Journal of Cleaner Production 253 (2020) 119719

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Conjunction of *Vetiveria zizanioides* L. and oil-degrading bacteria as a promising technique for remediation of crude oil-contaminated soils

Z. Kiamarsi [a], M. Kafi [a, *], M. Soleimani [b], A. Nezami [a], S. Lutts [c]

[a] Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, 91779-48974, Iran
[b] Department of Natural Resources, Isfahan University of Technology, Isfahan, 84156-83111, Iran
[c] Life Sciences Institute, Catholic University of Louvain, Louvain-la-Neuve, 1348, Belgium

ARTICLE INFO

Article history:
Received 21 September 2019
Received in revised form
23 November 2019
Accepted 13 December 2019
Available online 14 December 2019

Handling editor: Prof. Bing-Jie Ni

Keywords:
Degradation efficiency
Hydrocarbon degrading bacteria
Physiological traits
Phytoremediation
Tolerance index

ABSTRACT

Oil pollution is a great threat to all forms of aquatic and terrestrial life. Vetiver (*Vetiveria zizanioides* L.) is a C_4 perennial grass which can grow on diverse environments and may have the potential to be used for remediation of contaminated areas. Therefore, a controlled greenhouse experiment was conducted to study growth performance, petroleum tolerance and total petroleum hydrocarbons (TPHs) removal potency of vetiver and hydrocarbon-degrading bacteria over a period of 120 days in contaminated soils with various oil concentrations (0, 2, 4, 6, 8, 10, and 12% w/w). The results showed that chlorophyll (Chl) *a* and Chl *b* content decreased in contaminated soils, although carotenoid content increased. Vetiver grass showed no sign of toxicity and thrived well in contaminated soils by applying survival approaches. Total antioxidant activity, malondialdehyde, and proline contents in root and shoot of vetiver increased in the presence of crude oil. The results revealed that decreasing of TPHs was in the range of 47–77% by vetiver, 53.3–87.4% by bacteria, and 57.5–84.6% by plant-bacteria treatments. Therefore, this plant could be used effectively for cleansing crude oil-contaminated soil, particularly in the presence of degrading bacteria. However, it needs more studies in field conditions where the physicochemical and biological characteristics of natural polluted soils may affect plant and bacteria remediation efficiency.

1. Introduction

Soil, as a part of the biosphere, is one of the main bedrocks for food production and environmental health. Fuel-contaminated soils imposed multiple forms of adversely health impacts on human, fisheries and agriculture ecosystems, and other organisms by oil seepage and spills as well as the emission of toxic contaminations and carbon dioxide (CO_2) (Perera, 2018). The pollution of air, aquatic and terrestrial ecosystems during producing, transporting, and refining crude oil as the most predominant forms of anthropogenic pollution is of great concern for environmentalists (Masnadi et al., 2018). Contaminated soil is a source of acute and chronic illnesses such as intoxication, cancer, congenital disabilities, preterm birth, and cardiovascular diseases. Crude oil affects the safety of soil ecological system and causes long-running, severe impacts on land devoted to agriculture, resulting in a negative impact on crop plants, and finally public human health (Athar et al., 2016; Ramirez et al., 2017; Gaur et al., 2018).

Acute and high innate toxicity of the most petroleum hydrocarbons and also the insufficient aeration conditions lead stress effects on root and dysfunction of plant growth (Ashraf and Rehman, 1999). In addition, petroleum molecules absorbed by plants grown on contaminated soil can alter in the structure and permeability of the cell membrane (Peña-Castroa et al., 2006). It was observed that oil pollution has some toxicity impacts on alfalfa (Martí et al., 2009). These effects may cause a reduction in root and shoot phytomass, declined photosynthesis pigments and antioxidant activity, dissolving cell membrane and changing the protective function of compatible solutions (Liao et al., 2015), as well as, increasing reactive oxygen species (ROS) production via oxidative stress (Zhou and Yu, 2010).

The remediation of contaminated soil via an effective technology can be imperative (Zhao et al., 2019). Therefore, biological alternatives such as bioremediation with highly effective options attract much attention for removal of oil (Pi et al., 2017). Phytoremediation is a more environment-friendly technology that has a promising role in the management and treating petroleum-contaminated soils (Luo et al., 2016; Wang et al., 2019). It has

* Corresponding author.
E-mail address: m.kafi@um.ac.ir (M. Kafi).

https://doi.org/10.1016/j.jclepro.2019.119719
0959-6526/

Abbreviations

BAF	bioaccumulation factor
BCF	bioconcentration factor
Chl	chlorophyll
MDA	malondialdehyde
MDA_r	malondialdehyde content of root
MDA_s	malondialdehyde content of shoot
PAHs	polycyclic aromatic hydrocarbons
PRO	proline
PRO_r	proline content of root
PRO_s	proline content of shoot
EL	electrolyte leakage
REL	root electrolyte leakage
SEL	shoot electrolyte leakage
ROS	reactive oxygen species
TAC	total antioxidant capacity
TAC_r	total antioxidant capacity of root
TAC_s	total antioxidant capacity of shoot
TF	translocation factor
TI	tolerance index
TI_r	tolerance index of root
TI_s	tolerance index of shoot
TPHs	total petroleum hydrocarbons

been reported that specified plants and microorganisms have a crucial ability to sequester and/or neutralize petroleum hydrocarbons under affordable and eco-friendly conditions (Cai et al., 2016). The potency of a remediator plant can be improved by conjunction with specific microbes or by adding some soil amendments such as biochar which has the ability to increase plant production and detoxification potential (Ebadi et al., 2018; Maroušek et al., 2019). The plant-microbe system regarded as a novel, effective, and low-cost techniques in the remediation of organic contaminated-soil (Ni et al., 2018; Sarma et al., 2019). Supplemented the phytoremediation technique with petroleum hydrocarbon-degrading bacteria can enhance the remediation of crude oil-contaminated soil (Oliveira et al., 2015).

Grasses with multiple ramified root structure are superior for phytoremediation of oil-contaminated soils, since they increase the rhizospheric zone and make a suitable condition for degradation process (Soleimani et al., 2010; Bramley-Alves et al., 2014). Vetiver grass (*Vetiveria zizanioides* L.) with a large phytomass and long extreme robust massive root system undergo the elevated and toxic amounts of acidity, alkalinity, salinity, heavy metal and agrochemical compounds (Banerjee et al., 2016; Badejo et al., 2017). Therefore, this grass could be used as an effective means for remedying both surface and deeper contaminants (Pandey and Singh, 2015; Panja et al., 2018).

Although there is a wide information about vetiver reactions in varieties of organic and inorganic contaminated areas, still a detailed and comprehensive study on possible damage and physiological response of this grass under different crude oil concentrations is lacking. Such analyses are considered to be a helpful measurement in demonstrating plant tolerance on polluted environments.

Accordingly, the current work was aimed to evaluate the capability of vetiver grass to withstand oil toxins by analyzing the growth, physiological and biochemical reaction of this plant grown on crude-oil contaminated soil. These reactions may differ in the presence of sole plant and oil-degrading bacterial consortium simultaneously and may also increase our knowledge of vetiver-bacteria cooperation in crude oil-spiked soils. We hypothesized that vetiver grass has a high potential to grow on oil-contaminated soils and the presence of hydrocarbon-degrading bacteria would enhance its growth as well as its remediation efficiency.

2. Material and methods

2.1. Experimental layout

The experiment was set up in a completely randomized design arranged in factorial scheme with three replicates. Crude oil at seven concentrations (W_{oil}/W_{soil}), including zero (C_0), 2.0 (C_2), 4.0 (C_4), 6.0 (C_6), 8.0 (C_8), 10.0 (C_{10}), and 12.0% (C_{12}) was used as main factor. Bacterial inoculant at two levels, with (B^+) and without (B^-) bacteria, was used as the second factor. Five-native bacterial isolates were used in one consortium in B^+ treatments and clean soil (C_0) considered as control. Therefore, three kind of treatments for each contamination level were used as phytoremediation, bacterial remediation, and phyto-bacterial remediation (combined treatments). Vetiver (*Vetiveria zizanioides* L.)-enhanced removal of crude oil was indicated in phytoremediation groups, crude oil-degrading bacteria was used as one consortium in bacterial remediation treatments, and the cooperation of vetiver and degrading bacteria was used in combined treatment.

