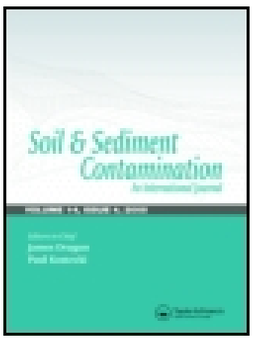

Soil and Sediment Contamination: An International Journal

ISSN: 1532-0383 (Print) 1549-7887 (Online) Journal homepage: http://www.tandfonline.com/loi/bssc20

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To cite this article: Javad Zamani, Mohammad Ali Hajabbasi, Mohammad Reza Mosaddeghi, Mohsen Soleimani, Mehran Shirvani & Rainer Schulin (2018): Experimentation on Degradation of Petroleum in Contaminated Soils in the Root Zone of Maize (Zea Mays L.) Inoculated with Piriformospora Indica, Soil and Sediment Contamination: An International Journal, DOI: 10.1080/15320383.2018.1422693

To link to this article: https://doi.org/10.1080/15320383.2018.1422693

Published online: 15 Jan 2018.

SOIL AND SEDIMENT CONTAMINATION
2018, VOL. 27, NO. 1, 1–18
https://doi.org/10.1080/15320383.2018.1422693

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Experimentation on Degradation of Petroleum in Contaminated Soils in the Root Zone of Maize (*Zea Mays* L.) Inoculated with *Piriformospora Indica*

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ABSTRACT

Plant-based methods such as rhizodegradation are very promising for the remediation of petroleum-contaminated soils. Associations of plants with endophytes can further enhance their phytoremediation potential. In this study, a rhizobox experiment was conducted to investigate whether inoculation with the root-colonizing fungus *Piriformospora indica* could further enhance the degradation of petroleum hydrocarbons in the root zone of maize (*Zea mays* L.). The rhizoboxes were subdivided into compartments in accordance with distance from the plants. After filling the boxes with soil from a petroleum-contaminated site, seedlings that had either been inoculated with *P. indica* or not were grown in the middle compartments of the rhizoboxes and grown for 64 days. A plant-free treatment was included for control. The presence of roots strongly increased the counts of total and petroleum-degrading soil bacteria, respiration, dehydrogenase activity, water-soluble phenols and petroleum degradation. All these effects were also found in the soil adjacent to the middle compartments of the rhizoboxes, but strongly decreased further away from it. Inoculation with *P. indica* further enhanced all the recorded parameters without changing the spatial pattern of the effects. Inoculated plants also produced around 40% more root and shoot biomass than noninoculated plants and had greener leaves. Together, the results indicate that the treatment effects on the recorded soil microbial and biochemical parameters including petroleum hydrocarbon degradation were primarily due to increased root exudation. Irrespectively of this, they show that maize can be used to accelerate the rhizodegradation of petroleum hydrocarbons in soil and that inoculation with *P. indica* can substantially enhance the phytoremediation performance of maize.

KEYWORDS

Rhizodegradation; rhizobox; endophyte; *Piriformospora indica*; petroleum-contaminated soil

Introduction

Soil contamination by petroleum hydrocarbons is a major environmental problem, especially in oil producing areas and around sites with petrochemical industries, impair soil

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fertility, adversely affect the nutritional quality of crops and is a source of water pollution (Soleimani *et al*., 2013). Phytoremediation, i.e., remediation based on the use of plants (Alkorta and Garbisu, 2001; Gerhardt *et al*., 2009), is an attractive alternative to classical engineering methods to treat petroleum-contaminated soil (Macek *et al*., 2000; Soleimani *et al*., 2010a; Liu *et al*., 2013). Plant roots can create conditions in the surrounding soil, i.e., the rhizosphere, under which the degradation of petroleum hydrocarbons is greatly accelerated (Njoku *et al*., 2009; Phillips *et al*., 2012), in particular through stimulating petroleum-degrading microbial populations, as reported for example by Nichols *et al*. (1997) for the rhizosphere of alfalfa (*Medicago sativa* L.) or by Cheema *et al*. (2009) and Soleimani *et al*. (2010a) for the rhizosphere of tall fescue. However, also direct biochemical effects of root exudates may be involved in this form of root-assisted soil remediation, which is also called rhizodegradation by some authors (Wang *et al*., 2011). Cheema *et al*. (2009) and Soleimani *et al*. 2010a for example observed in their studies that the enhanced degradation of petroleum hydrocarbons in the presence of plant roots was associated with increased dehydrogenase activity and concentrations of water-soluble phenols.

Recently it was found that associations of plants with endophytes can further enhance their phytoremediation potential (Wenzel, 2009; Weyens *et al*., 2009; Li *et al*., 2012; Stepniewska and Kuźniar, 2013), and Soleimani *et al*. (2010a) found that endophytes could in particular be used in combination with grasses to accelerate the degradation of petroleum hydrocarbons in contaminated soil. Here, we wanted to explore whether this approach could also be used for the remediation of petroleum-contaminated soil in combination with maize (*Zea mays* L.). Various studies suggest that maize could be a good choice for the remediation of soil polluted with organic contaminants including petroleum (Diab, 2008; Chouychai *et al*., 2009; Zand *et al*., 2010; Hechmi *et al*., 2013; Asadollahi *et al*., 2016; Liao *et al*., 2015). Based on work by Kumar *et al*. (2009) showing that maize is a suitable host plant and on preliminary tests (unpublished results), we selected the fungus, *Piriformospora indica*, for this purpose. *Piriformospora indica* (Sebacinales, Basidiomycota) is a cultivable root-colonizing endophytic fungus (Verma *et al*., 1998; Varma *et al*., 1999) that has been found to promote growth, biomass production and tolerance of inoculated plants to various biotic and abiotic stresses (Waller *et al*., 2005; Baltruschat *et al*., 2008; Sherameti *et al*., 2008; Alikhani *et al*., 2013). In contrast to arbuscular mycorrhizal fungi, which are obligatory biotrophs, *P. indica* is a saprobe and thus has the advantage that it does not only grow on a wide variety of natural and artificial media, but also that it can be propagated in axenic cultures (Franken, 2012).

While in a parallel study we focused on the response of maize root growth to soil contamination with petroleum in presence and absence of *P. indica* (Zamani *et al*. 2016), the aim of the study presented here was to investigate (i) how *P. indica* affects the degradation of petroleum hydrocarbons in the root zone of infected maize seedlings, (ii) how far away from the roots the effect would extend into the surrounding soil and (iii) how this spatial dependence would be related to soil microbial activity and biochemical parameters such as dehydrogenase activity and water-soluble phenol concentrations. For this purpose, we conducted a rhizobox experiment in which we compared treatments with and without inoculation of the plants and in which confining root growth to the middle section of the boxes allowed determining how the effects on hydrocarbon degradation and soil microbiological activity varied with distance from the roots. Our expectation was that inoculation with *P. indica* would markedly enhance

the degradation of the contaminating petroleum hydrocarbons in the root zone and that this enhancement effect would strongly decline with distance from the rooted soil compartment.

Materials and methods

Soil

The petroleum-contaminated soil used in this study was collected from a landfill nearby the Shahid Hasheminejad Gas Refinery Complex at Sarakhs in the Northeast of Iran. The site has an arid climate with a long-term mean temperature of 18.8°C and an annual rainfall averaging about 180 mm. The pollution was due to the application of diesel-contaminated sewage sludge produced by the refinery. Three samples were collected from the topsoil (0–30 cm depth), bulked, thoroughly mixed, air-dried and sieved through a mesh of 2 mm size. The concentration of total petroleum hydrocarbons (TPH) averaged 21.6 mg g^{-1} in the collected soil. Other characteristics of 3 replicate subsamples of the sieved soil are given in Table 1.

