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Effectiveness of the *Zea mays-Streptomyces* association for the phytoremediation of petroleum hydrocarbons impacted soils

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ABSTRACT

Restoring polluted sites by petroleum hydrocarbons is a challenge because of their complexity and persistence in the environment. The main objective of the present study was to investigate the performance of plant-actinobacteria system for the remediation of crude petroleum and pure-polycyclic aromatic hydrocarbons (PAHs) contaminated soils. The endophytic strain *Streptomyces* sp. Hlh1 was tested for its ability to degrade model PAHs (phenanthrene, pyrene and anthracene) in liquid minimal medium. *Streptomyces* sp. Hlh1 demonstrated the ability to grow on PAHs as sole carbon and energy source, reaching hydrocarbons removal of 63%, 93% and 83% for phenanthrene, pyrene and anthracene, respectively. Maize plant was chosen to study the impact of *Streptomyces* sp. Hlh1 inoculation on the dissipation of contaminants and plant growth. Thus, maize seedlings grown in soils contaminated with crude petroleum and pure-PAHs were inoculated with *Streptomyces* sp. Hlh1. Results showed that the endophyte inoculation increased contaminants removal. Maximum hydrocarbons removal (70%) was achieved in inoculated and planted soil contaminated with crude oil, while 61%, 59%, and 46% of hydrocarbons dissipation were registered for phenanthrene, pyrene and anthracene, respectively. These degradations rates were significantly higher compared to non-inoculated systems in all the treatments evaluated. Further, it was revealed that hydrocarbons (C_8–C_{30}) were efficiently degraded in plant-*Streptomyces* Hlh1 system. Moreover, the inoculation with the actinobacteria resulted significant plant development and enhanced photosynthetic pigments compared to plants grown in the other experimental conditions. The present study provide evidence that the inoculation of maize plants with *Streptomyces* sp. Hlh1 play a remarkable role in the removal of petroleum hydrocarbons, enhancing plant development in contaminated soils.

1. Introduction

Over the last decades there was an important increase of petrochemical industries due to the exponential growth of the worldwide population and the extensive use of petroleum-based products (Koshlaf and Ball, 2017). As a result, petroleum wastes have been released into the environment, where they can dissolve or float in water or accumulate in the soil causing several damages (Aydin et al., 2017). Certainly, petroleum wastes produce major concern besides consuming considerable economic resources (Lalande et al., 2003).

Crude petroleum is a mixture of hydrocarbon and non-hydrocarbon compounds. According to Speight (2014), hydrocarbon compounds can be classified as follow: paraffins (linear or branched chains), naphthenes or cycloparaffins (containing one or more rings) and aromatics compounds (containing one or more aromatic ring which may be linked up with naphthenic rings and/or paraffinic side chains). Those compounds are deleterious for the environment and living organisms because of their lipophilic, mutagenic and carcinogenic properties (Fatima et al., 2015). Especially pyrogenic polycyclic aromatic hydrocarbons (PAHs), which are generated from the incomplete incineration of crude petroleum throughout industrial process, has been recognized as priority persistent organic pollutants (POPs) and the most hazardous for the environment, due to their toxic, mutagenic, carcinogenic properties and their recalcitrance (Aydin et al., 2017). PAHs compounds

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contain petrogenic (2–3 fused aromatic benzene rings) and pyrogenic (≥4 rings) in various different configurations. Their persistence and bioavailability in the environment are both related to their molecular size. For instance, the half-lives of PAHs in soils and sediments may range from 16 to 126 days for phenanthrene (three ring molecule), whereas it may range from 229 to 1400 days for pyrene (five rings) (Kanaly and Harayama, 2000). Further, high molecular weight PAHs may be adsorbed onto deep soil or sediments where oxygen molecule is rare, significantly reducing their possibilities of being biodegraded since aerobic conditions are considered essential for PAHs degradation (Qin et al., 2017).

A number of restoring technologies including biological and physico-chemical processes are available to reduce petroleum hydrocarbon concentrations in the environment (Sannino and Piccolo, 2013). Significant attention has been given to bioremediation, that mainly relies on the use of organisms with specific metabolic abilities in order to remove or minimize the toxic effect of organic and inorganic pollutants (de Almeida et al., 2017). Certainly, biological remediation is considered as an efficient approach to cleanup petroleum contaminated soils (Tiralerdpanich et al., 2018). Phytoremediation based on the interactions between plants and microorganisms, appears to be especially effective for this purpose (Soleimani et al., 2010). Particularly, plant-bacteria association has been considered as an effective partnerships to cleanup hydrocarbons impacted soils (Khan et al., 2013).

Some plant species have shown the capacity to deal with relatively high concentrations of organic chemicals, which may be taken up, translocated, metabolized, and/or volatilized. In addition, plants may stimulate the degradation of xenobiotics in the rhizosphere (rhizodegradation) releasing plant root exudates (Alvarez et al., 2012, 2015). On the other hand, endophytic bacteria colonize the inner tissues of living plants establishing a harmonious relationship with the host and causing positive effects on plant health (Kidd et al., 2017). In a phytoremediation scene, endophytic bacteria with specific metabolic capabilities could be able to degrade organic pollutants and to reduce both phytotoxicity and evapotranspiration of volatile compounds. Certainly, plant-associated bacteria have exceptional ability to improve plant growth and biomass production in polluted soils, due to their ability to produce plant growth-promoting (PGP) molecules (Fatima et al., 2016;Simon Sola et al., 2017, 2019; Soleimani et al., 2010).

