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

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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.*

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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