Pesticide, chemicals, microorganisms, and culture media

Lindane (99% pure) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). It was dissolved in acetone (analytical grade, Sintorgan, Argentina) to prepare a stock solution (1 mg mL^{-1}). All other analytical grade chemicals used during the study were acquired from certificated manufacturers.

A defined actinobacteria consortium, composed by *Streptomyces* sp. A2, A5, A11, and M7, was employed in the study. This consortium was previously selected for its ability to degrade lindane and for the absence of antagonism among the strains (Fuentes et al., 2011).

Starch-Casein Agar (SC), used to maintain the strains and for antibiotic sensitivity tests, consisted of (g L^{-1}): starch, 10.0; casein, 1.0; K_2HPO_4, 0.5; agar, 15.0 (Hopwood et al., 1985).

Tryptic Soy Broth (TSB, Sigma-Aldrich), used to prepare the inoculum for removal assays in soils, was composed by (g L^{-1}): tryptone, 15.0; soy peptone, 3.0; NaCl, 5.0; K_2HPO_4, 2.5; glucose, 2.5.

Plate Count Agar (PCA, Britania), employed to enumerate total heterotrophic microorganisms, contained (g L^{-1}): yeast extract, 2.5; tryptone, 5.0; glucose, 1.0; agar, 15.0.

All media were adjusted to pH 7.0 ± 0.2 before autoclaving at 121 °C for 15 min.

2.2. Soil and sugarcane filter cake samples preparation

The bioremediation assays were carried out in microcosms formulated with three types of non-polluted soils, which were collected from different sites in Tucumán Province (Argentina). Soil samples were taken and conditioned following the methodology previously described by Aparicio et al. (2018a).

Sugarcane filter cake (SCFC) was a residue of the 2015 harvest and it was obtained from the Sugar Mill Cruz Alta (Tucumán, Argentina). SCFC was processed as described by Raimondo et al. (2020), in order to obtain two fractions with different particle sizes: 0.5 and 5.0 mm.

Main physico-chemical characteristics of soils and SCFC are shown in Table 1.

2.3. Bioremediation assays: experimental designs

Bioremediation experiments were performed in glass bottles containing 39.2 or 36.0 g of soil as appropriate, which was contaminated with lindane (final concentration: 2 mg kg^{-1}). Contaminated soils were biostimulated with SCFC particles (proportion 2 or 10%; size 0.5 or 5.0 mm) and bioaugmented with the actinobacteria consortium at a final concentration of 2 g kg^{-1}. Each consortium strain was previously cultivated in TSB, incubated at 30 °C during 72 h with agitation, and the biomass was harvested as described by Raimondo et al. (2019). Finally, the moisture content was adjusted with sterile distilled water to 20 or 30%. The whole system was vigorously mixed to ensure a homogeneous distribution of the components. The bioaugmented and biostimulated microcosms (BABS microcosms) were tested in triplicate at 30 °C for 14 days, with their respective controls: non-inoculated, non-contaminated, and biostimulated microcosms (natural soils or NAT soils) and non-inoculated, contaminated, and biostimulated microcosms (BS controls).

Three 2^3 full factorial designs were performed with BABS microcosms using MINITAB 17 (PA, USA) statistical software in order to identify the main effects and the interactions among the following factors on lindane bioremediation: SCFC proportion (2 or 10%), moisture content (20 or 30%), and size of SCFC particles (0.5 or 5.0 mm). The low and high levels of the factors were selected according to previous studies (García-Torres et al., 2011; Antonio-Ordaz et al., 2011; Aparicio et al., 2018a). The evaluated response was lindane

Table 1
Physico-chemical characteristics of soils and sugarcane filter cake employed in the study.

Parameters	Soil #1	Soil #2	Soil #3	Sugarcane filter cake
pH	7.6	7.3	6.2	
Organic carbon (%)	0.80	0.61	0.58	73.40
Oxidizable organic matter (%)	1.30	1.05	1.00	42.60
Total phosphorus (%)	0.002	0.004	0.002	0.880
Total nitrogen (%)	0.10	0.07	0.04	2.43
Clay (%)	14.3	62.5	2.5	
Silt (%)	59.8	13.8	4.0	
Sand (%)	25.9	23.7	93.5	
Texture	Silty Loam (SLS)	Clayey (CS)	Sandy (SS)	

Table 2
Twenty four runs of the factorial designs for optimization of lindane bioremediation process by the bioaugmentation with an actinobacteria consortium, in different soils biostimulated with sugarcane filter cake.

Conditions	A	B	C	Lindane removal (%): SLS		Lindane removal (%): CS		Lindane removal (%): SS	
				Exp	Pred	Exp	Pred	Exp	Pred
1	2	20	0.5	63.0	61.4	48.7	49.1	63.2	69.5
	2	20	0.5	58.9	61.4	50.4	49.1	74.6	69.5
	2	20	0.5	62.2	61.4	48.1	49.1	70.6	69.5
2	10	20	0.5	44.2	49.0	73.1	70.8	45.9	47.1
	10	20	0.5	54.8	49.0	69.0	70.8	52.7	47.1
	10	20	0.5	48.0	49.0	70.5	70.8	42.8	47.1
3	2	30	0.5	53.7	54.7	22.5	21.5	86.1	86.3
	2	30	0.5	58.9	54.7	22.0	21.5	85.9	86.3
	2	30	0.5	51.6	54.7	19.9	21.5	87.0	86.3
4	10	30	0.5	37.8	37.8	32.8	38.8	71.5	68.7
	10	30	0.5	38.2	37.8	41.1	38.8	66.6	68.7
	10	30	0.5	37.3	37.8	42.4	38.8	67.9	68.7
5	2	20	5.0	47.6	49.2	49.0	48.2	67.4	71.7
	2	20	5.0	53.4	49.2	47.4	48.2	72.3	71.7
	2	20	5.0	46.4	49.2	48.2	48.2	75.5	71.3
6	10	20	5.0	21.9	20.0	66.7	68.7	49.0	48.0
	10	20	5.0	20.2	20.0	71.6	68.7	46.9	48.0
	10	20	5.0	18.1	20.0	67.8	68.7	48.0	48.0
7	2	30	5.0	48.8	45.0	34.8	36.4	82.2	82.9
	2	30	5.0	40.8	45.0	37.6	36.4	83.6	82.9
	2	30	5.0	45.4	45.0	36.9	36.4	83.1	82.9
8	10	30	5.0	39.6	35.8	39.9	36.9	60.8	58.6
	10	30	5.0	29.7	35.8	40.6	36.9	56.5	58.6
	10	30	5.0	38.2	35.8	30.0	36.9	58.6	58.6

A: Proportion of sugarcane filter cake (%); B: Moisture content (%); C: Size of sugarcane filter cake particles (mm); SLS: Silty loam soil; CS: Clayey soil; SS: Sandy soil; Exp: Experimental values; Pred: Predicted values.

removal (%) at 14 days of incubation. The factorial designs generated 8 combinations, resulting in 24 runs (Table 2).

It was assumed that the response was approximately linear over the range of the factor levels considered and data from the designs were subjected to multiple regression analysis to obtain the parameter estimators of the mathematical models.

The effects of each independent factor and its interactions were detected with an analysis of variance (ANOVA). Significant factors were selected on the basis of the *F* test and the *p* value at a 95% significance level ($p < 0.05$). The accuracy and goodness of fit of final regression models were determined by using the coefficient of determination R^2. The conditions of maximum lindane removal (optimal bioremediation conditions) in each soil type were identified through a MINITAB response optimizer.

2.4. Analytical determinations

The bioremediation assays were carried out for 14 days, and soil samples were collected every 7 days, from BABS microcosms corresponding to the optimal conditions established by the factorial designs, and from their respective controls (NAT soils and BS controls). The samples were analyzed in order to determine lindane removal kinetics and half-life, total heterotrophic microorganisms, and soil enzymatic activities at 0, 7, and 14 days of incubation. Also, the survival of the strains constituents of the consortium was evaluated at the end of the bioremediation process.

The appropriate methodologies to extract, determine, and quantify lindane in soil samples were previously developed by our research group (Saez et al., 2015).

The total heterotrophic microorganisms were quantified by plating 100 μL of diluted soil suspensions on PCA through the surface spreading technique, and incubating the plates at 30 °C for 5 days (Raimondo et al., 2019).

Dehydrogenase activity (DH), fluorescein diacetate hydrolysis activity (FDA), acid and alkaline phosphatases activities (AP and AKP, respectively), urease activity (UR), and catalase activity (CAT) were determined by using traditional techniques, with slight variations in the methodologies as described by Raimondo et al. (2019).

