International Journal of Phytoremediation

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bijp20

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To cite this article: Keyvan Valizadeh Rad , Babak Motesharezadeh , Hossein Ali Alikhani & Arezoo Dadrasnia (2020): The potential use of *Cordia myxa* in the remediation of crude oil pollution, International Journal of Phytoremediation, DOI: 10.1080/15226514.2020.1825326

To link to this article: https://doi.org/10.1080/15226514.2020.1825326

Published online: 05 Oct 2020.

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
https://doi.org/10.1080/15226514.2020.1825326

The potential use of *Cordia myxa* in the remediation of crude oil pollution

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ABSTRACT
This study investigated the effects of hydrocarbon-degrading bacteria and organic matter on a crude oil-polluted soil by *Cordia myxa*. The treatments consisted of crude oil at two levels (3 and 6% w/w), municipal waste compost at two levels (5 and 10% v/v), and two different bacterial strains (*Pseudomonas* sp.141 and *Pseudomonas* sp. 27ps). At the end of the growth period, the plants were harvested and prepared for the laboratory analyses. The greatest population of oil degrading-bacteria (4.6×10^6 CFU/g soil) was observed in the treatment containing 10% compost, 6% crude oil, and *Pseudomonas* sp.141. The highest crude oil degradation (76.49%) was recorded in the soil polluted with 6% crude oil, amended with 10% compost, and inoculated with *Pseudomonas* sp.141. The investigation on the degradation of the chains of C10–C35 compounds indicated that, in various treatments, the most abundant compound was among those with fewer carbon atoms (C12–C25), so the application of organic matter boosted the degradation of crude oil. In conclusion, *C. myxa* seedlings has an acceptable efficiency in the remediation of the oil-contaminated soil affected by biological factors (compost and *Pseudomonas* bacteria), which is because of their high tolerance to the pollution and their ability to penetrate deeper soil layers.

KEYWORDS
Cordia myxa; *Pseudomonas*; Remediation; TPH; waste compost

Introduction

Petroleum hydrocarbon contamination of soil is a worldwide environmental problem, which results in environmental hazards and human health problems (Yang *et al.* 2017; Nwankwoala and Omofuophu 2019). Besides, it strongly affects soil chemical properties as well as the population and activity of soil microorganisms (Morais Leme *et al.* 2012). The hydrophobicity of oil hydrocarbons such as total petroleum hydrocarbons (TPH) reduces the ability of plants and microorganisms to absorb water and nutrients (Nie *et al.* 2011). Therefore, remediation of soils polluted with oil hydrocarbons is one of the most important subjects in environmental engineering (Varavipour and Mashal Soltani 2013). The phytoremediation of polycyclic aromatic hydrocarbons (PAHs) refers to the use of plants and associated soil microorganisms to reduce the concentrations of these contaminants in the environment. It is confirmed that many plants play crucial roles in the uptake and accumulation of PAHs from the atmosphere and soil (Dadrasnia and Agamuthu 2013; Huang *et al.* 2018). Plants belonging to the families Poaceae (grasses) and Fabaceae (legumes) have been chosen for their capabilities for the effective removal of PAH from contaminated soils.

Nowadays, the co-application of plants and bacteria is considered to be an effective approach to remediating crude oil-contaminated soils. In plant–bacteria symbiosis, there are a large number of bacteria in the rhizosphere and root and shoot of the host plant. The bacteria provide nutrients, while the host plant provides space for growth and colonization. Moreover, the bacteria degrade organic contaminants in various parts of the host plant (Fatima *et al.* 2017, 2018). The efficiency of phytoremediation depends on the toxicity of the pollutant itself and its effects on the availability of nutrients and soil texture. Another problem in such a contaminated soil is the inability of the microbial biomass to degrade the pollutant due to inadequate availability of nutrients and low bioavailability of the pollutant. Plant–bacteria symbiosis is considered to be one of the most efficient strategies employed for the remediation of PHs-contaminated soils due to the positive effects of bacteria on the plant growth and development and on the degradation of PHs (Kamathr *et al.* 2004; Cook and Hesterberg 2013). Plants inoculated with certain bacteria have more biomass and tolerance to pollutants compared to non-inoculated plants. A consortium of three endophytic bacteria was augmented to two kinds of grass, *Leptochloa fusca* and *Brachiaria mutica*, grown in an oil-contaminated soil (46.8 g oil kg^{-1} soil) in the vicinity of an oil exploration and production company (Fatima *et al.* 2018). This treatment improved plant growth, crude oil degradation, and soil health. The maximum degradation of oil (80%) was achieved with *B. mutica* plants augmented with the endophytes,

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which was significantly ($p < 0.05$) higher than with the use of plants or bacteria individually.

