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Full Length Research Paper

Effects of alfalfa, corn and sunflower with date palm on decreasing soil pollution

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Uptake of pollutants by plants, and entering the food chain can cause different diseases for humans. There are different methods for refining contaminated soils. One low-cost method for refining contaminated soils is using phytoremediation that has lower cost, compatible with nature and require fewer facilities than the other methods of refining. Khuzestan province in Iran has oil wells that cause soil pollution. We carried out an experiment in a completely randomized statistical design in greenhouse with treatments such as: T_1-sole date palm in non polluted soil (control), T_2-date palm with corn in non polluted soil, T_3-date palm with alfalfa in non polluted soil, T_4-date palm with sunflower in non polluted soil, T_5-sole date palm in polluted soil, T_6-date palm with corn in polluted soil, T_7-date palm with alfalfa in polluted soil and T_8-date palm with sunflower in polluted soil. Total plant, shoot and root weight was measured and TPH concentration in soil was determined. The results showed that there was significant difference between fresh and dry weight of total plant in the different treatments. T_3 had the highest weight and T_7 had the lowest weight. Alfalfa larger amounts of TPH concentration was removed more than corn and sunflower soil in polluted soil.

Key words: Phytoremediation, date palm, corn, alfalfa, sunflower, TPH.

INTRODUCTION

High concentrations of pollutants in soil, in addition to the vast effects of the regional ecosystem and the uptake of these pollutants by plants which enter the food chain, can cause different disease for humans and other organisms. This can also create pollutants in ground water and causes problems for human health and the environment. Due to the importance of subject study, refining oil compounds from soil is essential. Different methods for refining contaminated soils are mainly expensive and costly, so using low cost methods for removing soil contaminations can use refinement and optimization of these lands to help. One low-cost method for refining contaminated soils is using plants (phytoremediation). Phytoremediation is an emerging technology that uses plants and their associated rhizospheric microorganisms to remove, degrade, or contain contaminants located in the soil, sediments, groundwater, surface water, and even the atmosphere (Chappell, 1997). Scientists have found that plants can be used to treat most classes of contaminants, including petroleum hydrocarbons, chlorinated solvents, pesticides, metals, radio nuclides, explosives, and excess nutrients. These plants can be herbs, shrubs, and trees, and they can concentrate organics and heavy metals at levels much greater than normal (Brown, 1995; Ma et al., 2000). The advantages of phytoremediation compared to other approaches are: (1) it reserves the natural structure and texture of the soil; (2) energy is primarily derived from sunlight; (3) high levels of biomass in the soil can be achieved; (4) it is low in cost. Although, using plants for remediation of persistent contaminants may have advantages over other methods, many limitations exist for the large-scale application of this technology. For example, many plant species are sensitive to contaminants including PAHs so that they grow slowly and it is time consuming to establish sufficient biomass for meaningful soil remediation. In addition, in most contaminated soils, the number of microorganisms is depressed so that there are not enough bacteria either to facilitate contaminant degradation or to support plant growth. Cultivating plants together with plant growth-promoting bacteria allowed the plants to germinate to a much greater extent, and then to grow well and rapidly accumulate a large amount of