For the first five enzymatic activities, two controls were carried out: substrate without soil sample and soil sample without substrate, in order to measure the color from abiotic degradation of substrates and soil components, respectively. For CAT, controls were performed in the same way as soil samples, but without H_2O_2, while the blanks were performed with H_2O_2 but without soil sample.

The viability of the four *Streptomyces* strains was evaluated through biochemical and molecular approaches at the end of the assay. Suspensions of soils from BABS microcosms were seeded on SC supplemented with appropriate antibiotics for each actinobacteria. After 7 days of incubation, total DNA was extracted from the re-isolated colonies and unique genetic polymorphisms were detected by RAPD-PCR (annealing temperature: 50 °C) (Aparicio et al., 2018b; Raimondo et al., 2019).

2.5. Kinetic models

Kinetic studies were performed applying different kinetic models to investigate which of these equations fitted the mechanism of lindane removal. The mathematical models were evaluated by using the coefficient of determination R^2, in order to determine the best fit kinetic model (Ahmad et al., 2013). The lindane removal in microcosms was described by a first-order kinetic model, which is represented by the following equation:

$$\ln C_t = \ln C_0 - kt$$

where C_0 is lindane concentration in soils at initial time, C_t is lindane concentration in soils at time t, k is degradation rate constant (d^{-1}), and t is the degradation time (d). The half-life of lindane ($T_{1/2}$) for the first-order reaction was obtained by the equation:

$$T_{1/2} = \frac{\ln 2}{k}$$

2.6. Statistical analysis

Determinations were carried out in triplicate and the results were expressed as average values. One-way ANOVA (Tukey test, $p < 0.05$) was employed to compare among treatments, considering lindane removal, kinetic parameters, microbial counts, and soil enzymatic activities.

3. Results and discussion

3.1. Determination of the conditions of maximum lindane removal in bioaugmented and biostimulated soils

The analysis of variance (ANOVA) demonstrated that lindane removals were significantly higher in BABS microcosms than in their respective BS controls, at the end of the assay ($p < 0.05$) (Fig. 1).

The effects and interactions between SCFC proportion (A), moisture content (B), and size of SCFC particles (C) on lindane removal at 14 days of incubation in BABS microcosms were evaluated by using factorial designs. The studied factors were designated with letters in order to simplify their identification. The tested factors, their levels, and experimental and predicted responses are shown in Table 2. For the three soils, a high correlation was observed between the experimental values and those predicted by the statistical models, since the deviation between them was minimal (data not shown). In addition, the assumptions of normality in the distribution of residual and constant variance confirmed the validity of the results (data not shown).

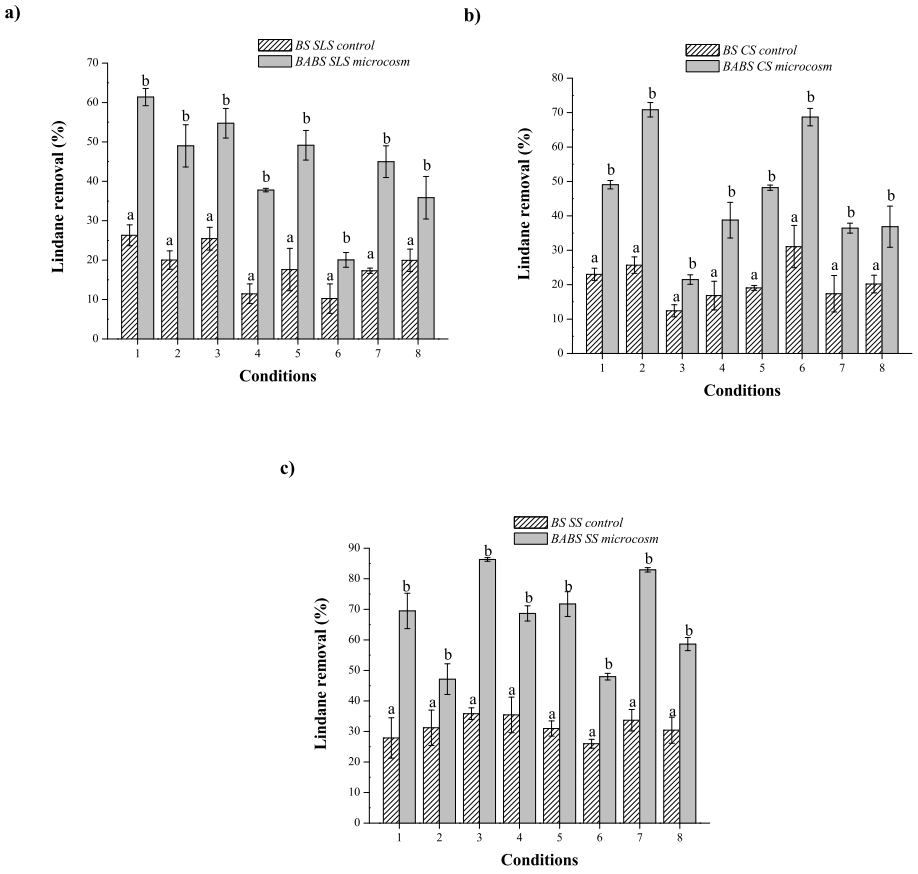


Fig. 1. Lindane removal (%) in soil microcosms for the eight evaluated conditions, at 14 days of incubation. a) Silty loam soil (SLS); b) Clayey soil (CS); c) Sandy soil (SS). Different letters indicate significant differences between treatments for each type of soil ($p < 0.05$, Tukey test). BS: Biostimulated; BABS: Bioaugmented and Biostimulated. References of the x axis in Table 2.

Significant main (individual) and interaction effects on lindane removal, at 14 days of incubation, were detected through ANOVA, and the results are presented in Table 3.

In BABS SLS microcosms, the main effects of SCFC proportion (A) and size of SCFC particles (C) on the response were significant and negative, indicating that increases in the levels of both factors would reduce the lindane removal, in the range between the evaluated levels. On the contrary, the response was insensitive to changes in moisture content (B) levels ($p > 0.05$) (Table 3). However, the 2-way interactions of moisture content with the other factors had a significant effect on the dependent variable ($p < 0.05$), being more important the B*C interaction. This interaction would indicate that an increase in the moisture content would reduce the lindane removal, when using SCFC particles of 0.5 mm. Also, the 3-way interaction had a significant effect on the response ($p < 0.05$) (Table 3).

In BABS CS microcosms, it was observed that not all the independent variables presented a significant main effect on the bioremediation process (Table 3). In the range between the evaluated levels, the moisture content had the most pronounced effect on the response and it was negative, meaning that lindane removal was lower when increasing this factor. The 2-way and 3-way interactions had a significant effect on the response ($p < 0.05$). The most pronounced effect on the bioremediation process was detected by the interaction A*B, which would indicate that an increase in SCFC proportion would improve the lindane removal, mainly when the moisture content is lower (Table 3).

In BABS SS microcosms, SCFC proportion and moisture content showed significant individual effects on the response ($p < 0.05$). SCFC proportion had a negative effect, meaning that higher removal percentages were detected at a lower value of this factor, while the moisture content had a less pronounced but positive effect on lindane removal, indicating a greater pesticide dissipation at higher humidity levels. Size of SCFC particles did not present a significant individual effect on the dependent variable ($p > 0.05$) (Table 3). Among 2-way and 3-way interactions, only B*C had a significant effect on the response ($p < 0.05$), which would indicate that an increase in moisture content would improve the lindane removal, mainly when using SCFC particles of 0.5 mm (Table 3).

Based on the results presented in Table 3, the factorial design corresponding to BABS SS microcosms was reanalyzed to obtain a mathematical model that involves only the significant predictors. It is important to note that, although size of SCFC particles did not have a significant effect on lindane removal ($p > 0.05$), one of its interactions with another factor did ($p < 0.05$). Therefore, this parameter was incorporated to the final model. The factorial designs corresponding to BABS SLS and BABS CS microcosms were not reanalyzed because, although some factors did not significantly affect the response, their interactions with other factors did, and therefore these parameters could not have been removed from the corresponding final models.