Research shows that an improvement in the plant growth conditions (*e.g.*, fertilization) enhances both plant growth and soil remediation. Wang *et al.* (2012) examined the phytoremediation of a pyrene-polluted soil using vermicompost and two plant species (*Lolium perenne* and *Medicago sativa*). They reported that the interaction between plant roots, microorganisms, and vermicompost improved the remediation process. The presence of organic matter in the soil improves microbial population and activity, water holding capacity and absorption, water movement in the soil, nutrient status, and aeration conditions, all of which alleviate the negative effects of hydrocarbons. For this purpose, organic and decomposable parts of wastes, which have a suitable content of microbes and nutrients, can be used (Semple *et al.* 2001).

Cordia myxa L. is a herb that grows in hot, arid regions, where there is a large oil site. The objective of this study was to investigate the effects of bacterial and waste compost treatments in the remediation of an oil hydrocarbon-polluted soil by *C. myxa* L.

Materials and methods

Material sources and trial design

A non-contaminated soil sample was used as a control. This soil sample was taken from a depth of 0–30 cm from the research station for the Faculty of Agricultural and Natural Resources, University of Tehran, Karaj (51°N, 36°E; 1312 m above sea level; Valizadeh Rad *et al.* 2016). The soil was passed through a sieve (2 mm), and the physic-chemical properties (Sparks 1996) were measured. Two factors were evaluated: compost at two levels (5 and 10%) and two bacterial strains. The compost was produced by Novin Bahar Company (Tehran, Iran). The crude oil was provided by the oil refinery of Tehran. After the preparation of the medium under sterile conditions, one bacterial loop was added into an Erlenmeyer flask for each bacterium. Erlenmayers were placed on a shaker at 120 rpm for 24 h. The optical density was read by a spectrophotometer (Unico 1100, Franksville, WI) at 600 nm, and the bacterial population was determined according to McFarland's table. The bacterial population was set at 1×10^8 cfu/ml (Heitkamp and Cerniglia 1988). Ten-milliliter inoculum was added to each pot. According to previous research, 10 ml inoculum (Colony Forming Unit) can provide a sufficient bacterial population for the degradation of oil and for a balanced bacterial activity (Heitkamp and Cerniglia 1988). Nutrients were available at ideal levels, and the soil was appropriate for the growth of *C. myxa*. To evaluate the efficiency of bacterial and organic treatments, crude oil contamination at 3 and 6% W/W were selected as medium and high pollution levels, respectively. The soil was polluted with oil compounds at two levels. These levels were selected based on previous trials (Shahriari *et al.* 2006). A factorial greenhouse experiment was arranged in a completely randomized design and carried out with three replications.

Treatment preparation

Five kilograms of the air-dried soil (clay-loam) was passed through a sieve (4 mm) and placed into the experimental pots. The pollutions were administered by spraying the oil onto the soil. The polluted pots were incubated at 30–35 °C near field capacity of 70–80% (irrigated with distilled water every week) for 3 months. During the incubation period, soil salts were allowed to go deep into the pots by irrigation and move up during evaporation. Thus, soil salts were evenly distributed throughout the soil. From the gene bank for the Department of Soil Science, University of Tehran, two bacterial strains (previously extracted from the polluted soil of Tehran oil refinery) with the best ability to degrade oil compounds were selected (Marquez-Rocha *et al.* 2001). After sampling from a contaminated area around the Tehran oil refinery, each strain of *Pseudomonas* was cultured in an agar medium to study its ability to decompose petroleum compounds (see details in the following paragraph). To administer the bacterial treatments, in each pot, 10 ml of bacterial inoculum with a population of 1×10^8 CFU/ml was added around the plant root. After 3 months of incubation, 6-month-old uniform seedlings of *C. myxa* with average height (35 cm), stem diameter (4 mm), and leaf number (10) were selected for the greenhouse experiment. The seedlings were provided from Isa Pareh nursery, Dezful, Khuzestan, Iran, transferred to the experimental greenhouse (Department of Soil Science, University of Tehran), and kept there for two weeks for adaptation. Seedlings were planted into 5 kg pots filled with contaminated or non-contaminated soil (depending on treatments), grown for 6 months, and then harvested. The pots were irrigated with distilled water until 70–80% of FC. The pots were kept under greenhouse conditions for 6 months. During this period, the temperature (20–35 °C for optimum conditions and no stress), light intensity (12,000 lux), and photoperiod (darkness 10 h, lightness 14 h) of the greenhouse were controlled every day. The plants were also monitored for any pests or diseases. Considering the soil analysis, 2.5 g complete fertilizer (N-P-K: 20-10-20 from Yara International Company, Oslo, Norway) dissolved in the irrigation water was added to each pot in four steps. The fertilizer was added to all pots at the same rate. Soil samples (250 g from each pot) were taken twice, one before plant cultivation (at the end of incubation period) and the other after plant cultivation. After that, the plants were harvested. In order to measure the level of oil hydrocarbons, one mixed sample (250 g) was provided from each pot.

Population of hydrocarbon-degrading bacteria in the pot soil

Firstly, the series of dilutions were provided from the soil suspension. Secondly, to determine the number of constituting units of the colony, 0.1 ml of dilution series was inoculated in Petri dishes containing crude oil + Agar (Biolife Italy) + the mineral basic culture media (Marquez-Rocha 2001) in three replications. After 7 days, bacteria were counted according to their mesophilic at 28 °C. Finally, the colonies grown on the surface of the culture media were detected (Stringfellow and Alvarez-Cohen 1999).

