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Enhanced lindane removal from soil slurry by immobilized *Streptomyces* consortium

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ABSTRACT

The aim of this work was to assess lindane removal from soil slurry by a *Streptomyces* consortium immobilized in cloth sachets, at different inoculum, lindane and slurry concentrations. In concentrated slurry (soil/water ratio of 2:3), the higher lindane removal (35.3 mg Kg^{-1}) was obtained with the medium inoculum (10^7 CFU g^{-1}) and the highest lindane concentration tested, at 7 days of incubation. Although, lindane removal was also detected in abiotic controls, probably caused by pesticide adsorption to soil particles. Thus, these parameters were selected for evaluating the pesticide removal in diluted slurry (soil/water ratio of 1:4). After 14 days of incubation, 28.7 mg Kg^{-1} of lindane were removed. Also, a phytotoxicity assay demonstrated that seeds growing on diluted slurries bioremediated during 7 and 14 days, showed an improvement in biological parameters, compared to those growing on non-bioremediated slurries. Thus, bioremediated slurries would not have toxic effects on lettuce seeds.

1. Introduction

Hexachlorocyclohexane (HCH) is a synthetic chemical compound consisting of eight isomers. The γ- isomer (γ-HCH or lindane) is the most toxic and the only one which exhibits strong insecticidal properties (Alvarez et al., 2012; Tor et al., 2013). Lindane has been widely used, mostly in developing countries, in agriculture to protect crops, and also in human health and veterinary medicine to control insect-borne diseases (Fuentes et al., 2010; Camacho-Pérez et al., 2012). Nowadays, its use has been limited or even banned, since it is considered one of the priority organic pollutants under the Stockholm Convention on persistent organic pollutants (Singh et al., 2011; Abdul Salam and Das, 2012). Besides, the International Agency for Research on Cancer has classified γ-HCH as possible carcinogenic to animals (ATSDR, 2011). Hence, it represents a serious risk since HCH-residues are still found in the environment (Carvalho et al., 2009; Fuentes et al., 2010; Gonzalez et al., 2010; Saadati et al., 2012).

In a bioremediation framework, microbial degradation is considered the primary mechanism responsible for pesticide mitigation in soil, and in this context, the development of relevant bioremediation tools is increasingly encouraged (Bazot and Lebeau, 2009). One of the greatest difficulties encountered in the microbial treatment of toxic compounds is the substrate inhibition of microbial growth and concomitant hindrance in the biodegradation (Halecky et al., 2014). To overcome these problems, immobilization of degrading microorganisms has been proposed as an effective strategy. Several advantages of using immobilized cells, rather than free suspended ones, have been reported, e.g. prevention of cell leaching, providing of high biomass concentration and easier solid–liquid separation (Poopal and Laxman, 2008; Ahamad and Kunhi, 2011). In addition, immobilized cultures tend to have a higher level of activity and higher tolerance to environmental perturbations, such as pH, temperature or exposure to toxic compounds, than suspended biomass cultures (León-Santiesteban et al., 2011). In fact, Saez et al. (2012) observed that actinobacteria immobilized in cloth sachets presented higher lindane removal efficiency than free cells. Besides all this, immobilized cells are easily reusable.

On the other hand, slurry bioreactors are one of the most important types of *ex situ* techniques for the bioremediation of polluted soils. In fact, the obtained results generally reflect the actual biological depuration potential of the soil, as first step before

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working on soil itself (Robles-González et al., 2008). Studies concerning the application of slurry bioreactors for the remediation of pesticides-polluted soils have been reported (Plangklang and Reungsang, 2010; Robles-González et al., 2012; Fuentes et al., 2013). Moreover, complete mineralization of pesticides or their transformation to non-toxic products is desirable, being consortia of microorganisms better than single isolates at this task, probably because of the metabolism diversity present in the first case (Yang et al., 2010). In this context, the present work focuses on evaluating lindane removal from soil slurry system by a defined consortium of *Streptomyces* immobilized in cloth sachets, at various inoculum sizes and different pesticide concentrations.

2. Materials and methods

2.1. Chemicals

Lindane (99% pure) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Solvents were of pesticide grade, and all other chemicals used throughout this study were of analytical grade and were purchased from standard manufacturers.