2.2. Preparation of experimental treatments

Uncontaminated soil with detectable neither organic nor inorganic pollution was collected from the research farm of Ferdowsi University of Mashhad, Iran. The soil was air-dried, sieved through a 2 mm mesh and identified as a sandy loam (with clay 11.6, silt 31.4, sand 57%) texture soil. Seven subsamples of soil, 48 kg each, laid out thinly in order to be spiked with crude oil. Different crude oil concentration was dissolved in acetone and were added to each group of soil samples gradually and mixed thoroughly. Treated soils covered with dark thick polyethylene bags and placed at greenhouse temperature within 16 weeks for aging. During the incubation time, the treated soils were blended twice a week and the moisture of soils were controlled in a stable condition to guarantee the homogeneity of pollutants. TPHs content and some characteristics of spiked soils were measured before transferring to experimental pots using standard methods (Carter and Gregorich, 2007) (Table 1).

Soil samples were inoculated with one consortium of five native oil-degrading bacteria in phyto-bacterial remediation groups. These bacteria species (*Pseudomonas resinovorans*, *Plantibacter auratus*, *Bacillus subtilis*, *Staphylococcus pasteuri*, and *Bacillus atrophaeus*) were isolated previously from aged crude oil-contaminated soils (Kiamarsi et al., 2019). Bacterial isolates were grown in broth medium (at 30 °C) on a rotary shaker (180 rpm), then they were washed with 0.85% sterile saline solution, and were suspended in sterile deionized water. These cultures were used as inoculums (4.0×10^8 CFU mL^{-1}) and the consortium was transferred into bacterial inoculation treatment pots to attain 10^8 cells g^{-1} of dry soil two weeks before planting. The schematic of the experimental design was shown in Fig. 1.

2.3. Experimental setup and sampling

The pots (4.0 kg DW soil pot^{-1}) were lined with gravel and sand and filled with treated soil samples. Forty-five days-old

Table 1
The concentration of petroleum hydrocarbon and some characteristics of soil samples contaminated with different crude oil concentrations and control (garden) soil before remediation (Control $_{To}$).

Analysis	Crude oil content						
	C_0	C_2	C_4	C_6	C_8	C_{10}	C_{12}
Petroleum hydrocarbon, mg kg^{-1}							
N-alkanes	ND	14902 ± 200	23057 ± 1492	27540 ± 1863	32108 ± 505	41592 ± 4216	50416 ± 1936
PAHs	ND	5.79 ± 2.59	10.77 ± 1.05	12.81 ± 2.09	15.03 ± 4.45	24.85 ± 2.84	28.70 ± 4.92
TPHs	ND	14908 ± 198	23068 ± 1491	27553 ± 1862	32123 ± 501	41617 ± 4219	50445 ± 1934
Soil pH	7.92 ± 0.14	7.95 ± 0.24	7.95 ± 0.24	7.95 ± 0.24	7.95 ± 0.24	7.95 ± 0.24	7.95 ± 0.24
Organic carbon, %	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09
Total nitrogen, %	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Phosphorous, mg kg^{-1}	116.30 ± 0.05	116.30 ± 0.05	116.30 ± 0.05	116.30 ± 0.05	116.30 ± 0.05	116.30 ± 0.05	116.30 ± 0.05
Sodium, mg kg^{-1}	95.60 ± 9.98	180.20 ± 17.30	415.40 ± 80.90	962.80 ± 30.70	981.08 ± 2.68	1028.50 ± 16.20	1007.50 ± 37.10
Potassium, mg kg^{-1}	491.46 ± 11.85	491.46 ± 11.85	491.46 ± 11.85	491.46 ± 11.85	491.46 ± 11.85	491.46 ± 11.85	491.46 ± 11.85
Iron, mg kg^{-1}	179.7 ± 18.20	920.5 ± 91.70	2155 ± 240.00	3166.50 ± 1.10	3202.00 ± 44.4	3498.70 ± 100.20	3167.70 ± 50.60
Trace elements, mg kg^{-1}							
Cu	6.39 ± 0.31	16.55 ± 1.56	35.30 ± 1.75	49.36 ± 0.02	54.55 ± 0.22	53.145 ± 1.09	50.95 ± 0.70
Zn	8.15 ± 1.05	19.24 ± 12.28	61.79 ± 10.55	85.14 ± 8.26	92.16 ± 8.79	104.10 ± 45.50	87.50 ± 17.70
Ni	ND	16.72 ± 1.51	23.61 ± 3.29	43.87 ± 4.61	50.91 ± 3.58	49.36 ± 1.64	51.29 ± 0.93
Pb	2.80 ± 0.45	40.13 ± 0.87	128.38 ± 0.57	87.9 ± 20.80	152.46 ± 11.14	152.60 ± 15.90	152.63 ± 6.01
Cd	ND	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
V	ND	ND	46.97 ± 3.23	64.9 ± 14.40	97.69 ± 4.69	95.91 ± 0.27	94.58 ± 0.96

Data are presented as Mean ± SD with three replicates (n = 3). C_0, C_2, C_4, C_6, C_8, C_{10}, and C_{12} are 0, 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil. PAHs: polycyclic aromatic hydrocrbons, TPHs: total petroleum hydrocarbons, ND: not detected.

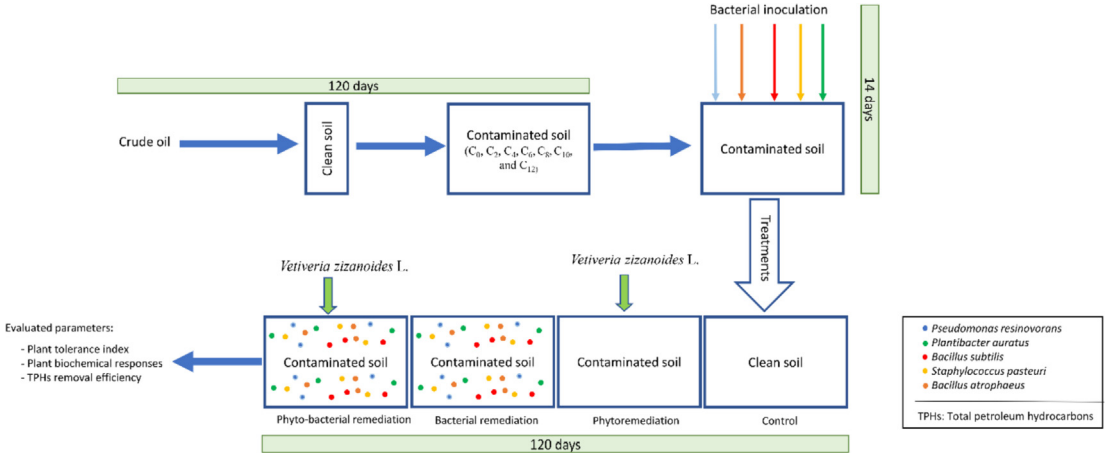

Fig. 1. Schematic description of experimental design.

vetiver plantlets were cultivated in planting materials to develop root system for two weeks, then cut to 25 cm length, and calibrated according to phytomass uniformity. Plants were cultured in crude-oil contaminated soils (two plants pot^{-1}) in a regulated greenhouse, with an average day/night temperature of 32 ± 3/22 ± 3 °C. The light/dark cycle was 14/10 h under irradiance of 500 ± 50 μmol m^{-2} s^{-1} PAR. All pots were watered (tap water) similarly just below field capacity, so no leaching was observed.

Plants were harvested after 120 days of growth. Shoots were cut at 2 cm above the soil surface, and washed with deionized water. Then, the pots were emptied and the roots were separated from the soil through washing with tap water. To take away soil particles, roots were rinsed three times with deionized water. All plant samples were dried using an oven drying cabinet at 70 °C to reach a constant weight and dry weight of root and shoot tissues were determined. All soils near the rhizosphere were sampled and kept at 4 °C until analysis in order to quantify crude oil hydrocarbons.

