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Running head: *P. indica* Enhanced Root Growth of Maize and Petroleum Remediation

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The effect of *Piriformospora indica* on the root development of maize (*Zea mays* L.) and remediation of petroleum contaminated soil

Abstract:

As the depth of soil petroleum contamination can vary substantially under field conditions, a rhizotron experiment was performed to investigate the influence of endophyte, *P. indica*, on maize growth and degradation of petroleum components in a shallow and a deep-reaching subsurface layer of a soil. For control, a treatment without soil contamination was also included. The degree in contamination and the depth to which it extended had a strong effect on the growth of the plant roots. Contaminated soil layers severely inhibited root growth thus many roots preferred to bypass the shallow contaminated layer and grow in the uncontaminated soil. While , the length and branching pattern of these roots were similar to those of uncontaminated treatment. Inoculation of maize with *P. indica* could improve root distribution and root and shoot growth in all three contamination treatments. This inoculation also enhanced petroleum degradation in soil, especially in the treatment with deep-reaching contamination, consequently the accumulation of petroleum hydrocarbons (PAHs) in the plant tissues were increased.

Keywords: endophyte, *P. indica*, root distribution, rhizotron, PAHs

Introduction:

Solis around petrochemical industries and petroleum distributors are generally prone to be contaminated by petroleum hydrocarbons. This can happen for example through leakage from tanks, accidental spillage, pipeline ruptures, or sometimes even through deliberate spreading of oily wastes as in landfilling operations. Many petroleum hydrocarbons or their metabolites are toxic to humans, plants and other organisms, and in addition, some, like benzene and benzo[a]pyrene, are highly mutagenic and carcinogenic. Thus, petroleum hydrocarbons contaminating soils, sediments, surface and ground waters are a severe environmental hazard, adversely affecting the quality of drinking water, crop plants and their products, and ultimately threaten human health, as they are transferred through the food chain (Hajabbasi et al. 2015).

Current research studies have shown that plants may play an important role in the bioremediation of petroleum-polluted soil (Palmroth, Pichtel and Puhakka 2002; Dominguez-Rosado and Pichtel 2004; Besalatpour, Hajabbasi and Khoshgoftarmanesh 2010; Soleimani et al. 2010; Edema, Idu and Edema 2011; Besalatpour et al. 2011; Li, Wei and Chen 2012). This provides an alternative to disruptive and expensive conventional methods such as thermal treatment, soil washing, or solidification (Macek, Mackova and Kas 2000). Plant-based methods proposing the treatment of petroleum-contaminated soil are including rhizodegradation and phytostimulation (Kasperl and Vontobel 2005; Merkl, Schultze-Kraft and Infante 2005; Besalatpour, Hajabbasi and Khoshgoftarmanesh 2010). In these methods, for providing soil conditions in a way that stimulate and promote degradation of petroleum hydrocarbons by rhizospheric microbial communities, plant roots play a crucial role (Kidd et al. 2008; Haritash and Kaushik 2009;

Soleimani et al. 2010). Hence, the use of plants and the associated microorganisms including endophyte are recently more considered as a promising green technology for soil remediation (Weyens et al. 2009).

Endophytes are bacterial or fungal endosymbionts, spending at least part of their life cycle in plants and increase the host plant's tolerance to biotic and abiotic stresses (Schulz and Boyle 2006). In this regard several studies have shown that endophytes can enhance the phytoremediation of organic contaminants (Newman and Reynolds 2004; Khan and Doty 2009; Soleimani et al. 2010). Soleimani et al. (2010) found that the endophytic fungus *Neotyphodium* promoted root and shoot growth of *Festuca arundinacea* Schreb. and *Festuca pratensis* Huds. and thus accelerated contaminant degradation of an aged petroleum-contaminated soil.

Piriformospora indica (Sebacinales, Basidiomycota) is described as a cultivable root-colonizing endophytic fungus (Verma et al. 1998; Varma et al. 1999). It was found to be promoting 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 the preliminary experiments we also found that *P. Indica* has the potential to promote phytoremediation of petroleum-contaminated soil (unpublished results).

Soil contamination with petroleum hydrocarbons can severely inhibit plant root growth, and consequently limit the efficacy of phytoremediation. Therefore, root growth and distribution is an important factor to be considered especially when the contamination is not evenly distributed in soil, usually the case in the real world situations. Many pot studies on the phytoremediation potential of candidate plants are generally performed under conditions of uniform contaminant

distribution (Spiares, Kenworthy and Rhykerd 2001; Ho, Applegate and Banks 2007; Besalatpour, Hajabbasi and Khoshgoftarmanesh 2010). Reports on root growth rate and distribution in soils with non-uniform petroleum contamination layers are rare (Merkl, Schultze-Kraft and Infante 2005; Kechavarzi et al. 2007). Of those, almost none have addressed the question whether and how endophytes influence the spatial distribution of plant root in petroleum-contaminated soil and if this influence depends on the spatial pattern of the contaminant distribution.

Thus, we performed a rhizotron experiment to investigate the effect of different types of layering and distribution of petroleum contaminants in soil on the root growth and distribution of maize (*Zea mays* L.) and the elimination of the contaminants in the presence and absence of *Piriformospora indica*. Maize was used in this study because it is known to be a suitable host for *P. indica* (Kumar et al. 2009) and because it was also found to be particularly effective in enhancing petroleum elimination from contaminated soil (Ayotamuno, Kogbara and Egwuenum 2006; Chouychai et al. 2009; Zand, Bidhendi and Mehrdadi 2010). Our expectation was that inoculation with *P. indica* would promote growth and root system development of maize in petroleum contaminated soil and thereby enhance phyto-stimulation of petroleum degradation. We further expected that these effects of the endophyte would be more pronounced with the deep-reaching than the shallow contamination.

Material and methods:

Soils

The contaminated soil was collected from a landfill nearby Shahid Hasheminejad Gas Refinery Complex at Sarakhs in northeastern Iran (36° 34' N; 60° 49' E). The uncontaminated soil samples were also taken from the vicinity of the landfill. Three soil samples (from 0 to 30 cm depth) of each location were bulked, thoroughly mixed, air-dried and sieved through a mesh of 2 mm size. Selected physical and chemical characteristics of the sieved soils are given in Table 1.