Experimental set-up

Fifteen rhizoboxes (200 mm × 200 mm × 100 mm) were constructed for the experiment following the design of Youssef and Chino (1988) with some minor modifications. Using nylon mesh of 25 μm pore size, each rhizobox was subdivided into seven adjacent compartments: A middle compartment for planting (denoted here as 'root' compartment also in the case of the plant-free treatment for simplicity) and three lateral compartments on both sides (denoted as 'near root', 'near bulk soil' and 'bulk soil' with increasing distance to the middle compartment). The bulk soil compartments were 20 mm wide, the others 10 mm as shown in Figure 1. All compartments were filled with air-dried polluted soil layer by layer using the same filling procedure in all cases.

The following three treatments were applied in 5 replicates to these rhizoboxes each: (1) planting 3 maize seedlings inoculated with *P. indica* into the middle compartment, (2) planting 3 noninoculated maize seedlings into the middle compartment, and (3) a plant-free control.

Table 1. Physical and chemical characteristics (mean ± standard error, $n = 3$) of the soil collected for this study

Parameter	Unit	Value	Method of analysis
Sand content	%	81±1.3	Gee and Bauder 1986
Silt content	%	11±0.7	
Clay content	%	8±1	
Texture	–	Loamy Sand	
pH	–	7.4±0.1	McLean, 1982
EC	dS m^{-1}	1.25±0.05	McLean, 1982
Total organic C	%	2.95±0.2	Nelson and Sommers, 1982
Total N	mg kg^{-1}	200±10.3	Bremner and Mulvaney, 1982
Available P	mg kg^{-1}	11.0±1.23	Olsen and Sommers, 1982
CEC	Cmol(+)kg^{-1}	9.2±1.1	Rhoades, 1982
TPH	g kg^{-1}	21.6±0.2	USEPA, 1998

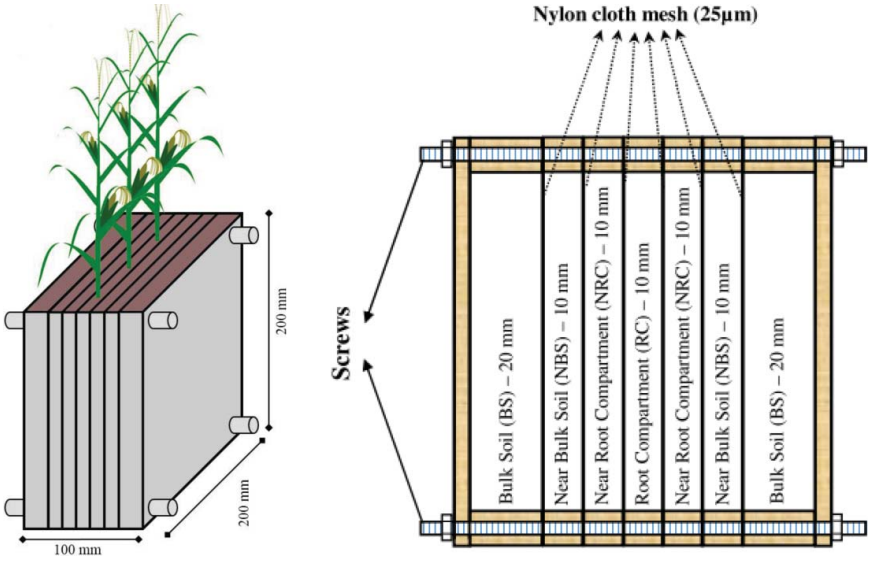

Figure 1. Schematic diagram and dimensions of the rhizoboxes used in the experiment.

Production of fungal inoculum

The fungal strain of *P. indica* used in this study was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSM 11827). In order to obtain large quantities of active spores, the inocula were produced in two steps. In a first step, mycelium plugs taken from the edge of a growing *P. indica* colony were cultured at 24°C in Petri dishes on solid complex medium. In a second step, spores were collected after 18 days by gently scratching the surface of the Petri-dishes with a spatula left to germinate for 10 days on a liquid medium at 28°C under gentle shaking at a frequency of 80 rpm. After each step, the spore suspensions were filtered through cheese-cloth to remove excess medium and washed three times with sterilized distilled water containing Tween-20 (0.02%). After each washing, the spores were collected by centrifugation at 4000 × *g* for 10 min. The composition of the solid medium was composed of glucose (20 g L^{-1}), peptone (2 g L^{-1}), yeast extract (1 g L^{-1}), Hy-Casamino Acid (1 g L^{-1}), salt solution (50 ml l^{-1}), microelement solution (1 mL L^{-1}) and agar (15 g L^{-1}). Salt solution consisted of $NaNO_3$ (120 g L^{-1}), KCl (10.4 g L^{-1}), $MgSO_4.7H_2O$ (10.4 g L^{-1}) and KH_2PO_4 (30.4 g L^{-1}) and microelement solution of $MnCl_2.4H_2O$ (6 g L^{-1}), H_3BO_3 (1.5 g L^{-1}) $ZnSO_4$ (2.65 g L^{-1}), KI (0.75 g L^{-1}), $Na_2MoO_4.2H_2O$ (2.4 mg L^{-1}). The composition of the liquid medium was the same as that of the solid medium, except that it did not contain agar.

Germination and inoculation of plants

Maize (*Zea mays* L. cv. SC704) seeds were surface-sterilized by rinsing for 30 s in 70% ethanol, 5 min in 5% sodium hypochlorite (NaOCl) and five times briefly with sterilized distilled water. After vernalization at 4°C the seeds were placed on agar surface medium in closed Petri-dishes (diameter, 120 mm) and incubated for 2 days at 25°C to germinate. After germination, two uniform sets of seedlings with radicles of about 1 cm length were selected for the

experiment, one of which was inoculated with *P. indica* by immersion of the seedlings in inoculum (adjusted to $\sim 2 \times 10^6$) for 3 h under gentle shaking and other one was the non-inoculated seedlings which just were dipped in sterilized distilled water containing Tween-20 (0.02%).

Cultivation of plants in the rhizoboxes and sampling

The seedlings were planted according to the experimental design at 1 cm depth into the 'root' compartments of the designated rhizoboxes. After planting, all rhizoboxes, including the plant-free controls, were placed in a glasshouse (temperature 28 ± 4°C, day/night cycle 13/11 h, and a 48 ± 7% relative humidity) in a randomized array. Soil moisture was kept approximately constant (near 70% field capacity) by periodical watering in order to replace consumed water. No pesticides were applied. Weeds were removed manually. Sixty-three days after transplantation, the amount of chlorophyll (SPAD-index) was determined using a SPAD 502 Plus Chlorophyll Meter. On the following day the rhizoboxes were dismantled, all plants harvested and soil samples taken from the center of all compartments. All soil samples were stored at 4°C until analysis. The plants were separated into shoots and roots, which were then washed separately in tap water followed by distilled water, dried at 60°C for 48 h and weighed.

