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Evaluation of a recovery technique for mangrove soils affected by oil spills, using as indicator plantules of *Rhizophora mangle* L. (Rhizophoraceae)

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Abstract. Experiments were performed *in situ* using (2^3) a factorial design to evaluate a technique that recovers mangrove soils affected by oil spills in coastal areas where a 100% of the mangrove forest did not survive and natural re-colonization had not been successful. The treatments were based on the natural and stimulated biodegradation of oil, including the effect of water saturation in it, where seedlings of "red mangrove" (*Rhizophora mangle* L.) were used as indicators of success. Results showed that stimulated biodegradation in a saturated environment can remove up to 78.5% of the spilt oil in the first two months, recovering soil quality of the degraded soil and obtaining a greater percentage of plantules survival (80%). It was concluded that stimulated biodegradation and the promotion of saturated environments are the most successful treatments.

Key words: hydrocarbons, soil, experiment, mangrove, Caribbean, Colombia

Resumen. Desarrollo y evaluación de una técnica de recuperación de suelos de manglar afectados por hidrocarburos utilizando *Rhizophora mangle* L. (Rhizophoraceae). Se realizó un experimento *in situ* de tipo factorial (2^3) en busca de una técnica para recuperar suelos de manglar afectados por hidrocarburos basándose en la biodegradación estimulada del hidrocarburo, y en la saturación con agua, utilizando como indicador al "mangle rojo" (*Rhizophora mangle*). Los resultados muestran una remoción del aceite de hasta 80% en los primeros dos meses con la biodegradación estimulada, una recuperación de la calidad del suelo y una supervivencia mayor al 80% en las parcelas con alta concentración de hidrocarburos. Se concluye que la biodegradación estimulada + agua, es la técnica que ofrece en el futuro los mejores resultados de recuperación natural.

Palabras claves: hidrocarburos, aceite, bioensayos, manglares, Caribbean, Colombia

Introduction

The mangrove ecosystem is a coastal wetland that provides a range of environmental services, which includes: the protection against tsunamis and storms, erosion, buffering of pollutants, and serves as an efficient carbon trap (Othman 1994, Chmura *et* *al.*2003, Moberg & Ronnback 2003, Valiela *et al.* 2004).It is recognized as a highly productive ecosystem, and are reported to play a major role in the export of carbon and nutrients to the coastal zone (Adame & Lovelock 2011, Kristensen *et al.* 2008).Therefore, it is not surprising that in turn it serves as feeding area, protection and reproduction to a wide range of organisms, many of which are of special commercial importance for humans (Lee & Shang-Shu 2004). While the importance of mangroves is worldwide recognized, the mangrove ecosystem is being degraded (Yap 2000), making its conservation a global problem that concerns associated ecosystems and humans.

The effects of oil spills on mangrove ecosystems have been studied for decades. Baker *et al.* (1980) make a compilation of spill accidents in mangrove areas, displaying the date, place, volume of the oil spill and the impacts on the mangrove forest. Lewis (1981) also examines the observations of different investigators about oil spills occurred from 1962 to 1978 finding that the cause of most damage is the toxic effect of oil in the sediments. Oliveira *et al.* (1983) studied the effects of oil spills in mangrove leaves and found that immediately after the spill occurs, a defoliation takes place, a decrease in the leaf area (spots, necrosis and / or holes in the leaves), as well as alterations in the pigmentation, texture and shape of leaves. Later, Martin *et al.* (1990) make a research in a chronically oil polluted area and its natural recolonization, concluding that to restore the soil it is enough to stop the pollution source. They demonstrated that fresh oil is the real cause of the small seedlings mortality, coinciding with the findings of Scherrer & Blasco (1989) for plantules of *R. mangle* in oil contaminated areas.

As for the physical and bacterial removal of oil, Oudot & Dutrieux (1989) performed their work on the Mahakam coast (Indonesia), finding that physical processes dominate the removal of oil (tidal activity in the area) and then the bacterial activity. Jackson *et al.* (1989), corroborate the above conclusion after a spill occurred in Panama, where mangrove areas with lower tidal energy impacts showed more damage and sublethal effects than areas with higher tidal energy. Yun *et al.* (2008), analyzed microorganisms isolated from mangrove soil and their potential to biodegradation polycyclic aromatic hydrocarbons, obtaining a removal of 32% after 63 days of incubation. Also, Tam & Wong (2008), studied the affectivity of degradation of polyaromatic hydrocarbons by bacterial inoculation on mangrove soils showing that native microorganism are capable to degraded naturally these hydrocarbons.