2.2. Streptomyces consortium and inoculum preparation

A defined consortium of actinobacteria consisting of four *Streptomyces* strains (A2, A5, A11, and M7) was used in this study. These strains were previously isolated from soils and sediments contaminated with organochlorine pesticides (Benimeli et al., 2003; Fuentes et al., 2010), and then selected based on its higher lindane removal compared with the single cultures of the four strains (Fuentes et al., 2011).

Tryptic Soy Broth (TSB) was used for the inoculum preparation. It consists of (g L^{-1}): tryptone, 15; soy peptone, 3; NaCl, 5; K_2HPO_4, 2.5; glucose, 2.5. The pH was adjusted to 7.0 and the medium was sterilized by autoclaving at 121 °C for 15 min.

Each microorganism was individually cultured in TSB for 72 h at 30 °C in a rotatory shaker (200 rpm). Then, the cells were harvested by centrifugation (9000 ×*g*, 10 min) and washed three times with sterile distilled water. Finally, the inoculum was prepared by combining equal amounts of each strain.

2.3. Cell immobilization

The *Streptomyces* consortium was immobilized in cloth bags, prepared with voile, in a size of 2.5 × 3.5 cm each one, following the methodology described by Saez et al. (2012). The bags, opened in one side, were individually sterilized in autoclave. Then, they were aseptically loaded with the appropriate size of inoculum, containing the same amount of each of the four strains. Finally, they were closed with sterile thread, by using sterile tweezers.

2.4. Soil slurries preparation

For the preparation of the slurries, a model loam soil, 5–15 cm-depth, was collected from an urban area in San Miguel de Tucumán, Argentina (26°48′35″ S 65°14′26″ W), free of pesticides contamination. Organic matter and nitrogen content of the soil were 2.6% and 0.14%, respectively, and the soil pH was 7.0. It contained 47.7% sand, 40% silt, and 12.3% clay.

Prior to use, soil was air-dried, lightly ground using mortar and pestle, and finally sieved through a 1-mm sieve. Subsequently, the soil samples were sterilized (three successive sterilizations at 121 °C for 15 min each, 24 h in between) (adapted from Fuentes et al., 2013).

The experiments were conducted in 250 mL-Erlenmeyer flasks, containing 40 g of sterile soil and 60 mL of sterile distilled water (soil/water ratio of 2:3, p/v) or 20 g of sterile soil and 80 mL of distilled water (soil/water ratio of 1:4, p/v; diluted slurry), as appropriate. The resulting mixture was autoclaved again for 15 min at 121 °C and 1 atm overpressure. The loss of liquid due to the sterilization process was calculated by difference in weight between the flasks before and after sterilization, and was then adjusted by adding sterile distilled water.

2.5. Assessment of the influence of inoculum size and lindane concentration in lindane removal

The consortium previously immobilized was inoculated into the slurry prepared as described above (see 2.4) at soil/water ratio of 2:3. The influence of the inoculum and lindane concentrations in the removal thereof was evaluated. For this purpose, three concentrations of inoculum (10^6, 10^7 and 10^8 CFU g^{-1}) and three concentrations of lindane (2, 10 and 50 mg Kg^{-1}) were tested, obtaining therefore nine treatments (1: 10^6 UFC g^{-1}, lindane 2 mg Kg^{-1}; 2: 10^6 UFC g^{-1}, lindane 10 mg Kg^{-1}; 3: 10^6 UFC g^{-1}, lindane 50 mg Kg^{-1}; 4: 10^7 UFC g^{-1}, lindane 2 mg Kg^{-1}; 5: 10^7 UFC g^{-1}, lindane 10 mg Kg^{-1}; 6: 10^7 UFC g^{-1}, lindane 50 mg Kg^{-1}; 7: 10^8 UFC g^{-1}, lindane 2 mg L^{-1}; 8: 10^8 UFC g^{-1}, lindane 10 mg Kg^{-1}; 9: 10^8 UFC g^{-1}, lindane 50 mg L^{-1}). The slurries were incubated in a rotary shaker at 30 °C and 200 rpm for 7 days. Samples were taken at 0, 3 and 7 days for residual lindane determinations.