Plant-bacteria association can be exploited for the remediation of polluted systems (Weyens et al., 2010) and is considered as an important component of phytoremediation technologies (Glick et al., 2007). Endophytes belonging to Gammaproteobacteria, Bacilli, Alphaproteobacteria, Flavobacteria and Actinobacteria have demonstrated potential to degrade different hydrocarbons (Afzal et al., 2014). In particular, actinobacteria play relevant ecological roles in the environment, and their ability to degrade complex polymers represents the reason why actinobacteria have received special attention as candidates for bioremediation of organic compounds (Alvarez et al., 2017). Thus, members of this phylum are being intensively studied by our research group in the Laboratory of Biotechnology of Actinobacteria because of their ability to bioremediate heavy metals, and petroleum hydrocarbons in single and mixed polluted systems (Alvarez et al., 2017; Aparicio et al., 2015; Baoune et al., 2018, 2019; Fuentes et al., 2010; Polti et al., 2009).

Previously, we have demonstrated that the endophyte *Streptomyces* sp. Hlh1 was capable of degrading light Algerian crude petroleum in both liquid and soil systems, and producing PGP molecules (Baoune et al., 2018). Based on these data, we hypothesized that the inoculation of maize plants exposed to petroleum hydrocarbons with *Streptomyces* sp. Hlh1, could be a successful phytotechnology to improve the phytoremediation process. In this context, the main purpose of the present study was to investigate the effectiveness of the plant-actinobacteria association to remove crude petroleum and pure-PAHs from contaminated soils.

2. Materials and methods

2.1. Chemicals and culture media

Light crude petroleum was obtained from refinery situated in Hassi Mesouad, City of Ouargla, Algeria (Baoune et al., 2018). PAHs used in this study were phenanthrene, anthracene and pyrene (> 99% purity, analytical grade), purchased from Sigma-Aldrich Co. (St.Louis, MO, USA). Stock solutions of PAHs (anthracene or phenanthrene 4.45 g L^{-1}; pyrene 5.81 g L^{-1}) were prepared by dissolving each PAH separately in acetone. Crude petroleum and pure-PAHs were filter sterilized prior to use.

Depending upon the objectives of the experiment, bacterial strain was cultured in one of the following media, all of which were sterilized by autoclaving at 121 °C for 20 min.

ISP2-agar medium containing (in g L^{-1}) malt extract, 10; yeast extract, 4; glucose, 4; agar, 20 (pH 7.0), was used for microbial maintenance.

Tryptic Soy Broth (TSB) containing (in g L^{-1}) tryptone, 15; soy peptone, 3; NaCl, 5; K_2HPO_4, 2.5; glucose 2.5, was used to prepare the bacterial inoculum.

Liquid minimal medium (MM) containing (in g L^{-1}) L-asparagine, 0.5; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; $FeSO_4$. H_2O, 0.01, was used for the biodegradation assays.

2.2. Polycyclic aromatics hydrocarbon degradation in liquid medium

Crude petroleum-degrading *Streptomyces* sp. Hlh1, previously isolated and characterized (Baoune et al., 2018), was cultured in Erlenmeyer flasks containing 30 mL of TSB and incubated for 3 days, at 30 °C in orbital shaker. Biomass was harvested by centrifugation (4000 × *g*, 15 min, 4 °C), washed two times and re-suspended in sterile distilled water.

Flasks with 20 mL of MM were supplemented with anthracene, phenanthrene (36 mg L^{-1}) or pyrene (46 mg L^{-1}) (final concentration). Prior to the microbial inoculation, flasks were left uncapped for 30 min to allow the evaporation of the acetone. Then, *Streptomyces* sp. Hlh1 was inoculated in the flasks at final concentration of 0.01 g L^{-1} and incubated for 7 days at 30 °C in orbital shaker. Non-inoculated flasks were used as control and three replicates of each treatment were done. At the end of the incubation period, centrifuged culture supernatants (9000 × *g*, 10 min, 4 °C) were used to determine contaminant concentrations as described below. Microbial biomass was estimated after centrifugation by washing the pellets with 25 mM Tris-EDTA buffer (pH = 8) and drying to constant weight at 105 °C.

2.3. Soil preparation and contamination

A model loam soil was collected from an urban area free of contamination at 5–15 cm depth. Prior to use, soil was air-dried, lightly ground, and finally sieved through a 2-mm sieve. Glass pots filled with 200 g of soil were sterilized (three successive sterilizations at 100 °C for 60 min each, whit 24 h intervals) (Polti et al., 2014). Sterilized soil facilitates the study of the effect of the *Streptomyces* strain on the hydrocarbons dissipation as well its effect on the growth of the plant, avoiding the possible interference of soil microbiota. Soil moisture was adjusted to 20% using sterile distilled water and then, each glass pot was artificially contaminated with filter-sterilized crude petroleum (20 g kg^{-1}) or pure-PAHs (anthracene, phenanthrene 36 mg kg^{-1}; pyrene 46 mg kg^{-1}) individually. Soil samples were thoroughly mixed and the pots were left uncovered for 6 h to allow evaporation of acetone. Then, pots were kept for 36 h at room temperature, so that water and hydrocarbons equilibrate in the soil.

