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Phytoremediation of soils polluted with crude petroleum oil using *Bassia scoparia* and its associated rhizosphere microorganisms

H.A. Moubasher [a, 1], A.K. Hegazy [a, b, *, 1], N.H. Mohamed [c], Y.M. Moustafa [c], H.F. Kabiel [a], A.A. Hamad [a]

[a] Department of Botany, Faculty of Science, Cairo University, Giza, Egypt
[b] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia
[c] Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt

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ABSTRACT

The ability of *Bassia scoparia* (L.) A. J. Scott to remediate petroleum-contaminated arid land sandy soil was studied with natural and sterilized soils, and with supplemental nutrients and water. The species showed good tolerance of petroleum hydrocarbons (PHs) in soils reaching 2–3% (oil:soil by mass) pollution levels. After five months of phytoremediation, the average degradation rate of petroleum hydrocarbons ranged between 31.2 ± 1.15–57.7 ± 1.29% for natural soil and 28.7 ± 1.04–51.1 ± 1.53% for pre-sterilized soil. The highest breakdown of PHs for both saturated and poly-aromatic fractions was achieved when plants were present. Changes in saturated and aromatic fractions were monitored and measured using gas chromatography and high performance liquid chromatography. Moderate concentrations of PHs activated specialized oil-degrading microorganisms which in turn promoted the efficiency of phytoremediation. Polluted soils planted with *B. scoparia* also showed a significant reduction in sulfur levels. The potential demonstrated for remediation of petroleum hydrocarbons and sulfur by *B. scoparia* suggests it may be a useful tool for remediation of arid land soils contaminated with crude oil.

Introduction

The environmental impacts of spills or seepage of crude petroleum oil include serious adverse effects on plants and soils (Hegazy, 1997; Dindar et al., 2013). Crude petroleum oil can be regarded as being composed of four major constituents; saturated hydrocarbons, aromatic hydrocarbons, asphaltenes and resins. Many polycyclic aromatic hydrocarbons (PAHs) and their epoxides are highly toxic, mutagenic and/or carcinogenic to microorganisms and higher organisms including humans (Dodor et al., 2004; Winquist et al., 2014). Bioremediation of PHs using microorganisms which degrade and/or detoxify organic contaminants has been established as an efficient and economic treatment for oil-contaminated areas (Dindar et al., 2013). Microorganisms possess oxygenase systems which oxidize aliphatic and/or aromatic hydrocarbon molecules to produce the corresponding alcohols (Boulton and Ratledge, 1984).

Bioremediation may not result in complete removal or bioavailability of petroleum contaminants, especially PAHs, which may be reduced by aging or weathering (Banks et al., 2003). Unlike microbial heterotrophs that metabolize organic contaminants to carbon dioxide and water, plants use detoxification mechanisms that transform parent chemicals to non-phytotoxic metabolites (Kamath et al., 2004). Phytoremediation has been used for treating many types of contaminants including heavy metals, radionuclides and PHs (Hegazy, 1995; Hegazy and Emam, 2011; Hegazy et al., 2013; Dindar et al., 2013; El-Khatib et al., 2014; Souza et al., 2014).

Bassia scoparia (L.) A. J. Scott (Chenopodiaceae) is an herbaceous bush that grows successfully in different soil types and has the potential to be used in revegetation or rehabilitation of sandy, alkaline and other poor soils (Hegazy et al., 1999). It has been suggested as phytoremediator as it has been shown to be a hyperaccumulator of chromium, lead, mercury, selenium, silver, zinc and uranium (Schmidt, 2003; Casey, 2009). Despite the importance of *B. scoparia*, only a few studies have been conducted on this species in arid regions. The present study aims to assess the potential use of *B.*

* Corresponding author. Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. Tel.: +966 541792717.
E-mail address: akhegazy@yahoo.com (A.K. Hegazy).
[1] These authors contributed equally to this work and considered as first authors.

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scoparia and associated rhizosphere microorganisms for bioremediation of soils contaminated with crude petroleum oil. The study addresses remediation of crude oil polluted soils of an arid land.

Materials and methods

Plant material and soil

Seeds of *B. scoparia* (L.) A. J. Scott were obtained from the Department of Ornamental Plants, Faculty of Agriculture, Cairo University, Egypt. To bypass the potential toxic effects of PHs at the seedling stage of plant growth (Günther et al., 1996), seeds were germinated and grown for three weeks in clean soils, in seedling trays, containing 50 mini pots. Parallel treatment groups were established, the first used untreated non-autoclaved soil and was irrigated with non-autoclaved water, and the other used untreated autoclaved soil and was irrigated with autoclaved water. The three weeks old seedlings were transplanted into the appropriate soil and crude oil treatment.

