International Journal of Plant & Soil Science
4(1): 61-71, 2015; Article no. IJPSS.2015.006
ISSN: 2320-7035

SCIENCEDOMAIN *international*
www.sciencedomain.org

Selection of the Most Effective Cultivar of Genus *Zinnia* Flowers for Phytoremediation of Oil-contaminated Soil

Suzuka Ozawa[1], Hiromi Ikeura[2], Etsuko Kaimi[3] and Masahiko Tamaki[4*]

[1]Department of Agriculture, Meiji University Graduate School, Kawasaki, Kanagawa, Japan.
[2]Organization for the Strategic Coordination of Research and Intellectual Properties, Meiji University, Kawasaki, Kanagawa, Japan.
[3]Department of Environmental Research and Development, Chugai-Technos Corporation, Hiroshima, Hiroshima, Japan.
[4]School of Agriculture, Meiji University, Kawasaki, Kanagawa, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MT, HI and EK designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SO managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors MT, HI and EK managed the experimental process. Author SO identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2015/13035
Editor(s):
(1) Marcello Iriti, Department of Agricultural and Environmental Sciences, Faculty of Agricultural and Food Sciences, Milan State University, Italy.
Reviewers:
(1) Qaisar Mahmood, Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan.
(2) Anonymous, Debub University, Ethiopia.
(3) Aisha Saleem Khan, Biological Sciences, Forman Chrisitian College, University , Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=651&id=24&aid=6032

Original Research Article

Received 30th July 2014
Accepted 21st August 2014
Published 10th September 2014

ABSTRACT

Low-cost and environmentally friendly removing processes for contaminated soil by oil, such as phytoremediation, are being evaluated in recent years. We aimed to select the most effective cultivar among 6 cultivars in 4 species of genus *Zinnia* (*Z. elegans* cultivars 'Uproar' and 'Zahara', *Z. angustifolia* cultivar 'Starbright', *Z. haageana* cultivars 'Sombrero' and 'Oldfashion' and *Z. hybrida* cultivar 'Profusion') that can be grown under Japanese environmental conditions for the phytoremediation of oil-contaminated soils. In addition, both total petroleum hydrocarbon (TPH)

**Corresponding author: E-mail: mtamaki@meiji.ac.jp;*

concentration of the soil and the soil dehydrogenase activity (DHA) were assessed over the growth period. The above-ground and under-ground dry matter weights of 6 cultivars in non-contaminated plot were significantly higher than those in contaminated plot. However, plant heights of 6 cultivars at 180 days after sowing in contaminated plot were as high as those in non-contaminated plot. Growth of 'Starbright' was the latest in all cultivars, and 'Uproar' and 'Sombrero' at 180 days after sowing in contaminated plot was curled up the above ground. These results were judged that 'Starbright', 'Uproar' and 'Sombrero' were not suited for growing in contaminated soil. The TPH concentration in soil significantly decreased by about 70% from an initial concentration of 13,191mg diesel kg^{-1} soil after 180 days in the contaminated soil for all 6 cultivars. In particular, the TPH concentrations of 'Sombrero', 'Profusion', and 'Starbright' decreased to 74%, 72%, and 72%, respectively. Soil DHA of 6 cultivars in contaminated plot at 180 days after sowing were significantly higher than that of irrigated plots, especially those of 'Starbright' and 'Profusion' were much higher compared to the other 4 cultivars. These results clarified that 'Profusion' and 'Starbright' are suited for high remediation in contaminated soil.

Keywords: Degradation; hydrocarbons; oil-contaminated soil; phytoremediation; zinnia; TPH.

1. INTRODUCTION

Petroleum is an essential material for daily life that is used for various purposes, including as a lubricant for machines and as a fuel for cars and heaters. However, various oils remain as substances in the soil. It is possible that soil contamination by oil occurs in many places; however, this issue has received limited research to date [1]. Soil and groundwater pollution by oil have a negative effect on human health and the environment [2-4], and may also cause decrease the value of the land deals [5]. Therefore, the Ministry of the Environment in Japan issued guidelines against oil pollution in 2006 [6], and established methods to counter soil contamination by oil.

Soil contamination by oil in Japan is often found in vacated gas stations and factories. It is treated by various processes, including excavation removal, thermal treatment, and liquid matter extraction. While such processes effectively remove the soil in a short period of time, they are problematic because they are expensive (requiring huge energetic inputs, manpower, and large-scale equipment) and place major stress on the environment, resulting in the alteration of soil quality [7,8].

Low-cost and environmentally friendly removal processes, such as those using plants (phytoremediation), have been assessed in recent years [8-12]. Among the various phytoremediation processes, phytostimulation is particularly effective at removing oil contamination [13]. Phytostimulation is a technique that applies knowledge gained from research on the impact of plant roots on microoganisms in the rhizosphere [13]. Root exudates which secreted from the root and root cells, such as carbon and nitrogen, are known to be a source of nutrients and grow TPH promoters for microoganisms [14-16]. Such rhizospheric processes are also expected to be effective in the organic removal of contaminated soil, with the remediation effect of polyaromatic hydrocarbons in the rhizosphere of plants that form fibers being recorded [17,18].