2.4. Plant measurements

2.4.1. Assessment of tolerance index (TI)

To evaluate the ability of the plant to grow on crude oil-contaminated soil, with respect to the control, the tolerance index was calculated according to Eq. (1) (Banerjee et al., 2018):

$$TI(\%) = DW\ of\ the\ treated\ plant\ parts / DW\ of\ the\ control\ plants \times 100 \quad (1)$$

2.4.2. Estimation of electrolyte leakage

Electrolyte leakage is generally considered as an indirect measure of cell membrane damage on various plant tissues which may also cause from injury of membrane components. Here, After 120 days of plant growth, 15 freshly cut discs (0.5 cm^2 each) of the youngest developed leaf and a subset of fresh roots (100 mg) from each treatment were rinsed two times with double-distilled water.

Then, the samples were floated on 20 mL of double-distilled water and placed on laboratory conditions at 25 ± 2 °C. After 24 h, the electrolyte conductivity in the solutions was measured using EC meter (JENWAY, 4510). The maximum electrical conductivity (EC_2) was measured after autoclaving samples at 121 °C with 1.2 bar pressure for 20 min. The results expressed as the percentage of electrolyte leakage in the shoot (SEL) and the root (REL) parts according to Eqs. (2) and (3) (Zhou and Yu, 2010):

$$SEL(\%) = (EC_{s1} - EC_w / EC_{s2} - EC_w) \times 100 \quad (2)$$

$$REL(\%) = (EC_{r1} - EC_W / EC_{r2} - EC_w) \times 100 \quad (3)$$

where EC_{s1} and EC_{r1} are initial conductance of shoot and root tissues, respectively; EC_{s2} and EC_{r2} are the maximal conductance (EC_{max}) of shoot and root tissues, respectively and EC_w is the deionized water conductance.

2.4.3. Biochemical assay

2.4.3.1. Chlorophyll and carotenoid assay. Chlorophyll and carotenoid as plant pigments are significant in the function of photosynthesis apparatus and also plant biomass production under biotic and abiotic stresses. The optical density of chlorophyll *a* (Chl *a*), Chl *b*, and carotenoid extraxts were spectrophotometrically (SP-3000 plus, OPTIMA INC. Tokyo, Japan) measured at 664, 648, and 470 nm, respectively (Lichtenthaler and Wellburn, 1983). The spectrophotometer readings convert to Chl and carotenoid contents using the following equations:

$$[\text{Chl } a] = [13.95 \times A_{665}] - [6.88 \times A_{649}] \quad (4)$$

$$[\text{Chl } b] = [24.96 \times A_{649}] - [7.32 \times A_{665}] \quad (5)$$

$$[\text{Carotenoid}] = [1000 \times A_{470}] - [20.5 \times Chl\ a] - [114.8 \times Chl\ b] \quad (6)$$

Here, A is absorbance value at the given wave length.

2.4.3.2. Assessment of malondialdehyde content. Malondialdehyde (MDA) is a by-product of the lipid peroxidation process, widely used as an indicator for assessing oxidative stress in biological fields. MDA content in the shoot (MDA_s) and the root (MDA_r) tissues was assayed by the method of Heath and Packer (1968).

2.4.3.3. Assessment of proline content. Proline (PRO), an α-amino acid, is regarded as an osmotic regulator in plants which can decrease osmotic damage. The content of PRO in shoot (PRO_s) and root (PRO_r) tissues was measured according to the procedure of Bates et al. (1973) and the absorbance read at 520 nm using a spectrophotometer.

2.4.3.4. Assessment of total antioxidant capacity. To determine the radical scavenging ability of plant, total antioxidant capacity in shoot (TAC_s) and root (TAC_r) was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) following Brand-Williams et al. (1995) method.

2.5. Contaminant analysis

The extraction process of TPHs in treated soil and plant samples was performed using the ultrasonication technique (Teng et al., 2011; Zhang et al., 2011). TPHs extraction of bacteria treatment was conducted based on the procedure of Mojarad et al. (2016). To assay the concentration of TPHs, an Agilent gas chromatography-mass spectrometry (GC-MS) was applied. The Thermo Scientific ISQ Q Trace 1310 gas chromatograph was fitted with a splitless injector, a fused-silica capillary column (Restek RXi: 5Sil-MS 30 m × 0.25 mmID × 0.25 μm). Helium at the flow rate of 1.2 mL min^{-1} was used as the carrier gas, and the aliquots of 1 μL were injected in splitless mode. The injector was set at 250 °C, and the GC oven temperature was programmed from 35 °C for 12 min and increased to 315 °C at 8 °C min^{-1}.

2.6. Accumulation and translocation of TPHs

The bioaccumulation factor (BAF), bioconcentration factor (BCF), and translocation factor (TF) were used to assess the plant phytoextraction ability for TPHs according to Eqs. (7)–(9) (Hu et al., 2019):

$$BCF = [Petroleum\ hydrocarbons]_{root} / [Petroleum\ hydrocarbons]_{soil} \quad (7)$$

$$BAF = [Petroleum\ hydrocarbons]_{shoot} / [Petroleum\ hydrocarbons]_{soil} \quad (8)$$

$$TF = [Petroleum\ hydrocarbons]_{shoot} / [Petroleum\ hydrocarbons]_{root} \quad (9)$$

2.7. Statistical analysis

In order to examine the differences among treatments, data were subjected to one-way analysis of variance (ANOVA) in SAS V9.4 software. Mean comparison was determined using the Tukey test ($P < 0.05$).

3. Results

3.1. Tolerance index (TI)

Crude oil contamination decreased shoot tolerance index (TI_s), conversely an increasing trend was observed on the root tolerance index (TI_r). The interaction of crude oil and bacteria enhanced TI_r significantly ($P < 0.05$) by 20.8, 49.4, 46.7, and 29.1% at C_4, C_6, C_8, C_{10}, and C_{12}, respectively relative to TI_s (Figs. 2 and 3).

3.2. Leaf chlorophyll and carotenoids content

Chl *a* content was increased in the non-inoculated contaminated soil (<C_{10}) compared to the control ($C_0 \times B^-$), while it was decreased at C_{10} and C_{12}. This parameter increased under all crude oil-contaminated soil inoculated with bacteria (Table 2). The content of Chl *b* increased 10.2–50.2% in all contaminated inoculated soil (except for C_{12}) in comparison with the respective non-inoculated one (Table 2). Crude oil concentration, bacterial consortium, and their interactions significantly affected the leaf carotenoid content. Generally, the carotenoid content in all contaminated inoculated and non-inoculated treatment was higher than that of the respective control ($P < 0.05$) (Table 2).

3.3. Proline content

PRO content, both in the root (PRO_r) and the shoot (PRO_s), was affected by crude oil concentration, bacterial inoculation, and crude oil × bacteria interactions (Table 3). The highest value of PRO_r (0.04 ± 0.01 mg g^{-1} FW) and PRO_s (0.41 ± 0.05 mg g^{-1} FW) was