Crude oil was obtained from Asal oil field, Ras-Sidr, West Sinai, Egypt. The composition of crude oil is shown in Tables 1 and 2. The untreated natural soil was collected from Wadi Sidr (Al-Haitan). The soil is sandy and characterized by a low content of organic matter (0.5%) and high concentrations of carbonates (22.2%). The soil pH is slightly alkaline (7.4) and the electric conductivity amounted 1185 μ mhos/cm (*cf*. Hegazy et al., 2014). Air dried soil was sieved through a 2-mm mesh to separate litter and gravel, and then mixed with the crude oil at rate of 40 g/kg dry soil (4.0%, oil:soil by mass). Appropriate natural or sterilized soil was added to obtain five crude oil concentrations: 0, 0.5, 1, 2, and 4% oil:soil by mass. The substrates were placed in plastic pots (12 pots for each concentration), 22 cm in diameter and 25 cm in depth, at a rate of 12 kg/pot.

Experimental design

Investigation of the remediation of petroleum treated soil using *B. scoparia* was conducted in a fenced area in the Faculty of Science, Cairo University over the course of 5 months under the prevailing natural environmental conditions during the period from May–September 2012. Pots with either natural or pre-sterilized soil were subdivided into three groups. The first group was planted with *B. scoparia* seedlings (four plants per pot) and regularly watered with Hoagland nutrition solution (components g/l double distilled water: 0.660, ammonium sulfate; 0.240, magnesium sulfate; 0.511, potassium nitrate; and 0.47, potassium monophosphate). The second group contained no plants but was watered with Hoagland nutrition solution at the same rate as the first group, and the third group received neither plants nor watering throughout the experiment.

Determination of petroleum hydrocarbons

Total petroleum hydrocarbons (PHs) were determined based on the US EPA 3550c, (2007) and US EPA 1664, (2007) methods. The PHs loss percent by using this method was 6.27%. The composition

Table 1
Changes in the abundance of different petroleum hydrocarbons extracted from 25 g soil before and after 5-months of phytoremediation following exposure to 1% PHs contamination. Values are mean ± standard error, $n = 4$.

Type of hydrocarbon				Fraction (%)
Saturated hydrocarbons	Crude oil			41.19 ± 3.01
	Natural soil	before treatment		42.15 ± 6.32
		after five months of plant culturing	Planted soil	22.29 ± 3.22
			Unplanted watered soil	25.79 ± 2.79
			Unwatered soil	34.88 ± 2.35
	Pre-sterilized soil	Before treatment		34.58 ± 1.47
		After five months of plant culturing	Planted soil	29.13 ± 2.10
			Unplanted watered soil	35.06 ± 4.32
			Unwatered soil	36.01 ± 2.32
Mono-aromatic hydrocarbons	Crude oil			5.06 ± 0.46
	Natural soil	Before treatment		5.69 ± 0.98
		After five months of plant culturing	Planted soil	16.16 ± 1.61
			Unplanted watered soil	11.29 ± 1.50
			Unwatered soil	7.25 ± 1.02
	Pre-sterilized soil	Before treatment		11.38 ± 1.52
		After five months of plant culturing	Planted soil	16.92 ± 0.57
			Unplanted watered soil	12.01 ± 1.45
			Unwatered soil	9.92 ± 1.37
Di-aromatic hydrocarbons	Crude oil			8.13 ± 1.53
	Natural soil	Before treatment		7.3 ± 0.50
		After five months of plant culturing	Planted soil	20.43 ± 2.34
			Unplanted watered soil	16.44 ± 2.17
			Unwatered soil	9.18 ± 1.40
	Pre-sterilized soil	Before treatment		13.69 ± 3.34
		After five months of plant culturing	Planted soil	15.02 ± 1.94
			Unplanted watered soil	13.04 ± 1.39
			Unwatered soil	14.59 ± 4.43
Poly-aromatic hydrocarbons	Crude oil			45.62 ± 5.52
	Natural soil	Before treatment		44.86 ± 4.19
		After five months of plant culturing	Planted soil	41.12 ± 3.16
			Unplanted watered soil	46.48 ± 4.44
			Unwatered soil	48.69 ± 2.32
	Pre-sterilized soil	Before treatment		40.35 ± 4.16
		After five months of plant culturing	Planted soil	38.93 ± 4.67
			Unplanted watered soil	39.89 ± 1.79
			Unwatered soil	39.48 ± 2.98

Table 2
Contents of PAHs (mg/l) after five months of phytoremediation following exposure to 1% PHs contamination. Nap = Naphthalene, A = Acenaphthylene, Ace = Acenaphthene, F = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Flu = Fluoranthene, Pyr = Pyrene, BaA = benzo (a) anthracene, BaP = benzo (a) pyrene, BbF = benzo (b) fluoranthene, BkF = benzo (k) fluoranthene, Chr = chrysene, DahA = dibenzo (a,h) anthracene, and BP = Benzo (g,h,i) perylene.