2.4. Plant-bacteria bioassay

Endophyte-free maize (*Zea mays*) seeds not treated with fungicide were surface-sterilized using 5% (*v/v*) of sodium hypochlorite for 2 min, followed by 2 min immersion in ethanol 70% (*v/v*). Seeds were thoroughly rinsed 5 times with sterile distilled water (Yousaf et al., 2011). The seeds were then placed into sterile Petri dishes with filter paper (Wattman No. 1) moistened with sterile distilled water, until germination, in a climate controlled room (25 °C, 16:8 light:dark, 65% relative humidity).

The rhizosphere zone of each germinated seed (three per pot) was inoculated with a cell suspension of *Streptomyces* sp. Hlh1 (final concentration of 1 g kg^{-1} of wet weight), which had been prepared as mentioned before. Non-inoculated plants and non-planted soils were used as controls in both, contaminated and non-contaminated systems. Three replicates were prepared for each treatment. Pots were incubated in a climate controlled room (25 °C, 16:8 light:dark, 65% relative humidity) for 15 days. Plants were watered with sterile water when needed. At the end of the experiment, soil samples were taken for determining residual petroleum and pure-PAHs.

2.5. Plant analyses

Maize plants were harvested at the end of the assay to determine the length of the shoots and roots (Calvelo Pereira et al., 2010); fresh and dry weight as well as carotenoid and chlorophyll content. For this purpose, the soil attached to the roots was removed by gently washing with distilled water. Afterwards, length of roots and shoots was measured using a millimeter scale and plant biomass was recorded as fresh weight. Chlorophyll content was determined according to the protocol of Arnon (1949). Briefly, 500 mg of fresh leaves were cut into small pieces, homogenized in 10 mL of 80% acetone and then centrifuged at 2400 rpm for 10 min, at 4 °C. The absorbance of the plant extract was measured at 645, 663 and 480 nm. Acetone 80% was used as blank. Detection and quantification limits of the method were 0.08 µg L^{-1} and 0.50 µg L^{-1}, respectively. Chlorophyll content was calculated according to the formula of Arnon (1949).

The carotenoid content in the plant extract was calculated according to the formula given by Kirk and Allen (1965). The remaining parts of plants samples were dried at 105 °C until constant weight to determine dry weight.

Tolerance Index (TI) was calculated as the mean dry weight of a plant grown on the presence of a hydrocarbon, divided by the mean dry weight of a plant grown on non-contaminated control soil (Diwan et al., 2010). TI values greater than 1 reflect a net increase in biomass and suggest that plants have developed tolerance, whereas TI values lower than 1 indicate a net decrease in biomass and a stressed condition by the plants. TI values equal to 1 indicate no difference relative to control treatments.

2.6. Hydrocarbons analysis

2.6.1. Polycyclic aromatic hydrocarbons analysis

Residual pure-PAHs were extracted from liquid and soil samples by adding 10 mL of acetone. The mixture was homogenized and filtered through a 0.22 µm – PTFE membrane (Microclar, Argentina). Filtered solutions were stored at −20 °C until analysis (Bourguignon et al., 2014).

PAHs determination was carried out by RP-HPLC using an HPLC equipment (Alliance e2695, Waters Co., MA, USA) coupled to a PDA 2998-detector operating at a fixed wavelength (λ = 254 nm). Samples were automatically injected into C18 µBondapak HPLC column (4.6 × 250 mm, 50 Å pore size, 5 µm particle size). The mobile phase was methanol:water (9:1) at a flow rate of 1 mL min^{-1} for 30 min (Manohar et al., 2001). The limits of detection (LOD) and quantification (LOQ) for the PAHs were determined from free samples (n = 5) as the lowest concentrations yielding signal-to-noise ratios of 3:1 and 10:1 or higher calculated for average baseline noise, respectively. The LOQ was subsequently determined by analysis of five spiked samples prepared at their respective concentrations. Stock solutions of phenanthrene, anthracene and pyrene (Sigma-Aldrich) were prepared by dissolving 1 mg of each in 100 mL of acetone. PAHs standards solutions were kept in the dark at 4 °C until analysis. Mixed working solutions of PAHs ranging from 0.5 to 2.0 ng L^{-1} were prepared by the appropriate dilution of aliquots of the stock solutions in acetone to prepare the calibration curves and calculate the LOD for all compounds. The LOD was: phenantrene (0.010 ng), anthracene (0.0009 ng) and pyrene (0.071 ng). The LOQ (10 S/N) was: phenanthrene (0.039 ng), anthracene (0.002 ng) and pyrene (0.209 ng). Both LOD and LOQ are expressed as ng per injection. The mean recovery from 10 mL of samples was in the range of 73–84%.

2.6.2. Petroleum crude hydrocarbons analysis

Ten grams of each individual soil sample were weighed into 20 mL glass vials, and 10 mL of pentane were added. The vials were shaken on a horizontal shaker at 120 oscillations per min for 1 h. The extracts were decanted overnight and the organic phase separated. For the determination of semi-volatile hydrocarbons, 1 µL of the extracts was injected, in the splitless mode, into an Agilent 7890 A gas chromatography equipped with a flame ionization detector (FID) and a HP5 capillary column (25 m, long; 0.25 mm, ID; 0.25 µm, film thickness) with nitrogen as carrier gas (3 mL min^{-1}). Temperatures of the injection port and detector were 285 °C and 325 °C, respectively. The oven temperature program was as follows: initial temperature of 30 °C for 3 min, increasing at 15 °C min^{-1} to 300 °C which was kept for 5 min, and then increasing at 15 °C min^{-1} to the final temperature of 325 °C. The OpenLab software was used for integrating chromatogram area to encompass straight chain hydrocarbons from C6 to C35. Quantification of the components was performed with the standards proposed by the methods EPA 8015 and TNRCC 1005. The ratios *n*-C17/pristane and *n*-C18/phytane were calculated. The quantification limit was 20 mg kg^{-1} and the detection limit was 5 mg kg^{-1}.