Residual level of oil hydrocarbons in soil

The method developed by the United States Environmental Protection Agency (EPA-3550) was used. Firstly, the soil was ground in a mortar, and then 1 g of the dried soil was transferred to gated centrifuge tubes, with 10 ml of dichloromethane and acetone solution. Next, the tubes were shaken for 4 min, and then centrifuged for 5 min at 3000 rpm in order for the sediments to deposit. After that, 1 ml of the upper liquid phase was used to measure the level of hydrocarbon compounds (Hutchinson *et al.* 2001; Minai-Tehrani *et al.* 2006). TPH was measured with gas chromatography and flame ionization (Agilent 7890 A model, Santa Clara, CA). After the incubation period was passed, the level of primary oil existing in the pots was measured and recorded for the final assessments. The reduction percentage of hydrocarbons was determined after 6 months of cultivation (Ezenne *et al.* 2014) as follows:

$$\text{Percentage of reduction} = (X_1 - X_2)/X_1 \times 100 \quad (1)$$

Where X_1 and X_2 are the primary concentration of crude oil and the concentration of crude oil in the soil after 6 months of cultivation, respectively. A factorial greenhouse experiment was arranged in a completely randomized design and carried out with three replications. The results were analyzed by using SAS 9.2 software, and the means were compared following Duncan's multiple-range test ($p < 0.05$).

Results and discussion

The experimental soil was calcareous with the following properties: textural class: clay loam; pH: 8; Ec: 1.68 dS/m; organic carbon: 0.58; total N: 0.1%; P: 11.93 mg/kg; K: 138.77 mg/kg; carbonate calcium equivalent (CCE): 7.01% (Valizadeh Rad *et al.* 2016). The soil had optimal levels of nutrients essential for plant growth. However, based on the soil analysis, 2.5 g complete fertilizer dissolved in the irrigation water was added to each pot in four steps (see details in Materials and Methods). Based on World Reference Base (WRB) for soil classification, the soil was classified as a *calcaric cambisol*. The soil family was Zeric Haplo Cambids, Clay Loam, Mixed, Active, and Thermic. Accordingly, the soil had suitable conditions for plant growth. Table 1 shows the analysis of the compost. The compost also had an appropriate content of organic materials meeting the bacterial requirements. The level of iron in the compost was due to its source (urban waste), an advantage for the calcareous soils of Iran. Table 2 shows the variance analysis of the population of crude oil-degrading bacteria in various treatments. The results showed that the main effects of crude oil, bacteria, and compost on the population of crude oil-degrading bacteria were significant ($p < 0.01$). Besides, the interactive effects of crude oil × bacteria, crude oil × compost, bacteria × compost, and oil × bacteria × compost were significant ($p < 0.01$) on the population of crude oil-degrading bacteria. The mean comparisons of the main effects of crude oil, compost, and bacteria on the population of crude oil-degrading bacteria showed a significant variation and rose with increasing the percentage of crude oil. Moreover, the results showed a significant increase in the population of bacteria with increasing the level of compost. On the other hand, in the treatments containing crude oil-degrading bacteria, the population was significantly higher than that of control (Table 3). Moreover, the largest population of the degrading bacteria, significantly higher than those of the other treatments, was observed in those treated with bacterium psu141 (Table 4). In addition, the mean comparisons of the interactive effects of crude oil × compost on the population of crude oil-degrading bacteria indicated that, at both levels of the pollution, it increased constantly with increasing the level of compost. Additionally, the largest population was observed in the treatment with the highest level of compost (10%), which was significantly larger than those of the treatments with no compost or 5% compost (Table 4).

The mean comparison of the interactive effects of bacteria × compost on the population of crude oil-degrading bacteria indicated that, at all levels of bacteria, the population

Table 1. Used compost properties.

Compost property	Value	Compost property	Value
pH 1:5	7	P (%)	0.3
EC 1:5 (dS/m)	8.68	K (%)	0.45
Total [*n* (%)]	0.85	Fe (mg/kg)	3500
O.M (%)	24.96	Zn (mg/kg)	276
O.C (%)	14.51	Mn (mg/kg)	223
C/N	17.07	Cu (mg/kg)	250
ρ b (g/cm^3)	0.712		

Table 2. Variance analysis results of effects of different levels of crude oil, bacteria and compost on population of crude oil decomposer bacteria and fresh/dry weight in soil cultivated with *Cordia myxa* L.