Most research on the phytoremediation of oil-contaminated soil use focuses on plants from the *Poaceace* [8,16,19] and *Fabaceae* [20,21] families, with few studies using ornamental flowers. The advantages of using ornamental flowers include the aesthetic of the environment and a therapeutic have healthy or good effects on humans of individuals, in addition to the fact that pollutants do not enter the food chain. Zhang et al. [22] reported that I*mpatiens balsamina* promoted the decomposition of persistent organic petroleum hydrocarbons (like resin and asphaltene) after being grown in oil-contaminated soil for 120 days. Peng et al. [23] grew *Mirabilis jalapa* in soil contaminated by less than 10,000mg kg^{-1} total petroleum hydrocarbon (TPH) concentration for 127 days, and found that TPH concentrations in soil significantly decreased in the test plot containing ornamental flowers compared to the plot without flowers, with no negative impact on flower growth compared to uncontaminated soil. Moreover, our previous study demonstrated that species belonging to the genus *Zinnia* were the most effective ornamental flower out of 33 species that grow under Japanese environmental conditions for the phytoremediation of oil-contaminated soils.

We aimed to identify the most effective cultivar out of 6 cultivars from 4 species belonging to the genus *Zinnia* that grow under Japanese environmental conditions for the phytoremediation of oil-contaminated soils. In addition, TPH soil concentrations and soil dehydrogenase activity (DHA) was assessed during the growth period.

2. MATERIALS AND METHODS

2.1 Plant Materials

We selected some dwarf and tall varieties in *Zinnia* genus having the highest remediation effect in 33 species can be grown under Japanese environmental conditions by result of preliminary experiment. *Zinnia elegans* cultivars 'Uproar' (Fig. 1-A) and 'Zahara' (Fig. 1-B), *Z. angustifolia* cultivar 'Starbright' (Fig. 1-C), *Z. haageana* cultivars 'Sombrero' (Fig. 1-D) and 'Oldfashion' (Fig. 1-E), and *Z. hybrida* cultivar 'Profusion' (Fig. 1-F) were purchased from Sakata Seed Corporation (Yokohama, Japan). The cultivars used in our previous study included the *Z. hybrida* cultivar 'Profusion' and an interspecific hybrid between *Z. elegans* and *Z. angustifolia*. 'Zahara', 'Starblight', 'Oldfashon' and 'Profusion' are dwarf variety, and 'Uproar' and 'Sombrero' are tall variety. 'Zahara' have about 5 cm of flower size and single petal, and its plant height is about 40cm. 'Starblight' have about 3 cm of flower size and single petal, and its plant height is about 40cm. 'Oldfashon' have 5 cm of flower size, and its plant height is about 40cm. 'Profusion' have about 5cm of flower size and single petal, its plant height is about 40cm. 'Uproar' have about 6-7cm of flower size and double petal, its plant height is about 70-90cm. 'Sombrero' have about 7cm of flower size and its plant height is about 70cm.

2.2 Preparation of Oil-contaminated Soil

Oil-contaminated soil was prepared by mixing 2% diesel oil by weight ratio per gram of soil that had been air-dried in a greenhouse to less than 1% water content for 2 weeks according to the method described by Kaimi et al. [8]. Kanto loamy soils sampled from test plots at Meiji University were used. To mix the oil evenly into the soil, it was gradually sprayed with a pump sprayer, while stirring the soil with a soil mixer. Contaminated soil was stirred once every 2 days in a greenhouse, and the oil particles were volatilized for avoiding affecting early growth by low molecular weight hydrocarbons. A 40% by volume ratio of leaf mould and perlite were also added as a soil conditioner to prevent the compaction of the Kanto loamy soils. The initial TPH concentration of the contaminated soil after volatilization was 13,191 mg diesel/kg soil. Non-contaminated soil followed the same treatment, but without the addition of diesel oil.

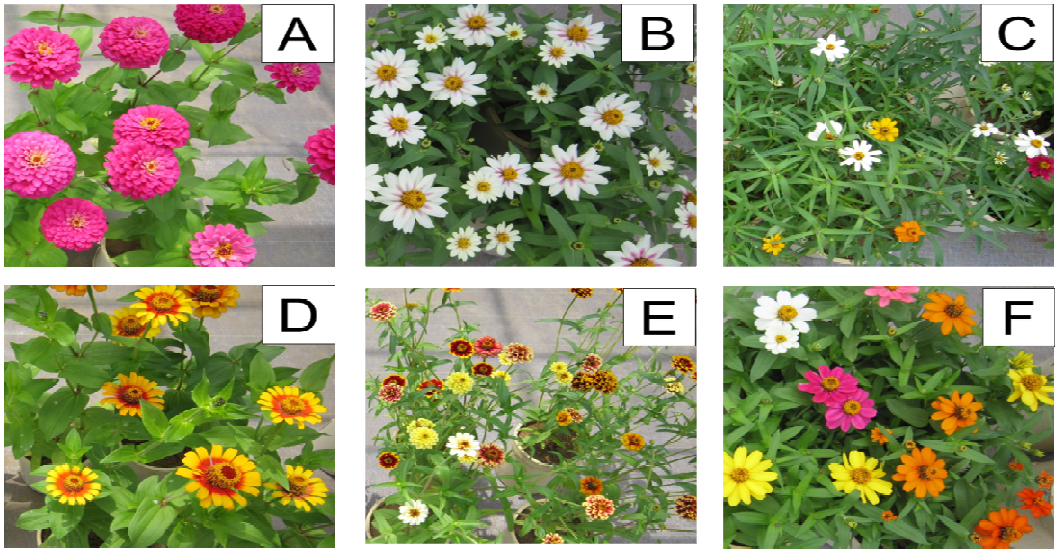

Fig. 1. Plant materials

A; Z. elegans 'Uproar', B; Z. elegans 'Zahara', C; Z. angustifolia 'Starbright', D; Z. haageana 'Sombrero', E; Z. haageana 'Oldfashion', F; Z. hybrida 'Profusion'