2.7. Statistical analysis

Statistical analysis was conducted using R software, version **3.5.3.** All data reported in this study were the means values of three independent replicates. Data were evaluated statistically by one-way analysis of variance (ANOVA), and significant differences between mean values were determined by Fisher post-test with a probability level of $p < 0.05$. The data normality was checked by using the Shapiro test.

3. Results

3.1. PAHs biodegradation in liquid medium

Growth and removal of pure-PAHs by *Streptomyces* sp. Hlh1 were evaluated in artificially contaminated liquid system. At the end of the assays, microbial growth ranged from 0.27 to 0.28 g L^{-1}, showing that *Streptomyces* sp. Hlh1 was able to use these pollutants as a sole source of carbon and energy, because no other carbon compound had been added to the culture medium. Consequently, no growth was observed in the absence of PAHs (Supp. Fig.1). Results of the quantitative assessment of the degradation of pure PAHs, showed degradation percentages of 64% (23 mg L^{-1}), 85% (30 mg L^{-1}) and 94% (44 mg L^{-1}) for phenanthrene, anthracene and pyrene, respectively.

3.2. Petroleum hydrocarbons removal in soil

The synergistic potential of *Streptomyces* sp. Hlh1 with maize plants to remove petroleum hydrocarbons was evaluated in artificially

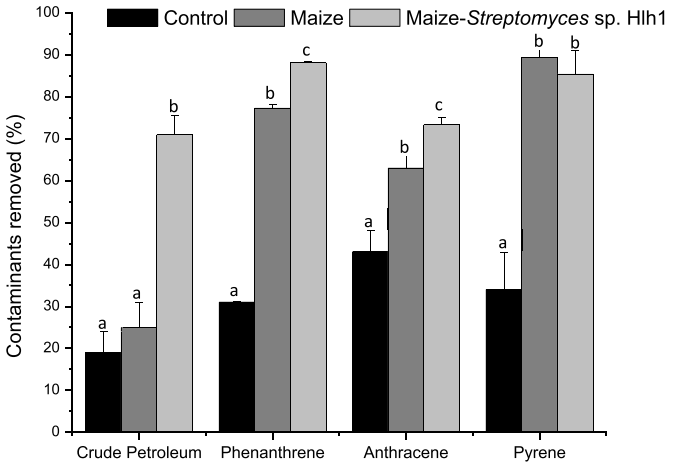

Fig. 1. Crude petroleum and pure PAHs dissipation from artificially contaminated soil after 14 days of treatment. Bars showing different letters indicate they were significantly different ($p < 0.05$, Fisher's post-test).

contaminated soils. Crude petroleum, phenanthrene, pyrene or anthracene dissipation was recorded after 14 days for soils assayed with the following treatments: Maize-*Streptomyces* Hlh1; Maize (non-inoculated) and Controls (non-inoculated and non-planted soil). In a previous work, *Streptomyces* sp. Hlh1 had been grown on petroleum hydrocarbon contaminated soil, so that its ability to degrade the contaminant from soils had been demonstrated (Baoune et al., 2019).

There was evidence for crude petroleum and PAHs removal since contaminants decreased at the end of the exposure period in all the biological treatments (Fig. 1). PAHs showed higher removal values (~50%) from soils implanted and non-inoculated, in comparison with control soil, strongly suggesting that maize seemed to be functional for the treatment of these PAHs. Moreover, statistically significant differences in the contaminants removal were found between biological treatments and controls ($p < 0.05$), except for the treatment with maize in the presence of crude petroleum. The presence of *Streptomyces* sp. Hlh1 accelerates the dissipation of petroleum hydrocarbonsfrom soils, under the tested conditions. Crude petroleum, phenanthrene and anthracene were dissipated more efficiently from soil implanted and inoculated (70%, 88% and 73%, respectively); (14,176, 31.4, 26.1 mg kg^{-1}, respectively) in comparison to soils implanted and non-inoculated (26%, 77% and 62%, respectively) (5217, 27.5, 22.3 mg kg^{-1}, respectively) (Fig. 1). Pyrene was removed up to 89% (41.5 mg kg^{-1}) and 85% (39.5 mg kg^{-1}) from soil with inoculated and non-inoculated plants, respectively, without statistically significant difference.

Fractions of crude petroleum hydrocarbons dissipated were also evaluated. Residual concentrations of crude petroleum components after the treatments are shown in Table 1. It was observed that hydrocarbons removal increased in the presence of inoculated plants, in comparison to non-inoculated plants and control pots (Table 1).

In the inoculated and planted soil, hydrocarbons dissipations were up to 77% (C8–C24) and 69% (C24–C30), while in the non-inoculated planted system were up to 34% (C8–C24) and 40% (< C24–C30).

