Bioremediation of petroleum-contaminated soil using landfarming technique: Influence on soil biological and chemical properties

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Abstract
Petroleum hydrocarbons are a carcinogenic group of contaminants which are widely distributed in the environment. In this study, bioremediation of two petroleum-contaminated soils around the Tehran Oil Refinery (S_1 and S_2) using landfarming technique was evaluated. The effect of this technique on some biological and chemical properties of investigated soils at the end of each month of the experimental period (4months) was also part of the goal. Results showed that about 50 and 57% of hydrocarbon contents in the landfarming plots were eliminated from soils S_1 and S_2 at the end of the experiment, respectively. Landfarming processes enhanced microbial respiration in both soils S_1 and S_2 at the end of all four months of the experiment. Furthermore, urease activity in the landfarming plots for soil S_2 was 21, 45, 26, and 23% higher than that in the control plots (without landfarming operations) at the end of the first to the 4th month of the experiment, respectively. There were also significant differences ($P<0.05$) in soil pH values between the landfarming plots and control. Soil electrical conductivity in the landfarming plots was lower than that in the control. It appears that improving soil aeration and exposing new layers of soil to sunlight as a result of landfarming operations, presumably, intensified the microbial activity and thus, facilitated degradation of petroleum hydrocarbons.
Keywords: Bioremediation; Landfarming; Microbial activity; Petroleum contaminants

1. INTRODUCTION

Large quantities of soil have been contaminated with petroleum hydrocarbons through transportation, leakage from tanks, accidental spillage, pipeline ruptures or in the case of deliberate spreading of oily wastes like in landfilling operations [1]. Presence of these contaminants in soil may be toxic to human, plants and soil microorganisms hence; there are urgent needs to find effective and low-cost technologies to clean up these contaminated soils. Physical, chemical, and biological methods can all be used for remediation of such contaminated soils, but landfarming has been recognized as a feasible and low cost-effective technique for removal of total petroleum hydrocarbons (TPHs) from soil [2]. Landfarming, known as land treatment or land application, is an above-ground remediation technology for soil that reduces concentration of petroleum constituents through biodegradation. This technology usually involves spreading excavated contaminated soil in a thin layer on the ground surface and stimulating aerobic microbial activity within the soils through aeration and/or the addition of mineral nutrients, and moisture [3].

There are several oil refineries in Iran (e.g. Tehran Oil Refinery) where environmental pollution has been growing as a great concern and thus; ecosystem has been faced serious challenges. Different approaches to reduce this problem have been failed so far and therefore, it seems that landfarming technique would be suitable to reduce environmental hazards of petroleum contaminants in these areas. The objective of this study was to evaluate the ability of landfarming as a useful and feasible technique for eliminating total petroleum hydrocarbons from contaminated soils around the Tehran

Oil Refinery Complex. Furthermore, the effect of landfarming technique on some microbial activity indicators and some chemical properties of the petroleum-contaminated soils was investigated.

2. MATERIALS AND METHODS

2.1 Soil preparation and characteristics

Bulk samples (about 500 Kg) of petroleum-contaminated surface (0-40 cm) soils were collected from oily wastes land fill soil (soil S_1) and petroleum-contaminated farm lands (soil S_2) around the Tehran Oil Refinery (35° 30' N, 51° 26' E), Iran. Soil samples were air dried, passed through a 4-mm sieve, land farmed and mixed at intervals every 3 days with a garden hoe for 21 days to ensure a homogenous distribution of the petroleum pollutants. After that, about 1 kg subsamples of the treated soils were sieved through a 2-mm sieve to be used for performing some chemical and physical analysis (Table 1.) Petroleum hydrocarbon-contents in the soil samples were also extracted by soxhlet using a 1:1 (v/v) dichloromethane and n-hexane (150 ml) mixture for 24 h [4]. Afterward, concentration of selected PAHs was determined in the extracts using gas-chromatograph (Table 2).

Table 1. Selected physical and chemical properties of soil sampled from oily waste landfill (soil S_1) and petroleum contaminated farm lands (soil S_2).

Characteristic	Soil	
	S_1	S_2
Clay (%)	20	25
Available-Mg (mg kg^{-1})	219	132
Available-Ca (mg kg^{-1})	620	532
Available-Na (mg kg^{-1})	51	39
Available-P (mg kg^{-1})	125	145
Available-K (mg kg^{-1})	200	230
DTPA- Mn (mg kg^{-1})	49	28
DTPA-Zn (mg kg^{-1})	18	15
DTPA-Cu (mg kg^{-1})	81	52
DTPA- Fe (mg kg^{-1})	71	85
DTPA- Ni (mg kg^{-1})	4	2

Table 2. Concentrations of measured PAHs and TPHs in soils S_1 and S_2 (ND: Not detected by gas chromatography).