2.6. Assessment of lindane removal by immobilized mixed culture of Streptomyces in diluted slurry

Lindane removal was evaluated in a diluted slurry system, prepared at water/soil ratio 1:4, by using the inoculum size and lindane concentration previously selected. It was incubated for 21 days at 30 °C and 200 rpm. Samples were taken at 0, 7, 14 and 21 days of incubation for determining the residual lindane concentration and for testing the phytotoxicity of bioremediated slurries. Afterwards, the reusability of the immobilized consortium was also tested in two additional cycles of 21 days each.

2.7. Lindane determination by gas chromatography

To recover lindane from slurries, an extraction with solvents was carried out. For this purpose, 5 mL of n-hexane, 1 mL of methanol and 4 mL of water for chromatography were added to 5 mL of slurry. The resulting mixture was stirred for 10 min and centrifuged at 9000 ×*g* for 10 min. Then, it was incubated at −4 °C for 5 min to enhance the separation of the organic phase, and subsequently 2 mL thereof were taken. The extract was evaporated to dryness and finally suspended in an appropriate volume of n-hexane (Adapted from Quintero et al., 2005).

Lindane concentration in extracts was quantified in a Gas Chromatograph Agilent 7890A equipped with a HP5 capillary column (30 m × 0.53 mm × 0.35 m) and ^{63}Ni micro-electron capture detector, a split/splitless Agilent 7693B injector and Agilent ChemStation software. The chromatographic conditions were as follows: carrier gas (nitrogen) flow rate: 25 cm s^{-1}, initial oven temperature: 90 °C increasing to 180 °C at 30 °C min^{-1}, and increasing to 290 °C at 20 °C min^{-1}, detector temperature: 320 °C and injection volume: 1 μl. Quantitative sample analysis was performed using appropriate calibration standards (AccuStandard).

2.8. Phytotoxicity test

To assess the possible presence of toxic intermediates in the bioremediated slurries, a toxicity test using seeds of *Lactuca sativa* (lettuce) was performed. Seeds were grown in Petri dishes

containing sterile filter paper (Wattman No. 1), moistened with 2 mL of diluted slurry bioremediated at 0, 7, 14 and 21 days. Thirty lettuce seeds were placed into each plate and incubated in darkness for 5 days under controlled environmental conditions (22 °C and 70% of relative humidity). At the end of the incubation period, the number of germinated plants was determined, and the length of the root and hypocotyl of each one was measured by using a millimetre scale. The vigour index of the seedlings (VI) was also calculated, by using the following formula: $VI = (LR + LT) \times G/10$, where LR is the average root length, LT is the average hypocotyl length and G is the percentage of germinated seeds (Ajithkumar et al., 1998).

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 slurries, and also to test significant differences in the number of germinated lettuce seeds. Nested-ANOVA was carried out to test the significant differences in the length of roots and hypocotyls. 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. Selection of the optimum inoculum size and lindane concentration for lindane removal

Slurry bioreactors can be used for bioremediation of problematic sites like soils highly contaminated with recalcitrant and toxic pollutants, which display hysteretic behaviour, such as lindane (Robles-González et al., 2012). Such characteristics make *in situ* bioremediation difficult to be applied (Robles-González et al., 2012). In view of this, and since biodegradation of a compound is often a result of the actions of multiple organisms (Robles-González et al., 2008), a defined consortium consisting of *Streptomyces* A2, A5, A11 and M7 immobilized in cloth bags, was inoculated into soil slurry (water/soil ratio 2:3) in order to evaluate the influence of the inoculum size and the lindane concentration on its removal. Thus, three inoculum concentrations (10^6, 10^7 and 10^8 CFU g^{-1}) and three initial lindane concentrations (2, 10 and 50 mg Kg^{-1}) were assayed. Results are shown in Fig. 1 A and B.