Number of rings	PAHs	Non-sterilized soil				Sterilized soil				Crude oil
		Initial level	After five months of treatment			Initial level	After five months of treatment			
			Planted soil	Unplanted watered soil	Unwatered soil		Planted soil	Unplanted watered soil	Unwatered soil	
2	Nap	0.200	4.820	4.714	3.954	4.259	3.991	4.034	4.327	0.312
% of 2 rings/total PAHs		0.045	4.617	2.240	0.977	1.271	2.173	1.330	1.328	0.060
3	A	–	1.304	6.882	–	5.627	–	2.564	–	–
	Ace	–	0.136	–	–	1.985	1.171	1.536	1.000	–
	F	–	–	–	–	0.109	–	–	–	–
	Phe	0.956	1.226	–	–	1.991	0.584	0.912	–	–
	Ant	0.962	0.819	1.051	2.832	0.061	–	0.065	–	1.260
% of 3 rings/total PAHs		0.433	3.338	3.770	0.700	2.916	0.956	0.829	0.307	0.243
4	Flu	1.106	4.919	–	1.535	4.041	3.301	1.048	1.623	6.320
	Pyr	–	0.453	1.558	2.419	–	–	–	–	6.000
	BaA	0.860	3.821	4.677	1.671	4.921	2.657	2.254	4.690	5.870
	Chr	–	1.221	2.585	–	–	0.611	0.002	0.095	1.080
% of 4 rings/total PAHs		0.444	9.975	4.190	1.390	2.674	3.577	1.090	1.967	3.722
5	BbF	1.872	–	–	0.344	0.106	1.011	–	–	1.720
	BkF	3.258	1.117	3.961	1.800	5.998	2.400	1.022	–	2.400
	BaP	–	–	–	–	1.433	1.017	0.016	1.045	–
	DahA	0.280	–	–	0.296	0.665	0.479	0.006	–	1.560
% of 5 rings/total PAHs		1.222	1.070	1.882	0.603	2.447	2.672	0.344	0.321	1.097
6	Bp	433.272	84.564	184.998	390.488	303.942	166.416	289.805	313.000	489.960
% of 6 rings/total PAHs		97.900	81.000	87.916	96.503	90.692	90.622	95.562	96.077	94.645
Total PAHs		442.566	104.4	210.426	404.637	335.138	183.638	303.264	325.780	517.680
% degradation of total PAHs			76.410	52.549	8.570		45.205	9.511	2.792	

of PHs was analyzed using a column chromatography technique (Mishra et al., 2001) where a glass column (1 cm in diameter and 60 cm height) was filled with 25 g of activated silica gel Merck (mesh 60–120). According to the refractive index of the collected fractions at 20 °C, elutes were categorized as: saturates, mono-aromatic, di-aromatic and poly-aromatic hydrocarbons (Mair and Rossini, 1958).

Gas chromatography

The GC apparatus used was a Perkin Elmer (Clarus 500), equipped with a hydrogen flame ionization detector and fused silica capillary column (60 m length × 0.32 mm i.d), packed with poly (dimethyl siloxane) HP-1 (non-polar packing) of 0.5 μm film thickness.

Polycyclic aromatic hydrocarbons analysis

The apparatus used was model Waters HPLC 600E, equipped with dual UV absorbance detector Waters 2487 and auto sampler Waters 717. This was attached to a computerized system with Millennium 3.2 software. PAH standards were obtained from Supelco. The conditions of separation were according to Lal and Khanna, (1996).

Determination of sulfur

The sulfur concentration in soil and plant tissues was measured using an X-ray fluorescence sulfur meter (ASTM D-4294-98).

Microbial plate counts

Viable microbial populations including bacteria, fungi and actinomycetes were enumerated in the soil samples collected after five months from the beginning of the experiment. A serial dilution method was used. For bacteria, 1 ml of three dilutions was inoculated on sterile plates with a nutrient agar medium (components g/L: beef extract, 3; peptone, 5; sodium chloride, 5; agar, 15 and the pH was adjusted to 7). For actinomycetes, 1 ml of three dilutions was placed on a starch agar medium (components g/L: soluble starch, 20; potassium nitrate, 1; di-potassium hydrogen phosphate, 0.5; magnesium sulfate hepta-hydrate, 0.5; sodium chloride, 0.5; ferrous sulfate hepta-hydrate, 0.01; agar, 20 and pH was adjusted to 7). For fungi, 1 ml of three dilutions was placed on the Czapek's Dox agar medium (components g/L: sucrose, 30; sodium nitrate, 2; potassium chloride, 1; magnesium sulfate hepta-hydrate, 0.5; ferrous sulfate hepta-hydrate, 0.01; di-potassium hydrogen phosphate, 1; agar, 20 and the pH value was adjusted to 5.5). The plates were incubated at 28 °C for fungi and actinomycetes and 37 °C for bacteria. The number of bacterial, actinomycete and fungal colonies were counted after 2, 5 and 7 days respectively and expressed as colony forming unit (CFU)/g dry soil.

The same procedure was repeated with selective media for oil-degraders. Czapek's Dox agar medium without sucrose, and starch agar medium without starch, were used to count the subset of the viable fungal and actinomycete populations, respectively. For bacteria, M9 Minimal Medium without glucose (components for 1 L: 64 g $Na_2HPO_4.7H_2O$, 15 g KH_2PO_4, 2.5 g NaCl, 5.0 g NH_4Cl, 2 ml of 1 M $MgSO_4$, 100ul of 1 M $CaCl_2$ and pH was adjusted to 7) was used. The solid medium was covered with a layer of crude oil (0.3 ml) before inoculation with the two dilutions (10^{-2} and 10^{-3}). Plates were incubated at 28 °C for 7 days for fungi and actinomycetes, and at 37 °C for three days for bacteria. The number of colonies were counted after the incubation period and expressed as CFU/g dry soil.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in the SPSS-12 statistical software package for PCs. Multiple comparisons of means of experimental parameters of each treatment for each concentration including counts of fungi, bacteria and actinomycetes, percentage of PHs degradation (%), sulfur content in plant tissues (mg/g dry tissue) and sulfur content in soil (mg/g dry soil)

were made using Duncan's multiple range tests at $P < 0.05\%$. The results represent the average of four replicates and are presented as the mean ± standard deviation.