PAHs (mg kg^{-1})	Soil	
	S_1	S_2
Naphthalene	42	17
Phenantheren	31	15
Anthracene	2	0.5
Fluoranthene	26	21
Pyrene	18	10
Benzo[k]fluoranthene	ND	32
Benzo[a]pyrene	43	33
Benzo[e]pyrene	42	21
Benzo[g,h,i]perylene	7	9
2-methyl phenanthrene	21	11
TPHs (mg kg^{-1})	108966	73233

2.3 Landfarming experiments

Three plots with 30000 cm^3 volumes for carrying out the bioremediation processes (landfarming) and another three for control plots (without landfarming operations) were established in an open field. Landfarming processes were consisted of irrigation (near the 0.7 field capacity) and aerating the contaminated soils every 3 days by hand mixing with a garden hoe. The soils were turned over before the irrigation to expose a new layer of the soils to sunlight and air. Control plots were treated in the same way as landfarming plots but without irrigation and aerating. Conditions were being totally natural with no nutrients addition. The entire experiment was run for 4 months from October (2007) to January (2008) and at the end of each month (1st, 2nd, 3rd and 4th months) soil samples were taken from each plot before irrigation and aeration processes. The samples were brought to the laboratory on the same day and kept in refrigerator at 4^0C until they were analyzed (microbiological analysis were carried out 3–4 days after sampling).

2.4 Microbiological and chemical analysis

Basal soil microbial respiration and urease activity were measured using methods described by Alef and Nannipiery (1995) [5]. Soil electrical conductivity (EC) and soil pH were also determined in a 1/5 solid/liquid aqueous extract. Total nitrogen content in each sample was determined by the

micro-Kjeldahl method. Moreover, TPH-concentration in each soil sample was measured according to the procedure of Christopher et al. (1988) [4]. The experiment was arranged in a factorial trail with completely randomized block design and analysis of variance was performed using SAS statistical computer program.

3. RESULTS AND DISCUSSION

3. 1 Microbial respiration

The results of soil microbial respiration in the landfarming plots and control (without landfarming operations) showed that CO_2 emission due to microbial respiration in the landfarming plots for both soils S_1 and S_2 was significantly ($P<0.05$) higher than that in the control plots at the end of all four months of the experiment (Figure 1). There were 50, 36, 20, and 45% increases in microbial respiration for soil S_1 at the end of first to the 4th month of the experimental period in the landfarming plots compared to the control, respectively. The CO_2 evolution in the landfarming plots for soil S_2 was also about 39, 31, 38, and 40% higher than that in the control plots. It appears that improving soil conditions for microorganism activities due to landfarming operations (aerating, providing optimal soil moisture and exposing new layers of soils to sunlight) intensified soil microbial activity and thus, CO_2 emission in the soil increased respected to time [6].

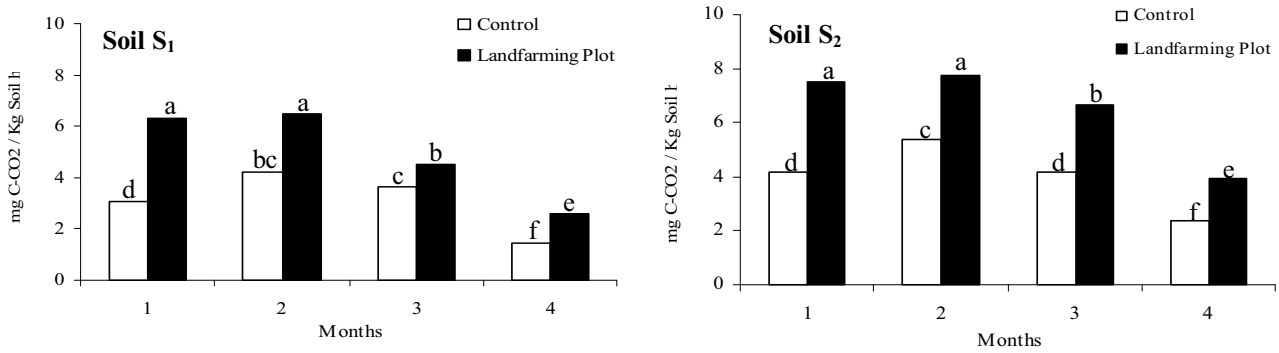

Figure 1. Microbial respiration (evolved CO_2) in the soils S_1 and S_2 during the landfarming experiment Different letters denote significant differences at $P<0.05$

3. 2 Urease activity

Measured urease activity in this study showed higher values in the landfarming plots than that in the control plots for both soils S_1 and S_2; however, no significant differences were observed between the landfarming and control plots for soil S_1 at the end of 4th month of the experiment (Figure 2). There were 16, 14, and 12% increases in urease activity in the landfarming plots as compared to the control plots for soil S_1 at the end of the first to the third month of the experiment, respectively. In addition, urease activity in the landfarming plots for soil S_2 was 21, 45, 26, and 23% higher than that in the control plots at the end of first to the 4th month of the experimental period, respectively. Moreover, figure 2 shows that urease activity in the investigated soils decreased by passing time especially at the end of the last 2 months of the experiment. It might be due to lower microbial activity during the last days of the experimental period (Figure 1). Furthermore, environmental restrictions for microbial activity during the last 2 months of the experiment such as lower temperature and daylight hours might be other reasons for diminishing of urease activity in the soils with time.