Comparing the lindane removed (mg Kg^{-1}) by using the different inoculum sizes, in all lindane concentrations tested, the lowest values were obtained with the maximum concentration of inoculum tested (treatments 7, 8 and 9) ($P < 0.05$). Conversely, the highest values of lindane removed were obtained at 7 days of incubation when the inoculum of 10^7 CFU g^{-1} was used ($P < 0.05$), removing more than twice than the higher inoculum tested. The maximum removal was achieved by treatment 6 at the end of the assay, with 35.3 mg Kg^{-1} of lindane removed (Fig. 1 B). Similar results were reported by Poopal and Laxman (2008), who found that chromate reduction was enhanced with the increase in the inoculum size up to a certain concentration (2 g wet cells); however, highest cell densities had no additional benefits. On the other hand, Jézéquel and Lebeau (2008), after testing different inoculum sizes of *Bacillus* sp. ZAN-044 and *Streptomyces* sp. R25 free and immobilized for cadmium removal, hypothesized that the metabolic state of the microorganisms has the most important effect on the pollutant removal, rather than the inoculum. Furthermore, cell-to-matrix ratio is also an important factor since it could affect substrate diffusivity and degradation ability of immobilized cells

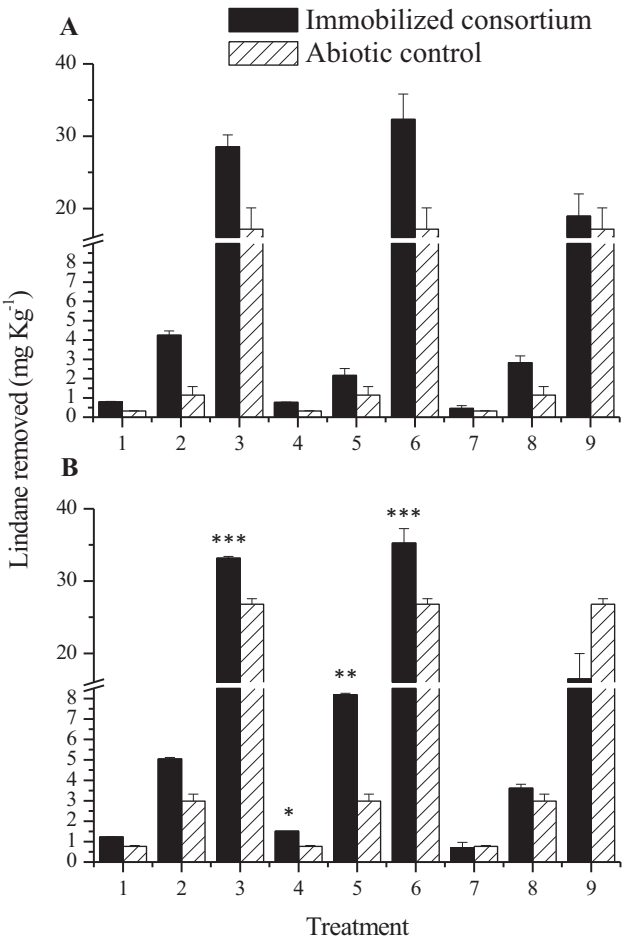

Fig. 1. Lindane removed (mg Kg^{-1}) by *Streptomyces* immobilized consortium in concentrated soil slurry, at (A) three days of incubation and (B) seven days of incubation. Bars showing asterisk indicate they were significantly different ($P < 0.05$, Tukey post-test). Treatments: 1: 10^6 UFC g^{-1}, lindane 2 mg Kg^{-1}; 2: 10^6 UFC g^{-1}, lindane 10 mg Kg^{-1}; 3: 10^6 UFC g^{-1}, lindane 50 mg Kg^{-1}; 4: 10^7 UFC g^{-1}, lindane 2 mg Kg^{-1}; 5: 10^7 UFC g^{-1}, lindane 10 mg Kg^{-1}; 6: 10^7 UFC g^{-1}, lindane 50 mg Kg^{-1}; 7: 10^8 UFC g^{-1}, lindane 2 mg L^{-1}; 8: 10^8 UFC g^{-1}, lindane 10 mg Kg^{-1}; 9: 10^8 UFC g^{-1}, lindane 50 mg L^{-1}.

(Siripattanakul et al., 2008). For all these reasons, 10^7 CFU g^{-1} was considered as the optimum inoculum for lindane removal in the conditions studied and thus selected for the subsequent studies.