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biomass. In addition, the plant growth-promoting bacteria in these experiments increased PAH removal; this is probably due to an alleviation of a portion of the stress imposed upon the plant by the presence of the PAHs. The plant growth-promoting bacteria increased seed germination and plant survival in heavily contaminated soils, decreased the plant dry weight to fresh weight ratio, increased the plant water content, helped plants to maintain their chlorophyll contents and chlorophyll a/b ratio, and promoted plant root growth. Grasses are often planted in tandem with trees at sites with organic contaminants or as the primary remediation method. They provide a tremendous amount of fine roots in the surface soil which is effective at binding and transforming hydrophobic contaminants such as TPH, BTEX, and PAHs. Grasses are often planted between rows of trees to provide for soil stabilization and protection against wind-blown dust that can move contaminants off-site. Legumes such as alfalfa, alsike clover, and peas, can be used to restore nitrogen to poor soils. Fescue, rye, and reed canary grass, have been used successfully at several sites, especially those contaminated with petrochemical wastes. The grasses are harvested periodically and disposed to compost or burned. Hydrophobic contaminants do not translocate appreciably, so the top portion of grasses is not contaminated. The system achieves phytoremediation via rhizosphere processes and sorption to roots (Ferro, 1996). Total petroleum hydrocarbons (TPH) comprise a diverse mixture of hydrocarbons that occur at petrochemical sites and storage areas, waste disposal pits, refineries, and oil spill sites. TPHs are considered persistent hazardous pollutants, and include compounds that can bioconcentrate and bioaccumulate in food chains (McElroy et al., 1986). TPHs are acutely toxic and some such as benzene and benzo[a]pyrene are recognized mutagens and carcinogens (Mortelmans, 1986). Since this group includes chemicals that have physical and chemical characteristics that vary over orders of magnitude, TPHs are divided into two categories. gasoline range organics (GRO) that corresponds to small chain alkenes (C6-C10) with low boiling point (60 to 170°C); diesel range organics (DRO) that includes longer chain alkenes (C10–C40) and hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAH). Research and application of phytoremediation for treatment of petroleum hydrocarbon contamination over the past fifteen years has provided much useful information that can be used to design effective remediation systems and drive further improvement and innovation (Gunther et al., 1996). Nedunuri et al. (2000) investigated on total petroleum hydrocarbon (TPH) removal at several field sites contaminated with crude oil, diesel fuel, or petroleum refinery wastes, at initial TPH concentrations of 1,700 to 16,000 mg/kg. Plant growth varied by species, but the presence of some species led to greater TPH disappearance than with other species or in unvegetated soil. At a crude oil-contaminated field site near the Gulf of Mexico, an annual rye-soybean rotation plot and a St. Augustine grass-cowpea rotation plot had significantly ($P < 0.05$) greater TPH disappearance than did sorghum-Sudan grass or unvegetated control plots, at 21 months. At a diesel fuel-contaminated Carney Island field site in Norfolk, Virginia, the fescue plot had significantly ($P < 0.10$) greater TPH removal than did an unvegetated plot. At a refinery waste site, statistical analyses were not presented due to the short time since establishment of the plots, but Nedunuri et al. (2000) reported that qualitatively, the vegetated plots had greater TPH removal than the unvegetated control plots. After investigating the potential to use phytoremediation at a site contaminated with hydrocarbons, the Alabama Department of Environmental Management granted a site, which involved about 1500 cubic yards of soil, of which 70% of the baseline samples contained over 100 ppm of total petroleum hydrocarbon (TPH), after 1 year of vegetative cover, approximately 83% of the samples contained less than 10 ppm TPH. Hecht and Badiane (1998) showed that soils with 2% TPH reduced alfalfa yield by 32%. Gunther et al. (1996) showed that soil with 5000 mg/kg hydrocarbons was reduced production of biomass by ryegrass, by 46%. Yateem et al. (1999) found that plants pre-grown in clean soil and subsequently transplanted to the contaminated soil, grew nearly as well as the control and showed that toxicity was associated with germination and/or early plant growth. Similarly, poor rooting of ryegrass compared to legumes, appeared to adversely affect the removal of TPH from Gulf War-contaminated soils. Wiltse et al. (1998) researched on greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes and showed that TPH concentration decreased to a minimum of 72% of the refrigerated control within 6 months and ranged from 67 to 44% after 12 months. The non-vegetated control soil concentration decreased to 54% of the refrigerated control. Two genotypes had significantly lower TPH concentrations, individually. The best performing genotype had 56% degradation while the non-vegetated control had 46% degradation. Although two alfalfa genotypes increased TPH degradation, the other genotypes had no effect or actually hindered TPH degradation.

MATERIALS AND METHODS

Ahwaz is the capital city of Khoozestan province in Iran and it is an industrial zone which lies between eastern longitude of 47º40' to 49º20' and 31º5', and northern latitude 32º20' at an elevation of 480 to 550m (a.s.l). It houses different petrochemical, industrial, and well oil units which spreads over 63,238 km² of land. A bulk soil from a field (0 to 20 cm) nearby well oil and industrial area around Ahwaz city was taken and analyzed for EC, pH, %O.C, Texture, and TPH (total petroleum hydrocarbon) (Table 1). Non-contaminated soil was taken from farm fields. The soils were air-dried and passed through < 2 mm stainless steel sieve. Each of

Table 1. Soil characteristics in applied treatments.