2.3 Flower Growth Test in Oil-contaminated Soil

Eight seeds from each of the 6 cultivars were planted on April 4, 2012, in two 1/5000 a Wagnar pots (φ159 mm ×190 mm) prepared by irrigation with 3 L of x mg/kg TPH of oil-contaminated (test pot) and non-contaminated (control pot) soil. The flowers were grown for 180 days after sowing under natural conditions in a greenhouse, and were thinned out after 3 individuals of each cultivar emerged. To evaluate oil volatilization, the test plots (4 separate pots) were divided into (1) cultivation in oil-contaminated soil (contaminated plots), (2) cultivation in non-contaminated soil (non-contaminated plots), (3) contaminated soil with no cultivation and only irrigation for comparison (irrigated plots), and (4) contaminated oil with no cultivation and no irrigation (non-irrigated plots). The plots were irrigated with 300mL of water every 2–3 days, and 300mL of HYPONeX distilled 500-fold by tap water (normal concentration for ornamental plants, N:P_2O_5:K_2O=6:10:5, HYPONeX JAPAN CORP.,LTD., Osaka, Japan) was provided instead of irrigated water once a week. Soil was extracted from the rhizosphere of plant specimens every 60 days after sowing, to measure the TPH concentration of the soil and DHA oxidation activity by the microorganisms in the soil, above-ground dry matter weight. In addition, the under-ground dry matter weight and plant height were also measured every 60 days for 180 days growing period after sowing. The cultivations were repeated four times for each samples.

2.4 Measurement of a Number of Flower, Plant Height and Dry Matter Weight

A number of flowers was measured when flowering in each cultivars. Plant height was measured from the soil surface to the shoot tip of 3 individuals per pot. The dry matter weight of the above-ground and washed root of the plants was measured after oven drying for 3 days at 80℃.

2.5 TPH Concentration in Soil

The TPH concentration of the soil was measured according to a method by the Ministry of the Environment in Japan issued guidelines against oil pollution in 2006 [6] for the extraction and analysis of soil TPH. To extract TPH, 30g of soil from the centre of the soil surface in a pot was dried at 30ºC for 4 days. Then, 5g of soil was placed in a 50mL conical flask, and then 15mL carbon disulphide was added and the mixture was shaken for 30 min. The supernatant was recovered 2h after shaking, and the residue was mixed again before extraction. The solution was kept stationary for 1h between the second shaking and the onset extraction, which was repeated 3 times. The extract was diluted to 50mL. The extract was separated with a membrane filter (pore diameter, 0.45μm). Then, 1μL of the filtrate was injected into a gas chromatograph hydrogen flame ionization detector (GC-FID) for analysis. The analysis was conducted using a GC (GC-2010, Shimadzu Corp., Ltd., Kyoto, Japan) equipped with an Intercap 1MS capillary column (liquid phase, 5% phenylmethyl silicon; i.d., 30 m × 0.32 mm; and film thickness, 0.25μm; GL Science Inc., Japan) and FID. For the analysis, the injection temperature and detector temperature were set at 320℃, and the heating program was set to maintain 35℃ for 5 min, and was then increased to 320℃ at a rate of 10℃ min^{-1}. Helium was used as the carrier gas in a splitless mode. The analysis was repeated twice for each sample.

2.6 Soil DHA

Soil DHA was determined according to the method described by Hayase [24]. One millilitre of 0.25 mmol Tris-hydrochloric buffer solution (pH 6.8), 200μL of 0.4% 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT), and 50μL of 1% glucose were added per 1 g of soil into a 100-mL test tube. The mixture was then tightly sealed and incubated for 24 h in the dark at 30℃. Then, 10mL of methanol was added to stop the enzyme reaction and stirred for 1 min by a vortex mixer. Subsequently, the supernatant was filtered after being left stationary for 10-15 min. The filtrate was measured using a spectrophotometer (UV-1700, Shimadzu Corp., Ltd., Kyoto, Japan) at a wavelength of 485 nm, and the amount of change from INT to iodo-nitro-tetrazolium formazan was analysed. The analysis was repeated 3 times for each sample. The contaminated soil was sterilized using an autoclave, and then subjected to the same treatment as the oil-contaminated soil for comparison.

2.7 Statistical Analysis

Data analysis for plant growth, TPH concentration and DHA in soil was carried out by using the statistical software (Excel Statistics 2008 for Windows, Social Survey Research

Information Co., Ltd., Tokyo, Japan). The means [± standard error (SE)] were compared using one-way analysis of variance followed by Turkey's Test for mean comparison at the 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Plant Growth