Furthermore, regarding the initial lindane concentrations added to the slurries, the removal profile was similar in the three inocula, i.e. inhibition was not observed in the pesticide removal when the concentration of lindane increased up to 50 mg Kg^{-1}. In fact, the maximum removal values were obtained in the slurries spiked with the lindane higher concentration ($P < 0.05$). Other authors reported different results. For instance, Cuozzo et al. (2012) observed no considerable differences in the removal of chlordane, another organochlorine pesticide, by *Streptomyces* A5 at different initial concentrations of the xenobiotic. In contrast, Park et al. (2013) reported that phenol degradation showed a concentration-dependence, increasing the removal efficiency when phenol concentration decreased.

It is important to note that lindane removal was also detected in abiotic controls (slurries without microorganisms) reaching higher removal values (17.0 and 27.0 mg Kg^{-1} at 3 and 7 days of assay, respectively) in treatments with the higher pesticide concentration assayed (50 mg Kg^{-1}) (Fig. 1 A and B). This could be due to the physical phenomenon of adsorption of the xenobiotic to soil

particles. In case of lindane adsorption, Van der Waals forces and hydrogen bonding were considered as the most probable adsorption mechanisms (Rama Krishna and Philip, 2008). Fuentes et al. (2013) also reported that a fraction of methoxychlor disappeared from the uninoculated controls of slurry and soil, probably because of the aggregation and/or adsorption of the pesticide to soil particles. Besides, it should be considered that although sorption capacity of organic compounds to soil and sediments is usually related with the octanol–water partition coefficient (K_{ow}) and the Log D value of the compound at a given pH, numerous physical-chemical characteristics of the soil can also affect the sorption affinity (i.e. clay and organic matter content, among others) (Rama Krishna and Philip, 2008; Monsalvo et al., 2014). As the organic carbon fraction of soil tends to decrease the bioavailability of organic compounds (Becerra-Castro et al., 2013), and, due to the presence of organic matter in the soil assayed (2.6%), it is not surprising that lindane removal observed in abiotic controls corresponds to lindane sorbed to organic matter of soil. In this connection, Vlcková and Hofman (2012) reported that a decrease in the organic matter content of soils consistently led to an increase in lindane bioaccumulation in *Eisenia fetida* in spiked soils with different ageing times and even in non-aged soils.

Nevertheless, it is worth to highlight that in this case, lindane sorption may not necessarily be accountable for the removal of the pesticide. Sorption alone would only result in a temporary build-up of lindane to soil particles and/or the immobilization matrix. However the removal achieved during extended operation greatly depends on the biodegradability of the xenobiotic (Monsalvo et al., 2014). Desorption process of lindane is also possible and is important to consider too, since it determines the release rate and potential mobility of the pesticide in soil (Rama Krishna and Philip, 2008).

3.2. Lindane removal in diluted slurry by an immobilized *Streptomyces* consortium

Taking into account the results presented above, and considering that the adsorption phenomenon of lindane to soil would depend mainly on the content of organic matter thereof, and hence in slurries, it could depend on its concentration, a more diluted slurry was assayed. Lindane removal was evaluated by using the inoculum previously selected as optimum (10^7 CFU g^{-1}) and the higher lindane concentration previously tested (50 mg Kg^{-1}).

The slurry consisted of 80% of water and 20% of soil (water/soil ratio 4:1), and the incubation time was extended to 21 days, taking samples every 7 days. This was done considering that most of the treatments presented above on non-diluted slurries achieved removal efficiencies significantly higher ($P < 0.05$) at 7 days of incubation respect to 3 days. Besides, it should be noted that higher concentrations of pesticide could require longer incubation periods to achieve best removal values (Cuozzo et al., 2012; Park et al., 2013).

The results showed that after 7 days of incubation, 19.5 mg Kg^{-1} of lindane was removed by the immobilized consortium, which represents almost 40% of lindane initially added. This value significantly increased at the 14th day ($P < 0.05$), up to 28.7 mg Kg^{-1} of lindane removed. However, no additional improvement was obtained at 21 days of incubation (Fig. 2). Fuentes et al. (2011) studied the bioremediation of soil microcosms contaminated with 1.66 mg Kg^{-1} of lindane during 28 days by the same actinobacteria consortium. They demonstrated that the consortium was able to remove 28.2% of the initial lindane at 7 days of incubation, but this percentage remained almost constant until the end of the experiment, with a maximum of 31.5% of removal at 21 days. In this connection, it is not surprising that