The simplified first-order lineal models for SLS (1), CS (2), and SS (3), involving only predictors with significant influence on the response and showing the relationship between lindane removal at 14 days of incubation (Y) and the independent variables, are presented below:

$$Y = 75.60 + 0.51A - 0.51B - 0.16C - 0.09A*B - 1.83A*C - 0.08B*C + 0.07A*B*C \quad (1)$$

$$Y = 100.92 + 3.44A - 2.86B - 8.88C - 0.03A*B + 0.83A*C + 0.44B*C - 0.04A*B*C \quad (2)$$

$$Y = 34.43 - 2.75A + 2.01B + 4.01C - 0.18B*C \quad (3)$$

The coefficient of determination R^2 is used to determine how well the mathematical models fit the data. The calculated R^2 values were

Table 3
Analysis of variance and estimated effects of factors on lindane removal (%) in bioaugmented and biostimulated microcosms, at 14 days of incubation.

Source	BABS SLS						BABS CS						BABS SS					
	ANOVA			Estimated effects			ANOVA			Estimated effects			ANOVA			Estimated effects		
	DF	*F*-value	*p*-value	Effect	*t*-value	Remarks	DF	*F*-value	*p*-value	Effect	*t*-value	Remarks	DF	*F*-value	*p*-value	Effect	*t*-value	Remarks
Model	7	35.58	0.000				7	85.47	0.000				7	57.65	0.000			
Linear	3	66.86	0.000				3	181.33	0.000				3	130.20	0.000			
A	1	123.82	0.000	−16.90	−11.13	S	1	136.37	0.000	15.00	11.68	S	1	263.66	0.000	−22.03	−16.24	S
B	1	1.07	0.317	−1.57	−1.03	I	1	403.80	0.000	−25.81	−20.09	S	1	123.30	0.000	15.06	11.10	S
C	1	75.69	0.000	−13.21	−8.70	S	1	3.82	0.068	2.510	1.95	I	1	3.64	0.075	−2.59	−1.91	I
2-Way Interaction	3	10.72	0.000				3	15.05	0.000				3	4.02	0.026			
A*B	1	6.42	0.022	3.85	2.53	S	1	22.94	0.000	−6.153	−4.79	S	1	0.58	0.458	1.03	0.76	I
A*C	1	2.14	0.163	−2.22	−1.46	I	1	12.45	0.003	−4.533	−3.53	S	1	2.21	0.157	−2.02	−1.49	I
B*C	1	23.59	0.000	7.37	4.86	S	1	9.74	0.007	4.010	3.12	S	1	9.27	0.008	−4.13	−3.04	S
3-Way Interaction	1	16.32	0.001				1	9.20	0.008				1	0.92	0.351			
A*B*C	1	16.32	0.001	6.14	4.04	S	1	9.20	0.008	−3.896	−3.03	S	1	0.92	0.351	−1.30	−0.96	I
Error	16						16						16					
Total	23						23						23					

SLS: Silty loam soil; CS: Clayey soil; SS: Sandy soil; BABS: Bioaugmented and Biostimulated; DF: Total degrees of freedom; A: SCFC proportion (%); B: Moisture content (%); C: Size of SCFC particles (mm); S: Significant; I: Insignificant.

Table 4
Optimal conditions for lindane removal in bioaugmented and biostimulated microcosms, obtained through factorial designs after run the response optimizer (Minitab 17).

Microcosms	SCFC proportion (%)	Moisture content (%)	Size of SCFC particles (mm)
BABS SLS	2	20	0.5
BABS CS	10	20	0.5
BABS SS	2	30	0.5

SCFC: Sugarcane filter cake; SLS: Silty loam soil; CS: Clayey soil; SS: Sandy soil; BABS: Bioaugmented and Biostimulated.

close to 1, therefore the first-order lineal mathematical models were very reliable for studying lindane removal in the different systems. In the case of BABS SLS microcosms (1), the model presented a R^2 value of 0.9396, meaning that 93.96% of the response variation is attributed to the variation of the independent variables. Furthermore, the predicted R^2 was 0.8642, which implies that the model would be adequate to predict 86.42% of lindane removal in new experiments. For BABS CS microcosms (2), the model would explain 97.40% of the observed data and predict 94.14% of the response in new experiments. These observations result from the R^2 and predicted R^2 values obtained for the model: 0.9740 and 0.9414, respectively. Finally, for BABS SS microcosms (3), the high R^2 value (0.9530) confirms that 95.30% of the response variability was explained by the statistical model, while the predicted R^2 value (0.9250) means that 92.50% of the lindane removal in new experiments would be predicted by this first-order lineal equation.

When bioremediation protocols are applied, one of the main objectives is to determine the conditions in which the process is more efficient. At the present work, a response optimizer tool identified the combination of the studied factors and levels that allowed maximizing the response in BABS microcosms, which are presented in Table 4 for each soil type.

The SCFC proportion needed to obtain the optimal lindane removal was variable among the different soil types. In BABS SLS and BABS SS microcosms, the application of 2% SCFC was convenient to reach the maximal removal, while in BABS CS microcosms, 10% was the proportion adequate. The application of high organic amendment contents is convenient when working with clayey soil, where the addition of these organic materials is necessary to improve the structure, porosity, and oxygen diffusion in this soil type (García et al., 2012).

Another important observation that results from Table 4 is that the maximum lindane removal was obtained when using SCFC particles of 0.5 mm in the three soils. This is not surprising, since it was already reported that small particles of organic amendments present a higher surface area, which favors the microbial colonization and the microorganisms-contaminant contact and promotes the bioremediation activity. On the contrary, large particles of organic amendments have smaller specific surface area, so the use of these particles in bioremediation assays reduces the number of potential sites for microbial activity (Chang et al., 2009).

Microorganisms require water for the growth and nutrient diffusion across the cell wall during the bioremediation processes. Low moisture contents could limit this movement, while high moisture contents could saturate the pores between soil particles and reduce oxygen transport, especially in soils with high contents of clay and silt particles (Niti et al., 2013; Haghollahi et al., 2016). In the present study, the highest lindane removal in soils with predominance of small particles was obtained by using a moisture content of 20%, while the highest lindane removal in the soil with predominance of large particles was obtained by using a moisture content of 30% (Table 4).

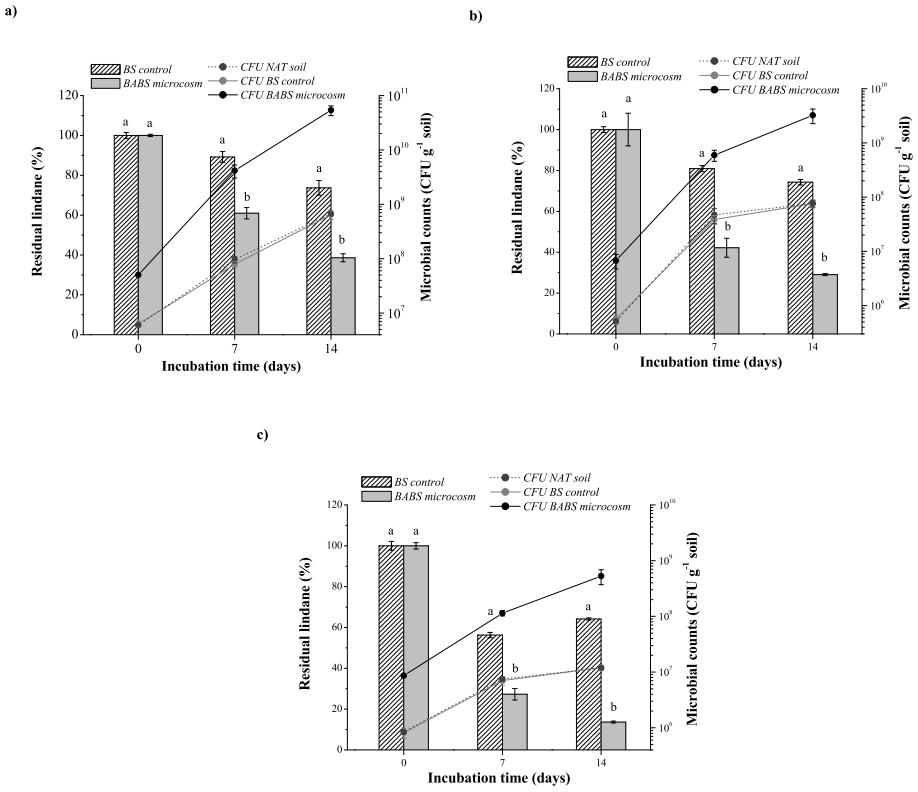


Fig. 2. Residual lindane (%) and heterotrophic microbial counts (CFU g^{-1} soil) in soil microcosms. a) Silty loam soil (SLS); b) Clayey soil (CS); c) Sandy soil (SS). Different letters indicate significant differences between treatments for each type of soil ($p < 0.05$, Tukey test). Nat: Natural; BS: Biostimulated; BABS: Bioaugmented and Biostimulated.