Rhizotrons and soil packings

The rhizotrons had a wooden frame and back plate, and a removable front cover made of a 4 mm thick glass plate. The inner space was 30 cm high, 20.5 cm wide and 1.5 cm thick (Figure 1a). The rhizotrons were placed on a rack with a 45^{o} inclination to induce roots growing along the front glass to enable visual growth monitoring. The front glass plate was covered with an opaque black plastic to prevent light entering except for the times of observation.

Three different patterns of soil-petroleum contamination layering were generated in the packings of the rhizotrons (Figure 1b): (a) a shallow layer of 2.5 cm thick petroleum-contaminated soil, underlying of a 2.5 cm and above a 22.5 cm layer of uncontaminated soil (in the following denoted as 'treatment with narrow subsurface contamination' or 'NSC'), (b) 25 cm of contaminated soil below a 2.5 cm layer of uncontaminated soil ('treatment with deep-reaching subsurface contamination' or 'DSC'); and (c) 27.5 cm of uncontaminated soil ('control'). The packing procedure was layer by layer using uniform filling in all three cases. The contaminated soil layers were covered with a 2.5 cm layer of uncontaminated soil in order to facilitate plant establishment, following Kechavarzi et al. (2007). Applying such a layer on a contaminated site

in practical remediation situations is a realistic option also in field applications of phytoremediation.

The experiment with three replication was included as the packing methods each for growing maize plants inoculated with and without *P. indica,* and plant-free controls. An additional set of rhizotron was established in the same way with all three types of packing and planted with inoculated and non-inoculated seedlings to check for root colonization by *P. indica*. The root systems of the latter rhizotrons were sampled after 20 days of growth and analyzed microscopically for fungal spores and hyphae.

Production of P. indica 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). Inoculum for the experiments was prepared in the Soil Biology Laboratory of Isfahan University of Technology. In order to obtain high quantities of active spores, inoculum was produced in two steps. First, the mycelium plugs were taken from the edge of a growing colony of *P. indica,* andcultured at 24 °C in Petri-dishes on a solid medium 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}). The 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}) and $CuSO_4.5H_2O$ (130 mg L^{-1}). At the second step, spores were collected after 18 days by gently

scratching the surface of the Petri-dishes with a spatula and allowed to germinate for 10 days on a liquid medium at 28 °C under gentle shaking at a frequency of 80 rpm. The composition of the liquid medium was identical to the solid medium, but with no agar. 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 4000g for 10 minutes.

Plant cultivation and experiment

Maize (*Zea mays* L. cv. SC704) seeds were surface-sterilized by rinsing for 30 seconds in 70% ethanol, 5 min in 5% sodium hypochlorite (NaOCl) and five times briefly with sterilized distilled water. After vernalization at 4 $^{\circ}$C the seeds were placed on an 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 for 3 hours in inoculums (adjusted to~2×10^6) under gentle shaking. The non-inoculated seedlings were dipped in sterilized distilled water containing Tween-20 (0.02%). Single inoculated or non-inoculated seedlings, according to the experimental design, were then planted at a depth of 1cm in the uncontaminated top soil layer in the center of each rhizotron. All rhizotrons, including the plant-free controls, were placed in a glasshouse (temperature 28±4 $^{\circ}$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% of field capacity) by periodical watering in order to replace consumed water.

Following the procedures described by Schwartz et al. (1999) and Kechavarzi et al. (2007), root development was recorded 12, 16, 22, 26, 33 and 45 days after transplanting by tracing all roots that were visible through the front glass on acetate transparencies. Then the transparencies were scanned at 300 dpi to obtain digitized images and analyzed for parameters such as root length, number of root tips and depth of rooting using the SmartRoot plugin of the software package ImageJ (Lobet, Pages and Draye 2011). At the same dates, also the shoot height of each seedling was measured using the soil surface as a datum. After the last recording (45 days after transplanting), the experiment was terminated. Roots and shoots were separated after harvesting, weighed and oven-dried. Rhizosphere soil samples were taken from the layer 2.5-5.0 cm below the soil surface. The soils of the plant-free rhizotrons were sampled and then analyzed in the same way as the samples from the planted rhizotrons. The root samples were boiled in 5% KOH solution for 1 minute, washed three times with sterile distillated water and stained with aniline blue solution for 1 minute and washed with sterile distillated water again and analyzed under a light microscope.

TPH and PAHs 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). 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).

Root and shoot samples from the DSC treatments were analyzed for PAHs to assess their potential uptake by maize from petroleum-contaminated soil. After several rinsing with distilled water, they were dried for 48h at 40°C. The PAHs were extracted from sub-samples of the dried tissues using the same Soxhlet-extraction procedure and 1:1 dichloromethane/acetone mixture as for the soil samples. The 16 USEPA target PAH were determined in the extracts by means of high-performance liquid chromatography (HPLC) using a KnauerC18-S5ODS1 column (250 mm×4.6 mm) and spectrophotometric UV detection at λ= 280 nm. The mobile phase contained methanol with a 1.5 mL/min flow rate. The same procedure was used to determine PAH concentrations in untreated soils used for packing the rhizotrons.

Statistical analysis

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

Results

Overall, the inoculated plants had larger and stronger appearance compared to the non-inoculated ones. Based on the microscopic analyses, *P. indica* could efficiently colonize the maize roots in

all treatments as indicated by production of the number of chlamydospores and hyphae (Figure 2). No colonization was observed in the non-inoculated plant roots. Microscopic analyses of 20-days old roots of inoculated plants showed that the infection percentages of the control, NSC and DSC treatments were respectively 72±8.2, 65±5.3 and 55±3.5%.

Contaminated soils had significantly lower shoot and root biomass compared to the control (Figure 3 and Table 2). Although all plants could generally survive, but the negative effect on the growth increased with the extent of contamination. Inoculation with *P. indica* reduced the adverse petroleum contaminating effect. Inoculating could also shift the ratio between shoot and root biomass towards the roots, while the contamination treatment had no effect on the root:shoot biomass allocation ratio (in both with or without endophyte) (Figure 3). In all three treatments, root biomass was about 35% larger in the inoculated than the non-inoculated plants. Shoot biomass was on average 15% larger in the inoculated than non-inoculated plants, but this was only a trend and not a statistically significant effect.