		Mean square Value		
Source of variation	DF	Population of crude oil decomposer bacteria	Shoot fresh weight (g)	Shoot dry weight (g)
Crude oil (P)	1	5.04×10^{13}**	2234.87**	97.15**
Compost (C)	2	6.12×10^{12}**	1851.20**	72.11**
Bacteria (B)	2	4.73×10^{12}**	898.05**	66.69**
P × C	2	2.10×10^{12}**	355.35**	7.73*
P × B	2	5.98×10^{12}**	30.19 ^{ns}	3.61^{ns}
B × C	4	4.17×10^{11}**	60.78^{ns}	1.93^{ns}
P × B × C	4	3.13×10^{11}**	42.70^{ns}	1.48^{ns}
Residual	36	2.47×10^{9}	43.69	2.55
CV (%)		3.48	13.39	14.88

Note: Marks "*" and "**" indicate significant effect in levels of 5% and 1%, respectively, and "ns" indicates non-significant effect.

of crude oil-degrading bacteria increased constantly with increasing the compost level. However, this increase was far more in treatments containing bacteria, and the largest population, significantly larger than those of the other treatments, was observed in the treatment with bacterium psu141 and 10% compost (Table 5). The interactive effects of crude oil × bacteria × compost on the population of crude oil-degrading bacteria indicated that at both levels of crude oil, the population of crude oil-degrading bacteria increased constantly with increasing the level of compost. At the first level of pollution, the population of bacteria was much larger in the treatment with no additional bacteria than in the treatments that received additional bacteria, but at the second level of pollution, the population of the degrading bacteria was much larger in the treatments that received additional bacteria than in the treatment with no additional bacteria (control). The largest population of the degrading bacteria, significantly larger than those of the other treatments, was observed in the soil polluted with 6% oil and treated with bacterium psu141 and 10% compost (Figure 1).

Effects of treatments on biomass

As shown in Table 2, the main effects of petroleum pollution, compost, and bacteria on shoot fresh and dry weights were significant. Regarding the mean comparisons (Table 6), the highest fresh and dry weights were recorded in the treatment with no pollution as well as in treatment with compost and in treatment with bacteria, which were significantly different from those of the control treatment. This indicates the positive effects of these treatments. Palmroth *et al.* (2002) reported that oil pollution at 0.5% resulted in a 43–64% reduction of biomass production in a number of plants belonging to Fabaceae and Graminae. Razmjoo and Adavi (2012) also observed that with increasing the level of hydrocarbon pollution, the dry weight of vegetative organs of *Cynodon dactylon* reduces. They reported that pollution at 6% generally resulted in 61% reduction of the dry weight. The negative effect of oil pollution on biomass production is also evident in Merkl *et al.* (2005). Guenther *et al.* (1995) already found that the presence of organic pollution (5 g/kg soil) led to a 64% reduction of ryegrass plant (ryegrass) growth, compared to the control plants. Moreover, a high concentration of contaminants in the soil could significantly inhibit plant growth, including root growth, in part due to oxidative stress, limiting the rate of phytoremediation. Other limiting factors for plant growth and phytoremediation efficiency are the lack of nutrients and low microbial diversity, often associated with polluted soils (Mcguinness and Dowling 2009). It seems that the nutrients have indirect effects on bacteria, which could be one of the reasons why bacteria degrade hydrocarbons at a higher rate in the presence of compost (Mcguinness and Dowling 2009). The amount of primary crude oil in the soil presented in Table 7.

Percentage of degradation

The variance analysis is shown in Table 8. Based on the results, the different levels of crude oil, bacteria, and

Table 3. Mean comparison results of main effects of crude oil, bacteria and compost on population of crude oil decomposer bacteria in soil cultivated with *Cordia myxa* L.

	Bacteria population (CFU/g soil × 10^6)
Crude oil (W/W)	
3%	0.46^b
6%	2.39^a
Compost (V/V)	
Control (0%)	1.02^c
5%	1.41^b
10%	1.84^a
Bacteria	
Control	0.87^c
psu141	1.88^a
psu27ps	1.51^b

Note: Means by the same letter are not significantly different according to DMRT ($p \leq 0.05$).

Table 5. Means comparison interaction effects of bacteria × compost on population of crude oil decomposer bacteria in soil cultivated with *Cordia myxa* L.

Treat	Bacteria (CFU/g soil × 10^6) population
B × C	
$B_0 \times C_0$	0.73^h
$B_0 \times C_5$	0.84^g
$B_0 \times C_{10}$	1.04^f
$B_1 \times C_0$	1.23^e
$B_1 \times C_5$	1.84^c
$B_1 \times C_{10}$	2.58^a
$B_2 \times C_0$	1.09^f
$B_2 \times C_5$	1.53^d
B2 × C10	1.91^b

Note: means by the same letter not significantly different according to DMRT ($p \leq 0.05$). P: crude oil; C: compost; B: bacteria. C5: 5% compost; C10: 10% Compost; B1: psu141; B2: psu27ps.

Table 4. Means comparison interaction effects of crude oil × bacteria and crude oil × compost on population of crude oil decomposer bacteria in soil cultivated with *Cordia myxa* L.