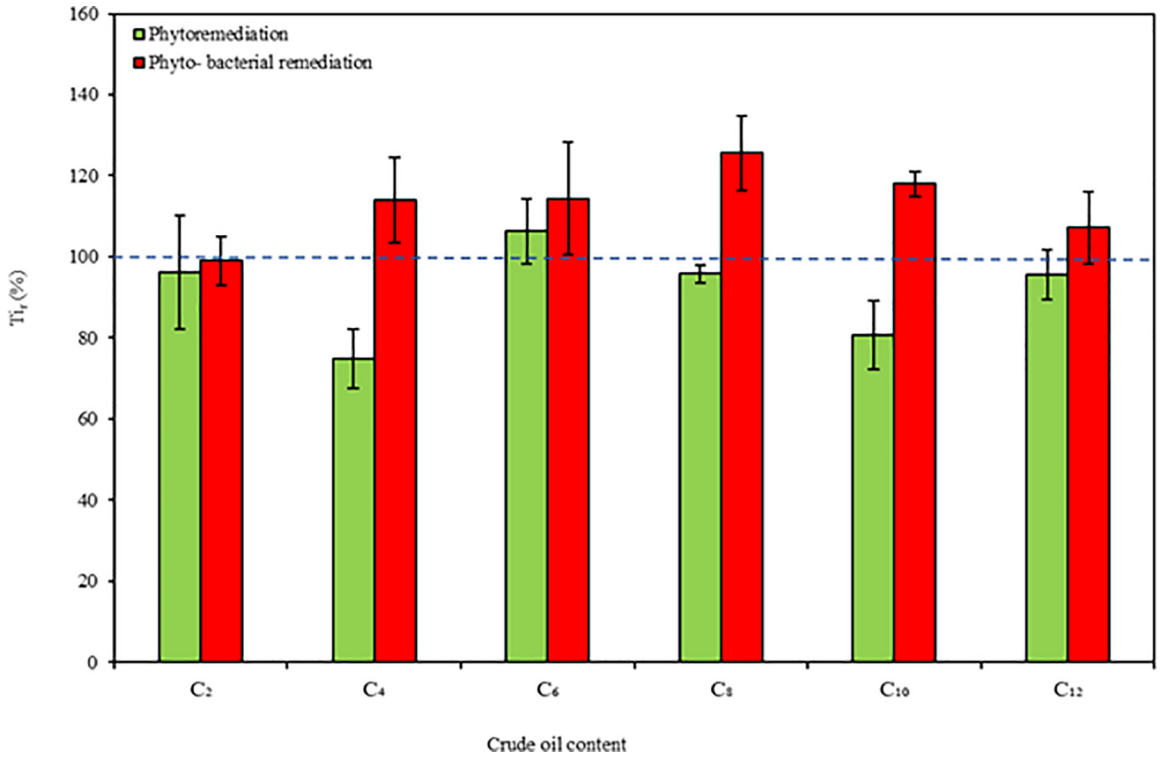

Fig. 2. Tolerance index of vetiver roots (TI_r) grown on different crude oil-contaminated soil in phytoremediation and phyto-bacterial remediation treatments bacteria for 120 days. Dashed line shows control (non-spiked soil). Bars on the top of each column are SEM. C_2, C_4, C_6, C_8, C_{10}, C_{12}, are 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

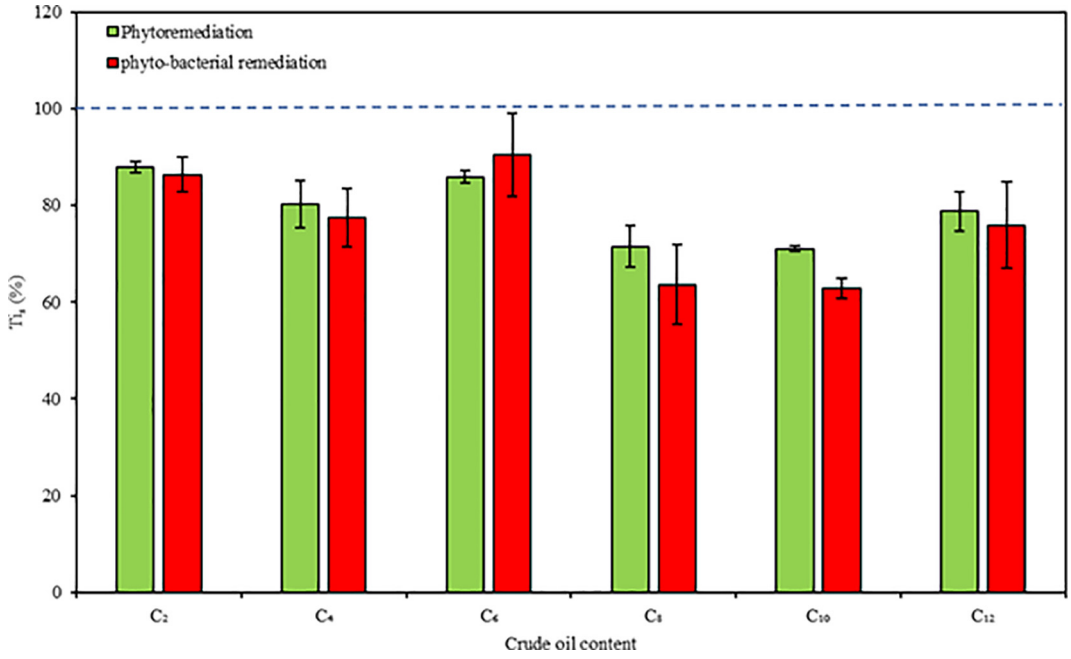

Fig. 3. Tolerance index of vetiver shoots (TI_S) grown on different crude oil-contaminated soil in phytoremediation and phyto-bacterial remediation treatments bacteria for 120 days. Dashed line shows control (non-spiked soil). Bars on the top of each column are SEM. C_2, C_4, C_6, C_8, C_{10}, C_{12}, are 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

observed at C_{12} and C_{10}, respectively. The results showed that the interaction between treatments caused an increase in the content of PRO_r and 11.6% and PRO_s to 8.5% compared to the respective control treatments.

3.4. Malondialdehyde content

Similar to proline, crude oil, bacteria inoculation, and their interactions caused a significant result ($P < 0.05$) on MDA expression in root (MDA_r) and shoot (MDA_s) of vetiver grass (Table 3). As a

Table 2
Leaf chlorophyll (Chl), Chl *a* and Chl *b*, and carotenoid contents (mg g^{-1} Fw) of vetiver grass grown on different crude oil-contaminated soil inoculated (B^+) and non-inoculated (B^-) with oil-degrading bacteria for 120 days.

Treatments	Chl *a*	Chl *b*	Carotenoid
Crude oil (*C*)			
C_0	0.39 ± 0.07^{bcA}	$0.30 \pm 0.07^{a-c}$	0.01 ± 0.00^{b}
C_2	0.59 ± 0.08^{a}	0.40 ± 0.15^{a}	0.03 ± 0.00^{b}
C_4	0.53 ± 0.12^{a}	0.37 ± 0.03^{a}	0.04 ± 0.00^{b}
C_6	0.53 ± 0.07^{ab}	0.33 ± 0.10^{ab}	0.03 ± 0.01^{b}
C_8	$0.53 \pm 0.09^{b-d}$	$0.23 \pm 0.06^{b-d}$	0.09 ± 0.02^{a}
C_{10}	0.46 ± 0.09^{cd}	0.19 ± 0.07^{cd}	0.11 ± 0.06^{a}
C_{12}	0.36 ± 0.16^{d}	0.16 ± 0.01^{d}	0.11 ± 0.01^{a}
Bacteria (*B*)			
B^-	0.43 ± 0.12^{b}	0.25 ± 0.09^{b}	0.07 ± 0.05^{a}
B^+	0.54 ± 0.10^{a}	0.32 ± 0.12^{a}	0.05 ± 0.03^{b}
B × C			
$B^- \times C_0$	0.40 ± 0.11	$0.33 \pm 0.09^{a-c}$	$0.01 \pm 0.00d^{e}$
$B^- \times C_2$	0.60 ± 0.10	$0.27 \pm 0.02^{b-e}$	$0.03 \pm 0.00^{c-e}$
$B^- \times C_4$	0.45 ± 0.08	0.36 ± 0.05^{b}	$0.04 \pm 0.00^{c-e}$
$B^- \times C_6$	0.48 ± 0.06	0.31 ± 0.09^{bc}	0.02 ± 0.00^{de}
$B^- \times C_8$	0.46 ± 0.04	$0.18 \pm 0.03^{c-e}$	0.11 ± 0.01^{b}
$B^- \times C_{10}$	0.38 ± 0.04	0.14 ± 0.02^{e}	0.17 ± 0.03^{a}
$B^+ \times C_{12}$	0.23 ± 0.04	0.16 ± 0.00^{de}	0.11 ± 0.01^{b}
$B^+ \times C_0$	0.37 ± 0.00	$0.27 \pm 0.03^{c-e}$	0.01 ± 0.00^{e}
$B^+ \times C_2$	0.59 ± 0.09	0.54 ± 0.06^{a}	$0.03 \pm 0.00^{c-e}$
$B^+ \times C_4$	0.62 ± 0.10	0.38 ± 0.01^{b}	$0.04 \pm 0.00^{c-e}$
$B^+ \times C_6$	0.58 ± 0.06	0.35 ± 0.12^{b}	$0.04 \pm 0.00^{c-e}$
$B^+ \times C_8$	0.60 ± 0.07	$0.28 \pm 0.05^{b-d}$	0.06 ± 0.01^{c}
$B^+ \times C_{10}$	0.54 ± 0.04	$0.25 \pm 0.07^{c-e}$	0.05 ± 0.01^{cd}
$B^+ \times C_{12}$	0.48 ± 0.14	0.16 ± 0.02^{de}	0.11 ± 0.01^{b}
C	**	**	**
B	**	**	**
C × *B*	*NS*	**	**