Figure 4 reveals that root growth was not only inhibited in the zones of contaminated soil, but also in the shallow uncontaminated surface layer above the contaminated layer, but the denser root system was developed in the surface layer close to the stems as compared to the control treatments. In deep-reaching contamination treatment, the roots penetrated only very superficially, i.e. up to 7.5 cm, into the contaminated soil for the *P. indica* inoculated plants. In the narrow subsurface contamination treatments, some roots (with low branching) passed through the contaminated layer and continued to grow without further retardation and with an apparently normal branching pattern down to the bottom of the rhizotrons. The quantitative

analysis of the digitized root distribution tracings confirmed the impression that inoculation enhanced root growth in all contamination treatments, i.e. including the treatment without contaminated soil, especially at the lower depths (Figure 5). Although root growth still showed strong inhibition in the contaminated soil in comparison to the uncontaminated soil, roots of inoculated plants produced many more laterals and show to grow much deeper into the contaminated soil than without inoculation.

Figure 6 provides further evidence of the strong inhibitory effect of the petroleum contaminants on root growth and of the mitigation effect of *P. indica* inoculation. It shows that root growth was particularly sensitive to shallow petroleum contamination and that inoculation with *P. indica* nearly doubled total root length in all treatments. Rooting depth was sensitive to inoculation only in the deeply contaminated soil. Figure 6 furthermore shows that the pattern of treatment effects on root length growth was mirrored by a similar pattern of weaker effects (or at least trends towards effects) on shoot height growth.

Comparing Figure 3 and Figure 6c shows that the effect of petroleum was stronger on shoot biomass than on its height, suggesting that shoot thickness was strongly reduced under petroleum stress. In contrast, there was no such discrepancy in the petroleum effects on root biomass and root length. However, the endophyte treatment revealed no effect on the ratios between length and biomass of shoots and roots.

Root activity could significantly ($p< 0.05$) eliminate (degrade) larger amounts of petroleum contaminants in soil when comparing plant with no plant-free treatments (Figure 7). Inoculation with *P. indica* slightly enhanced petroleum elimination from the deeply contaminated soil, but

had no significant effect on petroleum elimination from the soil with shallow contamination (Figure 7). In the presence of inoculated maize plants 30% of the initially present petroleum hydrocarbons had disappeared at the end of the experiment compared to 13% in plant-free soil.

Table 3 shows that 6 out of the 16 analyzed PAH (Phen, Pyr, BaA, Chry, BbF and BaP) were detected at low concentrations in the plant root samples in the deep contamination treatment, and 3 of these (Phen, Pyr and BaA) at even lower concentrations in the shoots. In total, shoots accumulated only a third of the PAH found in the roots. Inoculation had no significant effect on PAH accumulation, except for a decrease in the concentration of chrysene in root tissues and a slight increase in pyrene concentrations in the shoots. The root chrysene concentrations of the non-inoculated plants were in average 1.7 times higher than of the inoculated maize plants.

Discussion

The results confirmed the expectation that *P. indica* can mitigate the negative effects of soil petroleum contamination on root and shoot growth in maize. Kechavarzi et al. (2007) found similar inhibitory effects of diesel-contaminated soil on root and shoot development in perennial ryegrass (*Lolium perenne*), however, they did not include an endophyte treatment in their study. Enhancing maize root and shoot growth by *P. indica* even in the uncontaminated soil and that these effects were of very similar relative magnitude in all treatments even in the treatments with petroleum contaminated soil, suggests that they were not primarily due to reduced exposure or increased tolerance to soil petroleum toxicity, but more likely resulted from a benefit such as proper soil nutrient supply. This is in line with the root growth enhancement which was higher at

the lower depths only in the shallow contamination treatment. The increase in root-to-shoot ratio indicates that the endophyte was able to induce larger allocation of assimilates to the belowground parts of the plants, and the shifts in root length distributions towards lower soil depths indicate that a similar influence on assimilate allocation also occurred within the root system favoring apical over basal roots.

The results in agreement with previous studies confirmed that maize has the potential to eliminate soil petroleum contaminants (Dominguez-Rosado and Pichtel 2004; Ayotamuno, Kogbara and Egwuenum 2006; Chouychai et al. 2009; Zand, Bidhendi and Mehrdadi 2010; Li, Wei and Chen 2012). However, the rate of petroleum elimination was much less in our study than observed in some other studies. Ayotamuno, Kogbara and Egwuenum (2006) observed that more than three-quarters of petroleum hydrocarbon were lost from an agricultural field soil within only 2 weeks of maize culture, and Chouychai et al. (2009) found that around 40% of both phenanthrene and pyrene disappeared from a contaminated soil within 10 days in presence of maize, as compared to less than 10% in unplanted soil. As postulated, inoculation with *P. indica* further accelerated petroleum hydrocarbon elimination in the treatment with deep contamination. The lack of a significant endophyte effect on petroleum hydrocarbon elimination in the shallow contamination treatment could be related to the fact that the endophyte boosted root growth more in the bottom than in the top parts of the root systems.

The results in this study did not indicated to what extent the contaminants were actually degraded and that degradation may have occurred within the plants. To some degree at least, the observed plant and endophyte effects may also be explained with adsorption to root surfaces and

accumulation within plant tissues, as they were roughly proportional to root length in the contaminated soil layer from which the samples had been taken. The accumulation of PAHs in the root and shoot tissue samples were low enough to be not exceeded the detection limit for most of those analyzed.

Plant PAH uptake of contaminated soil found in previous studies was comparable to our findings (Wennrich, Popp and Zeibig 2002; Gao and Zhu 2004; Watts, Ballestero and Gardner 2006; Besalatpour, Hajabbasi and Khoshgoftarmanesh 2010; Wu et al. 2011). However, little is known about the influence of symbiotic microorganisms on plant uptake of petroleum hydrocarbons. Wu et al. (2011) found that inoculation with the arbuscular mycorrhizal fungus, *Glomus mosseae*, significantly increased root uptake of phenanthrene and pyrene and root-to-stem translocation of phenanthrene in maize. Their findings are in line with the trend towards higher root and shoot concentrations of these compounds in the treatments with *P. indica* observed in our study. Although the overall effect of *P. indica* on PAH accumulation was not substantial, it provides further evidence for the above conclusion. The beneficial effects of the endophyte on maize growth and contaminant elimination were not primarily due to increased plant tolerance or reduced exposure to the contaminants. It also means that even more caution is required in using maize grown on petroleum-contaminated soil as feed or for food production in presence of root-colonizing fungi.