Usually when an oil spill occurred either on land or sea, the companies responsible for undertaking contingency actions have a principal priority: stop the oil spill, especially when it heads towards the sea, water supplies or to the coast where it can penetrate the mangrove areas. This kind of action often affects the natural flow and mangrove hydrodynamics causing more degradation. Given this, and the importance of recovering mangrove areas, the main objective of this study was to describe the effects of water on the recovery of mangrove soils using natural and stimulate technics of biodegradation. A second objective was to describe the trends of hydrocarbon degradation under the same conditions, as of natural and stimulated biodegradation.

Material and Methods

Study area

The Cartagena Bay is located on the Caribbean Sea, in the northern part of the Bolivar State; (Fig. 1), with an area of approximately 82km^2 (Pagliardini *et al.* 1982). It is considered as a bay, but it has a significant source of freshwater coming from the Canal del Dique, constituting an estuarine ecosystem (Álvarez-León 1986, Polanía *et al.* 1991). The average annual temperature is of 27.9°C and the annual rainfall is of 920mm (Pagliardini *et al.*1982).

The study site is located the coastal zone of Cartagena Bay, and currently do not show mangrove forests (Fig. 1), but historically registers a mangrove zone until 1968. The study area presents a degraded zone of high degrees of erosion and soil compression, with a physical limit where the natural re-colonization of the mangrove has not been able to advance. Despite the absence of official records, local people said that about twenty years ago (Figure 2), this area was affected by an oil spill that penetrated the mangrove soil. Actually this area is not covered by tides, but historically it was. There isn´t water supplies.

The soil profile of 50 cm depth confirmed that this area was impacted by oil. The profile presented a sand layer of 0-10 cm depth on top, followed by an oil layer of 30 cm thickness, and in the deeper layer, we observed mangrove debris and fibrist, a typical organic soil of the *R. mangle* forest (Fig. 2).

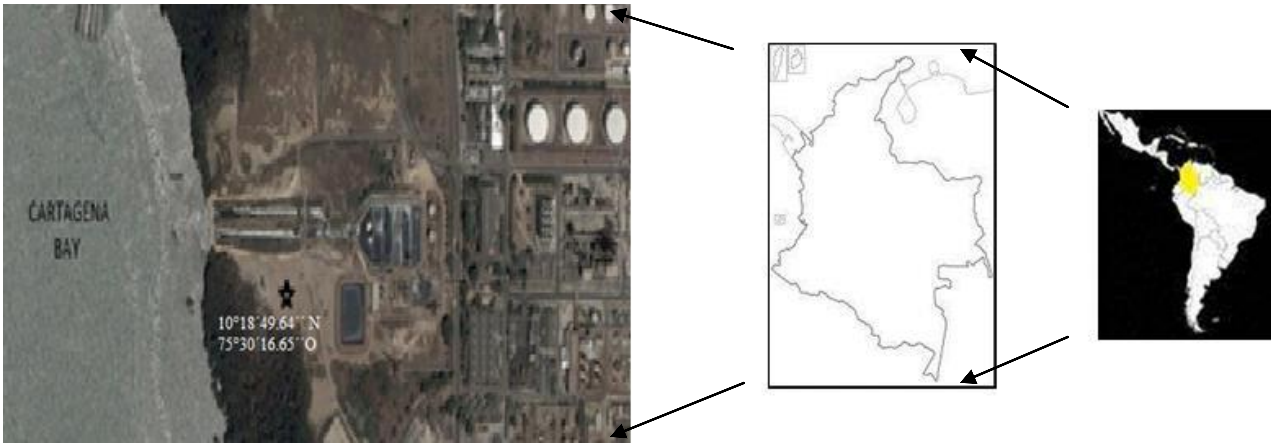

Figure 1. Study area (black star): Satellite image Geoeye 2005.Google Earth. True Color.

Experiments

The experiments were performed *in situ* using a factorial (2^3) design to evaluate a technique to recover mangrove soils affected by oil spills, where 100% of the forest cover did not survive and natural re-colonization has not been successful (Figure 1). The treatments were based on biodegradation of soil hydrocarbon, including the effect of water saturation using the seedlings of "red mangrove" (*R. mangle* L) as indicators of soil recovery. The experiment was based on the recovery of mangrove by the removal of oil content and the promotion of a saturated soil with water. The treatments were: (1) Hydrocarbons con centration of 4.5% and 8.5%; (2) Saturation of soils – with and without water; and (3) Stimulated biodegradation – with and without a bacteria that promotes the biodegradation of oil (Fig. 3).