Microscopic analysis of root inoculation

In addition to the 15 rhizoboxes used for the main experiment, we also prepared some rhizoboxes in which plants with and without inoculation were grown to check for successful root infection by *P. indica*. Root samples of these plants were collected 20 days after transplanting, boiled for 1 min in 5% KOH solution, washed three times with sterilized distilled water, stained for 1 min in aniline blue solution, washed again with sterilized distilled water and analyzed under a light microscope.

Analysis of soil samples

Microbial numbers

Aerobic heterotrophic bacteria and aerobic petroleum-degrading bacteria were counted using the drop plate method with nutrient agar and oil agar, respectively, as described by Soleimani *et al.* (2010a). Composition of oil agar media (g l^{-1}) was 990 ml sterile solution of agar (15), KH_2PO_4 (1), K_2HPO_4 (1), NH_4NO_3 (1), $MgSO_4.7H_2O$ (0.2), $FeCl_3$ (0.05) and $CaCl_2.H_2O$ (0.02) adjusted to pH 7.0 and 10.0 mL of filtered sterilized diesel. Plates were incubated for 2–4 days at 30°C in the dark prior to counting the numbers of colony forming units (CFU).

Soil microbial respiration

Soil microbial respiration was measured using the method described by Alef (1995). Briefly, samples of about 15 g soil were incubated for 7 days at 27°C in 500-mL glass containers closed with rubber stoppers in three replicates. A test tube containing 10 mL of a 0.5 M NaOH solution was placed into the containers to trap the evolving CO_2. The trapped CO_2

was determined by titrating the excess in alkali with HCl. Three glass containers without soil were incubated in the same way as controls.

Dehydrogenase activity

Dehydrogenase activity was analyzed by measuring the reduction of triphenyl-tetrazolium chloride (TTC) to triphenyl-formazan (TPF) (Cheema *et al.*, 2009; Soleimani *et al.*, 2010a). 5-g soil samples were incubated for 24 h at 37°C in 5 mL of TTC solution (0.5% buffer, pH 7.4). Two drops of concentrated H_2SO_4 were added immediately after the incubation to each sample in order to stop the reaction. The sample was then blended with 5 mL of toluene to extract the reaction product (i.e., TPF) and shaken for 30 min at 250 rpm, followed by centrifugation at 5000 rpm for 5 min and spectrophotometric analysis of absorbance at 492 nm. Soil dehydrogenase activity was expressed as μg TPF g^{-1} dry soil d^{-1}.

Water-soluble phenols

Water-soluble phenols were quantified colorimetrically using Folin–Ciocalteau reagent as described by Soleimani *et al.* (2010a). Soils were extracted by shaking with 25 mL distilled water for 4 h at 100 rpm, followed by centrifugation for 15 min at 5000 rpm. A 10-mL aliquot of extract or standard was placed in a test tube, and 3 ml of Na_2CO_3 solution and 1 mL of Folin-Ciocalteau reagent were added. The solution was mixed well and allowed to stand for 1 h at room temperature, before the light absorbance of the blue complex that formed during this time was determined spectrometrically at 750 nm. Vanillic acid was used as standard, and the amount of phenolic compounds was expressed as vanillin acid equivalents (μg vanillic acid g^{-1} soil).

TPH analysis

Soxhlet was used to extract petroleum hydrocarbons from homogenized soil samples (10 g) in a 1:1 (v/v) mixture of HPLC-grade dichloromethane and *n*-hexane (125 mL) (Christopher *et al.*, 1988). The extracts were sequentially purified and cleaned up using silica gel 60 (0.063–0.200 mm, Merck) to adsorb the polar compounds. The residues obtained (and weighed) after evaporation of the solvents in a rotary evaporator were considered as total petroleum hydrocarbons (TPH) according to USEPA (1998).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) in combination with the t-test and post hoc analysis of Least Significant Differences. All statistical analyses were performed by means of the statistical software package SAS-Version 9.1.3 (SAS Institute Inc., 2005).

Results

Efficiency of maize seedling inoculation with P. indica

Based on the microscopic analyses, 64 ± 13% of the roots of inoculated plants were colonized by *P. indica* 20 days after spore inoculation, while no colonization was observed in noninoculated plants. Figure 2 shows typical images of chlamydospores and hyphae

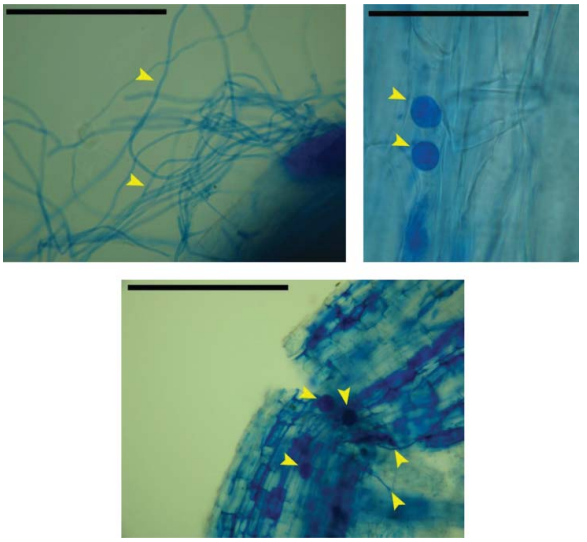

Figure 2. Colonization of maize roots with *P. indica* 20 days after inoculation. The yellow arrows point at chlamydospores and hyphae (length of black bar = 100 μm).

colonizing infected roots. Alikhani *et al.* (2013) described in detail how *P. indica* infects and colonizes plant roots, and our observations were in full agreement with their findings.

Effects of P. indica on plant growth and leaf chlorophyll content

Inoculation with *P. indica* visibly promoted the growth of the experimental maize plants (Figure 3). The inoculated plants were clearly larger and greener than the noninoculated plants throughout the experiment. The results of visual inspection were confirmed by the biomass and SPAD measurements at the end of the experiment (Figure 4). The total harvested biomass of roots and shoots was 40% larger in plants that had been inoculated than in noninoculated plants. With an average height of about 35 cm, inoculated plants were also

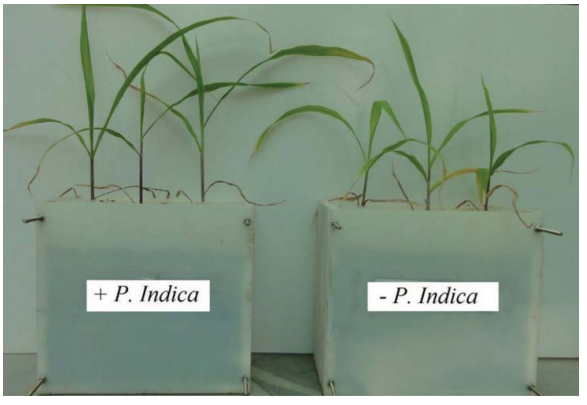

Figure 3. Growth of maize seedlings inoculated (+*P. indica*) with *P. indica* and of noninoculated (-*P. indica*) seedlings in petroleum-contaminated soil.