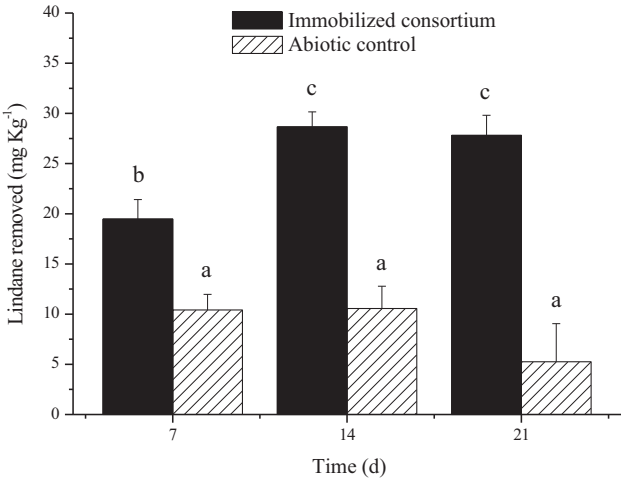

Fig. 2. Lindane removed (mg Kg^{-1}) by actinobacteria immobilized consortium in diluted soil slurry. Bars showing different letters indicate they were significantly different ($P < 0.05$, Tukey post-test).

slurries are more efficient for the remediation of soils contaminated with this kind of xenobiotic, since being an aqueous extract of soil, it increases the pesticide-microorganism contact. On the other hand, Plangklang and Reungsang (2010) investigated the effectiveness of bioremediation technology in the removal of carbofuran (20 mg Kg^{-1}) by using a bioslurry phase sequencing batch reactor augmented with *Burkholderia cepacia* PCL3 immobilized on corncob. Their results revealed a 97% of carbofuran removed in 80 h. In the present study, a higher concentration of xenobiotic was assayed obtaining favorable results, although longer incubation time was required.

It is important to point out that lindane removal registered in the abiotic controls of diluted slurries (Fig. 2) was a half the pesticide removal obtained in the abiotic controls of non-diluted ones (Fig. 1). In view of this, slurry consisting of a soil/water ratio of 1:4 seems to be the more efficient system for lindane dissipation. These results support the important influence of soil properties on bioremediation processes, and the fact that a bioremediation strategy development has to be site-specific (Vangronsveld et al., 2009).

Moreover, emphasizing that an advantage of cell immobilization consists on the possibility of reusing the cells (Poopal and Laxman, 2008), lindane removal was determined in two additional cycles of 21 days each, in order to evaluate the reusability of the immobilized consortium. Lindane relative removal was calculated considering the removal obtained by the cells immobilized in the cloth bag in the first use, as 100%. Lindane relative removal improved in the first and second reuse cycle, achieving relative removal values of 117 and 128%, respectively, while the abiotic control remained stable through time ($P > 0.05$) (Fig. 3). We have already demonstrated the possibility to use the cloth sachets containing the immobilized consortium for lindane removal in liquid medium, for three successive cycles of 96 h, obtaining 73% of lindane relative removal in both reuse cycles, respect to the first use (Saez et al., 2012). Dey and Roy (2009) reported that immobilized cells could be reused three or five times, depending on the immobilization matrix. Other study revealed that *Streptomyces* sp. immobilized in agar were efficiently used for acrylonitrile bioconversion during twenty cycles, without much loss of activity, although 35% of activity was lost after 25 cycles (Nigam et al., 2009). In this regard, further and deeper studies should be performed around the reuse of immobilized cells in cloth bags.