Results

Degradation of PHs

The relative degradation rate of petroleum hydrocarbons (PHs) in the soil, planted with *B. scoparia* and the corresponding controls varied depending on the concentration of crude oil treatment as shown in Fig. 1. The highest degradation rates were usually achieved in the 1% oil:soil (by mass) treatment. Over a five month period the degradation of PHs in the presence of plants reached 57.7 ± 1.29% (5.77 mg/kg) for natural soil and 51.1 ± 1.53% (5.11 mg/kg) for pre-sterilized soil. Breakdown in the watered soil without plants and unwatered soil reached 46.3 ± 2.31% (4.63 mg/kg) and 9.2 ± 0.52% (0.92 mg/kg) respectively for natural soil, while values 22.00 ± 0.54% (2.20 mg/kg) and 3.20 ± 0.13% (0.32 mg/kg) respectively were recorded for the pre-sterilized soil. At higher PHs concentration (40 g/kg dry soil), PHs degradation in planted soil, watered soil without plants and unwatered soil was significantly inhibited compared to degradation at lower PHs concentrations (5 and 10 g/kg dry soil).

Changes in the mixture of PHs

The composition of PHs extracted from different treatments contaminated with 1% PHs is shown in Table 1. In the presence of plants the percentage of saturates and poly-aromatic hydrocarbons decreased from 42.15% and 44.86% at the beginning of experiment to 22.29% and 41.12% respectively after five months of phytoremediation for natural soil. For planted pre-sterilized soil, the percentage of saturates and poly-aromatic hydrocarbons decreased from 36.58% and 40.35% to 29.13% and 38.93% respectively. There is an increase in the percentage of mono- and di-aromatic hydrocarbons in planted soils for both pre-sterilized and natural soils in comparison to watered soil without plants and unwatered soils.

The decrease in poly-aromatic hydrocarbons and increase in mono- and di-aromatic hydrocarbons percentages when 1% PHs were added were measured using HPLC analysis (Table 2). The HPLC data showed a shift from higher high-molecular weight PAHs to higher concentrations of low-molecular weight PAHs in the phytoremediated samples in both natural and pre-sterilized soils planted with *B. scoparia*. Meanwhile, the degradation of high-molecular weight PAHs was higher in natural planted soil (76.41%) than in the pre-sterilized planted soil (45.21%).

The relative percentages of n-paraffins and iso-paraffins in saturated fractions of the 1% PHs treatment, before and after remediation were determined using gas chromatography (Fig. 2). There was a significant reduction of n-paraffinin planted soil compared to the other treatments. Both natural and pre-sterilized planted soils has relatively low n-paraffins to iso-paraffins ratio values (2.65 and 2.99 respectively) compared to their natural and pre-sterilized controls (before phytoremediation) (4.15 and 4.20 respectively), while watered samples without plants and unwatered samples showed relatively high ratios of 3.06 and 3.39 respectively for natural soils and 3.46 and 3.78 respectively for pre-sterilized soil.