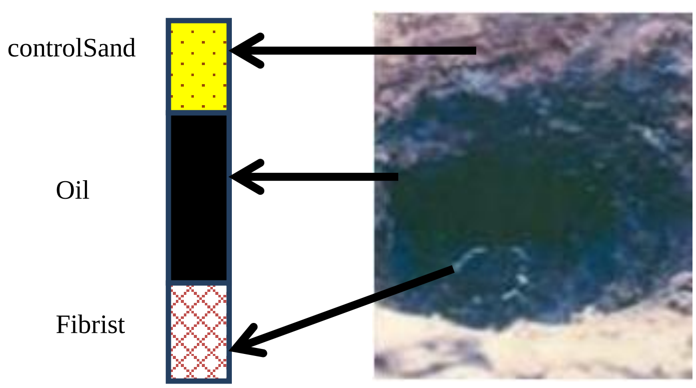

Figure 2. Soil Profile of the study area. The photo is a 50 cm profile taken in 1992

The experimental area has approximately 500 m^2 and consists of eight plots of 50 m^2 (10m x 5m). Additionally, we used two control plots without any oil spill, both of them with the same environmental and soil conditions (Fig. 3). The treatment plots were separated by a 2 m wide corridor with drainage canals to prevent mixing of treatments.

The saturated treatment was performed applying water to the plots until a surface film of about 1 cm thick was reached, avoiding washing out the ground. Because the soil's area had higher concentration of hydrocarbons (8.5% dried weight of soil), we used a mixture of sand to obtain plots with 4.5%and 8.5%W. The application of the stimulated biodegradation treatment was done with a strain of bacteria *Pseudomonas putrida,* that was enriched with nitrogenates compounds and for eight days we applied 200ml/pond using a sprinkler on the surface of each experimental plot. This strain of *P. putrida* was isolated at the Colombia Petroleum Institute (ICP), but Polanía *et al.*, (1991) reported *P. putrida* naturally in mangrove soils too.

For this experiment, we used 200 plantules of *R. mangle* (40 ± 4 cm height) like sampling units. They were extracted in different sites from the natural environment (Barbacoas Bay), following the methodology of Cintrón-Molero (1984). Before sowing, we began an acclimatization process to final conditions: full sun light, higher temperatures and lower humidity presented at the experimental area. After this process, groups of 20 plantules were randomly chosen and sown within each plot following a random pattern, which was imitated in the rest of the plots. In addition, for nine months we went monthly to the field to collect data: we registered how many plantules were alive, measured their increase in high per month, and counted monthly the number of total leaves in each plantules in order to determine their foliar variation.

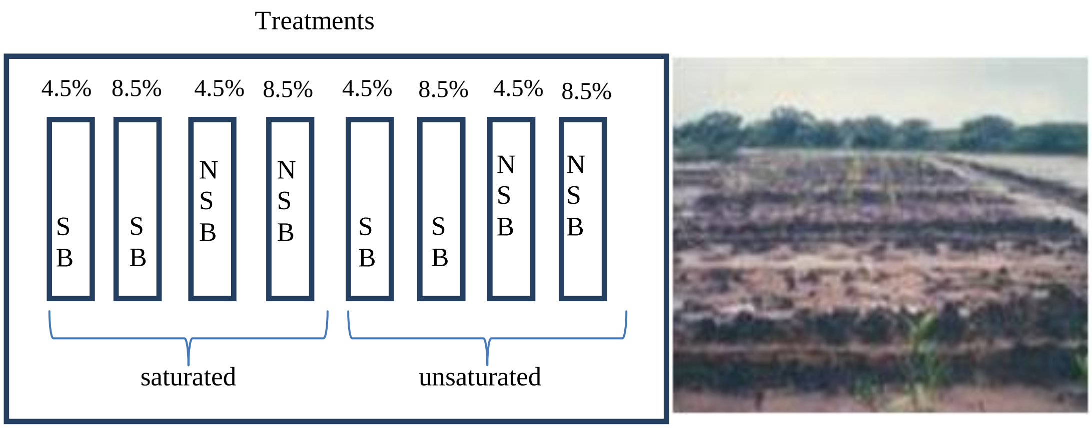

Figure 3. Applied Treatments. SB:Stimulated Biodegradation; NSB:Non Stimulated Biodegradation. Percentage values indicate the concentration of oil in the soil.