3.2. Kinetic and biological parameters involved in lindane bioremediation process, in the optimal conditions

3.2.1. Determination of total heterotrophic microorganisms, lindane removal, and Streptomyces strains survival

When analyzing the microbial counts in BABS microcosms in the optimal conditions established by the factorial designs, as well as in their respective controls, statistically significant differences were found between the values recorded at initial and final times, both in bioaugmented and biostimulated soils and in their respective controls ($p < 0.05$) (Fig. 2). In each soil type, CFU g^{-1} soil values were significantly higher in BABS microcosms than in controls, during all the assay ($p < 0.05$). In addition, no significant differences were found between microbial counts obtained in the two controls at the end of the assay (NAT soils and BS controls) ($p > 0.05$). This result suggests that the microbiota of each system (edaphic microorganisms plus those coming from SCFC) could tolerate the lindane concentration, without presenting considerable variations in its population density. Furthermore, in all contaminated soils, lindane removal was significantly higher in BABS microcosms than in BS controls ($p < 0.05$).

In SLS, final microbial enumerations of (5.37 ± 1.09) x 10^{10}, (6.44 ± 1.20) x 10^{8}, and (6.70 ± 0.21) x 10^{8} CFU g^{-1} soil were recorded for BABS microcosms, BS controls, and NAT soils, respectively (Fig. 2a). Regarding the lindane bioremediation, it was observed that the pesticide dissipation was gradual and greater in BABS microcosms, where a 61.4% removal was reached at the end of the assay. In the respective controls, a 26.3% removal was detected for the same period of time (Fig. 2a).

In BABS CS microcosms, the microbial counts increased throughout the assay, reaching a value of (3.24 ± 0.97) x 10^{9} CFU g^{-1} soil at the end of the incubation period. This value was significantly higher than those obtained in both controls: (7.59 ± 1.21) x 10^{7} CFU g^{-1} soil for BS controls and (7.66 ± 1.10) x 10^{7} CFU g^{-1} soil for NAT soils ($p < 0.05$) (Fig. 2b). Regarding the bioremediation process, a marked decrease in the initial lindane concentration was observed in BABS microcosms during the first 7 incubation days. In these systems, the final pesticide removal was 70.8%, while in the respective controls, the final pesticide removal was 25.7% (Fig. 2b).

In BABS SS microcosms, the initial population density increased until reaching a value of (5.29 ± 1.59) x 10^{8} CFU g^{-1} soil, at 14 days of incubation. The corresponding controls, BS controls and NAT soils, showed final values of (1.20 ± 0.13) x 10^{7} and (1.19 ± 0.10) x 10^{7} CFU g^{-1} soil, respectively (Fig. 2c). When analyzing the residual lindane concentration, a significant decrease was observed during the first 7 days, both in BABS microcosms and in BS controls. At 14 days of incubation, the pesticide removal was 86.3% and 35.8% for BABS microcosms and BS controls, respectively (Fig. 2c).

Previous results demonstrated that the pesticide degradation depends on features of the soil types, including organic matter, clay contents, pH, textural class, nutrients, water contents, autochthonous microbiota, among others, which are factors that determine the adsorption of the pesticides and their bioavailability for microbial degradation (Fuentes et al., 2017; Saez et al., 2018; Raimondo et al., 2019). These observations would explain the different removal percentages obtained in each type of soil.

In the different treatments, data of lindane removal were well fitted to a first-order kinetic model. The values of the kinetic parameters (k constant, lindane $T_{1/2}$, and coefficient of determination R^2) are presented in Supplementary Table 1. It is important to note that BABS microcosms showed lower $T_{1/2}$ and higher k values than BS controls ($p < 0.05$). Minimum value of lindane $T_{1/2}$ (4.9 d) and maximum kinetic rate constant (0.142 d^{-1}) were obtained in BABS SS microcosms, where the highest lindane removal (86.3%) was detected.

In BABS microcosms, the presence of the actinobacteria consortium increased the removals by 35.1%, 45.1%, and 50.5% and reduced the lindane $T_{1/2}$ by 22.8, 25.1, and 17.5 days (corresponding to decreases of 69%, 76%, and 78%), for SLS, CS, and SS, respectively.

These results indicate a positive interaction between the inoculated consortium and the autochthonous microbiota of each soil, which enhanced the bioremediation process. In addition, they confirm the hypothesis that the actinobacteria consortia constitute an adequate tool for the treatment of non-sterile amended soils contaminated with

organochlorine pesticides.

The biostimulation of the soils with SCFC presented a positive effect on lindane bioremediation by the actinobacteria consortium. This observation resulted from comparing the data of this work with a previous one, where the lindane removal was studied in the same soils bioaugmented with the quadruple consortium but without biostimulation, and lindane removals of 36.3%, 30.7%, and 70.3% were detected for SLS, CS, and SS, respectively, at 14 days of incubation (Raimondo et al., 2019). At the present work, the removal percentages obtained in BABS microcosms were significantly higher (SLS: 61.4%, CS: 70.8%, SS: 86.3%) ($p < 0.05$). This finding is very important, since it shows that the organic amendments coming from industrial activities increase the bioremediation activity of the actinobacteria. The same behavior was observed in the non-bioaugmented contaminated controls, where the SCFC application increased the pesticide removal regarding the non-bioaugmented and non-biostimulated contaminated controls (data not shown) (Raimondo et al., 2019).

SCFC is rich in organic matter and nutrients, particularly phosphorus and nitrogen. These nutrients are essentials for synthesis of proteins and nucleic acids, so they would promote microbial activity and growth (Santos et al., 2012). In addition, SCFC diversifies the soil microbiota by providing microorganisms that, together with the native ones, could metabolize xenobiotic substances (García-Torres et al., 2011). In the literature, it has been reported that the use of SCFC favors the physical-chemical soil properties: SCFC reduces the apparent density and increases the cation exchange capacity, the moisture retention, and the porosity, which in turn improves the oxygen diffusion and the mineral nutrient availability (Santos et al., 2012). These advantages make SCFC an interesting alternative when the objective is to improve the efficiency of soil bioremediation processes. Similar results were obtained by other researchers (García-Torres et al., 2011; Fundora-Tellechea et al., 2016; Cuevas-Díaz et al., 2017).

The higher microbial biomass detected in BABS microcosms would indicate the presence of viable cells of *Streptomyces* strains, at 14 days of incubation. This finding might be attributed to the fact that the studied actinobacteria were isolated from environments contaminated with organochlorine pesticides, so they can proliferate in different contaminated soils (Benimeli et al., 2003; Fuentes et al., 2010; 2017; Raimondo et al., 2019). However, these results do not indicate which of the inoculated *Streptomyces* strains would remain viable at the end of the assay. Because of this, the survival of the four actinobacteria members of the consortium was evaluated in BABS microcosms, based on their differential characteristics, previously detected (Raimondo et al., 2019). The analysis of polyacrylamide gels showed that the genetic profiles of the colonies re-isolated from the BABS microcosms (lanes 5, 6, 7, 8) were identical to the profiles of the pure strains used as controls (lanes 1, 2, 3, 4) (Supplementary Fig. 1). These results demonstrated the presence of the four *Streptomyces* strains at the end of the bioremediation assay, which reveals the ability of the actinobacteria to grow and adapt easily in different soils, even in presence of lindane, native microorganisms, and organic amendments such as SCFC. This might be explained because the actinobacteria have a significant degree of physiological and genetic adaptability and an important capability to degrade xenobiotics, which play an essential role in their survival and proliferation in harsh environments (Alvarez et al., 2017).

In addition, it is important to note that the SCFC used to biostimulate the soils would not exert toxic effects on the studied actinobacteria. SCFC comes from the sugarcane juice clarification process. Although it is rich in a large amount of nutrients, SCFC could also contain some heavy metals and toxic compounds potentially harmful to crops and the environment (Ramos-Anacleto et al., 2017).

3.2.2. Soil enzymatic activities

The enumeration of total heterotrophic microorganisms and the evaluation of actinobacteria survival do not provide information about the metabolic state of microbial populations. Therefore, soil enzymatic activities are often used to evaluate the metabolic microbial activity (Margesin et al., 2000). Moreover, these biological parameters allow studying changes that occur in the soil environment in response to the introduction of xenobiotic substances, such as pesticides. The most studied enzymatic activities are oxidoreductases and hydrolases, since they participate in several biochemical reactions that take place in the environment (Baćmaga et al., 2017). In the present work, the evaluated soil enzymatic activities varied between the different soil types and treatments.