In summary, the results of this study showed that inoculation of maize with *P. indica* has the potential to increase the ability of this plant to grow in petroleum contaminated soil and to

enhance phytostimulated elimination of petroleum hydrocarbons in contaminated soil, with only marginally increasing plant uptake of contaminating PAHs.

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Table 1. Physical and chemical characteristics (mean ± standard deviation of three replicate samples in each case) of the two soils (with and without petroleum contamination) used in this study

Parameter	Unit	Soil		Method of analysis
		Uncontaminated	Contaminated	
Sand content	%	86±5	81±4	Gee and Bauder 1986
Silt content	%	6±2	11±2	
Clay content	%	8±4	8±3	
Texture	---	Loamy Sand	Loamy Sand	
pH	---	7.2±0.2	7.4±0.2	McLean, 1982
EC	dS m^{-1}	1.28±0.16	1.25±0.05	McLean, 1982
Total organic C	%	0.67±0.10	2.95±0.56	Nelson and Sommers 1982
Total N	mg kg^{-1}	135±13	200±31	Bremner and Mulvaney 1982
Available P	mg kg^{-1}	31.2±4.9	11.0±3.7	Olsen and Sommers 1982
CEC	$Cmol^{+}kg^{-1}$	32.9±5.7	9.2±3.1	Rhoades, 1982

Table 2. Two-way analysis of variance for various measures of root and shoot growth

Variable†	Source of variability‡					
	Pollution		Inoculation		Pollution×Inoculation	
	MS	*p*-value	MS	*p*-value	MS	*p*-value
RW	8.2	<0.0001	2.1	0.0023	0.30	0.1631
ShW	15.7	<0.0001	0.68	0.1162	0.17	0.5141
TRL	3260.4	<0.0001	1697.7	<0.0001	440.1	<0.0001
RD	43.4	<0.0001	0.99	0.2124	0.01	0.98
NRT	233.6	0.0013	264.5	0.0030	56.0	0.0925
ShL	0.30	0.9746	9.5	0.3864	2.7	0.7999

† RW: root weight, ShW: shoot weight, TRL: total root length, R: rooting depth, NRT: number of root tips, ShL: shoot length

‡ Degrees of freedom for Pollution, Inoculation and Pollution×Inoculation are 2, 1 and 2 respectively.

Table 3. Total petroleum hydrocarbon content in soils and concentration of PAHs in initial contaminated soil and plant tissues in the treatments with deep subsurface contamination (DSC treatment). Values with the same letters in a row are not significantly different at $P < 0.05$.

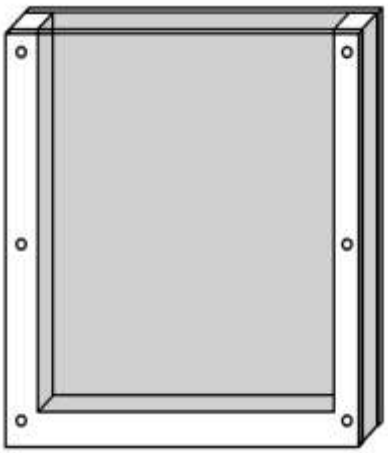
Compound (abbreviation, total rings number)	**Initial Soil (µg kg^{-1})**		**Plant Tissues (µg kg^{-1})**			
			Root		**Shoot**	
	Uncontaminated	contaminated	- *P. indica*	+*P. indica*	- *P. indica*	+ *P. indica*
Naphthalene (Nap, 2)	---	63.3±10.0	nd[†]	nd	nd	nd
Acenaphthene (Ace, 3)	---	59.7±9.5	nd	nd	nd	nd
Fluorene (Flu, 3)	---	20.3±4.5	nd	nd	nd	nd
Phenanthrene (Phen, 3)	---	380.3±28.0	3.4±1.1 [b]	6.8±1.2 [a]	1.4±0.6 [A]	2.1±0.6 [A]
Anthracene (Anth, 3)	---	2.1±0.8	nd	nd	nd	nd
Fluoranthene (Flt, 4)	---	29.3±5.9	nd	nd	nd	nd
Pyrene (Pyr, 4)	---	190.3±19.7	14.0±3.9 [a]	17.7±2.4 [a]	6.7±2.2 [B]	8.2±2.1 [A]
Benzo(a)anthracene (BaA, 4)	---	77.3±7.02	10.7±2.0 [a]	11.6±2.5 [a]	6.2±1.6 [A]	7.3±1.1 [A]
Chrysene (Chry, 4)	---	349.7±41.0	14.5±3.7 [a]	8.4±2.4 [b]	nd	nd
Benzo(b)fluoranthene (BbF, 5)	---	50.7±12.1	2.5±1.1 [a]	3.6±0.9 [a]	nd	nd
Benzo(k)fluoranthene (BkF, 5)	---	6.3±2.1	nd	nd	nd	nd
Benzo(α.)pyrene (BaP, 5)	---	19.7±4.0	0.5±0.2 [a]	0.6±0.2 [a]	nd	nd
Dibenzo(a,h)anthracene (dBahAn, 5)	---	9.7±2.1	Nd	nd	nd	nd
Benzo(g,h,i)perylene (BghiP, 6)	---	17.7±4.0	Nd	nd	nd	nd
Indeno(1,2,3-c,d)pyrene (InPy, 6)	---	3.8±1.1	Nd	nd	nd	nd
∑PAHs	---	1280.2±93.	45.7±7.3	48.7±5.4 [a]	15.3±1.8	19.2±3.3 [A]

		5	[a]		[A]	
TPH (mg g^{-1})	< 0.050	21.6±0.6	---	---	---	---

†nd = not detected

(a)

Dimension, mm			
	Length	**Width**	**Thickness**
Outer	310	235	23
Inner	300	205	15