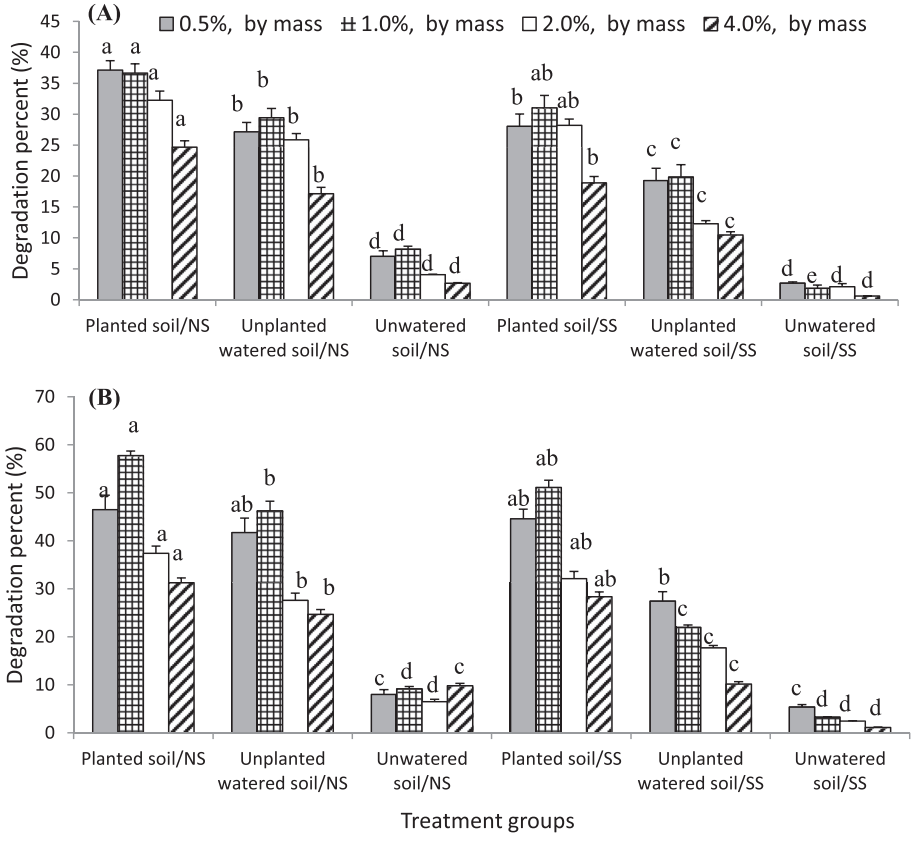

Fig. 1. Degradation of total petroleum hydrocarbons in soils treated with crude oil and planted with *Bassia scoparia* for (A) three months and (B) five months compared to unplanted controls. NS = natural soil; SS = pre-sterilized soil. Vertical bars above the means indicate the standard error in each case. The different letters above columns indicate significant differences between treatments in terms of percentage degradation, at each of two separate sampling dates for each concentration at $P < 0.05$ according to Duncan's multiple range test.

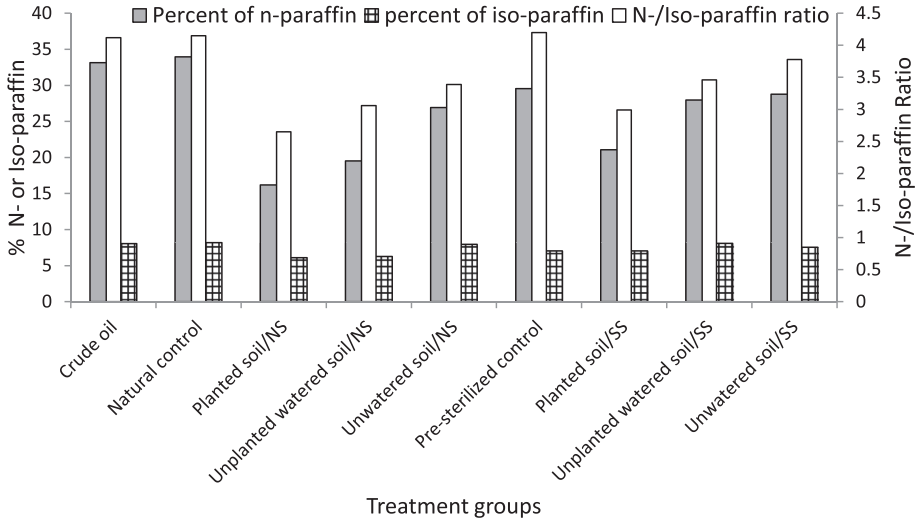

Fig. 2. GC analysis of crude oil and saturated fractions extracted from soil contaminated with 1% PHs before (natural and pre-sterilized control) and after remediation processes (treatment groups: natural and pre-sterilized planted soil, unplanted watered soil and unwatered soil). NS = natural soil; SS = pre-sterilized soil.

Sulfur remediation

The roots of *B. scoparia* accumulate more sulfur per unit dry mass than do shoots. Plants raised in natural soil accumulate more sulfur than those raised in pre-sterilized soil (Fig. 3). Accumulation of sulfur increased significantly in roots and shoots of plants grown in soil treated with 1% PHs compared to those raised in clean soils. In terms of soil levels of sulfur, after the phytoremediation period, in the presence of plants in both pre-sterilized and natural soil, there was a significant reduction in soil sulfur content compared to that in watered treatments without plants and unwatered treatments (Fig. 4). There was a significant difference between sulfur levels in pre-sterilized and natural soil; the pre-sterilized soil retained significantly more sulfur.

Response of microbial populations to soil pollution with PHs

Where plants were present, we saw the highest counts of fungi, bacteria and actinomycetes. In contrast, where there was no water or nutrient added, we saw the lowest colony counts in both pre-sterilized and natural soils (Fig. 5). The counts of fungi, bacteria and actinomycetes were higher at the 0.5% PHs concentration than in soil with no oil contamination. However when petroleum contamination was equal to or higher than 2%, the counts of fungi, bacteria and actinomycetes were significantly lower than in uncontaminated soil. Fig. 6 shows the numbers of fungi, and pooled numbers for actinomycetes and bacteria, that were capable of growing on media selective for microorganisms capable of metabolizing oil (oil-degraders). Oil-degraders were much more abundant when plants were present, and significantly more abundant in natural soils than when soils were pre-sterilized.