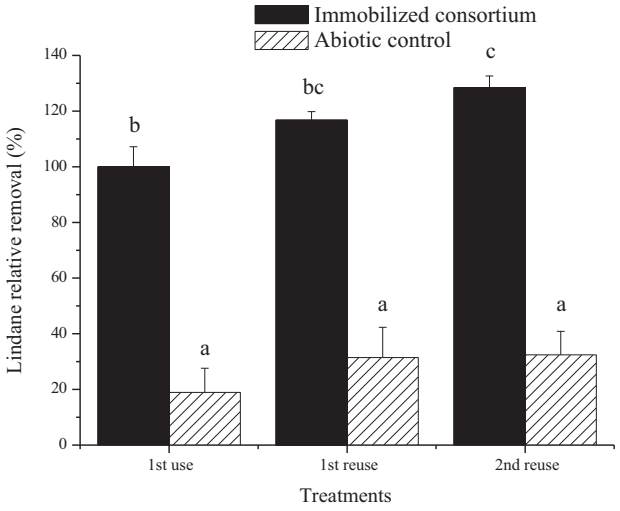

Fig. 3. Lindane relative removal (%) of the *Streptomyces* immobilized consortium in cycles of 21 days each. Different letters indicate significant differences ($P < 0.05$, Tukey post-test).

3.3. Assessment of the efficacy of the bioremediation

Phytotoxicity tests are versatile tools for monitoring the success of a remediation process (Chiochetta et al. 2013). For this purpose, lettuce seeds were cultured in aliquots of bioremediated diluted slurries, taken at different times. This bioremediation system was selected to be tested because it presented the best results from a biotechnological point of view. Tested seeds were found to be adversely affected by the addition of the contaminated slurry, since lower values in roots and hypocotyls lengths ($P > 0.05$) as well as in the vigour index (VI) were observed at the beginning of the experiment (Fig. 4A and B; Table 1), respect to non-contaminated slurries (VI = 32.03, data not shown). This is not surprising considering that phytotoxic effects of pesticides in general, and of lindane in particular, have already been described in several studies. In terms of germination, such effects are manifested as an imbalance in biochemical processes in the seed (Bidlan et al., 2004; Calvelo Pereira et al., 2010; Fuentes et al., 2013). However, this tendency was reversed with increasing the bioremediation time (7 and 14 days), since tested biological parameters on lettuce seedlings increased in general (Fig. 4A and B; Table 1). This was specially true for the VI wich was higher in the bioremediated slurry than abiotic control (Table 1). These results are in accordance with the high values of lindane removed at 7 and 14 days (Fig. 2), suggesting that the pesticide was effectively removed by the consortium. On the other hand, differences between root and hypocothyl lengths were found, since lettuce roots showed less development than hypocothlys, suggesting that the capacity for exploring the substrate and obtaining resouces for correct growth was reduced (Calvelo Pereira et al., 2010). However, roots developed on bioremediated slurry were longer than those developed on the abiotic control ($P < 0.05$), supporting the effectiveness of the microbial remediation.

VI was lower for lettuce seeds cultivated on slurry bioremediated during 21 days, than the previously obtained (Table 1). These results may be interpreted as a confirmation that the parent compound was not completely biodegraded or it could be transformed into toxic intermediates. In effect, pollutant removal sometimes may be accompanied by the transformation of the mother contaminant into more toxic metabolites that could persist in soil and produce toxicity in certain species (Robles-González et al., 2008; Calvelo Pereira et al., 2010; Fuentes et al., 2013).