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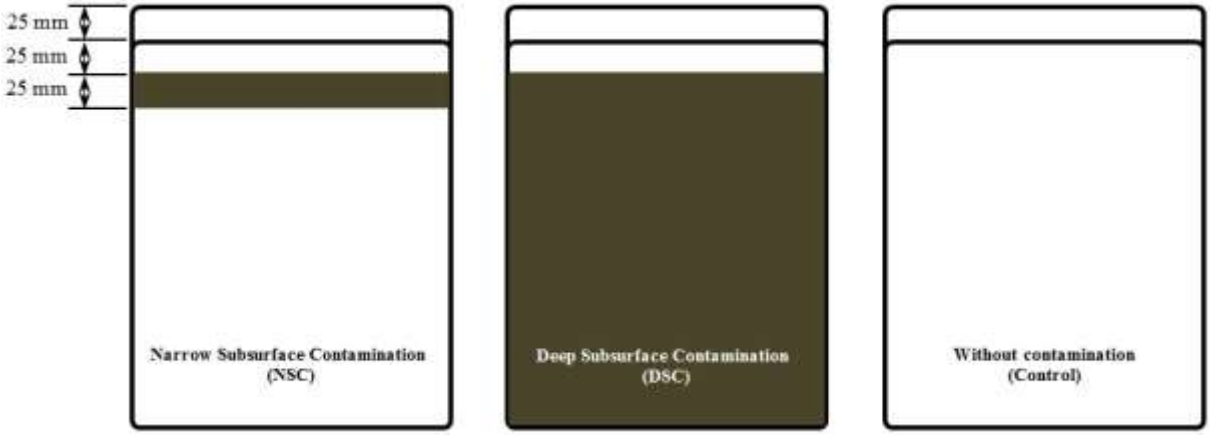


Fig. 1. Rhizotron dimensions (a) and applied soil contamination patterns (b).

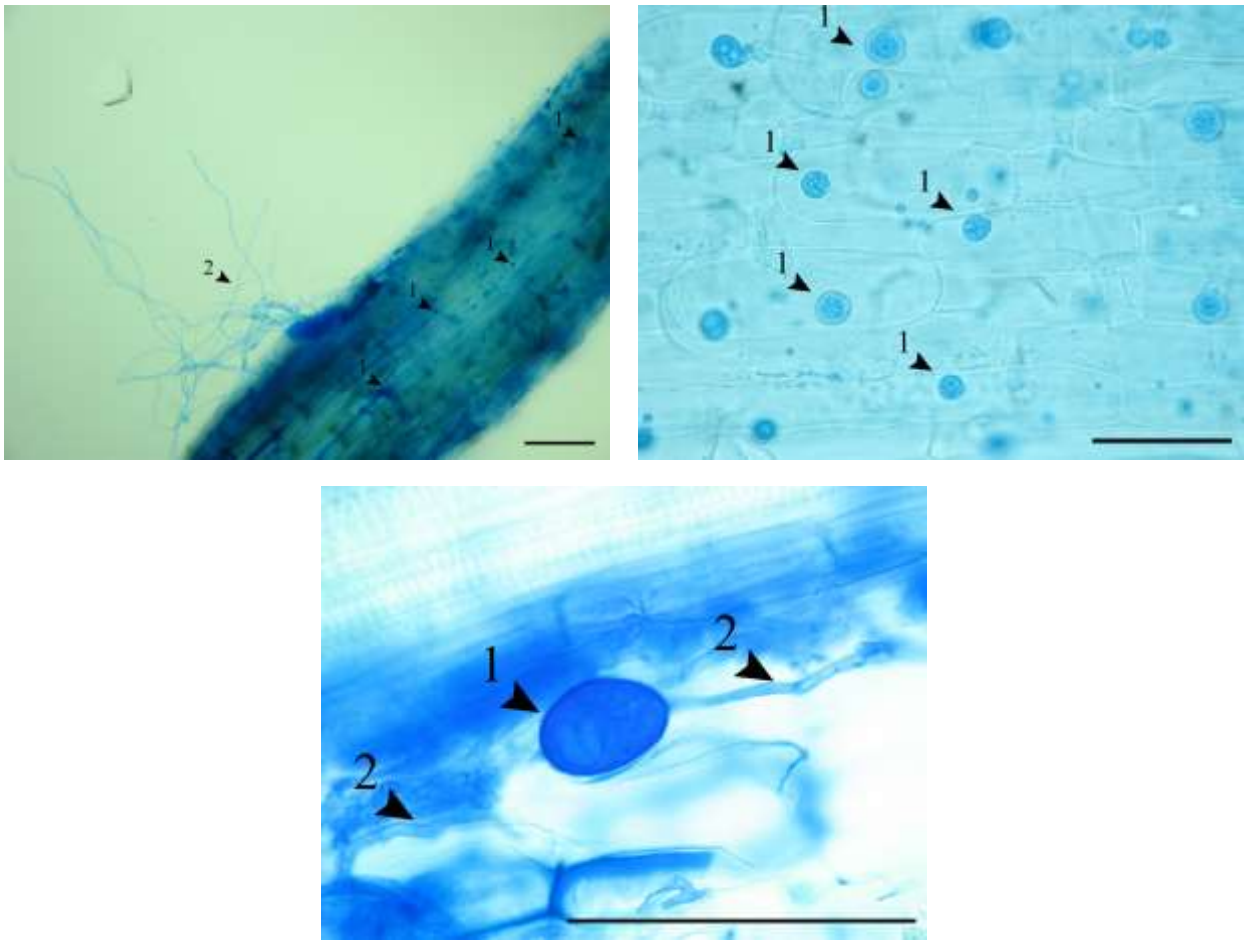


Fig. 2. Colonization of maize roots with *P. indica* at 20 days after inoculation. After spore germination, the fungus penetrates epidermal cells and spreads throughout the tissue and forms chlamydospores and inter- and intracellular hyphae. Arrows 1 and 2 indicate chlamydospores and hyphae, respectively (bar=100µm).