Data are presented as Mean ± SD with three replicates (n = 3). [A] Different letter in each row represent significant differences with the Tukey test ($P < 0.05$). *, **, and *NS* indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, and non-significant, respectively. C_0, C_2, C_4, C_6, C_8, C_{10}, and C_{12} are 0, 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

whole, the MDA content of the plant significantly increased with an increase in crude-oil concentration in both inoculated and non-inoculated soils by 3.3–59.2% on MDA_s and 10.1–57.6% on MDA_s, compared to non-spiked soil ($C_0 \times B^-$). The content of both MDA_r and MDA_s decreased >1.06 times in the presence of bacteria when compared to the same contaminated non-inoculated treatments.

3.5. Electrolyte leakage

Although treatments and their interactions influenced shoot EL (EL_s) not significantly, root EL (EL_r) changed significantly ($P < 0.05$) under crude oil concentration and crude oil × bacteria interactions (Table 4). The interaction of contaminated soil at < C_6 and bacteria caused an increasing trend on EL_r in inoculated groups when compared with non-inoculated ones. Non-inoculated treatments induced an increase on root EL_r by 22.8, 7.4, 19.5, and 21.4% at C_6, C_8, C_{10}, and C_{12}, respectively, compared to the respective bacterial inoculated levels.

3.6. Total antioxidant capacity

Crude oil-contaminated soil, bacterial inoculation, and their interactions influenced the production of total antioxidant capacity of roots (TAC_r) significantly ($P < 0.05$), while, no significant changes were observed on TAC_s (Table 4). The highest value of TAC_s (1.5 ± 0.06 mg g^{-1} FW) derived from the highest level of soil contaminated without bacterial inoculation (Table 4).

3.7. Accumulation and removal of TPHs

The RCF and SCF varied significantly among crude oil concentration (Table 5). RCF and SCF of TPHs varied from 0.08 to 0.16 and 0.01–0.04, respectively, at all crude oil concentrations. The lowest TF were observed at the highest contamination level with the value of 0.30. Bacterial inoculation considerably increased the accumulation of petroleum hydrocarbons in the both root and shoot tissues. Furthermore, the RCF, SCF, and TF showed a positive response in bacterial remediation treatments by 37.8, 48.4, and 13.9%, respectively, in comparison with non-inoculated treatments (Table 5).

The biodegradation of TPHs in planted soil was significantly ($P < 0.05$) higher than un-planted corresponding controls (Fig. 4). In general, TPH removal by vetiver was 46.3% greater than un-planted pots. The removal efficiency of petroleum hydrocarbons under crude oil × bacteria interactions was 6.2% and 16.8% higher than bacteria and plant treatment, respectively. As crude oil concentration increased, the role of bacteria on TPHs removal became greater (Fig. 5).

4. Discussion

In this study, the response of vetiver grass grown on crude oil-polluted soil was monitored through a period of 120 days. The biochemical and morpho-physiological traits of the cell intactness often provided useful informative indicators to estimate the degree of stress detriment or adaptation of plants. Present results showed that the TI_r and TI_s were >80.7% and >62.8%, respectively at all oil concentrations. On the other hand, our findings presented that root dry biomass and the root-shoot ratio of the vetiver enhanced on crude oil-contaminated soils (Data have not shown here). Therefore, it seems that vetiver translocated more assimilates to the belowground tissues in order to apply a survival approach under crude oil contamination because root has direct contact to soil pollution. This approach caused plant to increase root tolerance by 24.0% more than TI_s. Tolerance index regarded as an effective tool to select suitable plants for phytoremediation technology (Banerjee et al., 2018). According to Lux et al. (2004) as plant tolerance was more than 0.6 for root and shoot parts and in whole plant level, it could be concluded that vetiver can cope well with crude oil contamination and has a strong tolerance to grow on such these polluted soils.

Along with other abiotic stressors, chloroplasts are known as the first goal point of diesel toxicity (Xi et al., 2018). From the results, Chl content was higher than the control at < C_{10} for Chl *a* and at < C_8 for Chl *b*. It represented that the lower concentration of crude oil motivated the Chl expression in plant. Vetiver strategies at these concentrations might be described by a possible mechanism: The increase of vetiver root growth can be regarded as a benefit approach by providing a much greater sink for the accumulation of contaminants that restricted the transportation of crude-oil hydrocarbons from roots to shoots and keep more of them in the root tissues. The limitation entrance of contamination to leaves, as the location of photosynthesis reactions, is an effective tactic to protect nutrient synthesize system such as Chl in plants. On the other hand, by applying this approach plant would continue photosynthesis activity to produce more phytomass for survival and remediation process.

The intense toxicity impact of crude-oil hydrocarbons, especially PAHs, could be proposed to qualify the downward trend of Chl content at higher concentrations. Carotenoid content remained consistently higher than control in the plant exposed to oil pollution. Carotenoids as a group of indispensable pigments and non-enzymatic antioxidants in plants play an essential role in the

Table 3
Proline (PRO, mg g^{-1} Fw) and malondialdehyde (MDA, nmol g^{-1} Fw) contents of vetiver grass grown on different crude oil-contaminated soil inoculated (B^+) and non-inoculated (B^-) with oil-degrading bacteria for 120 days.