Treat	Bacteria population (CFU/g soil × 10)	Treat	Bacteria (CFU/g soil × 10^6) population
P × C		P × B	
$P_3 \times C_0$	0.53^d	$P_3 \times B_0$	0.39^f
$P_3 \times C_5$	0.41^e	$P_3 \times B_1$	0.45^e
$P_3 \times C_{10}$	0.43^e	$P_3 \times B_2$	0.53^d
$P_6 \times C_0$	1.21^c	$P_6 \times B_0$	1.65^c
$P_6 \times C_5$	3.36^a	$P_6 \times B_1$	2.36^b
$P_6 \times C_{10}$	2.60^b	$P_6 \times B_2$	3.16^b

Note: Means by the same letter not significantly different according to DMRT ($p \leq 0.05$). P: crude oil; C: compost; B: bacteria. P3: 3% crude oil pollution; P6: 6% crude oil pollution; C0: control treatment; C5: 5% compost; C10: 10% compost.

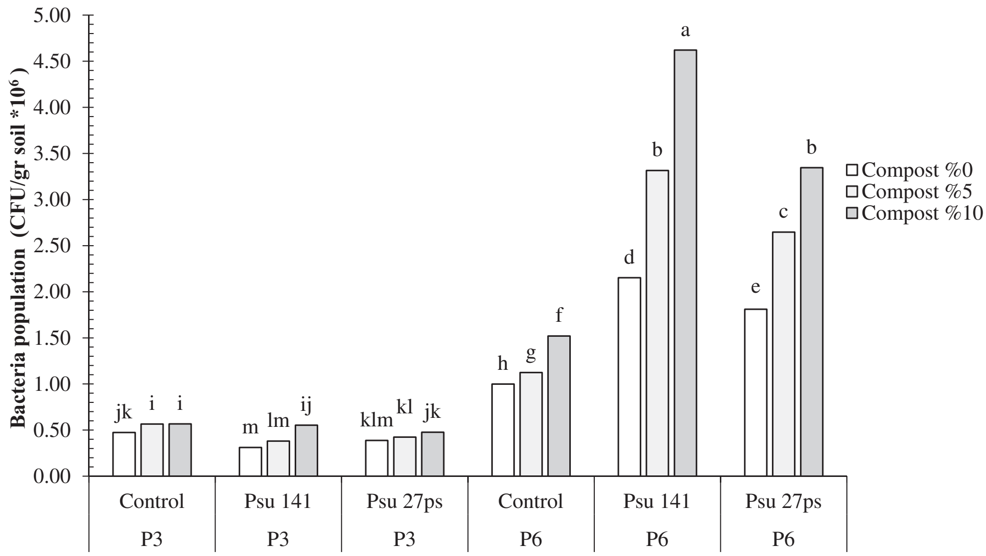

Figure 1. Means comparison interaction effects of crude oil × bacteria × compost on population of crude oil decomposer bacteria in soil cultivated with *Cordia myxa* L. P: crude oil; P3: 3% crude oil; P6: 6% crude oil; Control: without bacterial inoculation; psu 141: with *Pseudomonas* 141 inoculation; psu 27ps: with *Pseudomonas* 27ps inoculation.

Table 6. Mean comparison results of main effects of crude oil, compost, and bacteria on shoot dry/fresh weight in soil cultivated with *Cordia myxa* L.

Treatments	Shoot (g) Dry weight	Shoot (g) Fresh weight
Crude oil (W/W)		
Control (0%)	11.14^{b}	55.76^{a}
3%	12.40^{a}	53.37^{a}
6%	8.67^{c}	38.94^{b}
Compost(V/V)		
Control (0%)	8.99^{b}	43.96^{c}
5%	11.20^{a}	48.68^{b}
10%	12.03^{a}	55.43^{a}
Bacteria		
Control	9.22^{c}	41.40^{c}
psu141	12.47^{a}	57.92^{a}
psu27ps	10.52^{b}	48.75^{b}

Note: means by the same letter are not significantly different according to DMRT ($p \leq 0.05$).

Table 7. Amount of primary crude oil present in soil of different treatments.

Treatment	Crude Oil (mg/kg)
$P_3 \times C_0$	9,670
$P_3 \times C_5$	10,019
P3 × C10	9,343
$P_6 \times C_0$	21,883
$P_6 \times C_5$	21,883
P6 × C10	21,463

P: crude oil; C: compost. P3: 3% crude oil pollution; P6: 6% crude oil pollution; C0: control treatment; C5: 5% compost; C10: 10% compost.

Table 8. Variance analysis results of effects of different levels of bacteria and compost on decomposition value of different levels of crude oil in soil cultivated with *Cordia myxa* L.