Also, at the beginning and the end of the experiment we analyzed at the study area: climatological information (precipitation, temperature, humidity, radiation, wind speed) and soil variables. For soil analyses we used a composite soil sampling of 250 g/plot, where five sub-samples were collected from randomly points. Surface soil subsamples were collected at a depth of 0–10 cm using a stainless steel sampler and immediately be placed in a clean plastic bucket and mixed thoroughly and then refrigerated at 4°C. We analyzed total hydrocarbons (TH)by infrared spectrophotometry (IR), Organic Carbon (OC) was determined by high temperature combustion; samples were pre-treated with sulphurous acid to remove carbonates prior to C analysis (Ibañez,1992). Cation exchange capacity (CEC) were measured in 1:5 soil:1 M ammonium acetate (CH3COONH4) extracts buffered to a pH of 7. Soluble and exchangeable cations were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICPAES) and used Exchangeable Sodium Percentage formula, ESP= Na_{exch}/($Ca_{exch}{}^{2+}$, $Mg_{exch}{}^{2+}$ + $K_{exch}{}^{+}$ + Na_{exch})where Na_{exch}, $Ca_{exch}{}^{2+}$, $Mg_{exch}{}^{2+}$ and $K_{exch}{}^{+}$ are the amounts of exchangeable Na^{+}, Ca^{2+}, Mg^{2+} and K^{+} in cmol/kg soil. For this calculation, concentrations of Na, Ca, and Mg are measured in a saturated paste extract(Vanessa *et al*., 2009), pH were measured in a 1/5 solid/liquid aqueous extract (Marin *et al*. 2005)and salinity (S o/oo) was measured by saturated paste (Sanchez *et al*. 2010) .

All trends of response about the treatments: Saturation of soils (yes/no), Stimulated biodegradation (yes/no) and Hydrocarbons concentration (4,5and 8,5%) where analyzed in terms of biological variables like survival (%), growth (cm/month) and foliar variation (number of leaves/plant). Every month for nine months we registered these information and then we described it in graphics using the software Statistica V.7.

Results

The maximum average temperature per month was of 28.5°C on July, and the minimum average temperature was of 26.3°C on March, with a total average temperature of 27.5°Cfor the nine months (Table II). For sun radiation, we found that the months of highest luminosity were January and February. The relative humidity of 80% had varied +/- 1% throughout the sampling period. The maximum precipitation occurred during November and December. January, February and March were the driest months. The wind had an average speed of 11.4m/s and slowed down at the end of the experiment, coinciding with higher temperatures (Table I). The values obtained demonstrated that the experimental area is located in a semiarid environment.

Table I. Environmental variables of the experimental area during the study period.

	Months								
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**
Temperature (°C)	28.4	27.7	27.3	26.4	26.3	26.9	27.2	28.1	28.5
Radiation (h/month)	180	188	254	269	249	244	245	215	212
Relative humidity (%)	81	81	81	80	79	79	79	81	81
Wind speed (m/s)	13.1	10.0	9.8	12.2	10.8	10.9	11.3	13.1	3.6
Average precipitation(mm)	168.0	161.0	27.3	0.0	0.0	19.6	4.8	83.6	44.2

At the beginning of this experiment, all plots showed Cation Exchangeable Capacity (CEC) between 10.7 to 34 mequiv/100g; values of Exchangeable Sodium Percentage (ESP) from 20,1 to 32,2%, pH between 7.9 to 8.2 and salinity from 29,0 to 31,6o/oo. According to De la Salas (1992), these soils could be catalogued like saline-sodic soils (EPS>15), extremely saline (salinity> 25 ‰), moderately alkaline (pH between7.9and 8.4), with cation exchange capacity (CEC) high (CEC>20meq/100g) (De las Salas 1990). If we compared with arid soils in mangrove areas these soils presented a high percentage of organic matter (M.O> 4%) (Holguin *et al*, 2006).

Table II. Physical-chemical variables of the experimental area during the initial and final study period time. Treatments: HC (High Hydrocarbon Concentration - 8.5% W); LC (Low Hydrocarbon Concentration - 4.5% W); SB (Stimulated Biodegradation); NSB (Non Stimulated Biodegradation); S (Saturated); US (Unsaturated); C (Control).