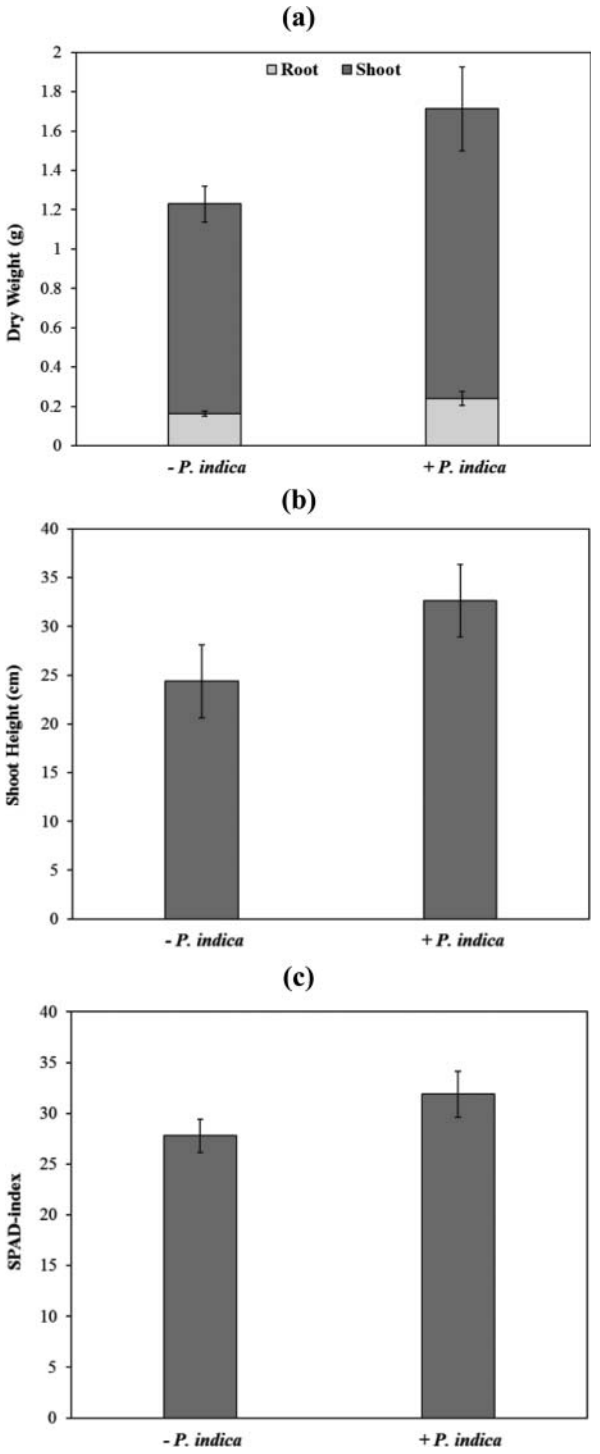

Figure 4. Root and shoot biomass (a), shoot height (b) and SPAD index (c) of maize seedlings with (+*P. indica*) and without (–*P. indica*) inoculation with *P. indica* after 9 weeks of growth on petroleum contaminated soil.

in average 1.4 times higher than noninoculated plants, and a 4 units higher SPAD index indicated that the leaves of the inoculated plants had a significantly higher leaf chlorophyll than the noninoculated plants.

Soil microbial counts, respiration, dehydrogenase activity, and water-soluble phenols

The total bacterial counts averaged about 0.6×10^6 CFU g^{-1} and the counts of petroleum-degrading bacteria 0.5×10^3 CFU g^{-1} in the plant-free control soils, with no significant difference between the various rhizobox compartments (Table 2). In the root compartment of the planted soils total bacterial counts were 30–40 times and the counts of petroleum-degraders 140–200 times higher than the respective counts in the plant-free soil (Table 2). Comparing values for given compartment categories, 50–60% higher counts were found throughout in the rhizoboxes with inoculated plants than in those with noninoculated plants. There was also a trend towards higher total bacterial counts in the rhizoboxes with inoculated plants, but the differences were not significant due to a larger degree of variability. Although the counts of both, total and petroleum-degrading bacteria significantly decreased with increasing distance from the root compartments, they were still higher in the 'bulk soil compartments' of the planted rhizoboxes than in the plant-free controls, in average 2–3 times for the total counts and even 10 to almost 20 times for the average counts of petroleum degraders.

At least in a qualitative sense, soil respiration, dehydrogenase activity and water-soluble phenols all showed similar patterns of treatment effects as the bacterial counts. Comparing respective soil compartments, significantly more CO_2 was always released from planted than from unplanted rhizoboxes. While there was no significant difference among the planted rhizoboxes between the treatments with and without inoculation, there was a clear root distance effect (Table 3): In the root and near-root compartments around twice as much CO_2 was produced with than without plants. In the bulk soil and near-bulk soil compartments the respective ratios ranged between 1.4 and 1.6.

Also soil dehydrogenase activity and water-soluble phenols showed clear plant and distance-to-root effects. Both were increased in the presence of plants and decreased with distance to the roots (Table 3). In contrast to soil respiration, both also showed an inoculation effect, with higher values for the treatment with than for that without *P. indica*. For the concentration of water-soluble phenols this inoculation effect was significant in all

Table 2. Counts of total and petroleum-degrading bacteria in different compartments of rhizoboxes with no plants (control) or after maize seedlings inoculated (+ *P. indica*) or not inoculated (–*P. indica*) with *P. indica* had been grown for 9 weeks in the root compartments

	Total bacterial counts ($\times 10^6$ CFU g^{-1} soil)			Petroleum-degrading bacterial counts ($\times 10^3$ CFU g^{-1}soil)		
Compartment	Control	– *P. indica*	+ *P. indica*	Control	– *P. indica*	+ *P. indica*
Root Compartment (RC)	0.4±0.2c[†]	20.0±4.6a	22.7±5.7a	0.5±0.3g	70.0±10.0b	100.0±10.0a
Near Root Compartment (NRC)	0.8±0.3c	8.7±2.1b	11.3±3.8b	0.3±0.1g	51.7±7.6c	73.3±5.8b
Near Bulk Soil (NBS)	0.5±0.2c	2.8±1.9c	2.5±1.3c	0.5±0.1g	16.3±5.5de	23.3±4.5d
Bulk Soil (BS)	0.6±0.1c	1.1±0.7c	1.4±0.6c	0.6±0.2g	6.2±2.3fg	10.7±3.8ef

[†]Values of a parameter marked with the same letter are not significantly different from each other ($p < 0.05$).

Table 3. Microbial respiration, dehydrogenase activity and water-soluble phenols in different compartments of rhizoboxes with no plants (control) or after maize seedlings inoculated (+ *P. indica*) or not inoculated (– *P. indica*) with *P. indica* had been grown for 9 weeks in the root compartments

	Microbial respiration (mg CO_2-C kg^{-1} day^{-1})			Dehydrogenase activity (μg TPF g^{-1} dry soil)			Water-soluble phenols (μg vanillic acid g^{-1} dry soil)		
Compartments	Control	– *P. indica*	+ *P. indica*	Control	– *P. indica*	+ *P. indica*	Control	– *P. indica*	+ *P. indica*
Root Compartment (RC)	45.4±5.9e[†]	95.4±4.9ab	102.5±8.6a	544±26e	643±26cd	794±29a	6.3±0.9f	8.4±0.4bc	9.9±0.7a
Near Root Compartment (NRC)	47.9±5.4de	91.8±7.6b	98.9±5.3ab	525±37e	607±30d	778±23b	6.4±1.0f	8.0±0.5cde	9.2±0.6ab
Near Bulk Soil (NBS)	50.4±8.8e	75.4±3.8c	79.3±6.7c	514±44e	528±41e	674±34c	6.2±0.8f	7.2±0.4def	8.5±0.6bc
Bulk Soil (BS)	56.8±2.8d	75.0±4.7c	77.5±5.4c	531±22e	516±26e	545±22e	6.4±1.0f	6.9±0.3ef	8.1±0.7bcd

[†]Values of a parameter marked with the same letter are not significantly different from each other ($p < 0.05$).

compartments and did not vary much with distance from the roots, while water-soluble phenol concentrations in the 'bulk soil' and 'near-bulk soil' compartments of the rhizoboxes planted with noninoculated plants decreased to the same level as in the plant-free controls. In the case of dehydrogenase activity, the inoculation effect disappeared with increasing distance to the roots, as did the plant effect without inoculation (Table 3). Thus, while dehydrogenase activity was between 15% and 20% higher in the root and near-root compartments of the noninoculated plants than in the plant-free soil and even by almost 50% for the inoculated plants, there was no difference in dehydrogenase activity between the three treatments in the bulk soil.