Dehydrogenase activity (DH) increased in all treatments during the incubation period (Fig. 3a). At 14 days, this enzymatic activity showed values of 8.70, 0.40, and 0.22 μg TPF g^{-1} h^{-1} for NAT SLS, CS, and SS soils, respectively. The lowest activities were registered in BS controls, with values of 6.86, 0.30, and 0.18 μg TPF g^{-1} h^{-1} for SLS, CS, and SS, corresponding to reductions of 22%, 25%, and 19%, respectively, regarding the values measured in NAT soils. On the contrary, the highest DH values were observed in BABS microcosms: 11.93, 0.58, and 0.29 μg TPF g^{-1} h^{-1}, for SLS, CS, and SS, respectively. The higher DH values in BABS microcosms might be attributed to the adaptation and reproduction of the lindane-degrading microorganisms with a consequent increase in microbial respiratory rate and biological oxidation of organic compounds. DH is typically linked to the presence of viable microorganisms in soils and it is considered an indicator of the oxidative metabolism (Achuba and Peretiemo-Clarke, 2008; Teng et al., 2010).

Catalase activity (CAT), at the end of the incubation process, was 1.38-fold, 1.22-fold, and 1.10-fold higher in BABS SLS, CS, and SS microcosms, respectively, than in their BS controls (Fig. 3b). The highest value (1.07 mmol H_2O_2 consumed g^{-1} h^{-1}) was detected in BABS SLS microcosms. BS SLS and BS CS controls also showed CAT values 29% and 4% significantly higher, respectively, that those obtained in NAT soils ($p < 0.05$), but there was no significant differences between NAT SS and BS SS controls, where CAT levels were 0.12 and 0.13 mmol H_2O_2 consumed g^{-1} h^{-1}, respectively, at 14 days of incubation ($p > 0.05$). CAT is an intracellular enzyme, whose activity constitutes an indicator of aerobic microbial activity and is associated to the number of aerobic microorganisms (Lee et al., 2008). So, the higher CAT values registered in BABS microcosms are probably due to the higher aerobic microbial biomass contents in these systems, as result of the soil bioaugmentation with the actinobacteria consortium.

Fig. 3c and Fig. 3d show that the levels of acid (AP) and alkaline (AKP) phosphatases significantly increased in all treatments during the incubation period. However, a negative effect on both phosphatase activities caused by lindane was observed in BS controls at the end of the assay, where AP was reduced by 9%, 15%, and 27%, while AKP decreased by 12%, 15%, and 12% in SLS, CS, and SS, respectively, regarding the values obtained in NAT soils. Highest AP and AKP values were observed in BABS microcosms, where AP presented values of 179.59, 68.33, and 55.44 μg *p*-Nitrophenol g^{-1} h^{-1}, while AKP showed levels of 176.38, 81.10, and 67.64 μg *p*-Nitrophenol g^{-1} h^{-1} for SLS, CS, and SS, respectively, at 14 days of incubation. Soil phosphatases play a critical role in the mineralization of organic phosphorus during the P cycle, so the higher values of AP and AKP in BABS microcosms would indicate an increase in fertility of bioremediated soils, compared to BS controls (Cuevas-Díaz et al., 2017).

Changes in urease activity (UR) during the bioremediation process are shown in Fig. 3e. At the end of the assay, the detected levels for UR ranged between 3.57 and 35.18 μg $N–NH_4$ g^{-1} h^{-1} for the three treatments and soil types. The highest UR values were observed in BABS microcosms, particularly in SLS, while the lowest values were observed in BS controls, where UR was slightly reduced in CS and SS by 8% and 6%, respectively, regarding the values obtained in NAT soils. There were no significant differences between the UR values registered in NAT SLS and BS SLS controls, at 14 days of incubation ($p > 0.05$). In the literature, it is reported that pesticides appear to have no or reduced effect on urease activity (Riah et al., 2014). This behavior can be explained by the fact that urease enzyme forms stable complexes with the

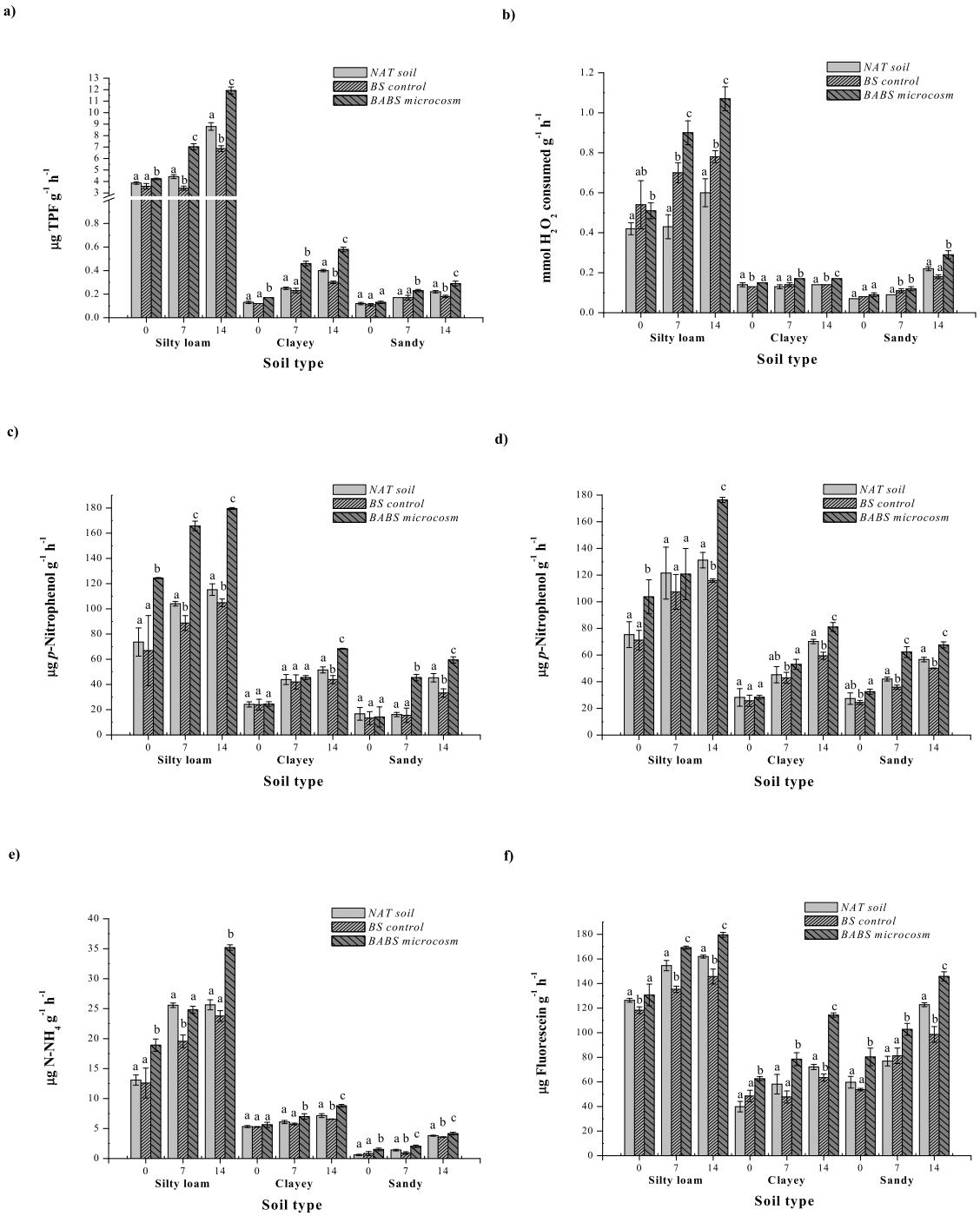


Fig. 3. Evolution of soil enzymatic activities in different treatments and soil types. a) Dehydrogenase activity; b) Catalase activity; c) Acid phosphatase activity; d) Alkaline phosphatase activity; e) Urease activity; f) Fluorescein diacetate hydrolysis activity. 0, 7, and 14: Incubation time (days). Different letters indicate significant differences between treatments for each type of soil ($p < 0.05$, Tukey test). Nat: Natural; BS: Biostimulated; BABS: Bioaugmented and Biostimulated.

humic substances and clay particles present in the soils, so it is generally protected from microbial degradation and toxic effects of xenobiotics (Pascual et al., 2002). The higher UR values detected in BABS microcosms might be result from the actinobacteria response to different nitrogenous substrates present in SCFC, which would have activated the enzyme synthesis (Pascual et al., 2002).