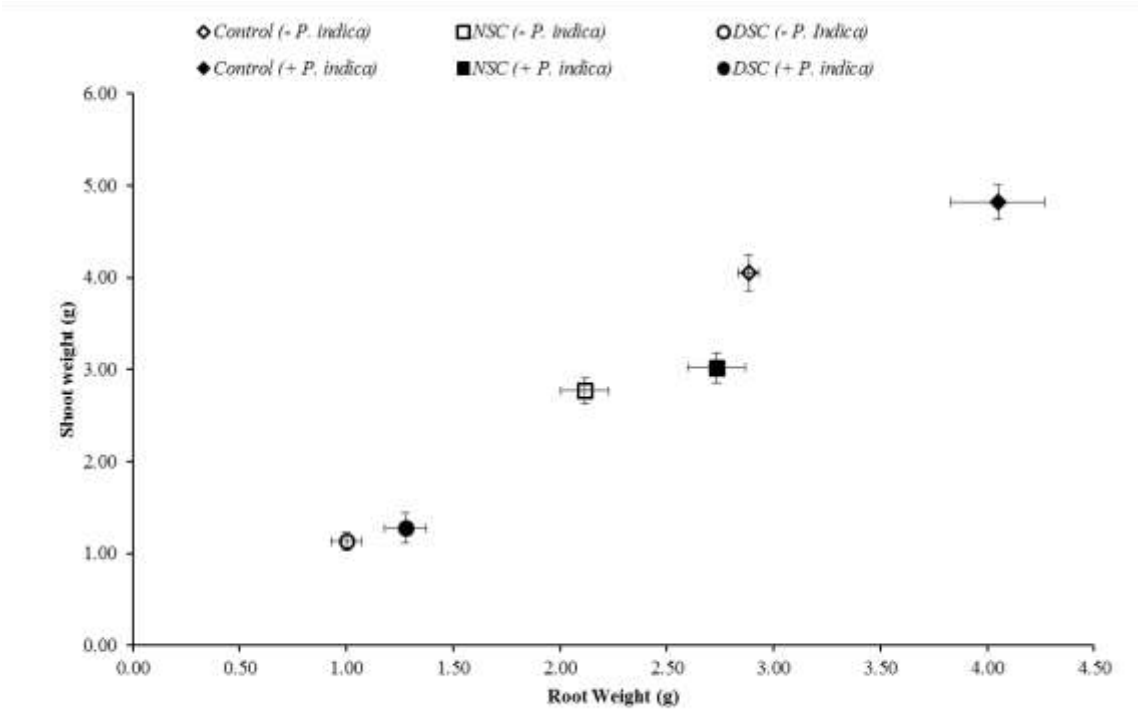
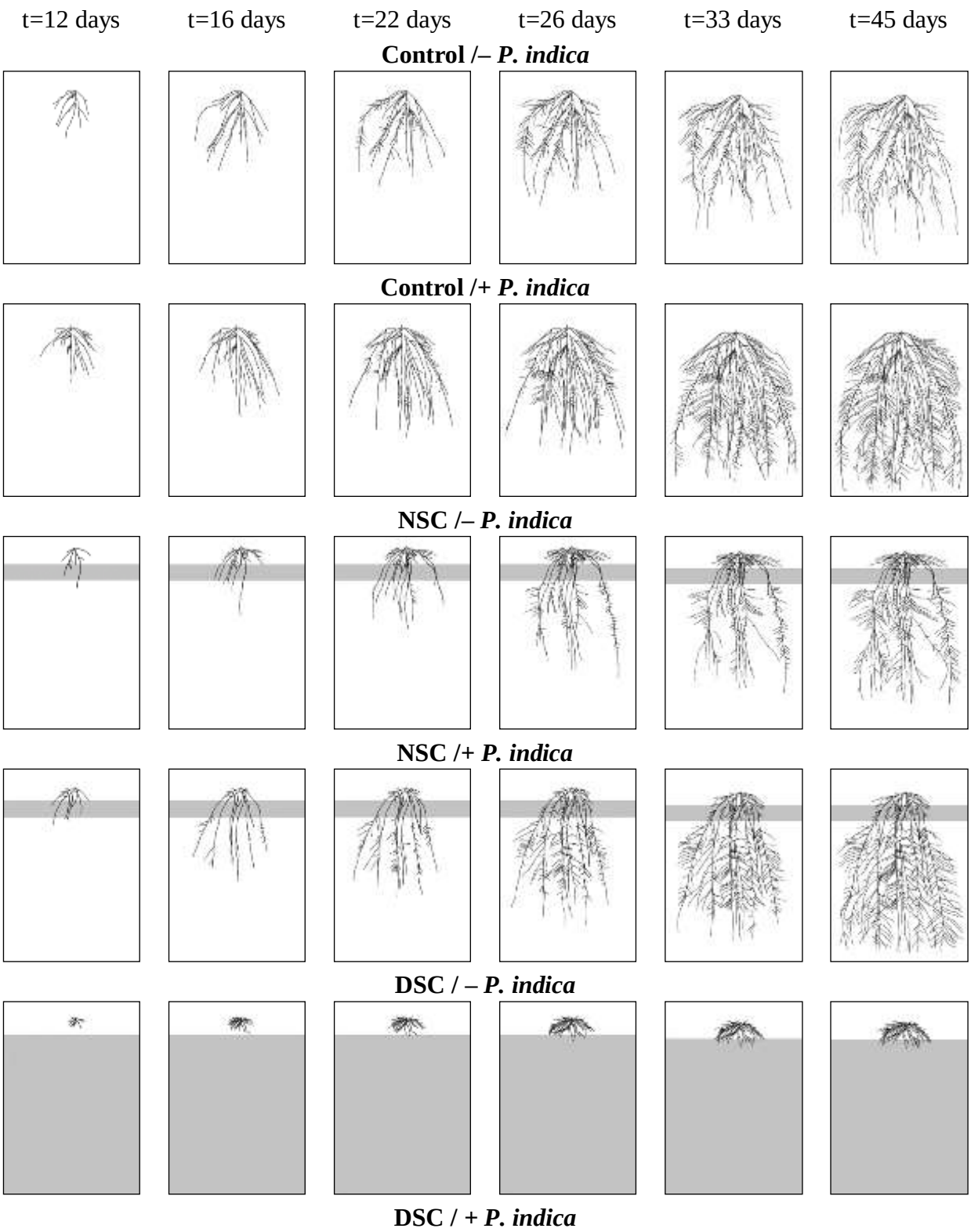


Fig. 3. Root biomass vs. shoot biomass of maize plants with (+ *P. indica*) and without (- *P. indica*) endophyte inoculation and grown for 45 days in uncontaminated soil ('Control'), soil with a narrow surface-near layer of petroleum contamination in otherwise uncontaminated soil ('NSC'), or deeply petroleum-contaminated soil below a superficial layer of uncontaminated soil ('DSC').