Treatments	Initial C.E.C meq/100g	Final C.E.C meq/100g	Initial pH	Final pH	Initial E.S.P%	Final E.S.P%	Initial Salinity o/oo	Final Salinity o/oo	Initial O.M %	Final 0.M %
LC SB US	10,7 (2,0)	28,4 (3,2)	8,2 (0,0)	8,1 (0,1)	20,1 (3,4)	31,6 (1,0)	31,9 (1,4)	40,0 (7,1)	4,5 (0,6)	5,7 (0,1)
LC SB S	14,3 (1,1)	30,9 (0,1)	8,1 (0,1)	7,8 (0,1)	25,3 (5,4)	3,5 (4,4)	34,0 (2,2)	16,0 (7,9)	4,6 (0,0)	6,5 (2,0)
LC NSB US	18,2 (4,4)	27,0 (8,0)	8,1 (0,0)	7,9 (0,0)	20,1 (4,2)	30,5 (1,7)	29,0 (0,0)	30,0 (1,4)	5,7 (2,9)	6,3 (2,1)
LC NSB S	14,3 (3,0)	34,0 (3,0)	8,1 (0,0)	8,0 (0,1)	26,5 (5,8)	10,8 (14,1)	29,0 (1,0)	10,0 (8,5)	6,4 (1,2)	7,4 (0,7)
HC SB US	17,5 (1,0)	24,9 (2,0)	8,2 (0,0)	8,0 (0,1)	24,3 (7,2)	30,1 (5,4)	29,0 (0,7)	30,0 (4,8)	4,2 (1,1)	5,7 (1,8)
HC SB S	17,1 (4,0)	28,7 (2,3)	8,1 (0,0)	7,9 (0,1)	19,9 (8,0)	8,7 (3,6)	30,0 (2,8)	9,0 (4,9)	4,0 (0,8)	7,4 (0,2)
HC NSB US	14,5 (3,7)	27,0 (3,8)	8,1 (0,0)	8,0 (0,1)	25,4 (4,2)	32,9 (1,7)	29,0 (0.0)	31,0 (0,1)	5,6 (1,3)	6,9 (0,8)
HC NSB S	16,2 (2,0)	30,0 (3,1)	8,2 (0,1)	8,1 (0,1)	23,5 (5,0)	6,6 (6,3)	31,0 (1,4)	7,0 (2,1)	5,3 (1,2)	7,4 (1,0)
C	17,9 (2,0)	20,0 (1,0)	8,0 (0,1)	8,0 (0,1)	32,2 (0,2)	37,7 (3,9)	27,0 (1,0)	32,0 (1,0)	4,4 (0,7)	4,4 (0,3)

Note: data in the parenthesis are standard deviation

At the end, all plots had an increase in CEC. The plots with saturation treatments (S) showed the highest values in C.E.C. Particularly. The plots with saturation showed the major decrease in Exchangeable Sodium Percentages with values between 3,5 to 10,8%. The rest of the experimental plots including control plots presented an increase in ESP values (between 30,1 to 30,7%). All experimental plots presented increase in the percentage of organic matter, (5,7 to 7,4%), the saturation treatments had the bigger values of organic matter and control plots the lower ones.

During the nine months, the survival behavior for *R. mangle* showed different results according with the presence (S) or absence (US) of saturation treatment. Plots with (+S) denoted the higher survival irrespective of what kind of hydrocarbon concentration was used and type of biodegradation treatments were applied. Control plots showed a higher survival percentage than those experimental plots that did not have saturation treatment (Fig. 3).