FDA hydrolysis is carried out by plentiful enzymes present in the soil environment, such as non-specific esterases, proteases, and lipases. This biological parameter is a measure of the total microbial activity, and it has been used as an indicator of soil health (Adam and Duncan, 2001). At the end of the bioremediation assay, the lowest FDA hydrolysis values (145.67, 63.50, and 98.60 μg Fluorescein g^{-1} h^{-1}) were detected in BS controls, which corresponded to decreases of 10%, 12%, and 20% in this activity for SLS, CS, and SS, respectively, regarding the values measured in NAT soils (Fig. 3f). In BABS microcosms, the greatest values (179.55, 114.35, and 145.75 μg Fluorescein g^{-1} h^{-1}, for SLS, CS, and SS, respectively) were detected, and they were 23%, 80%, and 48% higher than those observed in BS SLS, CS, and SS controls, respectively, at 14 days of experimentation. FDA was hydrolyzed at rates significantly higher in BABS microcosms than in their controls, indicating a greatest heterotrophic activity in these matrices, which would have resulted from soil bioaugmentation with the

actinobacteria consortium.

The different responses of the soil enzymatic activities to the presence of lindane in BS controls and to the actinobacteria bioaugmentation in BABS microcosms might be due to the different physical, chemical, and microbiological properties of the three evaluated soils (Raimondo et al., 2019). It is important to highlight that CAT was the only enzymatic activity stimulated by the pesticide in the BS controls, regarding the NAT soils. This stimulatory effect might be attributed to a microbial stress produced by soil contamination with lindane (Raimondo et al., 2019).

In addition, the introduction of the actinobacteria consortium into soils would have also stimulated the activity of the enzymes, because the consortium accelerated the lindane removal regarding the controls without bioaugmentation, creating favorable conditions and mitigating the negative effects of the pesticide on soil microbiota (Baćmaga et al., 2017).

Another important observation is that the variation tendency of enzymatic activities and microbial counts was similar. This is logical when thinking that lindane (or its metabolites) and the organic matter present in soils and SCFC, were likely used as substrates to increase the soil microbial biomass, which in turn increased the enzymatic activities (Teng et al., 2010).

By comparing the results of this work with those previously reported (Raimondo et al., 2019), it was observed that both microbial population densities and enzymatic activities in NAT soils, BS controls, and BABS microcosms were higher in soils biostimulated with SCFC than in non-biostimulated soils, in most cases. These results indicate that SCFC was capable of favoring the microbial growth with the consequent increase in the enzyme synthesis, and that the organic residue did not include toxic compounds for the actinobacteria and the soil microbiota. Furthermore, the SCFC addition to soils would have contributed with microbial cells or/and enzymes, which were present in this agroindustrial residue (Pascual et al., 2002). Similarly, other researchers reported that the values of enzymatic activities were higher in amended soils than in non-amended soils, during bioremediation processes (Lee et al., 2008; Cuevas-Díaz et al., 2017).

4. Conclusions

In the present study, the usefulness and effectiveness of factorial designs to identify the conditions of maximum lindane removal in different soil types by bioaugmentation with an actinobacteria consortium and biostimulation with sugarcane filter cake were demonstrated for the first time.

In optimal conditions, the inoculation with the actinobacteria consortium enhanced the bioremediation process, as well as increased heterotrophic microbial counts and enzymatic activities regarding the biostimulated controls. The higher values of these biological parameters indicated favorable conditions in bioaugmented and biostimulated soils and confirmed the efficiency of the bioaugmentation to improve soil microbiological properties. The survival of the *Streptomyces* strains at the end of the assay demonstrated their ability to adapt and grow in different soils, even in presence of lindane, native microorganisms, and sugarcane filter cake.

Sugarcane filter cake is a non-expensive alternative for the biostimulation of soils contaminated with lindane, by significantly improving the pesticide removal in the bioaugmented microcosms, regarding a previous study without biostimulation. Therefore, the simultaneous application of bioaugmentation with an actinobacteria consortium and biostimulation with sugarcane filter cake, in the previously optimized conditions, represents a promising biotechnological strategy for the restoration of lindane-contaminated soils. The next step would be scaling the process to achieve bioremediation of soil mesocosms contaminated with this pesticide.

CRediT authorship contribution statement

Enzo E. Raimondo: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Juan D. Aparicio:** Validation, Formal analysis, Investigation, Visualization. **Ana L. Bigliardo:** Investigation. **María S. Fuentes:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Claudia S. Benimeli:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Acknowledgements

The authors gratefully acknowledge financial support of Consejo de Investigaciones de la Universidad Nacional de Tucumán (PIUNT D626), Agencia Nacional de Promoción Científica y Tecnológica (PICT 2016-0493; PICT 2016-0234), Consejo Nacional de Investigaciones Científicas y Técnicas, and the technical supports of Mr. Leandro Coronel and Mr. Gonzalo Tapia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.110143.

References

Achuba, F.I., Peretiemo-Clarke, B.O., 2008. Effect of spent engine oil on soil catalase and dehydrogenase activities. Int. Agrophys. 22 (1), 1–4.
Adam, G., Duncan, H., 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol. Biochem. 33 (7–8), 943–951.
Ahmad, M., Lee, S.S., Oh, S.E., Mohan, D., Moon, D.H., Lee, Y.H., Ok, Y.S., 2013. Modeling adsorption kinetics of trichloroethylene onto biochars derived from soybean stover and peanut shell wastes. Environ. Sci. Pollut. Res. 20, 8364–8373.
Alessandrello, M.J., Juárez-Tomás, M.S., Raimondo, E.E., Vullo, D.L., Ferrero, M.A., 2017. Petroleum oil removal by immobilized bacterial cells on polyurethane foam under different temperature conditions. Mar. Pollut. Bull. 122 (1–2), 156–160.
Alvarez, A., Saez, J.M., Dávila-Costa, J.S., Colin, V.L., Fuentes, M.S., Cuozzo, S.A., Benimeli, C.S., Amoroso, M.J., 2017. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 166, 41–62.
Antonio-Ordaz, J., Martínez-Toledo, A., Ramos-Morales, F.R., Sánchez-Díaz, L.F., Martínez, A.J., Tenorio-López, J.A., Cuevas-Díaz, M.d.C., 2011. Biorremediación de un suelo contaminado con petróleo mediante el empleo de bagazo de caña con diferentes tamaños de partícula. Multiciencias 11 (2).
Aparicio, J.D., Raimondo, E.E., Gil, R.A., Benimeli, C.S., Polti, M.A., 2018a. Actinobacteria consortium as an efficient biotechnological tool for mixed polluted soil reclamation: experimental factorial design for bioremediation process optimization. J. Hazard Mater. 342, 408–417.
Aparicio, J.D., Saez, J.M., Raimondo, E.E., Benimeli, C.S., Polti, M.A., 2018b. Comparative study of single and mixed cultures of actinobacteria for the bioremediation of co-contaminated matrices. J. Environ. Chem. Eng. 6 (2), 2310–2318.
Baćmaga, M., Wyszkowska, J., Kucharski, J., 2017. Bioaugmentation of soil contaminated with azoxystrobin. Water Air Soil Pollut. 228 (1).
Ballav, S., Dastager, S., Kerkar, S., 2012. Biotechnological significance of Actinobacterial research in India. Recent Res. Sci. Technol. 4 (4).
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., González, A.J., Chaile, A.P., Amoroso, M.J., 2007. Temperature and pH effect on lindane removal by *Streptomyces* sp. M7 in soil extract. J. Basic Microbiol. 47 (6), 468–473.
Chang, B.V., Lu, Y.S., Yuan, S.Y., Tsao, T.M., Wang, M.K., 2009. Biodegradation of phthalate esters in compost-amended soil. Chemosphere 74 (6), 873–877.
Chishti, Z., Hussain, S., Arshad, K.R., Khalid, A., Arshad, M., 2013. Microbial degradation of chlorpyrifos in liquid media and soil. J. Environ. Manag. 114, 372–380.
Cuevas-Díaz, M.d.C., Martínez-Toledo, Á., Guzmán-López, O., Torres-López, C.P., Ortega-Martínez, A.d.C., Hermida-Mendoza, L.J., 2017. Catalase and phosphatase activities during hydrocarbon removal from oil-contaminated soil amended with agro-industrial by-products and macronutrients. Water Air Soil Pollut. 228 (4).
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., Sáez, J.M., Benimeli, C.S., Amoroso, M.J., 2011. Lindane biodegradation