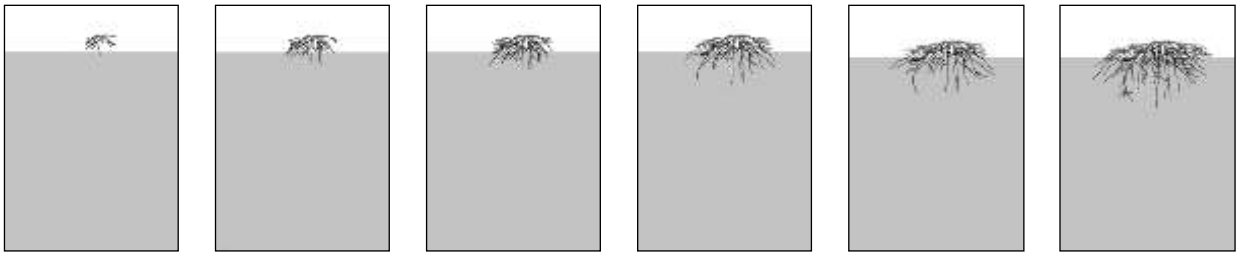
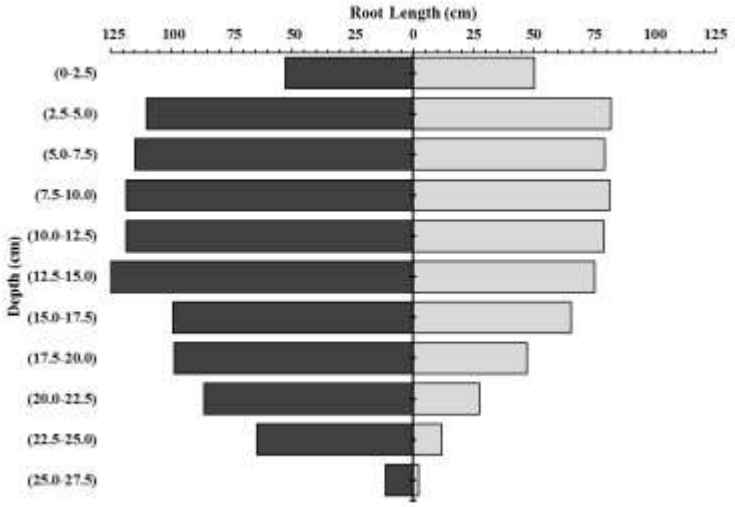


Fig. 4. Rooting patterns of maize plants with (+ *P. indica*) and without (- *P. indica*) endophyte inoculation and grown in either uncontaminated soil ('Control'), soil with a narrow surface-near layer of petroleum contamination in otherwise uncontaminated soil ('NSC'), or deeply petroleum-contaminated soil below a superficial layer of uncontaminated soil ('DSC') after different times of growth (12, 16, 22, 26, 33 and 45 days).

(a)



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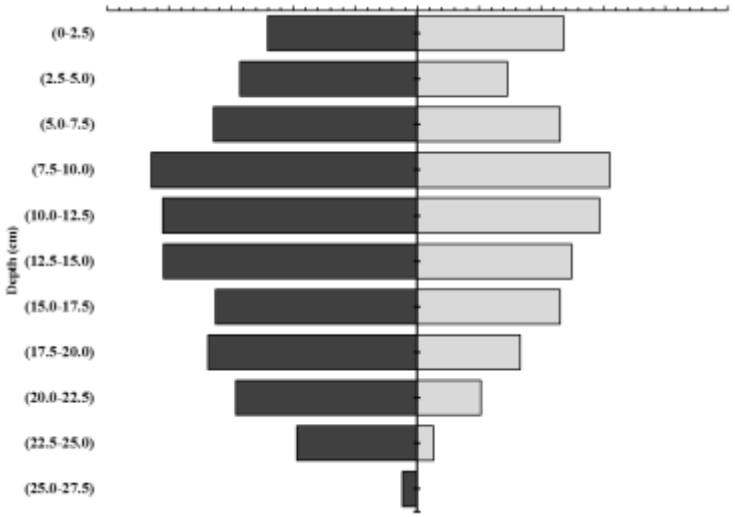

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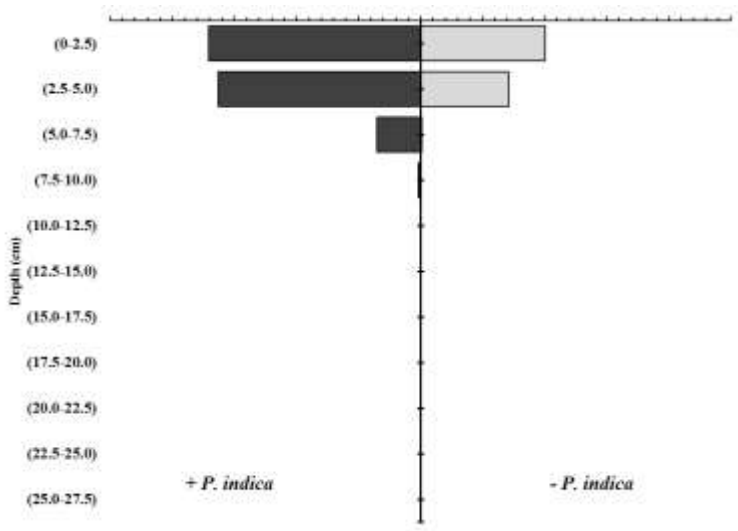

Fig. 5. Depth distribution of root length in maize plants with (+ *P. indica*) and without (- *P. indica*) endophyte inoculation and grown for 45 days in either uncontaminated soil (a), soil with a narrow surface-near layer of petroleum contamination in otherwise uncontaminated soil (b), or deeply petroleum-contaminated soil below a superficial layer of uncontaminated soil (c).