Treatments	Shoot		Root	
	PRO	MDA	PRO	MDA
Crude oil (*C*)				
C_0	0.12 ± 0.01^{cA}	76.18 ± 5.75^{b}	0.011 ± 0.00^{c}	26.12 ± 3.34^{d}
C_2	0.12 ± 0.02^{c}	76.13 ± 15.87^{b}	0.018 ± 0.00^{b}	31.22 ± 8.43^{cd}
C_4	0.21 ± 0.06^{b}	126.09 ± 13.18^{a}	0.019 ± 0.00^{b}	32.38 ± 3.77^{cd}
C_6	0.21 ± 0.09^{b}	127.66 ± 19.66^{a}	0.031 ± 0.00^{a}	37.21 ± 3.82^{b-d}
C_8	0.24 ± 0.07^{ab}	140.00 ± 43.10^{a}	0.031 ± 0.00^{a}	40.26 ± 6.69^{bc}
C_{10}	0.23 ± 0.10^{b}	121.30 ± 35.10^{a}	0.038 ± 0.00^{a}	54.09 ± 13.73^{a}
C_{12}	0.30 ± 0.13^{a}	143.50 ± 48.90^{a}	0.032 ± 0.00^{a}	44.90 ± 9.82^{ab}
Bacteria (*B*)				
B^-	0.26 ± 0.10^{a}	128.95 ± 45.36^{a}	0.029 ± 0.01^{a}	40.19 ± 13.92^{a}
B^+	0.15 ± 0.03^{b}	102.73 ± 24.41^{b}	0.023 ± 0.00^{b}	35.86 ± 8.12^{b}
B × C				
$B^- \times C_0$	0.12 ± 0.00^{d}	79.15 ± 4.90^{ef}	0.008 ± 0.00^{e}	26.96 ± 2.13^{c}
$B^- \times C_2$	0.12 ± 0.02^{d}	64.14 ± 12.06^{f}	0.01 ± 0.00^{cd}	27.91 ± 2.16^{c}
$B^- \times C_4$	0.13 ± 0.01^{bc}	119.52 ± 16.31^{c-e}	0.02 ± 0.00^{cd}	29.78 ± 1.70^{c}
$B^- \times C_6$	0.17 ± 0.04^{b}	131.90 ± 29.80^{b-d}	0.02 ± 0.00^{ab}	39.60 ± 2.06^{bc}
$B^- \times C_8$	0.19 ± 0.05^{b}	173.14 ± 8.85^{ab}	0.03 ± 0.00^{ab}	41.68 ± 8.30^{bc}
$B^- \times C_{10}$	0.3 ± 0.04^{b}	148.20 ± 18.40^{a-c}	0.3 ± 0.00^{ab}	66.21 ± 5.29^{a}
$B^+ \times C_{12}$	0.25 ± 0.03^{a}	186.68 ± 13.70^{a}	0.03 ± 0.00^{a}	49.21 ± 5.57^{bc}
$B^+ \times C_0$	0.12 ± 0.01^{d}	73.22 ± 5.69^{ef}	0.02 ± 0.00^{de}	25.28 ± 4.61^{c}
$B^+ \times C_2$	0.12 ± 0.05^{d}	88.12 ± 7.27^{d-f}	0.01 ± 0.00^{de}	34.52 ± 11.85^{bc}
$B^+ \times C_4$	0.17 ± 0.06^{cd}	73.22 ± 5.69^{b-d}	0.01 ± 0.00^{bc}	25.28 ± 4.61^{bc}
$B^+ \times C_6$	0.12 ± 0.02^{d}	132.66 ± 6.22^{b-e}	0.02 ± 0.01^{bc}	34.97 ± 3.52^{bc}
$B^+ \times C_8$	0.30 ± 0.06^{cd}	106.90 ± 35.70^{c-f}	0.03 ± 0.00^{b-d}	38.83 ± 6.08^{bc}
$B^+ \times C_{10}$	0.32 ± 0.16^{d}	94.40 ± 24.10^{d-f}	0.04 ± 0.01^{bc}	41.97 ± 1.63^{bc}
$B^+ \times C_{12}$	0.41 ± 0.05^{cd}	100.41 ± 14.25^{c-f}	0.03 ± 0.01^{bc}	40.60 ± 12.43^{bc}
C	**	**	**	**
B	**	**	**	*
C × *B*	**	**	**	**

Data are presented as Mean ± SD with three replicates (n = 3). [A] Different letter in each row represent significant differences with the Tukey test ($P < 0.05$). *, **, and *NS* indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, and non-significant, respectively. C_0, C_2, C_4, C_6, C_8, C_{10}, and C_{12} are 0, 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

photosynthesis and photoprotection process (Sun et al., 2018). The defensive functions of these natural pigments against abiotic stresses by quenching mechanisms of the electronically excited state of Chl, ROS-scavenging, and harmless dissipation of excess energy was referred by Ke et al. (2019). Therefore, it could be stated that vetiver increased the production of cartenoid under oil-polluted soil as a protective tool to reduce stress severity on the plant.

Biochemical features of the cell intactness provide very revealing and informative measures for estimating of stress adaptation or damage. In this study, the simultaneous increase in MDA and EL with an increase in concentration of crude oil content revealed that pollution lead to lipid peroxidation of the cell membrane and over-production of ROS in the vetiver, particularly in root tissues. The lower production of SEL relative to REL indicated that possibility leaf sustained less ROS production and oxidative damage. Here, crude oil increased production of organic solute (proline) in the leaf and root tissues. Several genes that contribute to the synthesis and catabolism of proline are regulated by the several stresses to have protection properties (Zegaoui et al., 2017). Besides, proline, which acts as an antioxidant, protects cell membranes against degradation process and stabilize photosynthesis mechanisms (Li et al., 2016; Pidatala et al., 2018).

In the present study, root antioxidant activity was increased in plants grown on crude oil contamination. The highest root antioxidant was observed at the highest level of crude oil concentration, while vetiver leaves showed no significant effect in the total antioxidant production. Crude oil can induce production of superoxide radicals and oxidative stress in different plant tissues. Therefore, the higher accumulation of ROS in roots at the higher crude oil concentrations stimulated production of total antioxidant activity defense system.

Microorganisms-plants partnership in the bioremediation technique attracted lots of attention over recent years. In this cooperation, plant roots and their exudates have a central role in organic polluted-soil which favored the environments for endophytic or rhizosphere microorganisms with degradation ability (Teng et al., 2011). This is an advantageous factor for degrading bacteria to colonize and survive in contaminated soil and consequently increased the removal efficiency of organic compounds significantly. The results can reveal the inoculation treatments significantly decreased the amounts of carotenoid pigment, TAC_r, MDA, PRO_s, and PRO_r under crude oil-contaminated soil. It could be speculated that bacterial consortium led to limit ROS production and its consequences on plant health. Besides, these bacteria are capable of using hydrocarbons as carbon and energy source, and thereby transform perilous hydrocarbons to harmless substances with less or no phytotoxicity effects.

Plants and rhizosphere microorganisms offer as potential tools to remediate chemical compounds biologically from the soil. In the current study, it was found that the degradation of TPHs in phytoremediation, bacterial remediation, and phyto-bacterial remediation treatments were comparatively higher than the control. High relative degradation ability of bacterial consortium suggests that combination of individual enzymes present in each strain allows to improve remediation efficiency.

The increment of TPHs removal by vetiver grass might be associated with its deep fibrous root as a rhizosphere effect. This

Table 4
Electrolyte leakage (EL, %) and total antioxidant capacity (TAC, mg g^{-1} Fw) in the roots and shoots of vetiver grass grown on different crude oil-contaminated soil inoculated (B^+) and non-inoculated (B^-) with oil-degrading bacteria for 120 days.

Treatments	Shoot		Root	
	EL	TAC	EL	TAC
Crude oil (*C*)				
C_0	77.54 ± 8.59	2.47 ± 0.13	44.87 ± 5.68b^{cA}	0.05 ± 0.00
C_2	77.30 ± 8.35	2.53 ± 0.24	43.10 ± 3.59^{c}	0.10 ± 0.03
C_4	77.15 ± 13.05	2.23 ± 0.18	46.18 ± 9.40^{bc}	0.24 ± 0.01
C_6	79.64 ± 5.74	2.33 ± 0.34	47.94 ± 9.71^{bc}	0.18 ± 0.04
C_8	83.40 ± 5.02	2.19 ± 0.31	55.04 ± 5.24^{ab}	0.21 ± 0.10
C_{10}	85.52 ± 4.90	2.34 ± 0.34	55.24 ± 8.84^{ab}	0.33 ± 0.04
C_{12}	84.31 ± 3.22	2.17 ± 0.06	61.03 ± 8.27^{a}	0.85 ± 0.78
Bacteria (*B*)				
B^-	80.19 ± 7.38	2.26 ± 0.22	52.38 ± 11.81	0.40 ± 0.40
B^+	81.20 ± 8.38	2.38 ± 0.30	48.59 ± 5.75	0.16 ± 0.09
B × C				
$B^- \times C_0$	71.69 ± 8.85	2.46 ± 0.17	41.54 ± 4.86^{c}	0.05 ± 0.01^{e}
$B^- \times C_2$	73.35 ± 3.70	2.54 ± 0.20	52.90 ± 7.31^{c}	0.12 ± 0.01^{d-e}
$B^- \times C_4$	79.59 ± 3.98	2.13 ± 0.22	42.47 ± 12.87^{c}	0.24 ± 0.01^{b-d}
$B^- \times C_6$	82.90 ± 5.82	2.22 ± 0.16	61 ± 13.13^{a-c}	0.20 ± 0.01^{cd}
$B^- \times C_8$	85.43 ± 7.11	2.09 ± 0.16	57.17 ± 1.24^{a-c}	0.28 ± 0.08^{bc}
$B^- \times C_{10}$	85.67 ± 7.14	2.27 ± 0.26	61.21 ± 6.52^{ab}	0.32 ± 0.06^{b}
$B^+ \times C_{12}$	82.72 ± 4.00	2.15 ± 0.05	68.37 ± 3.00^{a}	1.56 ± 0.06^{a}
$B^+ \times C_0$	83.40 ± 1.81	2.48 ± 0.12	48.19 ± 4.88^{bc}	0.06 ± 0.00^{e}
$B^+ \times C_2$	81.25 ± 10.66	2.51 ± 0.33	44.45 ± 5.10^{bc}	0.07 ± 0.01^{e}
$B^+ \times C_4$	74.70 ± 19.80	2.33 ± 0.08	49.90 ± 3.75^{bc}	0.24 ± 0.01^{b-d}
$B^+ \times C_6$	76.38 ± 4.07	2.43 ± 0.48	41.75 ± 0.88^{c}	0.15 ± 0.05^{de}
$B^+ \times C_8$	81.37 ± 0.35	2.28 ± 0.44	54.13 ± 10.97^{a-c}	0.13 ± 0.01^{de}
$B^+ \times C_{10}$	85.36 ± 3.01	2.42 ± 0.46	49.20 ± 9.59^{bc}	0.34 ± 0.01^{bc}
$B^+ \times C_{12}$	85.90 ± 1.51	2.18 ± 0.08	53.68 ± 0.96^{a-c}	0.13 ± 0.03^{de}
C	*NS*	*NS*	**	*
B	*NS*	*NS*	*NS*	*NS*
C × *B*	*NS*	*NS*	*	*