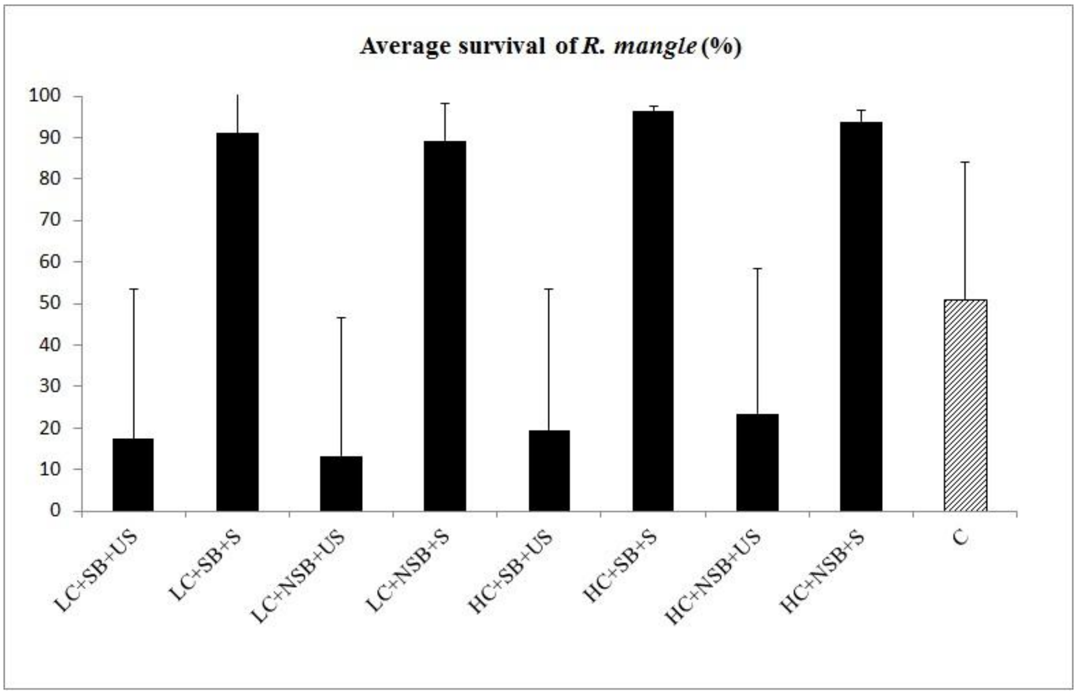

Figure 4. Average survival of *R. mangle* (%) in each combination of treatments during experimental period. Treatments: HC (High Hydrocarbon Concentration -8.5% W); LC (Low Hydrocarbon Concentration - 4.5% W); SB (Stimulated Biodegradation); NSB (Non Stimulated Biodegradation); S (Saturated); US (Unsaturated); C (Control).

Experimental plots with water and stimulated biodegradation (SB+S) showed the highest leaves/plantules irrespective of their hydrocarbon concentration. Those plots overcame the control plots (C) and plots with water treatment (S) too, although there was not significance difference between them. Regardless of the hydrocarbon concentration (HC/LC) plots without saturation (US) showed smaller increases than Control plots.

Regardless to the hydrocarbon concentration, all treatments that had saturation presented the highest increments in height, in contrast with the rest of the experimental plots. Control plots were more similar to those plots that did not have saturation treatment (Fig. 6).

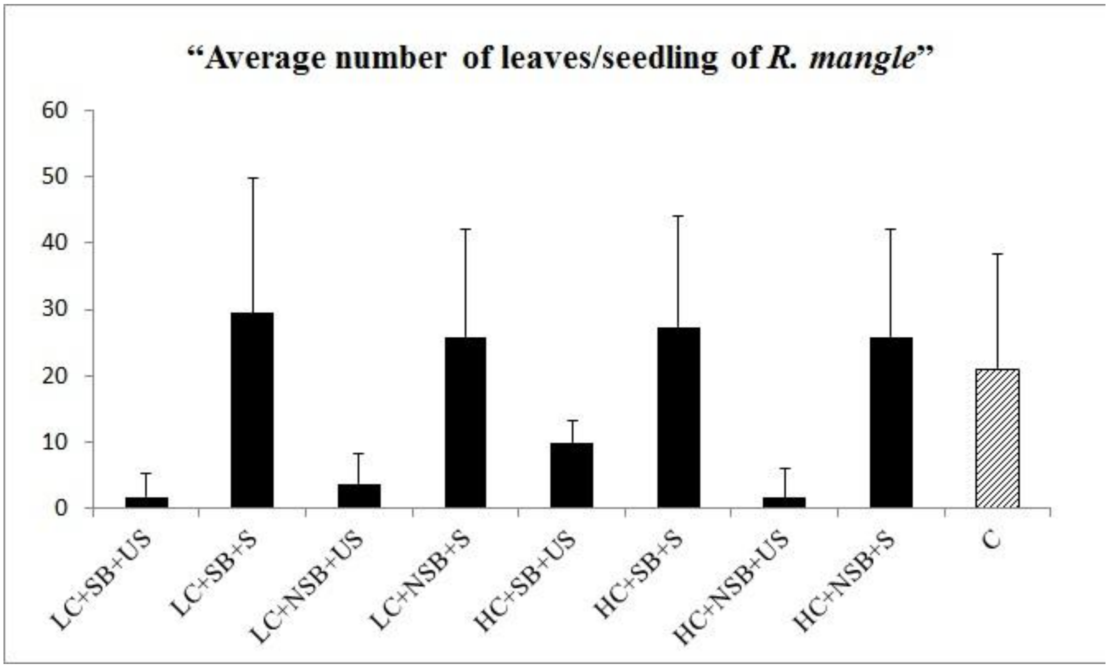

Figure 5. Average number of *R. mangle* leaves/seedling during experimental period. Treatments: HC (High Hydrocarbon Concentration - 8.5% W); LC (Low Hydrocarbon Concentration - 4.5% W); SB (Stimulated Biodegradation); NSB (Non Stimulated Biodegradation); S (Saturated); US (Unsaturated); C (Control).