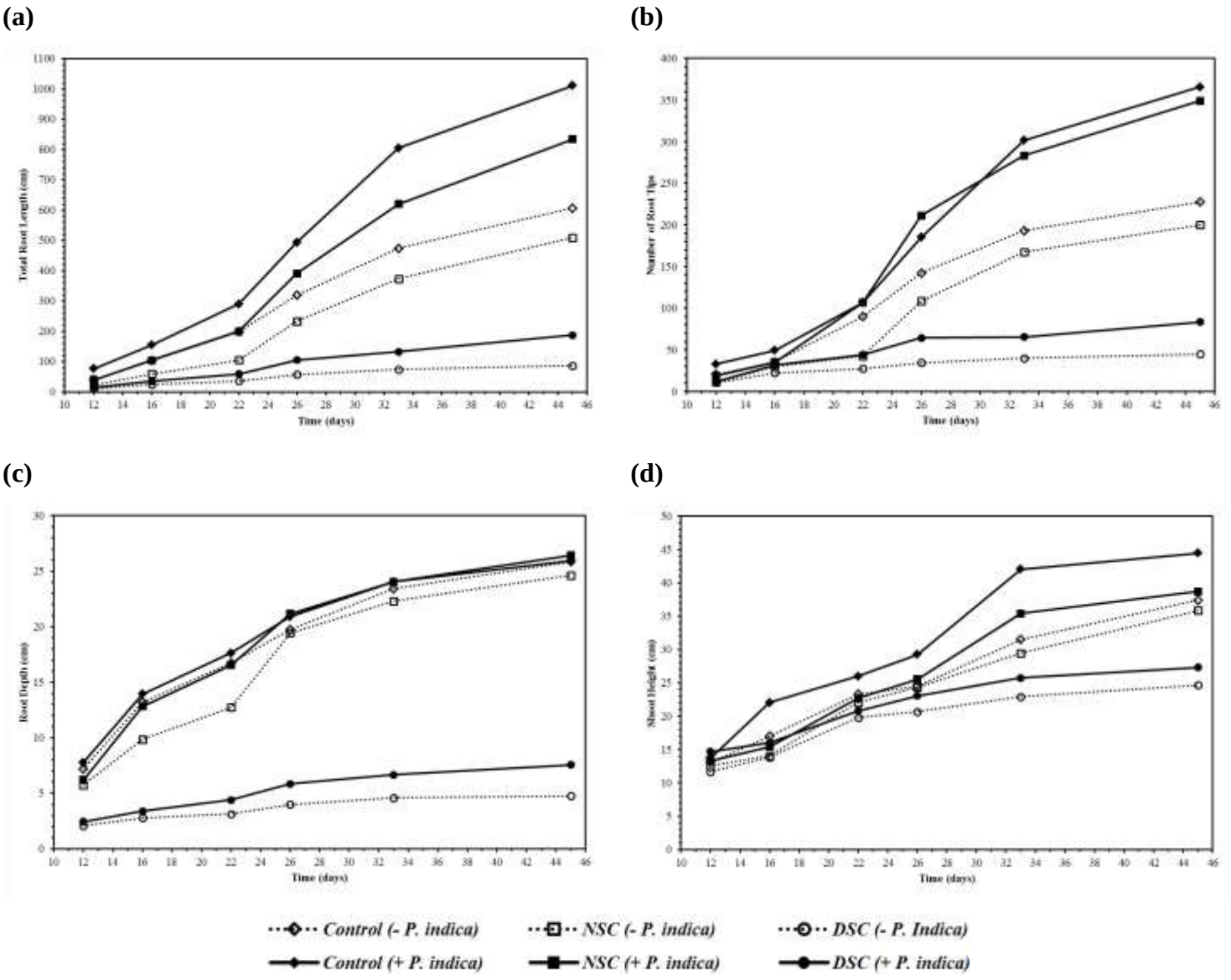

Fig. 6. Development of total root length (a), number of root tips (b), root depth (c) and shoot height (d) of maize plants with (+ *P. indica*) and without (- *P. indica*) endophyte inoculation and grown in either uncontaminated soil ('Control'), soil with a narrow surface-near layer of

petroleum contamination in otherwise uncontaminated soil ('NSC'), or soil deeply petroleum-contaminated soil below a superficial layer of uncontaminated soil ('DSC').

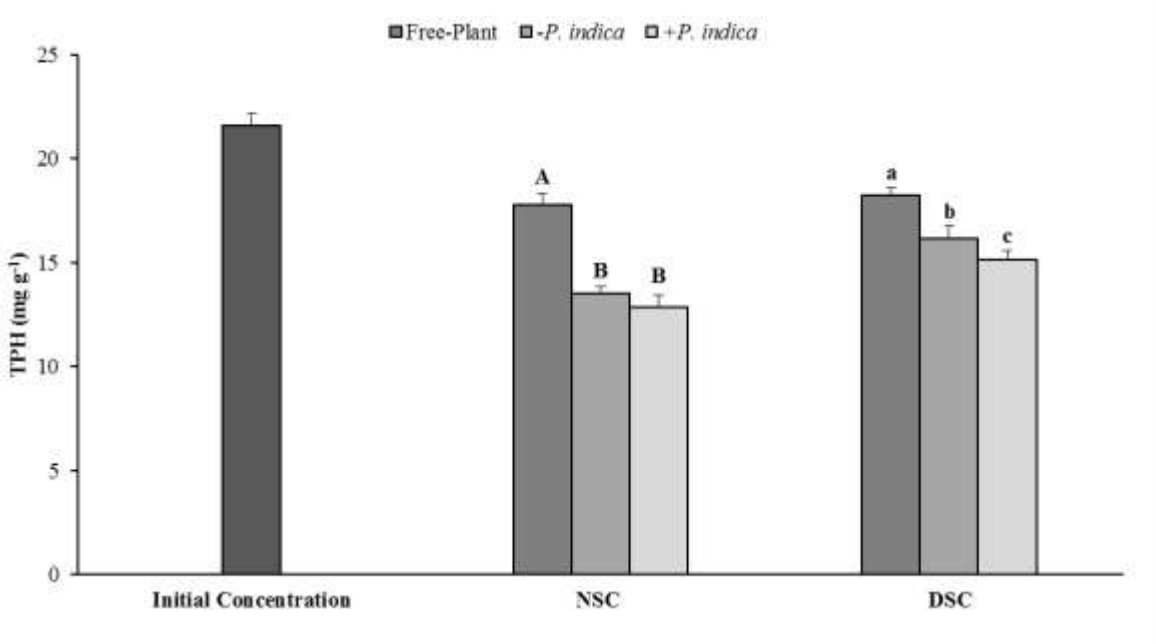

Fig. 7. TPH concentration in the contaminated soil layers of the two treatments with petroleum contamination at the beginning of the experiment (same initial concentration in both treatments) and after 45 days growth of maize plants with (+ *P. indica*) and without (- *P. indica*) endophyte inoculation in soil with a narrow surface-near layer of petroleum contamination in otherwise uncontaminated soil ('NSC'), or deeply petroleum-contaminated soil below a superficial layer of uncontaminated soil ('DSC') in comparison to unplanted soil ('plant-free') in both treatments.