Table 1 shows the time-dependent transition in plant height and above-ground and root dry matter weight for the 6 cultivars at 180 days after sowing. At 60 days after sowing, the plant height of the 6 cultivars in the non-contaminated plot was significantly higher (by 2-3 fold) compared to those in the contaminated plot. However, at 180 days after sowing, the plant height of 'Old fashion' was significantly higher in the contaminated plot compared to the non-contaminated plot, while the plant height of the other 5 cultivars was similar in both the contaminated and non-contaminated plots. Fig. 2 shows the picture of 'Old fashion' at 180 days after sowing. 'Old fashion' which is dwarf varieties and are characterized by a great number of branching and flower with increasing growth, but that in contaminated plot could not to branch with increasing the plant growth due to be not able to increase root growth. Whereas 'Old fashion' in non-contaminated plot surely branched and flowered with increasing root growth. Thus it is thought that plant height of 'Old fashion' in contaminated plot was expanded by scrimpy nutrition absorbed from root instead of increasing branching and flowering compared with that in non-contaminated plot. By the way, plant height of 'Uploar' and 'Sonbrero' was the highest of other cultivars, because their cultivars is tall variety and a little succulent growth. The above-ground and root dry matter weight of the 6 cultivars were lower in the contaminated plot compared to the non-contaminated plot throughout the cultivation period. At 60 days after sowing, the above-ground dry matter weight of the 6 cultivars was significantly lower (80%) in the contaminated plot compared to the non-contaminated plot. These results indicate that initial growth was delayed in plants that grew in the contaminated soil. In particular, at 60 days after sowing, the above-ground dry weight of 'Starbright' was 4.5% lower in the contaminated soil compared to the non-contaminated soil, and represented lowest value of all 6 cultivars. This finding indicated that 'Starbright' exhibited the slowest initial growth rate in contaminated soil. However, at 180 days after sowing, the above-ground dry weight of all 6 cultivars was around 50% higher in the contaminated plot compared to the non-contaminated plot. Therefore, even though the growth of all cultivars was initially delayed in the contaminated soil, cultivation was successful. Between 120 and 180 days after sowing, the root dry weight of 'Uproar' and 'Sombrero' was 2-fold higher in the non-contaminated plot compared to the contaminated plot, while it was 30% higher at 180 days in the contaminated plot. At 180 days, the above-ground part of 'Uproar' and 'Sombrero' fell over in the contaminated plot. This event occurred because it was estimated that the root mass was not sufficient to support the above-ground part, because the plant height was similar in both contaminated and non-contaminated plots, whereas the root dry weight was significantly lower in the contaminated plot compared to the non-contaminated plot. Contaminated soil influences plant growth, with Peng et al. [23] suggesting that changes in plant biomass are dependent on the level of petroleum contaminants in the soil. In contrast, another study reported that a relationship between root exudation and bioremediation efficiency was not apparent for 4 sorghum (*Sorghum bicolor* L.) genotypes, although the presence of all 4 *Sorghum* genotypes resulted in the significant removal of crude oil from impacted soils [25]. Some reports have shown the presence of petrogenic hydrocarbons significantly reduces plant growth and biomass, while other reports have found that the presence of hydrocarbon increases plant growth and biomass [26-28]. We observed both of these phenomena in this experiment, indicating that the response is dependent on the plant type in the contaminated soil. We found that 'Zahara', 'Starbright' 'Oldfashion' and 'Profusion' represent the most effective cultivars for growth in contaminated oil.

Table 2 shows a number of flower of 6 cultivars after sowing. The flowering date of 6 cultivars in contaminated plot delayed about 7 days compared with non-contaminated plot, and then a number of flowers of 'Zahara', 'Starblight', 'Oldfashon' and 'Profusion' in contaminated plot at the last day of measurement decreased about 60-80% compared with those in non-contaminated plot. Although Zinnia genus have compound umbel, dwarf varieties such as 'Zahara', 'Starblight', 'Oldfashon' and 'Profusion' breed for bedding plant are characterized by a great number of branching and flower. However, above-ground dry matter of these 4 cultivars in contaminated plot is about 50% than that in non-

contaminated plot, a number of branching could not increase with decreasing their growth, and therefore it is though that a number of flowering setting in contaminated plot decreased. 'Uploar' and 'Sonbrero' which is tall variety for cut flower and low branching and flower setting, they delayed flowering and decreased of a number of flowers. This is suggested to be a factor that above- and under-ground dry matters of their cultivars in contaminated plot were lower about 30% than those in non-contaminated plot, and those growth were bad compared to other 4 cultivars.

3.2 Soil DHA

Table 3 shows the transition of soil DHA over the 180-day period after sowing. During the 180 days growing period, the soil DHA for the 6 cultivars was equal to or higher in the contaminated plots compared to the non-contaminated and irrigated plots. In particular, the DHA of 'Starbright' and 'Profusion' was significantly higher in the contaminated plot compared to the other treatment plots. Kaimi et al. [21] showed that although the DHA of mature plants in all soils was significantly higher compared to the unplanted control, the values varied among plant species. Our data supported these results. These variations might result from differences in plant root morphology, root exudates, and microbial interactions in the rhizospheres of the different plant species [27,29,30]. In addition, exudate patterns are known to be influenced by a myriad of factors, including plant species, soil composition, environmental parameters, and the presence of xenobiotics [31]. These parameters were suggested because different exudate patterns and plant root morphology have different effects on microbes in contaminated and non-contaminated soils, with microbial activity being highly influenced by plant the cultivation environment. Therefore, 'Starbright' and 'Profusion' were judged to be cultivars that increase soil DHA in contaminated soils.