All experiments were done using sterile soil samples, so the losses of petroleum hydrocarbons and PAHs observed in controls could be attributed to abiotic factors such as volatilization, evaporation, photo-oxidation and irreversible sorption, among others (Soleimani et al., 2010).

In general terms, soils planted improved significantly ($p < 0.05$) PAHs removal comparing to the non-planted soils. This effect was not observed by crude petroleum, which could be due to the toxic effects of low molecular weight petroleum compounds (Iqbal et al., 2019).

The ratio *n*-C17/pristane and *n*-C18/phytane are considered as indicators of hydrocarbons biodegradation. In this study, both ratios were

Table 1
Residual concentrations (mg kg^{-1}) of crude petroleum components obtained from the soil after the treatments. Values sharing the same letter were not significantly different, among the treatments ($p < 0.05$, Fisher's post-test).

Hydrocarbons	Control	Maize	Maize-*Streptomyces* sp. Hlh1
Phenanthrene	25 ± 0 ^{a}	6 ± 0 ^{b}	2 ± 1 ^{c}
Anthracene	20 ± 2 ^{a}	8 ± 1 ^{b}	5 ± 0 ^{c}
Pyrene	31 ± 4 ^{a}	3 ± 1 ^{b}	2 ± 1 ^{b}
< C8	217 ± 54 ^{a}	141 ± 49 ^{a}	50 ± 24 ^{b}
C8 to < C10	2151 ± 146 ^{a}	1813 ± 87 ^{b}	641 ± 88 ^{c}
C10 to < C12	2680 ± 195 ^{a}	2728 ± 137 ^{a}	1038 ± 107 ^{b}
C12 to < C14	3086 ± 201 ^{a}	2780 ± 268 ^{a}	1172 ± 156 ^{b}
C14 to < C16	3175 ± 170 ^{a}	3000 ± 223 ^{a}	1221 ± 127 ^{b}
C16 to < C18	1753 ± 68 ^{a}	1566 ± 148 ^{a}	649 ± 47 ^{b}
C18 to < C20	1060.65 ± 63 ^{a}	996 ± 96 ^{a}	385 ± 27 ^{b}
C20 to < C22	848 ± 51 ^{a}	808 ± 83 ^{a}	306 ± 14 ^{b}
C22 to < C24	344 ± 10 ^{a}	368 ± 38 ^{a}	135 ± 43 ^{b}
C24 to < C26	350 ± 22 ^{a}	315 ± 32 ^{a}	121 ± 15 ^{b}
C26 to < C28	238 ± 23 ^{a}	200 ± 30 ^{a}	72 ± 4 ^{b}
C28 to < C30	116 ± 5 ^{a}	70 ± 19 ^{b}	35 ± 5 ^{c}
C30 to < C32	0	0	0
> C32	0	0	0
Total Hydrocarbons	16,019 ± 800 ^{a}	14,783 ± 969 ^{a}	5824 ± 541 ^{b}

Data are means with standard deviation presented after the observed value.

Table 2
Crude petroleum removal from soils after the biological treatment and *n*-C17/pristane and *n*-C18/phytane ratios calculated. Values sharing the same letter were not significantly different ($p < 0.05$, Fisher's post-test).

	Control soil	*Z. mays*	*Z. mays* -*Streptomyces* sp. Hlh1
Crude petroleum removal (%)	19.9 ± 4.0 ^{a}	26.1 ± 4.8 ^{a}	70.9 ± 2.7 ^{b}
Ratio *n*-C17/pristane	1.65 ± 0.21 ^{a}	1.48 ± 0.33 ^{a}	0.97 ± 0.09 ^{b}
Ratio *n*-C18/phytane	1.74 ± 0.24 ^{a}	1.57 ± 0.32 ^{a}	1.03 ± 0.16 ^{b}
n*-C17	168 ± 24 ^{a}	122 ± 26 ^{a}	36 ± 5 ^{b}
Pristane*	102 ± 2 ^{a}	83 ± 7 ^{b}	38 ± 8 ^{c}
n*-C18	177 ± 26 ^{a}	130 ± 22 ^{a}	38 ± 11 ^{b}
Phytane*	102 ± 2 ^{a}	84 ± 14 ^{a}	36 ± 6 ^{b}

Data are means with standard deviation presented after the observed value.

significantly lower ($p < 0.05$) in the presence of plants inoculated with *Streptomyces* sp. Hlh1, in comparison to soil without plants or implanted and non-inoculated (Table 2).

3.3. Effects of petroleum hydrocarbons on plant development

Maize plants were harvested at the end of the assay. Parameters such as roots and shoots length, plant biomass, and carotenoid and chlorophyll content were analyzed.

All plants survived and no mortality was noticed in either contaminated or non-contaminated soils. According to the tested contaminants, the length of roots and shoots were variable (Fig. 2) showing a strong tendency to reduction in comparison to root and shoots development of control plants grown on non-contaminated soil. Especially, crude petroleum seems to deeply influence plant development, due to roots length of plants grown on this contaminant was shorter in comparison to roots length of plants grown in pure-PAHs.

For most of the contaminants tested, plants inoculated with *Streptomyces* sp. Hlh1 exhibited higher roots and shoots length, as well as higher contents of chlorophyll and carotenoids in comparison to non-inoculated plants growing in contaminated soil (Fig. 3). Only for pyrene, contents of chlorophyll and carotenoids were similar for both inoculated and non-inoculated plants.