Degradation of petroleum hydrocarbons

The same basic pattern of treatment effects as on the microbial and biochemical soil parameters presented before can finally also be seen in the degradation of the contaminating petroleum hydrocarbons. Soil TPH contents decreased by 20% to 25% from the initial 22 g kg^{-1} during the 9 weeks of incubation even in the absence of plants. The presence of maize roots in the root compartment further increased the decline in TPH, especially in the root compartment itself and the compartment next to it, while it had only a small (with inoculation) or no significant (without inoculation) additional effect on TPH in the other two compartments (Figure 5). In all compartments, the additional decrease in TPH that was attributable to the presence of maize roots in the soil was almost twice as large for plants inoculated with *P. indica* than for noninoculated plants. As a result, the total degradation of petroleum hydrocarbons amounted to 35–40% of the initial TPH concentration in the root compartments of rhizoboxes with noninoculated plants and to even 50% in those of inoculated plants.

Correlations between TPH and the soil biological and chemical parameters

The matrix of scattergrams presented in Figure 6 shows that independent of treatment and distance to the roots there was a close linear relationship between TPH and count of petroleum-degrading bacteria. More scattered, but still quite close were the relationships between TPH and dehydrogenase activity, counts of petroleum-degrading bacteria and dehydrogenase activity, as well as between counts of petroleum-degrading bacteria and total bacterial

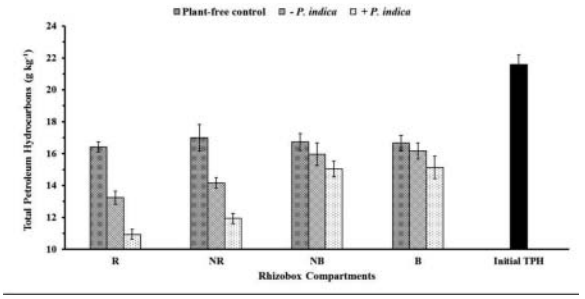

Figure 5. Total petroleum hydrocarbons in the various compartments of the rhizoboxes with no plants (◆), plants without inoculation (■), and plants with *P. indica* inoculation (▲).

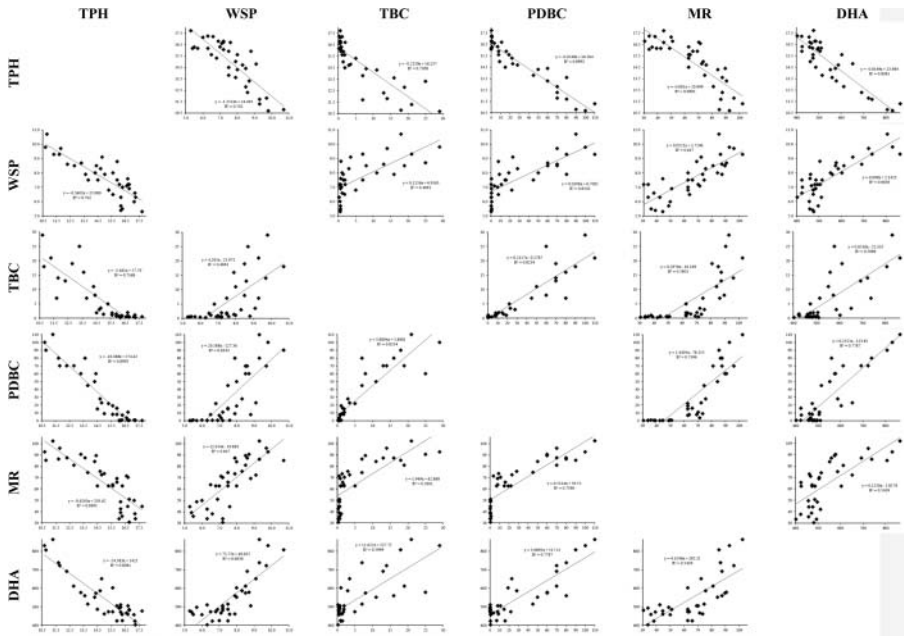

Figure 6. Scatterplot matrix showing the relationships between the investigated chemical and biological soil parameters over all treatments and rhizobox compartments. TPH: total petroleum hydrocarbons (g kg^{-1}), WSP: water-soluble phenols (μg vanillic acid g^{-1}), TBC: total bacterial counts ($\times$ 10^6 CFU g^{-1}), PDBC: petroleum-degrading bacterial counts ($\times$ 10^3 CFU g^{-1}), MR: microbial respiration (mg CO_2-C kg^{-1} day^{-1}), DHA: dehydrogenase activity (μg TPF g^{-1}).

counts. The latter was much more weakly correlated with dehydrogenase activity and TPH than the counts of petroleum-degrading bacteria, indicating that the relationships of TPH and dehydrogenase activity with total bacterial counts were of a more indirect nature. The weakest relationships to other parameters were found for water-soluble phenols and soil respiration rate, which may partially be attributed to nonlinearity in their relationships to bacterial numbers. Interestingly, the strongest relationship of both these parameters with other parameters was to TPH.

Discussion

The results confirmed our expectation that *P. indica* would enhance the degradation of the contaminating petroleum hydrocarbons in the rhizosphere of maize plants, which this effect was associated with increased shoot and root biomass of the plants. This observation indicate that inoculation inoculation with fungus mitigated any negative effect of the contamination on the growth of the plants. Petroleum contamination in soil can inhibit plant growth by direct toxic effects on plant cells but also in other more indirect ways, for example by decreasing nutrient availability or by impeding the movement of water and air into the soil (Adam and Duncan, 2002). This leaves many possibilities how *P. indica* enhanced the growth of the experimental plants. This result cannot be just due to reducing contaminant toxicity though, as enhanced growth of maize and other plants after inoculation with *P. indica* was also observed in noncontaminated soil (Varma *et al.*, 1999; Kumar *et al.*, 2009; Ray

and Valsalakumar, 2010; Krishnaveni *et al.*, 2015). Thus, we believe that also rather general effects on the host plant such as improved nutrient supply to the roots and increased plant hormone levels were involved in the growth-enhancing effect observed in our study. Such effects have been reported for other endophyte-host plant combinations (Sirrenberg *et al.*, 2007; Kuldau and Bacon, 2008; Kumar *et al.*, 2011). Independent of the mechanisms, our results show that inoculation with the endophyte *P. indica* is a promising approach to enhance the ability of maize and probably also other plants to grow in petroleum-contaminated soil. While many other studies found that endophytic fungi increased the ability of host plants to tolerate biotic and abiotic stresses (Malinowski and Belesky, 2000; Soleimani *et al.*, 2010a; Soleimani *et al.*, 2010b; Zarea *et al.*, 2012; Alikhani *et al.*, 2013), our study is the first to our best knowledge showing that maize plants inoculated with *P. indica* produce more biomass than noninoculated ones in petroleum-contaminated soils.