3.3 TPH Concentration in Soil

Table 4 shows the transition in TPH concentration in soil over the 180-day period after sowing. At 180 days after sowing, the TPH concentration in soil of the 6 cultivars significantly decreased in the contaminated plot compared to the irrigated plot. At 60 days after sowing, the TPH concentration was about 37% lower for the 6 cultivars in the contaminated plot compared to the initial concentration (13,191mg kg^{-1}), while this value was about 70% for all 6 cultivars after 180 days. 'Sombrero', 'Profusion', and 'Starbright' exhibited a 73.6%, 72.1%, and 72.0% decline in TPH concentration in soil after 180 days, respectively. Differences among plant species and plant genotypes are known to influence the degradation of petroleum oil by microbes in the rhizosphere [32]. Banks et al. [25] indicated that the growth rate of 4 *Sorghum* (*S. bicolor* L.) genotypes differed when cultivated in oil contaminated soil, despite there being no significant difference in TPH degradation ability among cultivars. Although Schwab et al. [33] showed that one female parent of the alfalfa cultivar 'Riley' tended to be associated with the lowest level of TPH degradation, another female from the same cultivar exhibited the highest degradation ability. Moreover, Razmjoo et al. [34] reported that 10 vegetatively propagated cultivars of bermudagrass reduced TPH in soil at different rates (0, 2, 4, 6, and 8% of TPHs) in oil sludge contaminated soil, with more than 40% reduction in TPH concentration in soil in the 10 cultivars. Thus, bermudagrass represents an efficient species for the phytoremediation of petroleum contaminated soil.

Furthermore, the phytoremediation of petroleum oil contaminated soil is associated with the growth of the plant root. Banks et al. [25] found that a *Sorghum* cultivar (*S. bicolor*) with many roots exhibited the highest decrease in TPH concentration in soil from the initial stage to the flowering stage. However, in this study, while 'Uproar' and 'Sombrero' had the heaviest of root dry weight and 'Starbright' and 'Profusion' had the lightest root dry weight, all 4 cultivars tended to decrease TPH concentrations in soil similarly. This result indicates that root mass is not linked to the decrease in TPH concentrations in soil, differing to the findings of previous studies. Whereas, Shirdam et al. [35] described that soil pH can affect the availability of nutrients and their absorption by plant roots. Moreover, they reported that no significant remedial effect was observed for the unfertilized sorghum treatment despite its successful growth in the contaminated soil, this could explain to cause the decreased microbial growth and metabolism caused by N deficiency in the root zone due to the plant's uptake of large amount of N from unfertilized soil. Thus, we suggest that the phytoremediation of petroleum oil contaminated soil by plants belonging to the genus *Zinnia* is associated with factors other than root mass.

Table 1. Plant height, above-ground and root dry matter weight of 6 cultivars

Treatments	Days after sowing	'Uproar'				'Zahara'			
		C[z]		NC[z]		C		NC	
Plant height (cm ± SE)	60	12.32±0.9	b	28.92±1.3	a	6.37±0.3	b	12.91±0.55	a
	120	66.38±2.5	b	83.29±3.8	a	28.83±0.	a	33.33±0.98	a
	180	106.33±4.	a	104.17±3.	a	35.64±1.	a	39.08±1.93	a
Above-ground dry matter weight (g pot^{-1} ± SE)	60	1.29±0.29	b	6.33±0.48	a	0.71±0.1	b	4.36±0.35	a
	120	15.41±0.6	b	29.79±1.3	a	9.88±0.8	b	21.68±0.61	a
	180	28.51±1.1	b	51.94±0.6	a	16.11±1.	b	32.59±0.56	a
Under-ground dry matter weight (g pot^{-1} ± SE)	60	0.34±0.09	b	2.20±0.23	a	0.21±0.0	b	0.69±0.16	a
	120	3.21±0.40	b	6.11±1.52	a	0.63±0.0	b	1.65±0.09	a
	180	3.49±0.89	b	12.39±1.9	a	1.00±0.0	b	2.31±0.17	a
Treatments	**Days after sowing**	**'Starbright'**				**'Sombrero'**			
		C		**NC**		**C**		**NC**	
Plant height (cm ± SE)	60	6.21±0.46	b	15.13±0.6	a	12.33±0.	b	27.42±1.85	a
	120	48.17±2.4	a	49.42±2.5	a	80.83±2.	b	91.75±2.05	a
	180	50.33±2.0	a	57.58±2.5	a	102.33±8	a	113.17±12.	a
Above-ground dry matter weight (g pot^{-1} ± SE)	60	0.12±0.01	b	2.65±0.39	a	0.82±0.1	b	4.64±0.18	a
	120	13.19±0.2	b	27.46±1.1	a	15.66±0.	b	29.17±0.69	a
	180	22.45±2.2	b	48.04±1.1	a	27.66±1.	b	52.76±4.87	a
Under-ground dry matter weight (g pot^{-1} ± SE)	60	0.10±0.02	a	0.31±0.03	a	0.27±0.0	b	1.87±0.06	a
	120	0.92±0.11	b	1.39±0.11	a	5.20±1.3	a	6.68±0.52	a
	180	1.02±0.10	b	1.91±0.07	a	3.94±0.8	b	13.27±0.57	a
Treatments	**Days after sowing**	**'Oldfashion'**				**'Profusion'**			
		C		**NC**				**NC**	
Plant height (cm ± SE)	60	13.65±1.4	b	30.42±1.7	a	5.50±0.3	b	12.51±0.62	a
	120	82.55±2.9	a	82.50±1.6	a	30.92±1.	a	31.33±1.54	a
	180	97.17±4.8	a	83.50±5.0	b	35.92±1.	a	37.50±2.22	a
Above-ground dry matter weight (g pot^{-1} ± SE)	60	0.73±0.07	b	4.89±0.47	a	0.87±0.2	b	4.18±0.47	a
	120	15.97±0.9	b	34.35±1.4	a	11.51±0.	b	20.12±0.75	a
	180	18.50±2.3	b	35.47±3.3	a	20.57±0.	b	36.85±1.18	a
Under-ground dry matter weight (g pot^{-1} ± SE)	60	0.35±0.09	b	1.66±0.06	a	0.28±0.0	b	1.34±0.14	a
	120	1.52±0.40	b	5.40±1.03	a	1.23±0.1	a	1.83±0.05	a
	180	1.76±0.74	a	2.43±0.50	a	1.50±0.1	b	3.26±0.26	a