Characteristics treatment	TPH (mg/kg)	EC (ds/m)	pH	Ca (meq/kg)	Mg (meq/kg)	K (mg/kg)	P (mg/kg)	OC%
T1	Non polluted soil	3.72	7.21	22.33	66.66	220	13.6	2.94
T2	Non polluted soil	3.12	7.38	26.1	66.47	199	9.2	1.96
T3	Non polluted soil	3.86	7.35	32.73	65.83	212	9.6	2.42
T4	Non polluted soil	3.84	7.35	32.83	60.33	222	13.0	2.17
T5	666.67	3.23	7.38	39.33	66.5	200	9.6	2.41
T6	715.67	3.16	7.35	20.93	60.1	185	9.8	1.66
T7	766.67	2.75	7.44	29.66	59.66	176	10.1	1.86
T8	766.67	3.08	7.39	30.43	62.83	185	9.4	1.59

Table 2. Analysis of variance of TPH concentration in the polluted soil before planting.

Prob	F	Mean square	Sum square	Degree of freedom	Source of error
0.318	1.397	140833.33	281666.667	2	Rep
0.975ns	0.068	6875.00	20625.0	3	Treat
Prob	F	100833.33	605000.00	6	Error

ns = non significant effects.

these soils with animal manure (10% volume) was mixed. Soils were transferred to polyethylene pots (30 cm length, 20 cm width and 15 cm depth) and each pot containing 10 kg soil. 12 pots were filled with non-contaminated soil and 12 pots were filled with contaminated soil. At the beginning of experiment 240, 120, and 150 mg kg^{-1} of N, P, and K were added to the pots as fertilizer, respectively. Experiment carried out in a completely randomized statistical design in greenhouse with two soil treatments (contaminated soil, non-contaminated soil), with 4 plant treatments (date palm *"Phoenix dactylifera"*, alfalfa "*Medicago sativa"*, corn "*Zea mays"* and sunflower "*Helianthus annus")*. The following treatments in three replications were applied:

T_1- sole date palm in non polluted soil (control);
T_2- date palm with corn in non polluted soil;
T_3- date palm with alfalfa in non polluted soil;
T_4- date palm with sunflower in non polluted soil;
T_5- sole date palm in polluted soil;
T_6- date palm with corn in polluted soil;
T_7- date palm with alfalfa in polluted soil;
T_8- date palm with sunflower in polluted soil.

In the first step, date palm was cultivated and then four seeds from each agronomic plant were sowed in the pots. This study was carried out in the greenhouse. Day and night temperatures in the greenhouse were 30 and 28°C, respectively, and natural sunlight was supplemented. The pots were irrigated at the field capacity condition by distilled water. After each plant species grew up for one growth cycle, whole plants were removed from soil and then the soil samples were taken from each of pot. Soil samples were air dried at room temperature, crushed and pulverized to pass through <2 mm stainless steel sieve and total petroleum hydrocarbons were determined. Root and shoot of each plant was separated and collected. Plant samples after washing were dried at 70°C. Total plant, shoot, and root weight was measured in different treatments and TPH concentration in pots were measured, and then TPH concentration in soil polluted in each treatment based on percent of TPH decrease was determined as shown thus:

$$\%D = \frac{(TPH_1 - TPH_2)}{TPH_1} \times 100$$

where %D =% TPH decrease in soil; TPH_1= initial TPH concentration; TPH_2 = final TPH concentration (after plants removal from soil).

Data analyzed by software SPSS 16 and mean comparison was done through Duncan's multiple range tests.

RESULTS

Before starting the experiment, soil were analyzed in applied treatments (Table1). Results showed that there were no significant difference between the concentration of initial TPH in the different treatments (Table 2), and Duncan test also showed that all these treatments were in same group (Table 3).

Date palm seedling weight

The results showed that there was significant difference between the fresh and dry total plant and shoot fresh weight of date palm seedlings in different treatments ($P < 0.05$). Date palm cultivated in non-polluted soil with alfalfa had the highest weight that is assigned to the A group by the mean of 157.35 g and date palm cultivated in polluted soil with alfalfa had the lowest weight that is assigned to the B group by the mean of 62.41 g (Table 4).

Average shoot dry weight also showed that T_3 with the highest amount (67/55 g) was placed in a group and the

Table 3. Mean of initial TPH concentration in polluted soil.