Discussion

The present study illustrates the significant role of both the plant, *B. scoparia*, and its associated rhizosphere microflora in the degradation of petroleum hydrocarbons, in that contaminant levels fall more in natural planted soils than in pre-sterilized planted soils where the initial “inoculum” of microorganisms is lower. The impact of different initial petroleum concentrations has also been

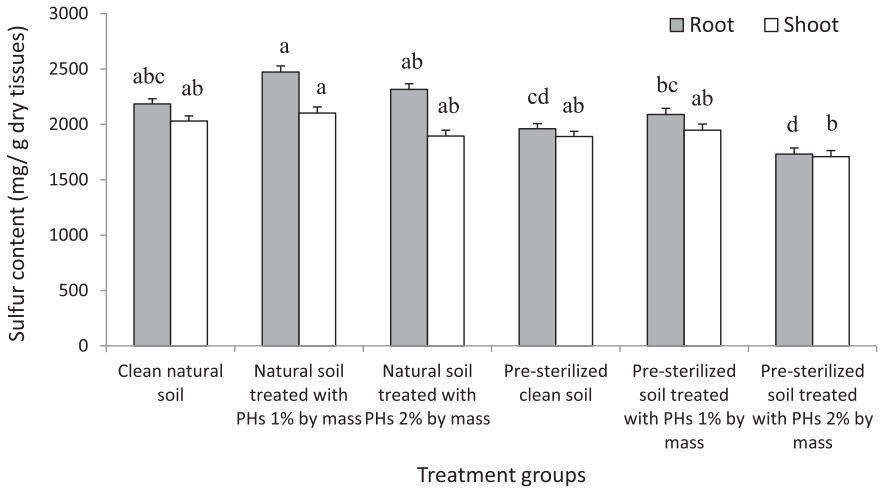

Fig. 3. Sulfur content in the root and shoot of *Bassia scoparia* plants grown for five months in soils treated with 0%, 1% and 2% concentrations of crude oil (by mass). Vertical bars above each mean indicate the standard error. The different letters indicate significant differences in sulfur content of plants grown in different media and concentrations of PHs at $P < 0.05$ according to Duncan's multiple range test.

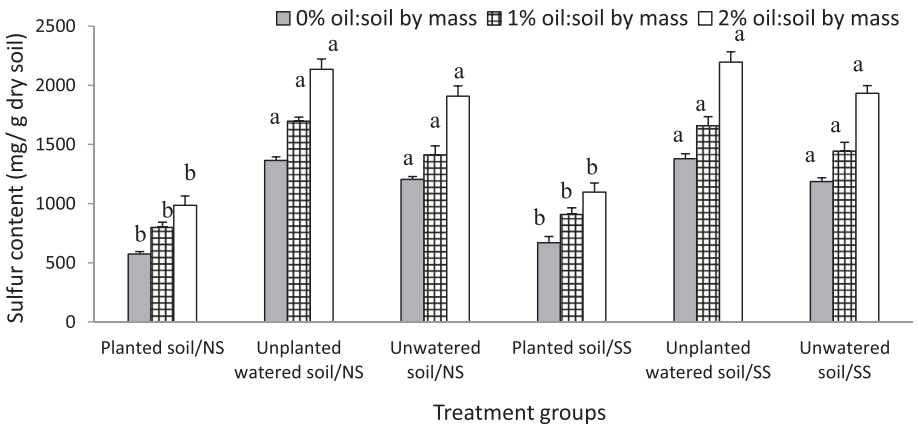

Fig. 4. Sulfur content in soils treated with 0%, 1% and 2% crude oil concentrations (PHs) after five months of phytoremediation. NS = natural soil; SS = pre-sterilized soil. Vertical bars above each mean indicate the standard error. The different letters indicate significant differences in sulfur content of natural and pre-sterilized soil planted with *B. scoparia*, watered soil, and unwatered soil at $P < 0.05$ according to Duncan's multiple range test.