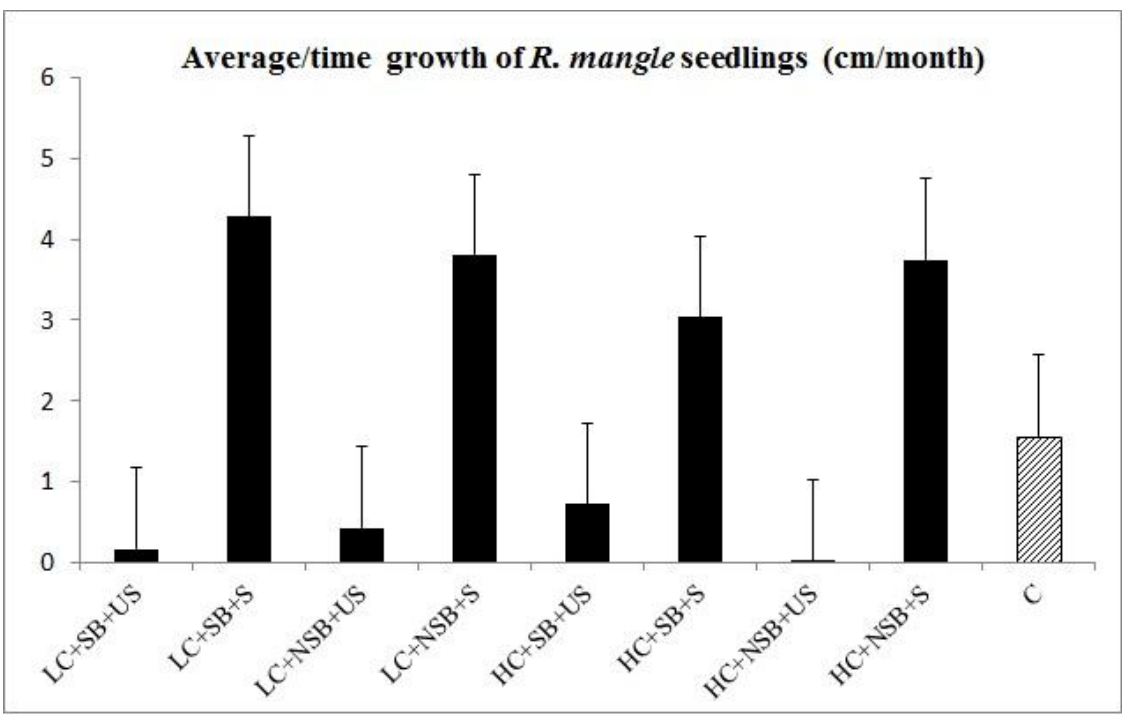

Figure 6. Average increasing seedlings growth (cm/month)during the experiment. Treatments: HC (High Hydrocarbon Concentration 8.5% W); LC (Low Hydrocarbon Concentration4.5% W); SB (Stimulated Biodegradation); NSB (Non Stimulated Biodegradation); S (Saturated); US (Unsaturated); C (Control).

Table III. Hydrocarbons degradation with Non Stimulated Biodegradation (NSB)/Stimulated Biodegradation (SB); Saturated (S)/Unsaturated (US); Low Concentration (LC)/High Concentration (HC).