Source of variation	DF	Mean square value Percentage of reduce TPH
Crude oil (P)	2	919.58**
Compost (C)	2	125.83**
Bacteria (C)	2	51.48**
P × C	4	24.71**
P × B	4	637.11**
B × C	4	157.35**
P × B × C	8	55.83**
Residual	54	0.19
CV (%)		0.72

Note: Marks "*" and "**" indicate significant effect in levels of 5% and 1%, respectively, and "ns" indicates non-significant effect.

compost had significant effects ($p < 0.01$) on the biodegradation process. In addition, the results showed the interactive effects of oil × bacteria, oil × compost, bacteria × compost, and oil × bacteria × compost were significant on the rate of crude oil degradation ($p < 0.01$; Table 8). The results of the mean comparison of the main effects of crude oil, bacteria, and compost demonstrated much more crude oil degradation at the higher pollution level (6%). Furthermore, the crude oil degradation increased with increasing the amount of compost, and the greatest value (63.81%) was attributed to the highest level of compost (10%), which had a significant difference from those of the other levels (Table 9). Besides, the results of the mean comparison of the main effects of bacteria demonstrated that the treatments that contained bacteria strongly reduced the crude oil content of the soil, which showed a significant difference from that of the treatment with no bacteria (control). Additionally, the greatest value (62.63%) was observed in the treatment that contained bacterium psu141. There was also a significant difference between the treatment that contained psu141 and the treatment that contained psu27ps (Table 9). The results of the mean comparison of the interactive effects of oil × bacteria on the rate of crude oil degradation indicated that the highest rate of crude oil degradation (72.63%) was observed at the highest level of pollution (6%) in the presence of bacterium psu141, which had a significant difference from those of the other treatments (Table 10). The results of the mean comparison of the interactive effects of crude oil × compost on the rate of crude oil degradation showed that it increased with increasing the amount of compost, the highest value of which (66.67%) was attributed to the treatment with the highest level of pollution (6%) that contained 10%

Table 9. Means comparison main effects of crude oil, bacteria and compost on decomposition value of crude oil in soil cultivated with *Cordia myxa* L.

	Reduce of TPH (%)
Crude Oil (W/W)	
3%	56.75[b]
6%	65.00[a]
Compost (V/V)	
Control (0%)	58.66[c]
5%	60.16[b]
10%	63.81[a]
Bacteria	
Control	59.25[c]
psu141	62.63[a]
psu27ps	60.76[b]

Note: means by the same letter not significantly different according to DMRT ($p \leq 0.05$).

Table 10. Means comparison interaction effects of crude oil × bacteria and crude oil × compost on decomposition value of crude oil in soil cultivated with *Cordia myxa* L.

Treat	Reduce of TPH (%)	Treat	Reduce of TPH (%)
P × B	61.15[c]	P × C	54.31[f]
$P_3 \times B_0$		$P_3 \times C_0$	
$P_3 \times B_1$	52.63[f]	$P_3 \times C_5$	54.99[e]
$P_3 \times B_2$	56.48[e]	$P_3 \times C_{10}$	60.95[d]
$P_6 \times B_0$	57.35[d]	$P_6 \times C_0$	63.02[c]
$P_6 \times B_1$	72.63[a]	$P_6 \times C_5$	65.34[b]
$P_6 \times B_2$	65.04[b]	$P_6 \times C_{10}$	66.67[a]

Note: means by the same letter not significantly different according to DMRT ($p \leq 0.05$). P: crude oil; C: compost; B: bacteria. P3: 3% crude oil pollution; P6: 6% crude oil pollution; C0: control treatment; C5: 5% compost; C10: 10% compost; B0: control, B1: psu141; B2: psu27ps.

Table 11. Means comparison interaction effects of bacteria × compost on decomposition value of crude oil in soil cultivated with *Cordia myxa* L.

Treat	Reduce of TPH (%)
B × C	
$B_0 \times C_0$	62.86[c]
$B_0 \times C_5$	58.05[f]
$B_0 \times C_{10}$	56.85[g]
$B_1 \times C_0$	56.85[g]
$B_1 \times C_5$	60.93[e]
$B_1 \times C_{10}$	70.11[a]
$B_2 \times C_0$	56.28[g]
$B_2 \times C_5$	61.52[d]
$B_2 \times C_{10}$	64.47[b]

Note: means by the same letter are not significantly different according to DMRT ($p \leq 0.05$). C: compost; B: bacteria. C0: control treatment; C5: 5% compost; C10: 10% compost; B0: control; B1: psu141; B2: psu27ps.

compost, which had a significant difference from those of the other treatments (Table10). The highest level of degradation (70.11%) was observed in the treatment with bacterium psu141 that contained 10% compost, which had a significant difference from those of the other treatments (Table 11). Moreover, the highest rate of crude oil degradation in the soil was observed in the treatment with the second level of pollution (6%) treated with 10% compost and bacterium psu141, which differed significantly from those of the other treatments (Figure 2).