Moreover, a significant increase in chlorophyll and carotenoids contents was registered in plants inoculated with *Streptomyces* sp. Hlh1,

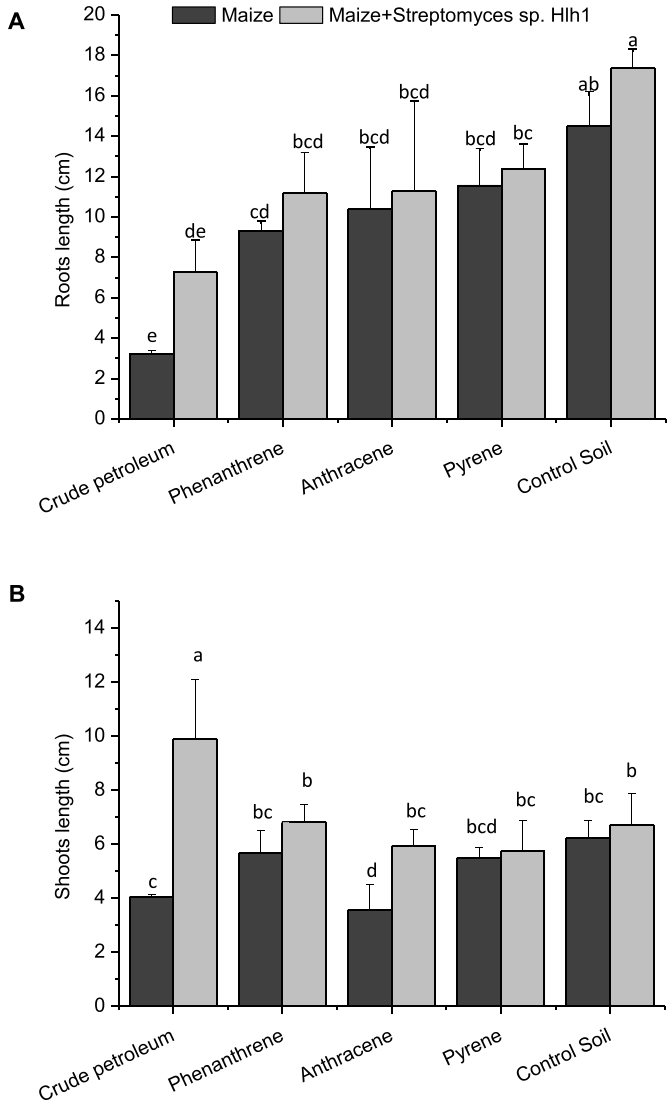

Fig. 2. Seedling development of *Zea mays* grown on soils artificially contaminated with petroleum hydrocarbons. (A) Root length; (B) Shoot length. Bars showing different letters indicate they were significantly different ($p < 0.05$ Fisher post-test).

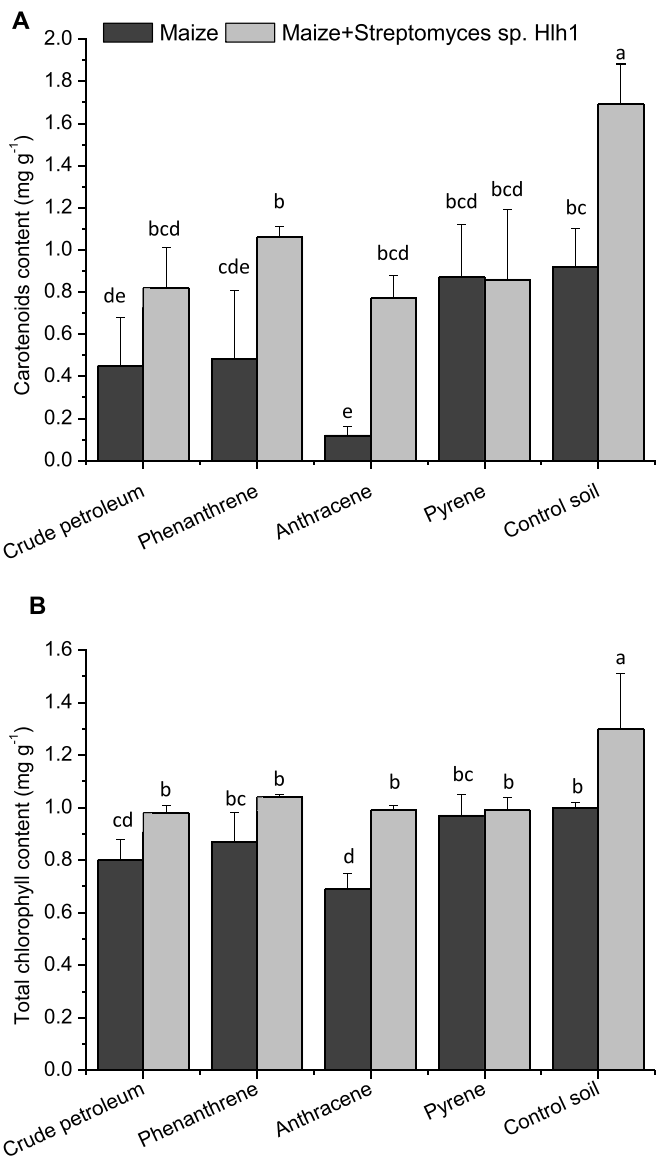

Fig. 3. (A) Carotenoids and **(B)** Chlorophyll content of *Zea mays* plants grown on soils artificially contaminated with petroleum hydrocarbons. Bars showing different letters indicate they were significantly different ($p < 0.05$, Fisher's post-test).

in absence of pollutants ($p < 0.05$).