by defined consortia of indigenous *Streptomyces* strains. Water Air Soil Pollut. 222 (1–4), 217–231.
Fuentes, M.S., Alvarez, 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 (4), 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 (2), 127–137.
Fuentes, M.S., Raimondo, E.E., Amoroso, M.J., Benimeli, C.S., 2017. Removal of a mixture of pesticides by a *Streptomyces* consortium: influence of different soil systems. Chemosphere 173, 359–367.
Fundora-Tellechea, F.R., Martins, M.A., da Silva, A.A., da Gama-Rodrigues, E.F., Leal Martins, M.L., 2016. Use of sugarcane filter cake and nitrogen, phosphorus and potassium fertilization in the process of bioremediation of soil contaminated with diesel. Environ. Sci. Pollut. Res. Int. 23 (18), 18027–18033.
García, M.G., Infante, C., López, L., 2012. Biodegradación de un crudo mediano en suelos de diferente textura con y sin agente estructurante. Bioagro 24 (2), 93–102.
García-Torres, R., Ríos-Leal, E., Martínez-Toledo, A., Ramos-Morales, F.R., Cruz-Sánchez, J.S., Cuevas-Díaz, M.d.C., 2011. Uso de cachaza y bagazo de caña de azúcar en la remoción de hidrocarburos en suelo contaminado. Rev. Int. Contam. Ambient. 27 (1), 31–39.
Goss, M.J., Tubeileh, A., Goorahoo, D., 2013. A review of the use of organic amendments and the risk to human health. In: Sparks, D. (Ed.), Advances in Agronomy, pp. 275–379 USA.
Grondona, S.I., González, M., Martínez, D.E., Massone, H.E., Miglioranza, K.S.B., 2019. Assessment of organochlorine pesticides in phreatic aquifer of Pampean Region, Argentina. Bull. Environ. Contam. Toxicol. 1–6.
Haghollahi, A., Fazaelipoor, M.H., Schaffie, M., 2016. The effect of soil type on the bioremediation of petroleum contaminated soils. J. Environ. Manag. 180, 197–201.
Hopwood, D.A., Bibb, M.J., Chater, K.F., Kieser, T., Bruton, C.J., Kieser, H.M., Lydiate, D.J., Smith, C.P., Ward, J.M., Schrempf, H., 1985. Genetic Manipulation of *Streptomyces*. A Laboratory Manual. John Innes Foundation, Londres.
Jastrzębska, E., 2011. The effect of chlorpyrifos and teflubenzuron on the enzymatic activity of soil. Pol. J. Environ. Stud. 20 (4), 903–910.
Lee, S.H., Oh, B.I., Kim, J.G., 2008. Effect of various amendments on heavy mineral oil bioremediation and soil microbial activity. Bioresour. Technol. 99 (7), 2578–2587.
Margesin, R., Zimmerbauer, A., Schinner, F., 2000. Monitoring of bioremediation by soil biological activities. Chemosphere 40 (4), 339–346.
Ministerio de Salud, 2017. Químicos prohibidos y restringidos en Argentina. Dirección Nacional de Determinantes de la Salud e Investigación. Departamento de Salud Ambiental. Buenos Aires, Argentina.
Niti, C., Sunita, S., Kamlesh, K., Rakesh, K., 2013. Bioremediation: an emerging technology for remediation of pesticides. Res. J. Chem. Environ. Sci. 17 (4), 88–105.
Pascual, J.A., Moreno, J.L., Hernández, T., García, C., 2002. Persistence of immobilised and total urease and phosphatase activities in a soil amended with organic wastes. Bioresour. Technol. 82 (1), 73–78.
Raimondo, E.E., Aparicio, J.D., Briceño, G.E., Fuentes, M.S., Benimeli, C.S., 2019. Lindane bioremediation in soils of different textural classes by an Actinobacteria consortium. J. Soil Sci. Plant Nutr. 19 (1), 29–41.
Raimondo, E.E., Saez, J.M., Aparicio, J.D., Fuentes, M.S., Benimeli, C.S., 2020. Coupling of bioaugmentation and biostimulation to improve lindane removal from different soil types. Chemosphere 238, 124512.
Rama-Krishna, K., Philip, L., 2011. Bioremediation of single and mixture of pesticide-contaminated soils by mixed pesticide-enriched cultures. Appl. Biochem. Biotechnol. 164 (8), 1257–1277.
Ramos-Anacleto, L., Mantuanelli-Roberto, M., Marin-Morales, M.A., 2017. Toxicological effects of the waste of the sugarcane industry, used as agricultural fertilizer, on the test system *Allium cepa*. Chemosphere 173, 31–42.
Ren, X., Zeng, G., Tang, L., Wang, J., Wan, J., Wang, J., Deng, Y., Liu, Y., Peng, B., 2018. The potential impact on the biodegradation of organic pollutants from composting technology for soil remediation. Waste Manag. Res. 72, 138–149.
Renella, G., Egamberdiyeva, D., Landi, L., Mench, M., Nannipieri, P., 2006. Microbial activity and hydrolase activities during decomposition of root exudates released by an artificial root surface in Cd-contaminated soils. Soil Biol. Biochem. 38 (4), 702–708.
Riah, W., Laval, K., Laroche-Ajzenberg, E., Mougin, C., Latour, X., Trinsoutrot-Gattin, I., 2014. Effects of pesticides on soil enzymes: a review. Environ. Chem. Lett. 12 (2), 257–273.
Saez, J.M., Aparicio, J.D., Amoroso, M.J., Benimeli, C.S., 2015. Effect of the acclimation of a *Streptomyces* consortium on lindane biodegradation by free and immobilized cells. Process Biochem. 50 (11), 1923–1933.
Saez, J.M., Alvarez, A., Fuentes, M.S., Amoroso, M.J., Benimeli, C.S., 2017. An overview on microbial degradation of lindane. In: Singh, S.N. (Ed.), Microbe-induced Degradation of Pesticides, pp. 191–212 Cham.
Saez, J.M., Bigliardo, A.L., Raimondo, E.E., Briceño, G.E., Polti, M.A., Benimeli, C.S., 2018. Lindane dissipation in a biomixture: effect of soil properties and bioaugmentation. Ecotoxicol. Environ. Saf. 156, 97–105.
Santos, D.H., Tiritan, C.S., Simoneti Foloni, J.S., 2012. Efeito residual da adubação fosfatada e torta de filtro na brotação de soqueiras de cana-de-açúcar. Agrarian 5 (15), 1–6.
Tejada, M., Gómez, I., del Toro, M., 2011. Use of organic amendments as a bioremediation strategy to reduce the bioavailability of chlorpyrifos insecticide in soils. Effects on soil biology. Ecotoxicol. Environ. Saf. 74 (7), 2075–2081.
Teng, Y., Luo, Y., Sun, M., Liu, Z., Li, Z., Christie, P., 2010. Effect of bioaugmentation by *Paracoccus* sp. strain HPD-2 on the soil microbial community and removal of polycyclic aromatic hydrocarbons from an aged contaminated soil. Bioresour. Technol. 101 (10), 3437–3443.
Vijgen, J., Abhilash, P.C., Li, Y.F., Lal, R., Forter, M., Torres, J., Singh, N., Yunus, M., Tian, C., Schaeffer, A., Weber, R., 2011. Hexachlorcyclohexane (HCH) as new Stockholm convention POPs- a global perspective on the management of lindane and its waste isomers. Environ. Sci. Pollut. Res. 18, 152–162.