Plants in combination with microorganisms can remediate soils contaminated with organic pollutants such as petroleum hydrocarbons (Afzal *et al.* 2012, 2014). Inoculation of plants with degrading bacteria is an approach to improve remediation processes but is often not successful due to the competition with resident microorganisms. It is therefore of high importance to address the persistence and colonization behavior of the inoculant. The primary reaction of soil microorganisms after the pollution of soil with oil is due to the decrease in their activity because of the reduction of soil aeration, and finally, after aerobic bacteria and fungi are removed, the resistant and compatible species remain (Shukry *et al.* 2013). It can also buffer the soil pH and improve the soil water holding capacity (Semple *et al.* 2001). Consequently, the growth and activity of biological biomass increase, thereby increasing the organic matter degradation (Semple *et al.* 2001). Ikuesan (2017) reported that the use of cow dung on crude oil-contaminated soils also protected the soil structure and provide utilizable nutrients to indigenous microorganisms, thereby enhancing their activities. They further took advantage of crude oil degradation. The rhizosphere has so many microorganisms that can increase the biological degradation of organic pollutants. The increased microorganism population is attributed to the root excretions and dead tissues, which supply carbon, energy, nitrogen, and other nutrients required for growth. Increased compost content can increase the availability of simpler nutrient resources for microorganisms' growth, thus reducing hydrocarbon degradation. Wang *et al.* (2012) assessed the effects of the application of alfalfa, rye, and compost on

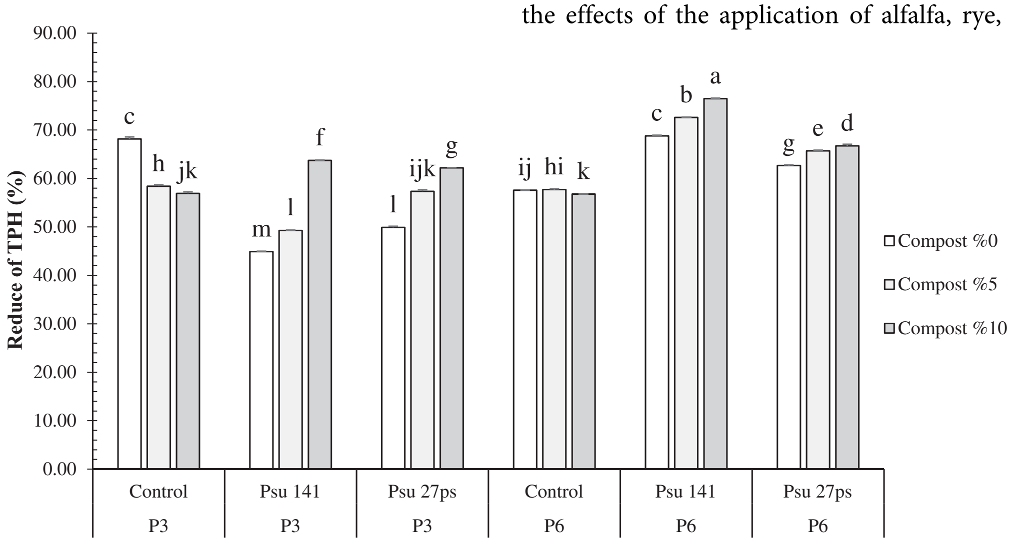

Figure 2. Means comparison interaction effects of crude oil × bacteria × compost on decomposition value of crude oil in soil cultivated with *Cordia myxa* L. P: crude oil; C: compost; B: bacteria. P3: 3% crude oil; P6: 6% crude oil; Control: without bacterial inoculation; psu 141: with *Pseudomonas* 141 inoculation; psu 27ps: with *Pseudomonas* 27ps inoculation.

Table 12. Results of determining C10–C35 values in soil cultivated with *Cordia myxa* L. under different treatments.

Concentration (mg/kg)

Treat	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
$P_3 \times B_0 \times C_0$	1	1	1	2	3	6	7	19	9	9	11	10	9	8	9	8	8	6	4	5	6	5	3	3	3	2
$P_3 \times B_0 \times C_5$	1	1	1	2	5	8	10	12	14	11	14	12	11	10	9	9	11	6	7	7	7	7	4	4	3	4
$P_3 \times B_0 \times C_{10}$	1	1	1	3	7	11	13	14	15	12	14	12	12	10	11	11	10	7	6	6	8	6	5	5	6	3
$P_3 \times B_1 \times C_0$	1	1	1	4	12	24	30	36	32	30	31	27	25	21	23	19	19	14	13	11	12	10	7	7	6	5
$P_3 \times B_1 \times C_5$	1	1	1	2	6	13	18	23	22	21	23	20	20	17	18	16	16	13	10	10	10	10	7	6	5	4
$P_3 \times B_1 \times C_{10}$	1	1	1	1	4	8	10	12	14	11	12	11	11	9	9	9	9	6	6	6	6	6	5	5	4	4
$P_3 \times B_2 \times C_0$	1	1	1	2	5	9	13	17	17	16	19	16	15	13	14	12	13	9	9	8	10	9	6	6	5	5
$P_3 \times B_2 \times C_5$	1	1	1	2	5	10	14	17	17	16	18	17	15	13	14	12	13	8	8	8	9	8	5	6	6	5
$P_3 \times B_2 \times C_{10}$	1	1	1	1	3	6	7	9	8	8	9	8	8	7	6	7	8	5	4	5	6	5	3	5	4	3
$P_6 \times B_0 \times C_0$	1	1	1	3	7	10	13	17	17	16	18	15	15	13	15	14	14	9	7	8	10	9	5	5	4	4
$P_6 \times B_0 \times C_5$	1	1	2	8	20	32	28	43	45	41	40	34	32	28	30	24	27	18	17	16	16	13	9	8	8	5
$P_6 \times B_0 \times C_{10}$	1	1	2	6	13	21	25	29	26	24	26	23	21	18	17	25	18	12	10	10	13	9	5	6	4	4
$P_6 \times B_1 \times C_0$	1	1	1	4	15	28	36	41	38	34	37	31	28	24	26	22	21	15	14	12	13	10	7	7	6	5
$P_6 \times B_1 \times C_5$	1	1	1	8	15	21	25	24	22	25	21	20	17	20	18	17	17	11	11	11	12	11	6	8	8	6
$P_6 \times B_1 \times C_{10}$	1	1	1	5	10	15	17	20	30	17	21	18	17	14	15	16	16	11	10	11	12	8	6	8	9	5
$P_6 \times B_2 \times C_0$	1	1	1	4	13	24	31	37	33	31	36	30	30	24	28	25	24	16	15	15	18	14	10	10	7	7
$P_6 \times B_2 \times C_5$	1	1	1	4	11	19	24	28	32	25	29	25	24	20	23	22	21	15	15	13	14	12	7	9	6	5
$P_6 \times B_2 \times C_{10}$	1	1	1	3	12	18	23	27	31	25	29	24	23	19	22	21	19	13	13	12	13	11	7	6	4	5