Fresh and dry weight of plants grown in different experimental conditions did not show significant differences, while inoculated plants showed slightly higher fresh weights in comparison to non-inoculated plants. Such differences were not detected when dry weights of the plants were obtained (Supplementary Table 1).

4. Discussion

Plants-endophytes associations are recognized as synergistic partnerships useful for cleaning contaminated soils (Newman and Reynolds, 2005; Ryan et al., 2008). Several aspects related to the implementation of this technology such as pollutants nature, soil type, plant species, inoculum density and inoculation methods have been intensively researched and deeply debated (Afzal et al., 2011, 2013; Khan et al., 2013; Zheng et al., 2018). In any case, there is a general consensus that plant-microbe association is a highly desirable component of any phyto-management program (Glick et al., 2007). Within Bacteria domain, Actinobacteria have received special attention to be active for bio/phyto-remediation (Alvarez et al., 2017). Particularly, the *Streptomyces* genus, whose members are distributed extensively in soil, water, and in association with plants (Balachandran et al., 2012; Baoune et al., 2018; Polti et al., 2007), have been reported to be able to degrade petroleum hydrocarbons (Bourguignon et al., 2016). In addition to their metabolic diversity, strains of the *Streptomyces* genus may be well suited for soil inoculation because of their production of spores that can persist and disperse. In this context, the present work is shedding light on the role of a petroleum-degrading endophytic *Streptomyces* strain to improve phytoremediation of petroleum hydrocarbons contaminated soil.

An intensive review about the success of several actinobacteria strains to bioremediate contaminated systems was compiled and analyzed by our research group (Alvarez et al., 2017). Nevertheless, no study has been reported about plant-endophytic *Streptomyces* as partnerships useful for petroleum hydrocarbons dissipation from soil.

Previously, Baoune et al. (2018) determined the ability of *Streptomyces* sp. Hlh1 for degrading crude petroleum in liquid culture medium. The degradation of phenanthrene, pyrene and anthracene from crude petroleum observed in that study, was lower in comparison to the degradation obtained in the present study using the pure-PAHs added individually into the culture medium. It seems quite evident that

hydrocarbons degradation was not only highly dependent on the type of petroleum hydrocarbons but also depends on their presence in a mixture or individually. Crude petroleum contains an extreme range of organic compounds of different molecular size. In general terms, crude petroleum oil has a complex composition of hydrocarbons, nitrogen, oxygen and sulfur compounds, and metallic constituents (Speight, 2014) which could interfere in the bioavailability of each compound. Interestingly, although pyrene (four-ringed compound) has been used as a model of high molecular weight PAH, *Streptomyces* sp. Hlh1 achieved the highest removal for pure-pyrene, which is considered more persistent and difficult to biodegrade than phenanthrene and anthracene (Ghosal et al., 2016). In addition, microbial growth was not inhibited by the contaminants. This ability to grow in the presence of petroleum hydrocarbons could response to selective evolutionary pressure that would have been exerted by the environment in which the microorganisms were isolated, leading to the acquisition of metabolic capabilities to survive and grow in polluted environments. Most studies have demonstrated that the use of microorganisms isolated from contaminated systems tend to function more effectively than using those not adapted to contaminants (Fingas, 2011). The capacity of *Streptomyces* strains to grow and degrade hydrocarbons was described in the literature as a common feature of the genus, although the studies were usually carried out on the determination of the degradation potential of *n*-alkanes or PAHs (Balachandran et al., 2012; Baoune et al., 2018; Barabás et al., 2001; Ferradji et al., 2014). To date, some actinobacteria including genera *Rhodococcus*, *Gordonia*, *Streptomyces* and *Amycolatopsis* have been shown to be predominantly PAHs degraders (Bourguignon et al., 2014; Isaac et al., 2013). Based on the chemical composition and the concentration of crude petroleum, the degrading effectiveness of *Streptomyces* strains has been reported from 50% to 99% (Balachandran et al., 2012; Baoune et al., 2018; Ferradji et al., 2014).

Microbial inoculation promotes soil enzyme activity, PAHs removal and plant growth. Endophytic bacteria may be of particular interest as bioinoculant since they have the advantage of proliferating within plant tissue thus facing less competition for nutrients and being protected from the high-stress environment of polluted soils (Sturz et al., 2000). Through roots and leaves, hydrocarbons can achieve vascular system and intercellular spaces, reducing plant growth or inducing plant mortality (Arellano et al., 2017). Despite the toxicity of hydrocarbons is a limiting factor for plants that cannot easily degrade them, some plants have tolerance mechanisms by which tolerate, immobilize and/or accumulate hydrocarbons in their different parts, and even degrade and eliminate them (Khan et al., 2013; Li et al., 2012). This response depends not only on the plants type but also on the concentration of hydrocarbons and the exposure time (Arellano et al., 2017). In the present study, maize plants were able to tolerate the concentrations of hydrocarbons used, and also contributed to dissipation of crude petroleum and PAHs. Moreover, significant degradation of crude petroleum and PAHs was observed in soil by inoculated plants since hydrocarbons dissipation was higher compared to non-inoculated plants and non-planted soils. It would be possible to hypothesize that the effect of *Streptomyces* sp. Hlh1 could be related to stimulation mechanisms to the plants, which excreted root exudates that attract rhizospheric microbes. Several works have demonstrated enhanced removal of organic contaminants in the rhizosphere because of the increase of microbial density and/or activity due to the release of plant root exudates (Becerra-Castro et al., 2013; Ying et al., 2011). For instance, Simón Solá et al. (2019)found that the addition of maize root exudates to the culture medium led to an important increase of *Streptomyces* strain biomass as well as a higher Cr(VI) and lindane dissipation.