It is reasonable to assume that the more vigorous growth resulting from the colonization of the roots with *P. indica* was a major factor in accelerating TPH elimination in the endophyte treatment. Substantial TPH degradation also occurred in the plant-free treatments, indicating that it was primarily due to microbial activity. Microbial activity greatly increased in the presence of roots, which can be attributed to abundant supply with assimilates through root exudation and turnover, and this in turn would certainly also have promoted TPH degradation (Thoma *et al.*, 2003). The endophyte effect on TPH elimination may thus simply have been due to enhanced root exudation (Bais *et al.*, 2006; Martin *et al.*, 2014)and turnover resulting from boosted plant growth (Leigh *et al.*, 2002).

Whether the roots or the endophyte also contributed more directly than through the stimulation of petroleum-degrading microbial activity via root exudation to the degradation of the contaminants cannot be concluded from the results of our study. But it is certainly a possibility. Such an effect could have operated in particular through increased release of water-soluble phenols (WSP) from the roots or increased WSP production in the rhizosphere resulting from the decomposition of exudates and decaying root cells. The concentration of WSP significantly increased in the presence of roots, but was much less closely correlated to the number of petroleum-degrading bacteria than dehydrogenase activity. Phenolic compounds can defend plants against biotic and abiotic stresses, and endophytic fungi have been found to increase their release into the rhizosphere of host plants (Schulz *et al.*, 2002). Cheema *et al.* (2009) and Lee *et al.* (2008) reported that the production of phenolic compounds tended to be enhanced in petroleum-contaminated soil, and Lee *et al.* (2008) moreover found that this production of phenolic compounds was closely related to root turnover. Furthermore, there is some evidence suggesting that by releasing phenolic compounds into the rhizosphere plants my not only enhance overall microbial activity in contaminated soil (Soleimani *et al.*, 2010b), but also selectively foster the growth of bacteria degrading organic contaminants such as PCB (Donnelly *et al.*, 1994), and Liste and Alexander (1999) showed that measuring the exudation of phenolic compounds into the rhizosphere can be a useful method to screen candidate plants for their potential to promote phenanthrene degradation in contaminated soil. However, roots certainly were not the main source of WSP or of their precursors, as WSP concentrations in the plant-free controls were much higher than the increase associated with the presence of roots, with and without endophyte; and although the relationship between TPH elimination and WSP was not as strong and linear as the relationship between TPH and the counts of petroleum-degrading bacteria, it is also possible that the majority of the WSP found in the soil were actually metabolites of

microbial petroleum hydrocarbon degradation. Various researchers found that significant amounts of phenolic compounds could result in petroleum-contaminated soil from PAH degradation through aromatic ring cleavage (Kraus *et al.*, 1999; Lee *et al.*, 2008; Cheema *et al.*, 2009; Soleimani *et al.*, 2010a).

Among the parameters measured in our study, TPH elimination showed the closest relationships to the number of petroleum-degrading bacteria and to dehydrogenase activity in soil, in agreement with the results of Soleimani *et al.* (2010a) who studied the potential use of endophyte-inoculated grasses to accelerate the remediation of oil-contaminated soil and also found that petroleum hydrocarbon degradation was more closely related to dehydrogenase activity and petroleum-degrading bacteria counts than to total bacteria counts. In line with previous studies, these findings also show that soil dehydrogenase activity assays may be used to determine the potential petroleum-degrading microbial activity that can be stimulated by plant roots through their rhizosphere effect in phytoremediation operations (Balba *et al.*, 1998; Mathew and Obbard, 2001; Lee *et al.*, 2008).

Conclusions

While there are still many questions open with respect to the way in which the factors measured in our study mechanistically relate to the observed treatment effects, the results clearly show that maize is a suitable plant to be used for the phytoremediation of petroleum-contaminated soil and that inoculation with the endophyte *P. indica* is a promising measure to further accelerate the rhizodegradation of petroleum hydrocarbon in the root zone of maize plants. This potential of *P. indica* deserves further exploration, as there may be soil and site conditions under which it is much larger still than in our experiment. In previous studies, *P. indica* was also found to protect plants against salt stress (Waller *et al.*, 2005; Sun *et al.*, 2010; Zarea *et al.*, 2012; Alikhani *et al.*, 2013; Bagheri *et al.*, 2013). Thus, since many petroleum contaminated sites are located in saline areas (Qin *et al.*, 2012; Wu *et al.*, 2012), inoculation of plants with *P. indica* may hold particular promise as a way to enhance phytoremediation treatments of petroleum-contaminated soils in salt-affected areas.

References

Adam, G. and Duncan, H. 2002. Influence of diesel fuel on seed germination. *Environ. Pollut.* **120**, 363–370. doi:10.1016/S0269-7491(02)00119-7.

Alef, K. 1995. Soil respiration. In: *Methods in Applied Soil Microbiology and Biochemistry*, pp. 214–216 (Alef, K. and Nannipieri, P., Eds.), London, Harcourt Brace & Company Publishers.

Alikhani, M., Khatabi, B., Sepehri, M., Nekouei, M. K., Mardie, M., and Salekdeh, G. H. 2013. A proteomics approach to study the molecular basis of enhanced salt tolerance in barley (*Hordeumvulgare* L.) conferred by the root mutualistic fungus *Piriformospora indica*. *Mol. Biosyst.* **9** (6), 1498–1510. doi:10.1039/c3mb70069k.

Alkorta, I. and Garbisu, C. 2001. Phytoremediation of organic contaminants in soils. *Bioresour. Technol.* **79**, 273–276. doi:10.1016/S0960-8524(01)00016-5.

Asadollahi, A., Zamani, J., Hajabbasi, M. A., and Schulin, R. 2016. Using maize (*Zea mays* L.) and sewage sludge to remediate a petroleum contaminated calcareous soil. *Soil Sediment Contam.* **25** (1), 26–37. doi:10.1080/15320383.2016.1085835.

Bagheri, A. A., Saadatmand, S., Niknam, V., Nejadsatari, T., and Babaeizad, V. 2013. Effect of endophytic fungus, *Piriformospora indica*, on growth and activity of antioxidant enzymes of rice (*Oryza sativa* L.) under salinity stress. *Int. J. Adv. Biol. Biomed. Res.* **1** (11), 1337–1350.

Bais H. P., Weir T. L., Perry L. G., Gilroy S., and Vivanco J. M. 2006. The role of root exudates in rhizosphere interations with plants and other organisms. *Annu. Rev. Plant Biol.* **57**, 233–266. doi:10.1146/annurev.arplant.57.032905.105159.

Balba, M. T., Al-Awadhi, N., and Al-Daher, R. 1998. Bioremediation of oil-contaminated soil: Microbiological methods for feasibility assessment and field evaluation. *J. Microbiol. Methods* **32**, 155–164. doi:10.1016/S0167-7012(98)00020-7.

Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., Kogel, K. H., Schäfer, P., Schwarczinger, I., Zuccaro, A., and Skoczowski, A. 2008. Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytol.* **180**, 501–510. doi:10.1111/j.1469-8137.2008.02583.x.