[z] C; contaminated, NC; non contaminated, [y] Means with different letters are significantly different at the 5% level of probability based on t-test for each observation day

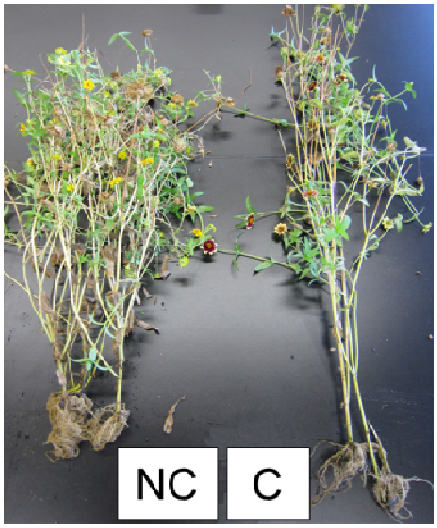

Fig. 2. Picture of 'Oldfashion' at 180 days
C; in contaminated soil, NC; in non-contaminated soil

Table 2. A number of flower of 6 cultivars after sowing

Treatments		Days after sowing													
		54		56		62		66		69		71		74	
		A number of flowers (flower pot^{-1})													
'Uproar'	C[z]	-		-		-		-		-		-		0.2	*
	NC	-		-		-		0.17		1.00		1.58		2.5	
'Zahara'	C	-		-		-		-		0.08	*	0.08	*	0.6	*
	NC	-		-		-		0.75		1.83		2.17		2.7	
'Starbright'	C	-		-		-		-		-		0.08	*	0.6	n.
	NC	-		-		-		-		0.08		0.50		1.5	s.[y]
'Sombrero'	C	-		-		-		-		-		-		0.4	*
	NC	-		-		0.50		1.33		1.67		2.25		2.4	
'Oldfasion'	C	-		-		-		0.42	*	0.83	*	1.17	*	2.0	*
	NC	0.17		0.42		2.00		3.83		5.42		6.75		9.4	
'Profusion'	C	-		-		-		-		-		0.17	*	0.5	*
	NC	-		-		-		1.00		1.00		2.25		2.5	

Treatments		Days after sowing											
		77		82		86		87		93		96	
		A number of flowers (flower pot^{-1})											
'Uproar'	C[z]	0.50	*	1.08	*	1.75	*	1.92	*	2.42	n.s	2.58	n.
	NC	2.75		2.92		2.92		2.92		2.92	.	2.92	s.
'Zahara'	C	1.42	*	3.00	*	3.75	*	3.75	*	5.67	*	7.33	*
	NC	3.33		8.00		12.5		14.2		18.5		21.1	
'Starbright'	C	0.67	*	1.42	*	2.75	*	3.33	*	7.00	*	9.00	*
	NC	2.33		7.50		11.8		14.5		25.0		31.4	
'Sombrero'	C	0.67	*	1.83	*	2.33	*	2.50	*	2.92	*	2.92	*
	NC	3.08		3.33		3.50		3.92		5.00		5.25	
'Oldfasion'	C	3.17	*	4.92	*	5.58	*	6.33	*	7.92	*	9.33	*
	NC	11.5		16.3		16.4		18.1		22.3		24.1	
'Profusion'	C	1.17	*	2.08	*	2.67	*	2.83	*	4.50	*	4.83	*
	NC	2.83		7.75		12.5		13.3		18.8		20.5	

z C; contaminated, NC; non contaminated, y; significantly different and n.s.; not significant at the 5% level of probability based on t-test for each observation day*

Table 3. The transition in soil DHA over the 180-day period after sowing

	DHA (mg INTF g DW^{-1}h^{-1} ± SE)							
	C[z]		NC[z]		C		NC	
	'Uproar'				'Zahara'			
60 days	14.65±0.61	b*	12.22±0.44	c	13.05±0.74	bc	12.87±0.86	bc
120 days	12.22±0.42	c	13.15±0.53	bc	13.65±0.56	b	15.47±0.39	a
180 days	15.78±0.73	cd	13.00±0.59	e	15.38±0.70	cd	14.00±0.42	cde
	'Starbright'				'Sombrero'			
60 days	18.93±1.01	a	12.31±1.11	c	18.93±1.01	a	12.31±1.11	c
120 days	13.27±1.41	b	12.39±0.28	b	13.37±0.33	b	13.81±0.41	b
180 days	20.00±0.80	a	14.97±0.71	cd	14.61±0.35	cde	14.54±0.33	cde
	'Oldfasion'				'Profusion'			
60 days	17.71±1.01	a	12.16±0.37	c	12.11±0.90	c	14.70±0.62	b
120 days	11.73±0.60	c	14.35±0.49	b	12.90±0.44	b	12.74±0.42	b
180 days	14.43±0.52	de	11.90±0.43	e	18.24±0.80	b	16.16±0.69	c
	irrigated				unirrigated			
60 days	14.61±1.27	b	-		4.233±0.40	d	-	
120 days	10.99±0.43	c	-		4.59±0.17	d	-	
180 days	11.63±0.39	f	-		5.37±0.37	g	-	