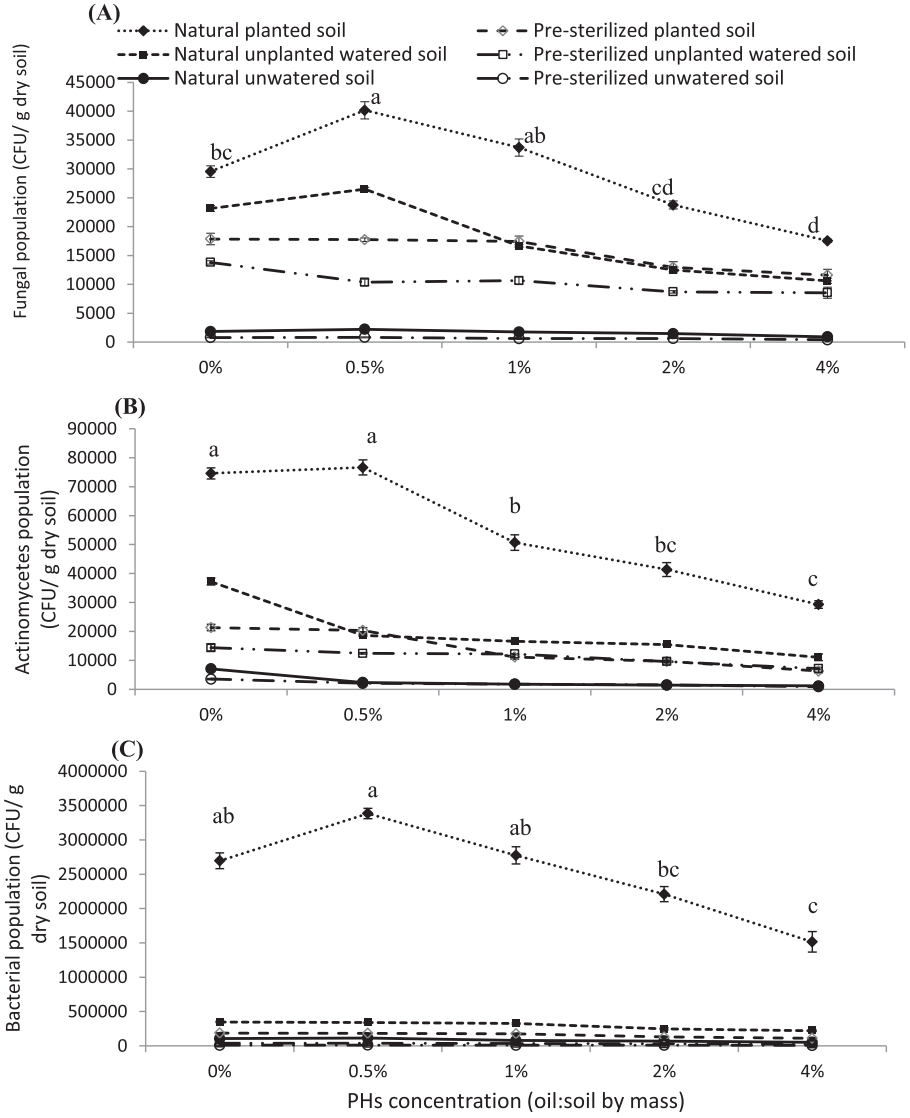

Fig. 5. Effects of soil treatment and different initial crude oil concentrations on the total counts of (A) fungi, (B) actinomycetes and (C) bacteria. The vertical bar around the mean indicates the standard error. The different letters indicate significant differences between different PHs concentrations in soil for each soil group at $P < 0.05$ according to Duncan's multiple range test. CFU = colony forming unit.

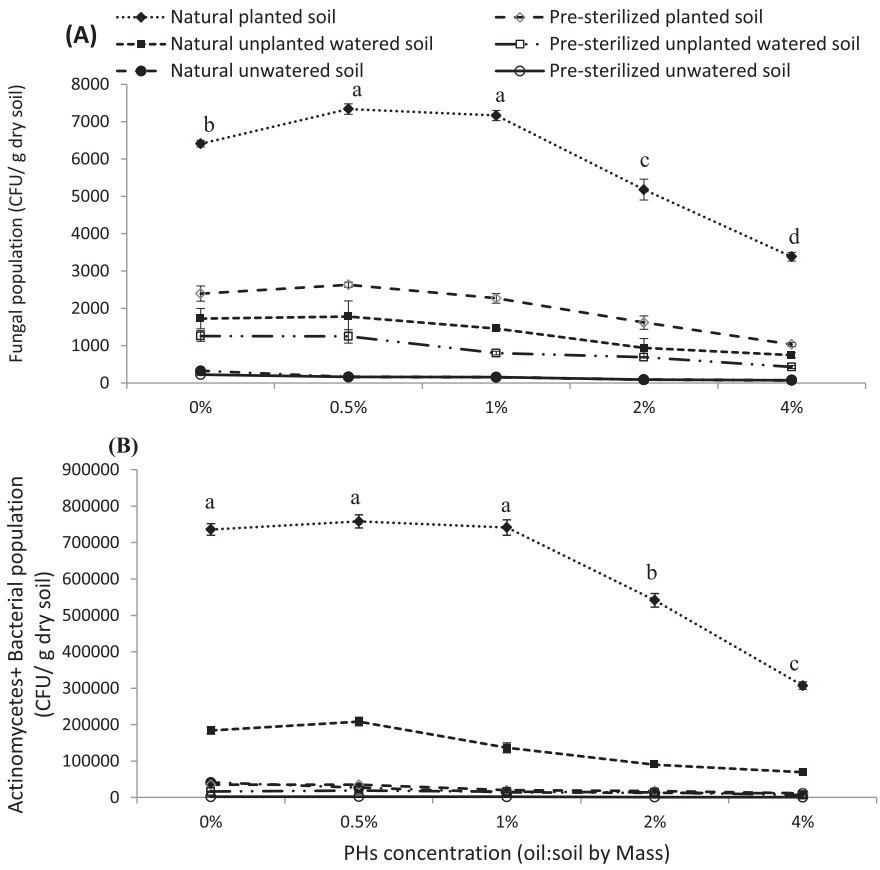

Fig. 6. Effects of soil treatment and different initial crude oil contamination on the total counts of (A) fungi and (B) actinomycetes + bacteria. These microorganisms were grown on media that are selective for microorganisms capable of breaking down oil. The vertical bar around the mean indicates the standard error. The different letters indicate significant difference between fungi, and bacteria + actinomycetes in different PHs concentrations in the soil at $P < 0.05$ according to Duncan's multiple range test. CFU = colony forming unit.

studied by Peng et al. (2009) using *Mirabilis Jalapa*. The greater level of contaminant degradation in the natural soil planted with *B. scoparia* may be explained by the rhizosphere effect supported by a fibrous root system. The presence of *B. scoparia* may greatly enrich the rhizosphere microbial flora by providing exudates, enzymes, and oxygen through its roots. Roots and mycelium can also create pores in the soil structure which can improve connectivity and diffusion (Young and Crawford, 2004). Any reduction in crude oil in the unwatered and unplanted controls could take place only by volatilization, photooxidation or the activity of its original microflora (Peng et al., 2009). In arid regions, with very low soil moisture and low nitrogen levels, there would typically be slow microbial growth. Under these conditions it would be expected that the natural breakdown would occur very slowly. In the soil that was watered with nutrient solution, but had no plants, the additional nutrients and water may stimulate microbial activity, resulting in some crude oil breakdown compared to what can occur in the unwatered treatments.