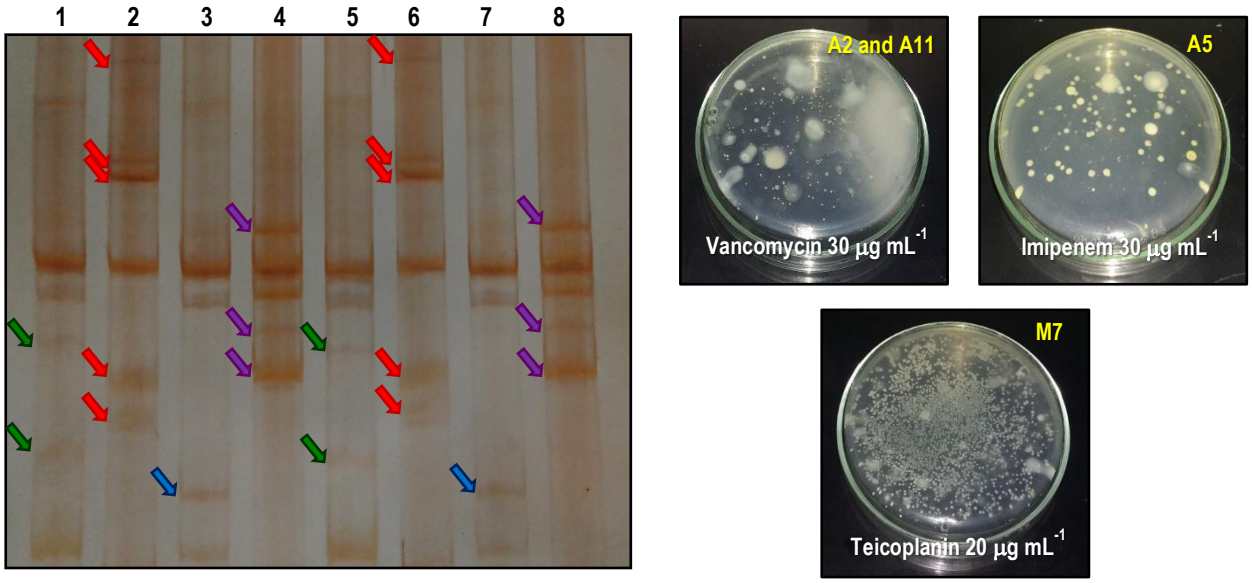
Supplementary Table 1. First-order kinetic parameters for lindane removal. Different letters indicate significant differences between treatments for each type of soil ($p < 0.05$, Tukey test).

Soils	Treatments	First-order kinetic parameters			
		Equation	R^2	k (d^{-1})	$T_{1/2}$ (d)
SLS	BS control	y = - 0.021x + 0.602	0.990	0.021 ± 0.003 [a]	33.0 ± 0.1 [a´]
	BABS microcosm	y = - 0.068x + 0.601	0.999	0.068 ± 0.002 [b]	10.2 ± 0.0 [b´]
CS	BS control	y = - 0.021x + 0.707	0.971	0.021 ± 0.005 [a]	33.0 ± 0.2 [a´]
	BABS microcosm	y = - 0.088x + 0.622	0.974	0.088 ± 0.020 [b]	7.9 ± 0.2 [b´]
SS	BS control	y = - 0.031x + 0.516	0.937	0.031 ± 0.029 [a]	22.4 ± 0.9 [a´]
	BABS microcosm	y = - 0.142x + 0.479	0.985	0.142 ± 0.025 [b]	4.9 ± 0.2 [b´]

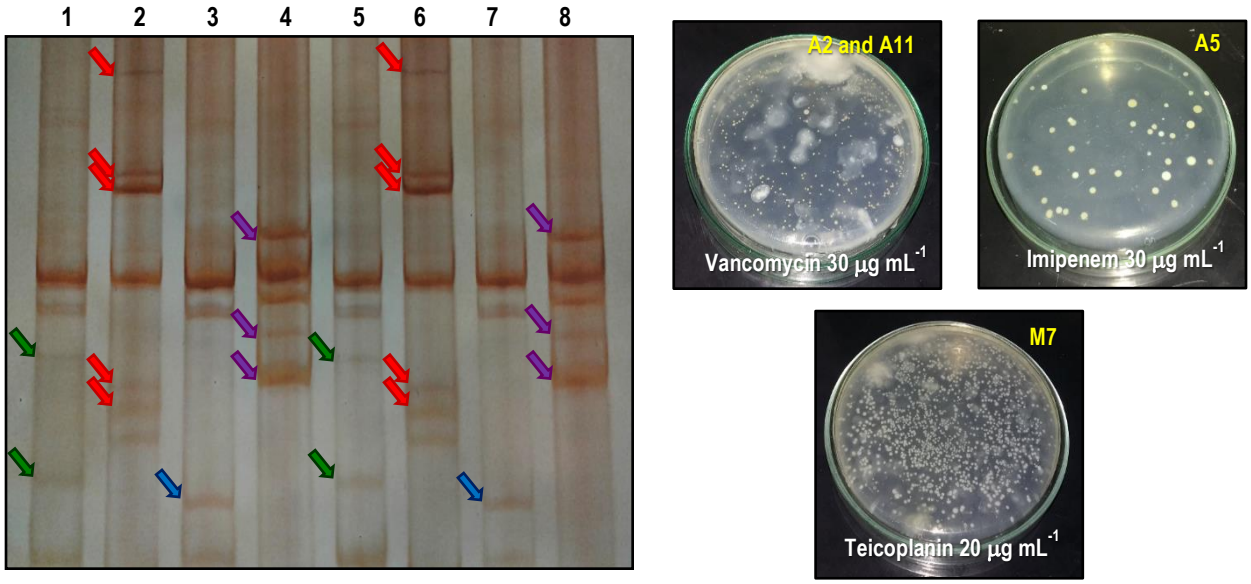
SLS: Silty loam soil; CS: Clayey soil; SS: Sandy soil; BABS: Bioaugmented and Biostimulated; BS: Biostimulated.

Supplementary Figure 1. Microbial development of soil samples on SC plates and polyacrylamide gels electrophoresis of the fragments amplified from: a) Silty loam soil (SLS); b) Clayey soil (CS); c) Sandy soil (SS). Lanes: 1: *Streptomyces* sp. A2, 2: *Streptomyces* sp. A5, 3: *Streptomyces* sp. A11, 4: *Streptomyces* sp. M7, 5: Colony A2 isolated from SC plate added with Vancomycin 30 µg mL^{-1}, 6: Colony A5 isolated from SC plate added with Imipenem 30 µg mL^{-1}, 7: Colony A11 isolated from SC plate added with Vancomycin 30 µg mL^{-1}, and 8: Colony M7 isolated from SC plate added with Teicoplanin 20 µg mL^{-1}.

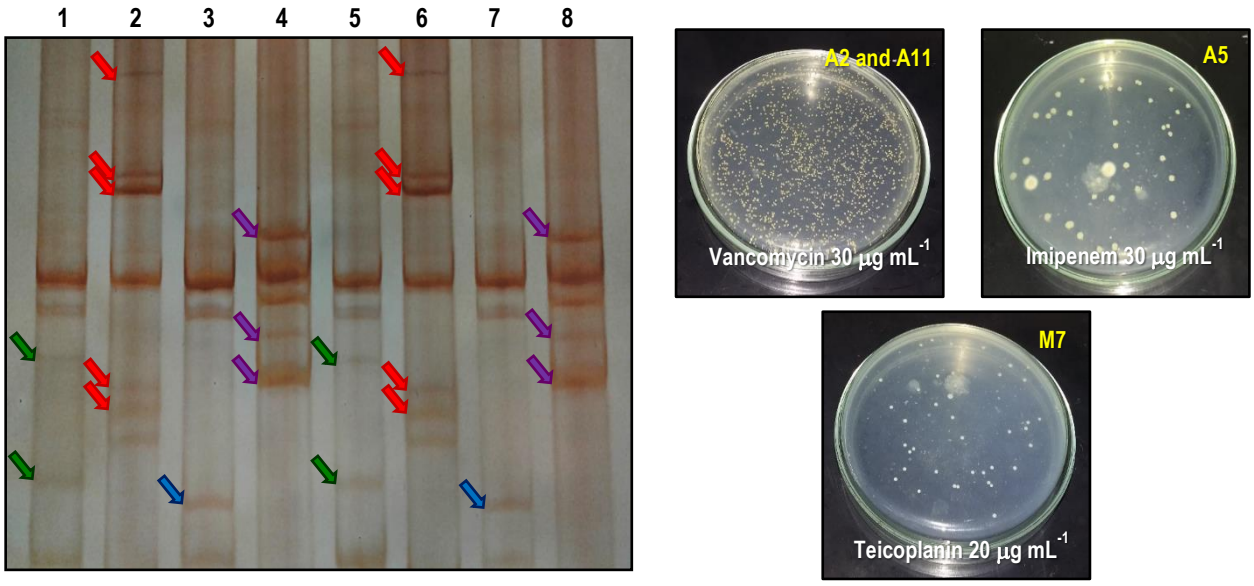
a)



b)


1 2 3 4 5 6 7 8
A2 and A11
Vancomycin 30 µg mL-1
A5
Imipenem 30 µg mL-1
M7
Teicoplanin 20 µg mL-1

c)


1 2 3 4 5 6 7 8
A2 and A11
Vancomycin 30 µg mL-1
A5
Imipenem 30 µg mL-1
M7
Teicoplanin 20 µg mL-1