[z] C; contaminated, NC; non contaminated.[y] Different letters are significantly different at the 5% level of probability based on Fisher's LSD between cultivars and treatments

Table 4. The transition of TPH concentration in soil over the 180-day period after sowing

Treatments	Concentration (mg/kg ± SE)					
	60 days		120 days		180 days	
'Uproar'	8786±757	b[z]	7047±308	cd	4501±603	cd
'Zahara'	7817±219	b	8078±582	bcd	5178±421	c
'Starbright'	8271±11	b	6464±1151	d	3694±148	de
'Sombrero'	8728±1033	b	6575±473	bc	3481±304	de
'Oldfasion'	7903±524	b	7700±859	bc	3866±126	cd
'Profusion'	8326±853	b	6516±426	d	3685±126	e
irrigated	9020±1150	b	9464±752	b	6633±118	b
unirrigated	11253±157	a	12291±279	a	9322±248	a

[z] *Means with different letters are significantly different at the 5% level of probability based on Fisher's LSD for each observation day*

Various degrading enzymes (such as esterase, cytochrome P450 and amidase) are excreted from the plant root, which decompose persistent organic pollutants, such as trinitrotoluene, trichloroethylene, polycyclic aromatic hydrocarbon, and polychlorinated biphenyl [36]. Moreover, plant exudates have been suggested to influence the pattern of bacterial gene expression, with differential bacterial responses to signals from different plant species potentially mediating the selection of specific rhizosphere populations, such as amino acid and phenol compounds [37].

4. CONCLUSION

In this study, we investigated the growth of 6 ornamental flower cultivars belonging to the genus *Zinnia*, which have aesthetic value and are independent of the food chain, in oil-contaminated soil. In addition, we evaluated the remediation of oil-contaminated soil by the 6 *Zinnia* cultivars by recording changes in TPH concentration and DHA in soil. We found that the growth of 'Zahara', 'Starbright', 'Oldfashion', and 'Profusion' increased during the 180 days after sowing. This finding indicates that these cultivars are particularly tolerant of oil-contaminated soil. The TPH concentration significantly decreased by about 70% from an initial concentration of 13,191 mg diesel kg^{-1} soil after 180 days in the contaminated soil for all 6 cultivars. In particular, the TPH concentrations in soil of 'Sombrero', 'Profusion', and 'Starbright' decreased to 74%, 72%, and 72%, respectively. In addition, soil DHA significantly increased in the contaminated plot compared to the irrigated plot after 180 days, verifying the depuration effect of all 6 ornamental flowers. The results of this study indicate that 'Profusion' and 'Starbright' represent the most appropriate cultivars for the phytoremediation of oil-contaminated soils, because of their depuration effects and ability to maintain favorable long-term growth. In near future, we need to investigate the internode length, change in coloration of stem, leaf growth, delay in lamina expansion etc., and then analyze soil microflora in 6 cultivars for elucidating the factors causing the different remediation of oil contaminated soil by 6 cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huang XD, El-Alawi Y, Gurska J, Glick BR, Greenberg BM. A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbon (TPH) from soil. Microchem J. 2005;81(1):139-147.
2. Peña-Castro JM, Barrera-Figueroa BE, Fernández-Linares L, Ruiz-Medrano R, Xoconostle-Cázares B. Isolation and identification of up-regulated genes in bermudagrass roots (*Cynodon dactylon* L.) grown under petroleum hydrocarbon stress. Plant Sci. 2006;170(4):724-731.
3. Phillips LA, Greer CW, Germida JJ. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biol Biochem. 2006;38(9):2823-2833.
4. Dowling DN, Doty SL. Improving phytoremediation through biotechnology. Curr Opin Biotechnol. 2009;20(2):204–206.