Data are presented as Mean ± SD with three replicates (n = 3). ^{A} Different letter in each row represent significant differences with the Tukey test ($P < 0.05$). *, **, and *NS* indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, and non-significant, respectively. C_0, C_2, C_4, C_6, C_8, C_{10}, C_{12} are 0, 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

Table 5
Bioaccumulation factor (BAF), bioconcentration factor (BCF), and translocation factor (TF) of petroleum hydrocarbons in vetiver grass grown on different crude oil-contaminated soil inoculated (B^+) and non-inoculated (B^-) with oil-degrading bacteria for 120 days.

Treatments	SCF	RCF	TF
Crude oil (*C*)			
C_2	0.041 ± 0.023^{aA}	0.061 ± 0.020^{c}	0.624 ± 0.182^{a}
C_4	0.042 ± 0.019^{a}	0.124 ± 0.041^{b}	0.340 ± 0.046^{b}
C_6	0.031 ± 0.011^{ab}	0.089 ± 0.026^{c}	0.345 ± 0.037^{b}
C_8	0.024 ± 0.012^{bc}	0.137 ± 0.051^{ab}	0.174 ± 0.026^{c}
C_{10}	0.017 ± 0.006^{bc}	0.137 ± 0.039^{ab}	0.126 ± 0.014^{cd}
C_{12}	0.014 ± 0.006^{c}	0.162 ± 0.042^{a}	0.085 ± 0.020^{d}
Bacteria (*B*)			
B^-	0.017 ± 0.006^{b}	0.089 ± 0.027^{b}	0.244 ± 0.149^{b}
B^+	0.039 ± 0.017^{a}	0.150 ± 0.041^{a}	0.321 ± 0.238^{a}
B × C			
$B^- \times C_2$	0.020 ± 0.001^{c-e}	0.043 ± 0.001	0.469 ± 0.057^{b}
$B^- \times C_4$	0.028 ± 0.000^{c-e}	0.084 ± 0.002	0.339 ± 0.016^{c}
$B^- \times C_6$	0.021 ± 0.000^{c-e}	0.067 ± 0.005	0.317 ± 0.033^{cd}
$B^- \times C_8$	0.014 ± 0.001^{de}	0.094 ± 0.001	0.153 ± 0.0153^{ef}
$B^- \times C_{10}$	0.012 ± 0.000^{de}	0.104 ± 0.004	0.116 ± 0.005^{ef}
$B^+ \times C_{12}$	0.008 ± 0.000^{e}	0.127 ± 0.007	0.068 ± 0.000^{f}
$B^+ \times C_2$	0.061 ± 0.003^{a}	0.078 ± 0.003	0.780 ± 0.006^{a}
$B^+ \times C_4$	0.056 ± 0.019^{ab}	0.163 ± 0.019	0.341 ± 0.077^{c}
$B^+ \times C_6$	0.0416 ± 0.004^{a-c}	0.111 ± 0.012	0.373 ± 0.000^{bc}
$B^+ \times C_8$	0.035 ± 0.003^{b-d}	0.179 ± 0.025	0.195 ± 0.007^{de}
$B^+ \times C_{10}$	0.023 ± 0.000^{c-e}	0.171 ± 0.011	0.137 ± 0.013^{ef}
$B^+ \times C_{12}$	0.020 ± 0.000^{c-e}	0.197 ± 0.0192	0.103 ± 0.007^{ef}
C	**	**	**
B	**	**	**
C × *B*	*	*NS*	**

Data are presented as Mean ± SD with three replicates (n = 3). ^{A} Different letter in each row represent significant differences with the Tukey test ($P < 0.05$). *, **, and *NS* indicate statistical differences at $P \leq 0.05$, $P \leq 0.01$, and non-significant, respectively. C_2, C_4, C_6, C_8, C_{10}, C_{12} are 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

kind of root system could spread over a large volume of soil and provide a desirable and strong rhizosphere establishment in soil. The usage of *V. zizanioides* for remediation of crude oil spilled-water showed that the more plants, the greater the oil content reduced (Effendi et al., 2017). The degradation of petroleum hydrocarbons in soils may occur through plant–microbe system activity. In an efficient phytoremediation system, plant root must increase not only the microbial activity but also the bioavailability of hydrocarbons by improving the contact between microorganisms and pollutants. A report by Liu et al. (2013) demonstrated that the remediation of oil-contaminated sludge by the cooperation of tall fescue and *Pseudomonas* sp. S–B was greater than single remediation. This result is consistent with our findings.

Here, vetiver grass took up crude oil hydrocarbons in a very small amount from the contaminated soil. The concentration factor of TPHs in root was more than 36.5% relative to shoot at all pollution levels. It shows that the concentration of petroleum hydrocarbons was far lower in shoot than in root tissues. The accumulated PAHs in the shoot are the total amounts originating from the air and transferring from the roots (Sivaram et al., 2018).

The results showed that RCF of TPHs were higher than SCF, indicating that vetiver restricted the transfer of hydrocarbons from roots to shoots. This finding is in parallel with Cheema et al. (2010), who reported that the concentration of PAHs in root was higher than shoot tissues. The lowest TF value was observed on the highest crude oil concentration, referring to a tolerance mechanism which vetiver applied in order to prevent damage of above-ground tissues from contaminants toxicity at higher pollution levels. Vetiver grass with narrow and waxy leaves could decrease evaporation rates which limit the transportation of pollution to shoot via xylem (Gautam and Agrawal, 2017). The overall findings of present study recommended that *V. zizanioides* could be a right choice for phytoremediation of organic pollution, especially in cooperation with mentioned degrading bacteria, although it needs to dominate limitations.

Hydrophobic nature of the most petroleum hydrocarbons could reduce nutrient availability for the plant. Therefore, nutrient deficiency could be regarded as one of the main limiting factors for plant growth and developments in a phytoremediation technology in oil-contaminated soil. Nutrient supplementations on different levels could be proposed to supply more nutrient elements for plant and thereupon improve the remediation efficiency. In connection with the point considered, addition of biochar to the soil could be a useful suggestion due to its strong chemical as well as physical sorption abilities to improve soil fertilities (Maroušek et al., 2017).

On the other hand, there is a need to improve present study tools toward industrial scales. Therefore, some field experiments as supplementary studies should be arranged for industrialization of such this process phytoremediation technique. Hence, our findings could be used as an introduction of industrialization and it might have potential to be applied on economic sectors. Besides, the usage of biofuel crops like vetiver in remediation process is affordable from industrial point of view since it concomitantly rehabilitates contaminated area and produce more biomass which is profitable for the possessor of the polluted lands.