Palmroth et al. (2002), detected small amounts of low molecular weight PAHs in root peels taken from grass roots and carrot respectively. Their results support our finding that the efficiency of poly-aromatics degradation in natural soil is significantly greater than that in pre-sterilized soil. They attributed the results to microbial degradation of poly-aromatic hydrocarbons to low molecular weight aromatic hydrocarbons (Dean-Ross et al., 2002), which in turn can be readily taken up by plant roots (Newman et al., 1997).

Large PAHs are insoluble in water, and therefore are not readily taken up by plant roots in significant amounts (White et al., 2006). The increase in the proportion of smaller mono- and di-aromatic hydrocarbons in planted soils is therefore probably attributable to the degradation of high molecular weight PAHs to low molecular weight PAHs by rhizosphere microorganisms. The change in molecular type composition of PHs seen in the sterilized controls in comparing to natural controls seems to be attributable to exposure of sterilized polluted soil to high temperature and pressure (121 °C and 1.2 Kg/cm^2 respectively) through sterilization process which affects the physical properties of the sandy soil (silica), that lead to activation of the soil particles (*cf.* Hegazy and Emam, 2011). In this process low melting saturates attached to aromatic ring are adsorbed on the surface of the activated soil particles and become more difficult to extract leading to change in the composition of extracted petroleum fractions.

Iso-paraffin hydrocarbons are generally more resistant to degradation than n-paraffins. The ratio of n-paraffins to iso-paraffins indicates the relative amount of degradation in each case. This ratio decreases as remediation proceeds (Moustafa, 2004). Our results showed that growing *Bassia* plants in oil-contaminated soil promotes degradation of saturated hydrocarbons. The relatively high ratios of n-paraffins to iso-paraffins in soil receiving no water or nutrients suggest that weathering alone is ineffective in the remediation of petroleum contamination.

Analysis of the sulfur content of soils and plant roots and shoots showed the important role of *Bassia* in remediation of the sulfur associated with petroleum contamination. The reduced sulfur content of planted soils matches the reduction of poly-aromatic hydrocarbons. Since sulfur is mainly attached to high molecular weight PAHs, as degradation of PAHs proceeds the availability and uptake of sulfur by *B. scoparia* plants readily increases. The sulfur content of planted soil differed between pre-sterilized and natural soil, as microorganisms play important role in PAHs degradation and therefore sulfur remediation. As reported in previous studies,

the abundance of microorganisms and their distribution among various physiological groups are important characteristics of the soil microflora, which make it possible to interpret the biochemical processes involving transformations of biogenic elements such as sulfur (Mishustin, 1984; Muratova et al., 2003).

The relationship between microorganism populations and petroleum contamination indicates that the three important microorganism groups (fungi, bacteria and actinomycetes) show different responses to the concentration of petroleum contaminants in soil. In general, oil-degraders were most abundant at 0.5% and 1% PH concentrations. These results agree with those of Saxtone and Atlas (1977), as they concluded that moderate pollution stress activates organisms' ability to resist the negative effects. The decrease in oil-degraders at higher PHs concentrations (2% and 4%) for planted and watered soils is related to the greater toxicity of the petroleum pollutants and inadequate aeration condition (Muratova et al., 2003; Liste and Prutz, 2006; Zhou and Song, 2004). Under the conditions of soil pollution, plants help sustain and/or increase the abundance of soil microorganisms, thereby intensifying the processes of polluted soil remediation (Jordahl et al., 1997). Growth of the plant root system increases microbial populations by secreting compounds favoring the development of oil-degraders and restoring the function of the microbial community, thus accelerating the biodegradation of crude oil. In turn, healthier microbial populations promote plant growth and increase the efficiency of phytoremediation (Cai et al., 2010).

Conclusions

Based on our results, we conclude that *B. scoparia* is a promising tool for bioremediation of soils polluted with petroleum hydrocarbons and associated sulfur pollution at levels ranging up to about 3% oil:soil by mass. The phytotoxicity of high concentrations of PHs (greater than 3%) limits the use of *B. scoparia* in the remediation processes to the edges of spills, or to areas that are only moderately contaminated. This phytoremediation process significantly reduced the toxicity of the high molecular weight PAHs. The present study specifies the critical role of the rhizosphere microflora as well as the plants in remediation of crude oil spills. Moderate concentrations of crude oil activate the oil-degrading microorganisms which in turn promote the efficiency of bioremediation.

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