Stimulated biodegradation				Natural biodegradation			
Plots	[C] initial (%)	[C] final (%)	Removal (%)	Plots	[C] initial (%)	[C] final (%)	Removal (%)
LC SB S	4.5	1.47	67.33	LC NSB S	4.5	2.3	48.88
HC SB S	**8.5**	**0.80**	**90.58**	HC NSB US	8.5	3.17	62.71
HC SB US	8.5	2.86	66.36	**HC NBS S**	**8.5**	**2.4**	**71.76**
LC SB US	4.5	1.89	58.0	LC NBS US	4.5	3.39	24.67
Average Removal (HC) %			**78.46%**	Average Removal (HC) %			**67.23%**
Average Removal (LC) %			**62.66%**	Average Removal (LC) %			**36.89%**

Note: [C]: concentration of hydrocarbon

The results of the removal of hydrocarbon biodegradation using stimulated treatment was greater than the results obtained by natural biodegradation, however this difference was more marked in those plots with low initial concentration (4.5%) than plots with high initial concentration (8.5%). When analyzing stimulated biodegradation and saturation treatment, we noted that plots with no water had removal lower than those that have saturation treatments. The natural degradation of hydrocarbons has a similar behavior of stimulated biodegradation, but lower removal efficiency, despite the differences between high and low hydrocarbon concentration, saturation treatment presented the best results, even when comparing plots with natural biodegradation (Table III).

According to the removal obtained in both treatments, the results showed the importance of stimulated biodegradation on hydrocarbons in contrast with natural degradation, especially in soils with low concentration of oil (4.5%W). Also, these results showed that the removal was less efficient in the absence of water saturation.

Discussion

Our experiment was located in semiarid coastal zone, which had a low water supply that could be the cause of a soil salinization process, according to the climatic variables and the initial state of the mangrove soil.The major issues arising from high sodium levels relative to other exchangeable cations are on the physical properties of soil. In surface soil horizons this imbalance in the ratio of cations resulted in poor soil structure. This is normally evidenced by surface soil crusts or the soil settings of large blocks drying out. As a result mineralization of organic matter, seedling germination, survival and plant growth are problematic (Vanessa et al, 2009).In addition, if these arid soils were affected by hydrocarbons it is easy to think that the native microorganisms or fungus could have their growth process strongly interrupted.

Experimental plots had adverse initial soil conditions represented in high sodium and salts contents (De las Salas, 1990), and were affected by hydrocarbon. When we compared control plots (C) with the experimental plots without remediation treatments (LC NSB US or HC NSB US), we found that control plots (C), presented similar results concerning to E.S.P; C.E.C; So/oo and pH, but when we analyzed biological results we found that the survival, foliar variation and the rate of growth were greater in control plots (C) than in experimental plots (LC NSB US or HC NSB US). This was corroborated by the adverse effects of hydrocarbons that are known to increase seedling mortality, leaf defoliation (Brito *et al.*, 2009; Melville, 2009) and inhibit plant growth (Afzal *et al.*, 2011).

However when we compare control plots (C) and plots with no remediation treatment (LC NSB US or HC NSB US)with plot with stimulate biodegradation (SB)without saturated (US) treatment (LC SB US or HC SB US),we found that the percentages of hydrocarbons decreased a little, but the survival, the foliar variation and the growth of *R. mangle* seedling was similar to plot with natural biodegradation (LC NSB US or HC NSB US) and this biological variables continued lower than control plots (C). The natural biodegradation of hydrocarbon can be explained by the effectiveness of dormant native microorganisms population that are adapted to sodic and saline soil conditions, that multiply rapidly when substrate is available (Vanessa et al., 2009). In this case, microbial activity was not able enough to be recover the quality of the soil to allow a favorable health status for seedlings.

When we analyzed the plot with saturation treatment (S), all of these results changed. The E.S.P and salinity considerably decreased, organic matter and C.E.C. increased improving oil quality. The survival, the foliar variation and the rate of growth of *R. mangle* seedlings increased and the percentage of degradation of hydrocarbons increased independently of the type of biodegradation and the initial hydrocarbon concentration.

Our results suggest that in semiarid coastal zones, saturation treatments led to favorable conditionsfor hydrocarbons degradation. The biodegrading products can be used by the seedlings to increase their survival, foliar development and their own growth. For example, Qureshi (1990) reported for *Rhizophora* sp. in natural conditions rate growth from 2.20 to 4.82 cm/month, in our study regardless hydrocarbon concentration, we reported for plots that had Stimulated Biodegradation and Saturation treatments (LC SB S, HC SB S), a growth ratefrom 3.04 to 4.28cm/month for *R. mangle* seedlings; in contrast of 1.57 cm/month obtained in control plots.

Conclusions

Water is the determining factor for the development of plants under the experimental conditions. However, the treatment of stimulated biodegradation + water is the most successful in recovering the mangrove soils affected by oil spills, since this minimizes the oil presence and recovers the soil quality.

Acknowledgements

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