Bremner, J. M. and Mulvaney, C. S. 1982. Nitrogen-total. In: *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties*, pp. 595–624 (Page, A. L., Miller, R. H., and Keeney, D. R., Eds.), Madison Wisconsin USA, American Society of Agronomy and Soil Science Society of America.

Cheema, S. A., Khan, M. I., Tang, X., Zhang, C., Shen, C., Malik, Z., Ali, S., Yang, J., Shen, K., Chen, X., and Chen, Y. 2009. Enhancement of phenanthrene and pyrenedegradation in rhizosphere of tall fescue (*Festuca arundinacea*). *J. Hazard. Mater.* **166**, 1226–1231. doi:10.1016/j.jhazmat.2008.12.027.

Chouychai, W., Thongkukiatkul, A., Upatham, S., Lee, H., Pokethitiyook, P., and Kruatrachue, M. 2009. Plant-enhanced phenanthrene and pyrene biodegradation in acidic soil. *J. Environ. Biol.* **30** (1), 139–144.

Christopher, S. H., Marsden, P. J., and Sharleff, A. S. 1988. Evaluation of methods 3540 (Soxhlet) and 3550 (Sonication) for evaluation of appendix IX analyses from solid samples. S-CUBED, Report for EPA contract 68-03-33-75, work assignment no. 03, document no. SSS-R-88-9436.

Diab, E. A. 2008. Phytoremediation of oil contaminated desert soil using the rhizosphere effects. *Global J. Environ. Res.* **2** (2), 66–73.

Donnelly, P. K., Hegde, R. S., and Fletcher, J. S. 1994. Growth of PCB degrading bacteria on compounds from photosynthetic plants. *Chemosphere* **28**, 981–988. doi:10.1016/0045-6535(94)90014-0.

Franken, P. 2012. The plant strengthening root endophyte *Piriformospora indica*: potential application and the biology behind. *Appl. Microbiol. Biotechnol.* **96**, 1455–1464. doi:10.1007/s00253-012-4506-1.

Gee, G.W. and Bauder, J. W. 1986. Particle-size analysis. In: *Method of Soil Analysis*, pp. 404–409 Klute, A. (Ed.), Madison Wisconsin USA, Agron. Monogr, Agronomy Society of America and Soil Science of America.

Gerhardt, K. E., Huang, X. D., Glick, B. R., and Greenberg, B. M. 2009. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Sci.* **176**, 20–30. doi:10.1016/j.plantsci.2008.09.014.

Hechmi, N., Aissa, N. B., Abdennaceur, H., and Jedidi, N. 2013. Phytoremediation potential of maize (*Zea mays* L.) in co-contaminated soils with pentachlorophenol and cadmium. *Int. J. Phytorem.* **15**, 703–713. doi:10.1080/15226514.2012.723067.

Kraus, J. J., Munir, I. Z., McEldoon, J. P., Clark, D. S., and Dordick, J. S. 1999. Oxidation of polycyclic aromatic hydrocarbons catalyzed by soybean peroxidase. *Appl. Biochem. Biotechnol.* **80**, 221–230. doi:10.1385/ABAB:80:3:221.

Krishnaveni, N., Geetha, R., Ranjitha, L., and Cibichakravarthy, B. 2015. Novel cultivable mycobiont *Piriformospora Indica* as plant growth promoting endophyte. *Int. Res. J. Biol. Sci.* **4** (1), 11–15.

Kuldau, G. and Bacon, C. 2008. Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biol. Control* **46**, 57–71. doi:10.1016/j.biocontrol.2008.01.023.

Kumar, M., Yadav, V., Kumar, H., Sharma, R., Singh, A., Tuteja, N., and Johri, A. K. 2011. *Piriformospora indica* enhances plant growth by transferring phosphate. *Plant Signaling Behav.* **6** (5), 723–725. doi:10.4161/psb.6.5.15106.

Kumar, M., Yadav, V., Tuteja, N., and Johri, A. K. 2009. Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. *Microbiol.* **155**, 780–790. doi:10.1099/mic.0.019869-0.

Lee, S-H., Lee, W-S., Lee, C-H., and Kim, J-G. 2008. Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes. *J. Hazard. Mater.* **153**, 892–898. doi:10.1016/j.jhazmat.2007.09.041.

Leigh, M. B., Fletcher, J. S., Fu, X., and Schmitz, F. J. 2002. Root turnover: an important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. *Environ. Sci. Technol.* **36**, 1579–1583. doi:10.1021/es015702i.

Li, H-Y., Wei, D-Q., Shen, M., and Zhou, Z-P. 2012. Endophytes and their role in phytoremediation. *Fungal Diversity* **54**, 11–18. doi:10.1007/s13225-012-0165-x.

Liao, C., Xu, W., Lu, G., Liang, X., Guo, C., Yang, C., and Dang, Z. 2015. Accumulation of hydrocarbons by maize (*Zea mays* L.) in remediation of soils contaminated with crude oil. *Int. J. Phytorem.* **17**, 693–700. doi:10.1080/15226514.2014.964840.

Liste, H-H. and Alexander, M. 1999. Rapid screening of plants promoting phenanthrene degradation. *J. Environ. Qual.* **28**, 1376–1377. doi:10.2134/jeq1999.00472425002800040044x.

Liu, W., Sun, J., Ding, L., Luo, Y., Chen, M., and Tang, C. 2013. Rhizobacteria (*Pseudomonas* sp. SB) assist phytoremediation of oily-sludge-contaminated soil by tall fescue (*Testuca arundinacea* L.). *Plant Soil* **371**, 533–542. doi:10.1007/s11104-013-1717-x.

Macek, T., Macková, M., and Káš, J. 2000. Exploitation of plants for the removal of organics in environmental remediation. *Biotechnol. Adv.* **18**, 23–34. doi:10.1016/S0734-9750(99)00034-8.

Malinowski, D. P. and Belesky, D. P. 2000. Adaptations of endophyte infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Sci.* **40**, 923–940. doi:10.2135/cropsci2000.404923x.

Martin, B. C., George, S. J., Price, C. A., Ryan, M. H., and Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. *Sci. Total Environ.* **472**, 642–653. doi:10.1016/j.scitotenv.2013.11.050.

Mathew, M. and Obbard, J. P. 2001. Optimisation of the dehydrogenase assay for measurement of indigenous microbial activity in beach sediments contaminated with petroleum. *Biotechnol. Lett.* **23**, 227–230. doi:10.1023/A:1005613406948.

McLean, E. O. 1982. Soil pH and lime requirement. In: *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties*, pp. 199–224 (Page, A. L., Miller, R. H., and Keeney, D. R., Eds.), Madison Wisconsin USA, American Society of Agronomy and Soil Science Society of America.

Nelson, D. W. and Sommers, L. E. 1982. Total carbon, organic carbon, and organic matter. In: *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties*, pp. 539–580 (Page, A. L., Miller, R. H., and Keeney, D. R., Eds.), Madison Wisconsin USA, American Society of Agronomy and Soil Science Society of America.

Nichols, T. D., Wolf, D. C., Rogers, H. B., Beyrouty, C. A., and Reynolds, C. M. 1997. Rhizosphere microbial populations in contaminated soils. *Water Air Soil Pollut.* **95**, 165–178. doi:10.1007/BF02406163.

Njoku, K. L., Akinola, M. O., and Oboh, B. O. 2009. Phytoremediation of crude oil contaminated soil: the effect of growth of Glycine max on the physico-chemistry and crude oil contents of soil. *Nat. Sci.* **7** (10), 79–87.