5. Hall C, Tharakan P, Hallock J, Cleveland C, Jefferson M. Hydrocarbons and the evolution of human culture. 2003;426(6964):318-322.
6. The Geo-Environmental Protection Center. The TPH test methods using GC-FID, Guidelines against Oil Pollution by the Ministry of Environment. Tokyo: The Chemical Daily Co., Ltd; 2006.
7. Zhou QX, Song YF. Principles and methods of contaminated soil remediation. Beijing: Chin Environ Sci Press; 2004.
8. Kaimi E, Mukaidani T, Tamaki M. Effect of rhizodegradation in diesel-contaminated soil under different soil conditions. Plant Prod Sci. 2007;10(1):105-111.
9. Susarla S, Medina VF, McCutcheon SC. Phytoremediation: An ecological solution to organic chemical contamination. Ecologic Eng. 2002;18(5):647–658.
10. Muratova AY, Bondarenkova AD, Panchenko LV, Turkovskaya OV. Use of integrated phytoremediation for cleaning-up of oil-sludge-contaminated soil. Applied Biochem Microbiol. 2010;46(8);789–794.
11. Wang Z, Xu Y, Zhao J, Li F, Gao D, Xing B. Remediation of petroleum contaminated soils through composting and rhizosphere degradation. J Hazard Mater. 2011;190(1–3):677–685.
12. Yi Z, Jian W, Yizhi S, Yuting L, Guanghe L. Microbial community and functional genes in the rhizosphere of alfalfa in crude oil-contaminated soil. Front. Environ Sci Eng. 2012;6(6):797–805.
13. Pilon-Smits E. Phytoremediation. Annual Reviews in Plant Biology. 2005;56:15-39.
14. Banks MK, Lee ES. Bioremediation of petroleum contaminated soil using vegetation a microbial study. J Environ Sci Health. 1993;28(10):2187-2198.
15. Koo SY, Sun HH, Hee WR, Kyung SC. Plant growth-promoting trait of rhizobacteria isolated from soil contaminated with petroleum and heavy metals. J Microbiol Biotechnol. 2010;20(3):587-593.
16. Sun HH, Hee WR, Jaisoo K, Kyung SC. Rhizoremediation of diesel-contaminated soil using the plant growth-promoting rhizobacterium *Gordonia* sp. S2RP-17. Biodegradation. 2011;22(3):593-601.
17. April W, Sims RC. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere. 1990;20(1):253-265.
18. Brandt R, Merkl N, Schultze-Kraft R, Infante C, Broll G. Potential of vetiver (*Vetiveria zizanioides* (L.) Nash) for phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. Int J Phytoremediat. 2006;8(4):273-284.
19. Korade DL, Fulekar MH. Effect of organic contaminants on seed germination of *Lolium multiflorum* in soil. Biol Medicine. 2009;1(1):28-34.
20. Amakiri JO, Onofeghara FA. Effects of crude oil pollution on the germination of *Zea mays* and *Capsicum frutescens*. Environ Pollut. 1984;35(2):159-167.
21. Yateem A, Balba MT, El-Nawawy AS, Awadhi NAI. Plants-associated microflora and the remediation of oil-contaminated soil. Int J Phytoremediat. 2000;2(3):183-191.
22. Zhang C, Qixing Z, Peng S, Kenan L. Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* L. with strong endurance. J Hazard Mater. 2010;183(1):731-737.
23. Peng S, Zhou Q, Cai Z, Zhang Z. Phytoremediation of petroleum contaminated soils by *Mirabilis Jalapa* L. in a greenhouse plot experiment. J Hazard Mater. 2009;168(2):1490-1496.
24. Hayase K, Measurement of enzyme activity in soil. Experimental Methods in Soil Microbiology-New Edition. Tokyo: Youken-dou; 1992.
25. Banks MK, Kulakow P, Schwab AP, Chen Z, Rathbone K. Degradation of crude oil in the rhizosphere of sorghum bicolor. Int J Phytoremediat. 2003;5(3):225-234.
26. Shahsavari E, Adetutu EM, Anderson PA, Ball AS. Tolerance of selected plant species to petrogenic hydrocarbons and effect of plant rhizosphere on the microbial removal of hydrocarbons in contaminated soil. Water Air Soil Pollut. 2013;224(4):1495-1504.
27. Gaskin S, Soole K, Bentham R. Screening of Australian native grasses for rhizoremediation of aliphatic hydrocarbon-contaminated soil. Int J Phytoremediat. 2008;10(5):378–389.
28. Smith M, Flowers T, Duncan H, Alder J. Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and

soil with aged PAHs residues. Environ Pollut. 2006;141(3):519–525.
29. Kaimi E, Mukaidani T, Tamaki M. Screening of twelve plant species for phytoremediation of petroleum hydrocarbon-contaminated soil. Plant Prod Sci. 2007;10(2):211-218.
30. Bowen GD. Nutrient status effects on loss of amides and amino acids from pine roots. Plant Soil. 1969;30(1):121-127.
31. Phillips LA, Greer CW, Farrell RE, Germida JJ. Plant root exudates impact the hydrocarbon degradation potential of a weathered-hydrocarbon contaminated soil. Applied Soil Ecology. 2012;52:56-64.
32. Chiapusio G, Pujol S, Toussaint ML, Badot PM, Binet P. Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (*Lolium perenne* L. and *Trifolium pratense* L.) in three spiked soils. Plant Soil. 2007;294(1-2):103-112.
33. Schwab P, Banks MK, Kyle WA. Heritability of phytoremediation potential for the alfalfa cultivar Riley in petroleum contaminated soil. Water Air Soil Pollut. 2006;177(1-4):239-249.
34. Razmjoo K, Adavi Z. Assessment of bermudagrass cultivars for phytoremediation of petroleum contaminated soils. Int J Phytoremediat. 2012;14(1):14-23.
35. Shirdam R, Zand AD, Bidhendi GN, Mehrdadi N. Phytoremediation of hydrocarbon-contaminated soils with emphasis on the effect of petroleum hydrocarbons on the growth of plant species. Phytoprotect. 2008;89(1):21-29.
36. Wang X, Li F, Sugisaki M. Phytoremediation of contaminated soil: Current Status and Perspective. J Environ Lab Associat. 2004;29(2):85-94. Japanese
37. Mark GL, Dow JM, Kiely PD, Higgins H, Haynes J, Baysse C, Abbas A, Foley T, Franks A, Morrissey J, O'Gara F. Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe–plant interactions. Proc Natl Acad Sci USA. 2005;102(48):17454–17459.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=651&id=24&aid=6032