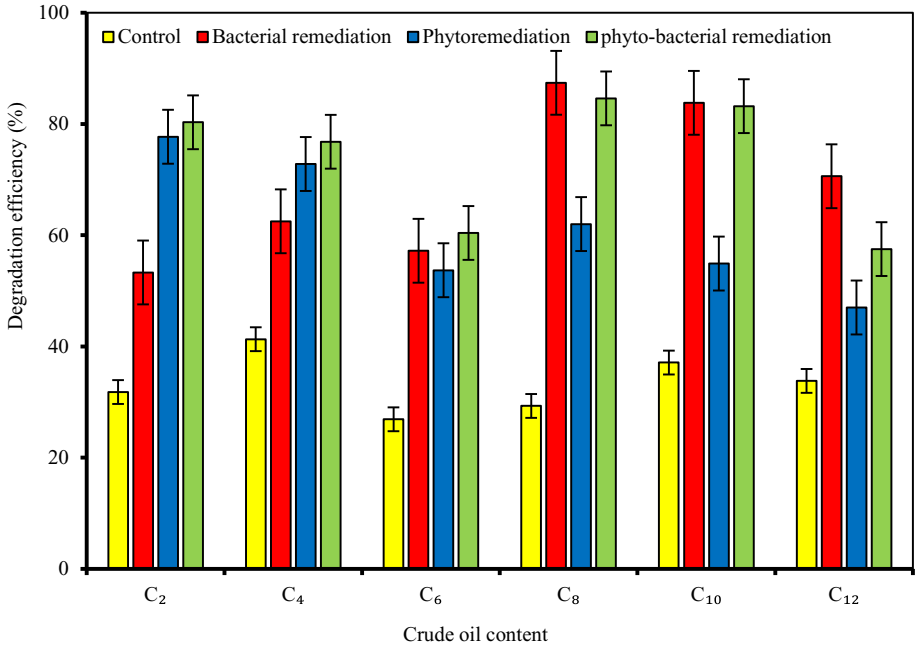

Fig. 4. Degradation efficiency of TPHs on different crude oil-contaminated soil under different treatments for 120 days. Bars on the top of each column are SEM. C_2, C_4, C_6, C_8, C_{10}, and C_{12} are 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

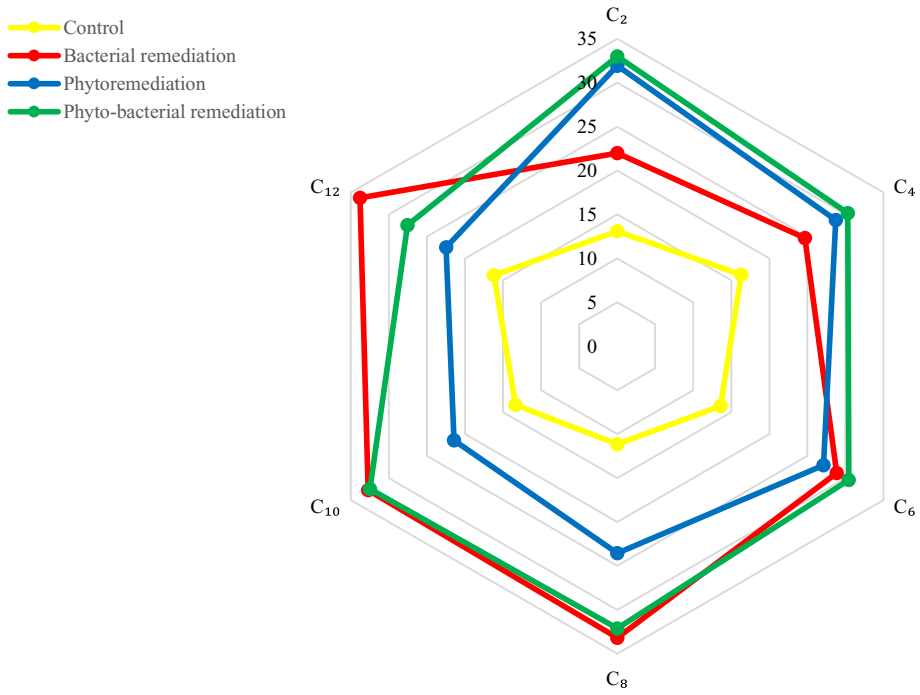

Fig. 5. The percentage contribution of each treatments in crude oil removal at different crude oil-contaminated soil. C_2, C_4, C_6, C_8, C_{10}, C_{12} are 20, 40, 60, 80, 100, and 120 g of crude oil in one kg of soil.

5. Conclusions

The present study was designed to explore the physio-biochemical responses of *Vetiveria zizanioides* on crude oil-contaminated soil and its potential to rehabilitate the contaminated media in conjugation with the bacterial consortium. Based upon the results of the present work, *V. zizanioides* possess the ability to grow well and effectively withstand crude oil concentration upto 120 g kg^{-1} of soil. Exposing this grass to oil pollution stimulated the root biomass and concomitantly induced the biochemical and physiological responses in this grass. Total antioxidant activity, carotenoid contents, the production of proline, and MDA motivated in an efficient way in both aboveground and belowground parts with increasing oil concentration in soil. These inductions cause *V. zizanioides* to tolerate hydrocarbon pollution and to mitigate ROS formation and relieve oxidative stress resulting

from pollutants. These conclusions guarantee the competency of physio-biochemical measurements to biomonitor the effects of environmental pollution on plant responses and to assess the endurance of plant grown on abiotic stressors.

In this context, *V. zizanioides* showed a remarkable ability for phytoremediation of petroleum hydrocarbons up to 47.0%. It is interesting to mention that the inoculation of bacterial consortium increased plant growth as well as hydrocarbon degradation. The presence of plant and oil-degrading bacteria increased the removal of TPHs >60%. The unusual potency of *V. zizanioides* to retain major hydrocarbon amounts in roots and translocate only a fraction to shoots is admirable. Such restricted translocation is ideal for phytoremediation process. Therefore, the utilizing of vetiver grass and specially the interaction of vetiver-degrading bacteria suggests an alternative strategy for effectual phytoremediation of soils contaminated with crude oil. The exact mechanisms and toxicological risk with the remediation process in this interaction and the possible effect of the polar fractions produced during the degradation process need further elucidation and also investigation. Furthermore, a lab-scale experimental system for phytoremediation purpose could be criticized since studies on this scale is much simpler and do not notify us with realistic time frames on remediation of contaminated areas. Hence, additional experiments need to be designed on field-scale of phytoremediation aims.

Author contributions

Zahra Kiamarsi: Conceived and designed the analysis, Carried out the experiment, Collected the data, Performed analysis, Wrote the paper (Wrote the manuscript in consultation with author 2 and author 3), Other contribution (Discussed the results, provided critical revision of the article, contributed to the conception and design of the study and interpretation of the data). Mohammad Kafi: Conceived and designed the analysis, Performed analysis, Other contribution (Supervised the project, conceived the study and were in charge of overall direction and planning, discussed the results and commented on the manuscript, co-wrote the paper, contributed to the design of the study and interpretation of the data, provided final approval of the version to publish, provided critical revision of the article). Mohsen Soleimani: Conceived and designed the analysis, Other contribution (discussed the results and commented on the manuscript, co-wrote the paper, provided critical revision of the article). Ahmad Nezami: Other contribution (Verified the analytical methods, contributed to the design of the study). Stanley Lutts: Other contribution (Guide and help with the GC-MS analysis and interpretation of the related results).

Declaration of competing interest

The authors confirm that there are no known conflicts of interest associated with this publication.

Acknowledgment

The authors gratefully acknowledge Mrs. Hélène Dailly at the Catholic University of Louvain for her worthful technical assistance on GC-MS analysis. We are also grateful to Ferdowsi University of Mashhad, Iran, for providing financial support for present research.

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