Olsen, S. R. and Sommers, L. E. 1982. Phosphorus. In: *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties*, pp. 403–430 (Page, A. L., Miller, R. H., and Keeney, D. R., Eds.), Madison Wisconsin USA, American Society of Agronomy and Soil Science Society of America.

Phillips, L. A., Greer, C. W., Farrell, R. E., and Germida, J. J. 2012. Plant root exudates impact the hydrocarbon degradation potential of a weathered-hydrocarbon contaminated soil. *Appl. Soil Ecol.* **52**, 56–64. doi:10.1016/j.apsoil.2011.10.009.

Qin, X., Tang, J. C., Li, D. S., and Zhang, Q. M. 2012. Effect of salinity on the bioremediation of petroleum hydrocarbons in a saline-alkaline soil. *Lett. Appl. Microbiol.* **55** (3), 210–217. doi:10.1111/j.1472-765X.2012.03280.x.

Ray, J. G. and Valsalakumar, N. 2010. Arbuscular mycorrhizal fungi and *Piriformospora indica* individually and in combination with Rhizobium on green gram. *J. Plant Nutr.* **33**, 285–298. doi:10.1080/01904160903435409.

Rhoades, J. D. 1982. Cation exchange capacity. In: *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties*, pp. 149–157 (Page, A. L., Miller, R. H., and Keeney, D. R., Eds.), Madison Wisconsin USA, American Society of Agronomy and Soil Science Society of America.

SAS Institute Inc. 2005. *SAS/Genetics TM 9.1.3 User's Guide.* Cary, NC: SAS Institute Inc.

Schulz, B., Boyle, C., Draeger, S., Romert, A-K., and Krohn, K. 2002. Endophytic fungi: A source of novel biologically active secondary metabolites. *Mycol. Res.* **106**, 996–1004. doi:10.1017/S0953756202006342.

Sherameti, I., Tripathi, S., Varma, A., and Oelmüller, R. 2008. The Root-colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress–related genes in leaves. *Mol. Plant-Microbe Interact.* **21** (6), 799–807. doi:10.1094/MPMI-21-6-0799.

Sirrenberg, A., Göbel, C., Grond, S., Czempinski, N., Ratzinger, A., Karlovsky P., Santos, P., Feussner, I., and Pawlowski, K. 2007. *Piriformospora indica* affects plant growth by auxin production. *Physiol. Plant.* **131**, 581–589. doi:10.1111/j.1399-3054.2007.00983.x.

Soleimani, M., Afyuni, M., Hajabbasi, M. A., Nourbakhsh, F., Sabzalian, M., and Christensen, J. H. 2010a. Phytoremediation of an aged petroleum contaminated soil using endophyte infected and non-infected grasses. *Chemosphere*, **81**, 1084–1090. doi:10.1016/j.chemosphere.2010.09.034.

Soleimani, M., Farhoudi, M., and Christensen, J. H. 2013. Chemometric assessment of enhanced bioremediation of oil contaminated soils. *J. Hazard. Mater.* **254–255**, 372–381. doi:10.1016/j.jhazmat.2013.03.004.

Soleimani, M., Hajabbasi, M. A., Afyuni, M., Mirlohi, A. F., Borggaard, O. K., and Holm, P. E. 2010b. Effect of endophytic fungi on Cd tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. *Int. J. Phytorem.* **12** (6), 535–549. doi:10.1080/15226510903353187.

Stepniewska, Z. and Kuźniar, A. 2013. Endophytic microorganisms – promising applications in bioremediation of greenhouse gases. *Appl. Microbiol. Biotechnol.* **97** (22), 9589–9596. doi:10.1007/s00253-013-5235-9.

Sun, C., Johnson, J. M., Cai, D., Sherameti, I., Oelmüller, R., and Lou, B. 2010. *Piriformospora indica* confers drought tolerance in Chines cabbage leaves by stimulating antioxidant enzymes, the expression of drough-related genes and the plastid-localized protein. *J. Plant Physiol.* **167**, 1009–1017. doi:10.1016/j.jplph.2010.02.013.

Thoma, G. J., Lam, T. B., and Wolf, D. C. 2003. A mathematical model of phytoremediation for petroleum-contaminated soil: model development. *Int. J. Phytorem.* **5** (1), 41–55. doi:10.1080/16226510390856466.

USEPA, 1998. Method 1664, Revision A. n-hexane extractable material (HEM; oil and grease) and silica gel treated n-hexane extractable material by extraction and gravimetry. EPA-821-R-98-002; 40 CFR Part 136 (July 1, 2000); *Federal Register* **64** (93):26315. U.S. Environmental Protection Agency, Washington, D.C.

Varma, A., Verma, S., Sudha, Sahay, N., Bütehorn, B., and Franken, P. 1999. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol.* **65** (6), 2741–2744.

Verma, S., Varma, A., Rexer, K-H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Butehorn, B., and Franken, P. 1998. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* **90** (5), 898–905. doi:10.2307/3761331.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., von Wettstein, D., Franken, P., and Kogel, K. H. 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl. Acad. Sci. U.S.A.* **102** (38), 13386–13391. doi:10.1073/pnas.0504423102.

Wang, Z., Xu, Y., Zhao, J., Li, F., Gao, D., and Xing, B. 2011. Remediation of petroleum contaminated soils through composting and rhizosphere degradation. *J. Hazard. Mater.* **190** (1–3), 677–685. doi:10.1016/j.jhazmat.2011.03.103.

Wenzel, W. W. 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. *Plant Soil* **321**, 385–408. doi:10.1007/s11104-008-9686-1.

Weyens, N., van der Lelie, D., Taghavi, S., and Vangronsveld, J. 2009. Phytoremediation: Plant–endophyte partnerships take the challenge. *Curr. Opin. Biotechnol.* **20** (2), 248–254. doi:10.1016/j.copbio.2009.02.012.

Wu, T., Xie, W. J., Yi, Y. L., Li, X. B., Yang, H. J., and Wang, J. 2012. Surface activity of salt-tolerant Serratia spp. and crude oil biodegradation in saline soil. *Plant, Soil Environ.* **58** (9), 412–416.

Youssef, R. A. and Chino, M. 1988. Development of a new rhizobox system to study the nutrient status in the rhizosphere. *Soil Sci. Plant Nutr.* **34** (3), 461–465. doi:10.1080/00380768.1988.10415701.

Zamani, J., Hajabbasi, M. A., Alaie, E., Sepehri, M., Leuchtmann, A., and Schulin, R. 2016. The effect of *Piriformospora indica* on the root development of maize (*Zea mays* L.) and remediation of petroleum contaminated soil. *Int. J. Phytorem.* **18** (3), 278–287. doi:10.1080/15226514.2015.1085831.

Zand, A. D., Bidhendi, G. N., and Mehrdadi, N. 2010. Phytoremediation of total petroleum hydrocarbons (TPHs) using plant species in Iran. *Turk. J. Agric. For.* **34**, 429–438.

Zarea, M. J., Hajinia, S., Karimi, N., Mohammadi Goltapeh, E., Rejali, F., and Varma, A. 2012. Effect of *Piriformospora indica* and Azospirillum strains from saline or non-saline soil on mitigation of the effects of NaCl. *Soil Biol. Biochem.* **45**, 139–146. doi:10.1016/j.soilbio.2011.11.006.