the phytoremediation of pyrene in three different soils and reported that compost application increased pyrene degradation significantly. The statistical analysis also indicated that there was an appropriate correlation between the percentage of TPH degradation and the total number of microorganisms (Ouvrard *et al.* 2014). Afzal *et al.* (2014) observed that root secretions significantly promoted the biological degradation of phenanthrene in the rhizosphere, which occurred either due to an increased bioavailability or increased soil microbial activity and population. Wang *et al.* (2008) reported that the cultivation of plants like *Festuca arundinaceu* reduced the amount of crude oil in the soil 3–4 times more effective compared to when there were no plants.

In general, by the production of root secretions, plants promote the biological degradation of oil hydrocarbons in two ways: (1) increasing the activity and growth of oil hydrocarbon-degrading microorganisms; and (2) increasing the bioavailability of oil compounds. However, root secretions also provide N, P, and C as well as micronutrients, which are required for the growth of hydrocarbon-degrading microorganisms. Moreover, root secretions may supply the primary carbon substrate for microorganisms, which are responsible for the biological conversions of metabolism.

C10–C35 compounds

The investigation of the level of C10–C35 compounds showed that C10–C13 compounds were present at small levels in the soil in all treatments. C32–C35 compounds were also present in the soil at small levels, but compounds with lower carbon chains (C15–C25) were most abundant in the soil studied (Table 11). With increasing the compost content at both levels of pollution and in all treatments, whether contained bacteria or not, carbon compounds reduced, and this decrease was more clear in compounds that contained fewer carbon atoms (Table 12). The presence of numerous compounds containing a moderate number of carbons in the soil is an indication of the breaking of long chains of the crude oil affected by plant or oil degrading-bacteria. It is presumed that bacteria at first break weaker bounds in long chains, which is the reason for the increased compounds containing fewer carbon atoms. A reason for the presence of compounds containing fewer carbon atoms is the more availability of more easily degradable materials and unsuitable conditions for the degradation of compounds that are more difficult to degrade, which may become more as time passes. A reason for the reduction of carbon compounds with increasing the compost content can be the absorption of these compounds by compost, and the level of this absorption increases with more short carbon chains. Feng *et al.* (2014) in a soil with *F. arundinaceu* plants assessed the effectiveness of different ratios of sludge compost (0, 10, 25, and 50% by weight) in the reduction of multi-chain aromatic hydrocarbons. They concluded that at different times of sampling, the residual values of multi-chain aromatic hydrocarbons in the planted soil were far less than in the non-planted soil. After 126 days, the lowest residual value in the soil was observed in the soil treated with a 10%

compost. These results indicate that both of the addition of compost and plant cultivation improve the degradation of multi-chain aromatic hydrocarbons in the soil and increase the activity of soil dehydrogenase enzyme, which is due to increased microbial activity and population.

Conclusion

The greatest level of degradation of crude oil was observed in the treatment with 6% pollution in the presence of bacterium psu141 and with the highest level of compost under *C. myxa* cultivation. Furthermore, with increasing the compost content, the level of carbon compounds reduced, because of their absorption on the surface of the compost, and this reduction increased with short carbon chains (due to more absorption on compost). Although compost did not have a direct role in the reduction of pollution, it supported the plant and bacterial growth with its nutrients, thereby indirectly increasing the rate of hydrocarbon degradation. Overall, the highest rate of remediation of TPH was recorded in the treatment containing *Pseudomonas* PSU 141 and 10% compost. Thus, the creation of suitable physical and chemical conditions for the microorganisms and the improvement of the permeability of the soil for water and air movements are important for the microorganism growth and therefore for the remediation.

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