Treatment (mg/ kg)	5	6	7	8
TPH	666.67^{A}	715.67 ^{A}	766.67 ^{A}	766.67 ^{A}

*mean in each cell with same letter are not significantly different (P < 0.05).

Table 4. Average date palm seedling weight (g) in different treatments.

Treatment	Root dry weight	Shoot dry weight	Total dry weight	Root fresh weight	Shoot fresh weight	Total fresh weight
T1	14.07 ^{a}	28.04 ^{b}	42.11 ^{a}	38.443^{a}	70.710^{ab}	109.15^{ab} *
T2	13.73 ^{a}	36.33 ^{b}	50.67 ^{ab}	36.137 ^{a}	84.663 ^{ab}	120.80^{ab}
T3	14.83 ^{a}	55.67 ^{a}	70.50 ^{a}	46.860 ^{a}	110.467 ^{b}	157.35^{a}
T4	12.91 ^{a}	30.25 ^{b}	43.16 ^{b}	34.103 ^{a}	70.317 ^{ab}	104.42^{ab}
T5	13.05 ^{a}	26.91 ^{b}	39.97 ^{b}	26.477 ^{a}	64.257 ^{b}	90.73^{ab}
T6	15.18 ^{a}	34.37 ^{b}	49.54 ^{ab}	34.910 ^{a}	67.773 ^{b}	102.68^{ab}
T7	9.75 ^{a}	43.42 ^{b}	35.67 ^{b}	18.958 ^{a}	43.423 ^{b}	62.41^{b}
T8	19.67 ^{a}	48.64 ^{b}	43.08 ^{b}	36.117 ^{a}	48.640 ^{b}	84.86^{ab}

*mean in each column with same letter are not significantly different (P < 0.05).

Table 5. Analysis of variance of TPH concentration decrease in different treatments.

Prob	F	Mean square	Sum square	Degree of freedom	Source of error
0.656	0.453	43.032	21.516	2	Rep
0.021*	7.08	1009.910	336.637	3	Tre
		285.195	47.533	6	Erorr

*significant effects (P < 0.05).

Table 6. Average of TPH concentration decrease in different treatments.

Treatment (mg/kg)	T5	T6	T7	T8
TPH	57.57^{c}	75.13^{bc}	82.17^{a}	67.07^{ab}

*mean in each cell with same letter are not significantly different (P < 0.05).

other treatments were the next group (Table4). There was no significant difference between root fresh and dry weight in the treatments (Table4).

TPH concentration in soil

Final TPH concentration was determined in soil after harvesting of plants, and then the percent of TPH decrease was calculated in the different treatments. Analysis of variance (Table 5) showed that there was significant difference between treatments in TPH decrease from soil (P < 0.05). So, T_7 (date palm planting with alfalfa) and T_5 (sole date palm) had maximum (82.17%) and minimum (57.57%) effect in the decrease of TPH, respectively. Sole date palm, date palm with corn, date palm with alfalfa and date palm with sunflower reduced TPH in polluted soil by 57.57, 75.13, 82.17 and 67.07%, respectively (Table 6).

DISCUSSION

Results showed that the presence of TPH in soil reduced date palm seedling fresh and dry weight. Seedling weight results (include : total plant and shoot weight) from this, project in coordination with obtained results from the other researchers, for example, Rangzan and Landy (2007) showed that the concentration of gasoline had significant effects on total plant and shoot dry weight and relationship was reversed, so that increased concentration of gasoline declined plant dry weight.

Wilste et al. (1998) showed that all factors of alfalfa in crude oil contaminated soil were reduced and the product harvest in contaminated soil was about 32% of non-contaminated soil. Shahriari et al. (2007) showed that with increasing oil, the number of leaves in the alfalfa was decreased.

Comparing the TPH removed by alfalfa, corn, and sunflower, showed significant deference. Date palm planted with alfalfa had most TPH decrease from soil than the other treatments, thus, date palm with alfalfa was more effective than corn or sunflower in TPH decrease from soil. This result is in coordination with the results of researchers like, Khatibi et al. (2009) who showed that alfalfa, white clover and grass, reduced the concentration of petroleum hydrocarbons in soil and alfalfa and grass decreased more concentration of total petroleum than white clover. Alikhani et al. (2009) showed that barley in high-density, reduced surface contamination of soil is about 46%.

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