According to Fatima et al. (2016), the persistence and the action of endophytes in the plant environment play a critical role to improve crude petroleum dissipation. Regarding this approach, maximum dissipation of hydrocarbons was achieved in inoculated and planted soil contaminated with 20 gkg^{-1} of crude petroleum. Similar results were informed by Andria et al. (2009), since the authors reported the use of 20 gkg^{-1} of diesel was the most efficient concentration for the microbial colonization of plants and degradation of hydrocarbons.

Streptomyces sp. Hlh1 was able to improve hydrocarbons degradation from crude petroleum in soil samples. The highest removal was observed in shorter chain hydrocarbons. This is in agreement with previous studies that showed that the shorter and intermediate hydrocarbons (C_{10}–C_{20}) are the most easily degradable, even if their higher solubility in water makes them more toxic (Liu et al., 2018). Moreover, the inoculated maize plants showed greater hydrocarbons degradation than the non-inoculated plants, indicating that *Streptomyces* sp. Hlh1 could be responsible of improving contaminants dissipation since the assays presented here were carried out under sterile conditions.

It is well known that in petroleum polluted soils, the reduction of plants development is due to toxic nature of hydrocarbons which reduce water and nutrient uptake (Kirk et al., 2005). A similar impact of other environmental pollutants such as heavy metals has been observed on maize plants (Polti et al., 2011). Crude petroleum and its by-products can reduce shoots and roots development which could be refer to the delay in cell expansion (Athar et al., 2016). According to Calvelo Pereira et al. (2010), the biomass distribution of plants grown in contaminated soil and also the physiological activity is determined not only by the level of soil contamination but also by the type of contaminant.

The results presented in this work strongly suggest that the inoculation of maize plants with the endophyte *Streptomyces* sp. Hlh1 reduced crude petroleum and PAHs by-products toxicity. In fact, *Streptomyces* sp. Hlh1 improve shoot and root length of maize plants, This could be refer to the production of PGP metabolites by *Streptomyces* sp. Hlh1 such as IAA and ACC deaminase activities, previously demonstrated for this strain (Baoune et al., 2018). It is well known that IAA and ACC deaminase contribute to the growth and development of plants under stress conditions. The ACC deaminase is a key enzyme responsible for reducing ethylene levels in plants and, therefore, promoting the growth and development of roots (Glick, 2014; Khan et al., 2013). According to Sheng et al. (2008), the endophytic *Enterobacter* sp. 12J1 could effectively remove pyrene and improve maize and wheat growth in pyrene contaminated soils. However, no significant differences were found in the fresh weight between inoculated and non-inoculated plants grown in presence of petroleum hydrocarbons.

Chlorophyll content of leaves is used as indicator of plants stress (Huang et al., 2004). In accordance with other studies, our results showed petroleum hydrocarbons caused significant reduction of chlorophyll and carotenoids contents in non-inoculated plants (Das and Kumar, 2016; Shabir et al., 2016). In opposite, plants inoculated with *Streptomyces* sp. Hlh1 presented higher levels of chlorophyll and carotenoids, make evident a better performance compared to non-inoculated plants.

The ratios *n*-C17/pristane and *n*-C18/phytane are usually used as index for the estimation of hydrocarbons biodegradation. Since *n*-C17 and *n*-C18 are easily degradable compounds and pristane and phytane are relatively less degradable compounds, these ratios are higher in the case of fresh inputs, whereas low ratios indicate significant degradation of petroleum hydrocarbons (Bajt, 2017; Rostami et al., 2019). In the present work, the ratios were lower in presence of *Streptomyces* sp. Hlh1, indicating higher biodegradation of crude petroleum.

To our knowledge, this study is the first to demonstrate the ability of an endophytic *Streptomyces* strain to remove petroleum hydrocarbons and improve plant growth in contaminated soils.

5. Concluding remarks

As summarized in this article, the data presents the evidence that *Zea mays* is able to remediate soil contaminated with petroleum hydrocarbons and its remediation ability is improved by the inoculation of the endophytic *Streptomyces* sp. Hlh1. Higher degradation level of both pure-PAHs and crude petroleum were observed in the inoculated plants

compared to non-inoculated ones. Similarly, better plant growth was demonstrated in inoculated plants grown in contaminated soils.

The data presented in this work provide evidence about the outstanding possibility to use maize plants inoculated with *Streptomyces* sp. Hlh1 as an important component of a phyto-management program of hydrocarbons contaminated soils. Further studies are needed to understand the microbial mechanisms involved for improve the plant performance and contaminants dissipation. In this context, the study of physico-chemical variations on soils along the phytoremediation process will be a complementary tool to better understand such mechanisms.

Conflicts of interest

The authors declare that they have no conflict of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109591.

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