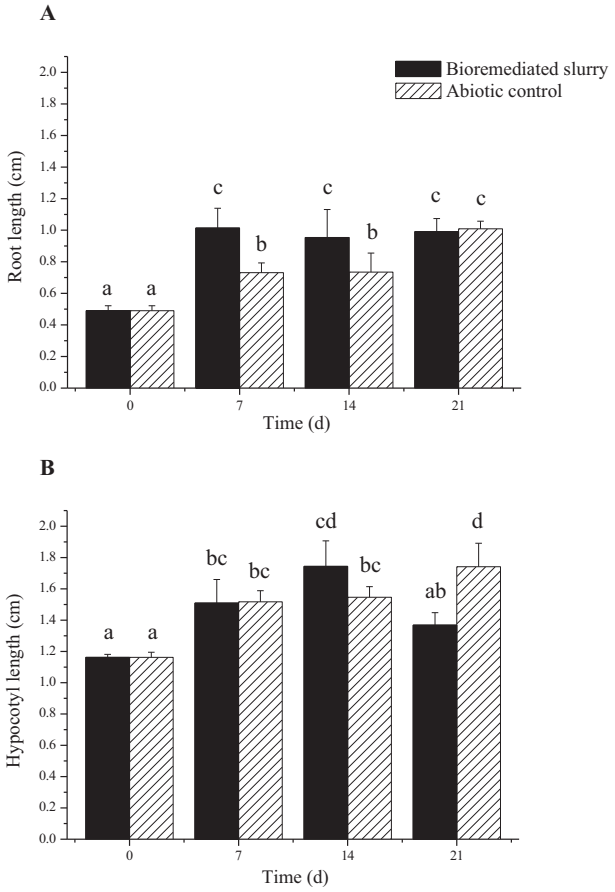

Fig. 4. Development of lettuce seedlings cultivated on bioremediated diluted soil slurry. (A) Root length and (B) Hypocotyl length. Bars showing different letters indicate they were significantly different ($P < 0.05$, Tukey post-test).

On the other hand, the VI of seedlings growing in the abiotic control was higher than the VI obtained in the slurry bioremediated during 21 days. In this connection, Calvelo Pereira et al. (2010) found in *Solanum nigrum*, a relative increase in the percentage of germination at low levels of contamination with lindane, i.e. the presence of the contaminant stimulates plant responses. Such overcompensation, known as hormesis, is a relatively common phenomenon in studies of environmental contamination which involves an additional activity of the plant in an attempt to recover from the stress to which it has been subjected (Calabrese, 2005). Another possible explanation is that the enhancement of biological parameters of lettuce cultivated on abiotic control could be due to a reduction of the contact between lindane and plants because of the ageing time of lindane in the soil. This "ageing effect" could reduce the bioavailability of the pesticide for the plants considering that hydrophobic organic compounds are well known to stablish

Table 1
Vigour Index (VI) of lettuce seedlings cultivated on bioremediated diluted slurry at different incubation times and its abiotic control. VI = (mean root length + mean shoot length) × percentage of germination/10.

Treatment	VI			
	Day 0	Day 7	Day 14	Day 21
Bioremediated slurry	14.31 ± 0.44	21.51 ± 2.13	24.55 ± 3.00	20.64 ± 1.00
Abiotic control	14.31 ± 0.44	19.21 ± 0.72	20.04 ± 1.47	23.49 ± 1.78

stronger bonds with the organic carbon fraction of soils as time goes on (Alexander, 2000; Reid et al., 2000). The same reason could explain higher roots and hypocotyls lengths in the abiotic control at 21 days of assay. However, results from toxicity monitoring are highly dependent on the test organisms used and it is therefore important to use several assays together (Moradas et al., 2008).

Taking all these results into account, the effectiveness of the bioremediation process could be improved by removing the toxic effect of lindane intermediates, after their identification by gas chromatography-mass spectrometry. The proposed strategy, in this case, should involve the use of multi-component system (Ledin, 2000). For instance, an important improvement of the bioremediation of contaminated areas has been found by using soil additives, like vegetable surfactants. Specifical surfactants applications were observed in the decrease of toxic intermediates accumulation during lindane degradation (Quintero et al., 2005; Baczynski et al., 2010). In this connection, some plants are excellent sources of surfactants by root exudates production (Alvarez et al., 2013). Also, successful applications of maize plants for the remediation of xenobiotics (phytoremediation) in combination with actinobacteria had been previously reported (Gao et al., 2010; Alvarez et al., 2013).

4. Conclusions

Lindane was more efficiently removed from a concentrated slurry by using an inoculum of 10^7 CFU g^{-1} of the *Streptomyces* consortium immobilized in a cloth sachet and 50 mg Kg^{-1} of lindane, obtaining 35.3 mg $Kg^{-1}$of lindane removed at 7 days of incubation. Although, high lindane removal was also registered in abiotic controls, probably because of the aggregation of the pesticide to soil particles. In a diluted slurry, lindane removed achieved by the immobilized consortium was slightly lower (28.7 mg Kg^{-1}) than in concentrated slurry, but adsorption significantly decreased respect to the concentrated slurry. Ecotoxicology assay demonstrated that seeds grown on diluted slurries bioremediated during 7 and 14 days showed an improvement in biological parameters, respect to those grown in non-bioremediated slurry. Thus, the results obtained in this work seem to indicate that this bioremediation strategy would represent a promising alternative from a biotechnological point of view, to bioremediate lindane contaminated sites.

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