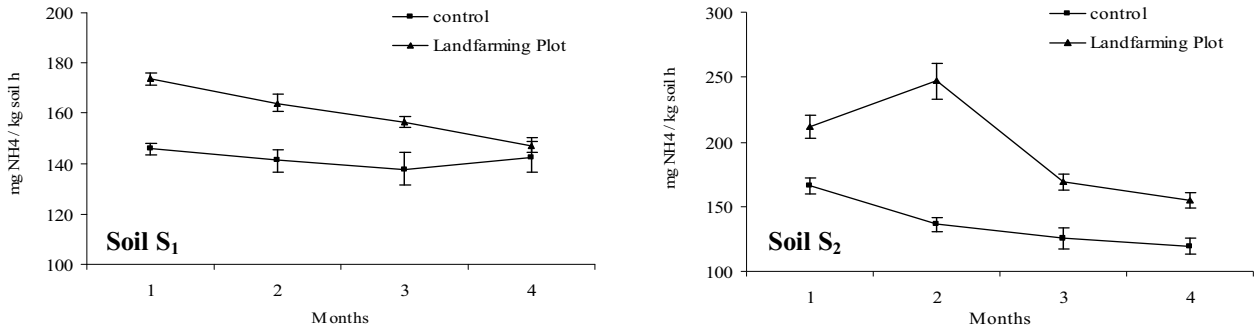

Figure 2. Urease activity in the soils S_1 and S_2 during the landfarming experiment

3.3 Soil pH

The results of measured pH values in the landfarming and control plots indicated a rising trend for both soils S_1 and S_2 (Figure 3). Soils with landfarming operations showed higher pH values than the controls so that significant differences ($P<0.05$) were observed between the landfarming treatment and control at the end of most times of the experimental period. It appears that successive landfarming operations would probably have contributed to increasing pH values in the landfarming plots as compared to the controls. On the other hand, bioremediation of petroleum hydrocarbons using landfarming technique is carried out by soil microorganisms hence; soil characteristics that influence soil microbial activity such as pH will also affect degradation of these compounds. In some studies, optimal pH values ranged from 7.5 to 8 have been reported for mineralization of petroleum hydrocarbons in soil [6]. Therefore, it seems that pH values in the landfarming plots had probably no prohibitive effects on microbial growth and activity.

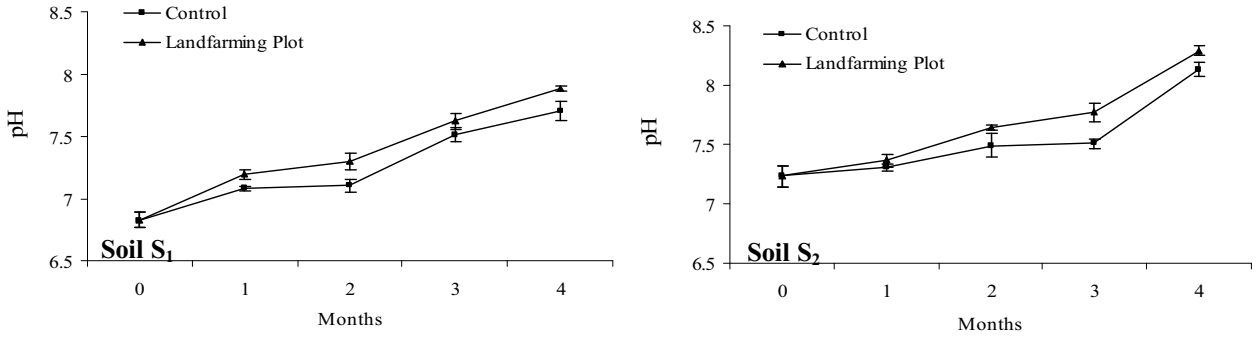

Figure 3. Measured pH values in the soils S_1 and S_2 during the landfarming experiment

3.4 Soil Electrical Conductivity

Soil electrical conductivity (EC) in the landfarming plots was lower than that in the control plots at the end of most times of the experiment (Figure 4). In addition, there was a diminishing trend for EC values with time for both landfarming and control treatments, however, EC values at the end of the first 2 months of the experiment were higher than that at the beginning of the study. During the landfarming operations a large volume of water was used to maintain the soil moisture content within optimum level for microbial activities. This apparently leads to the leaching of salts and reduction of the investigated soil salinity in the landfarming plots compared to the non-irrigated control plots.

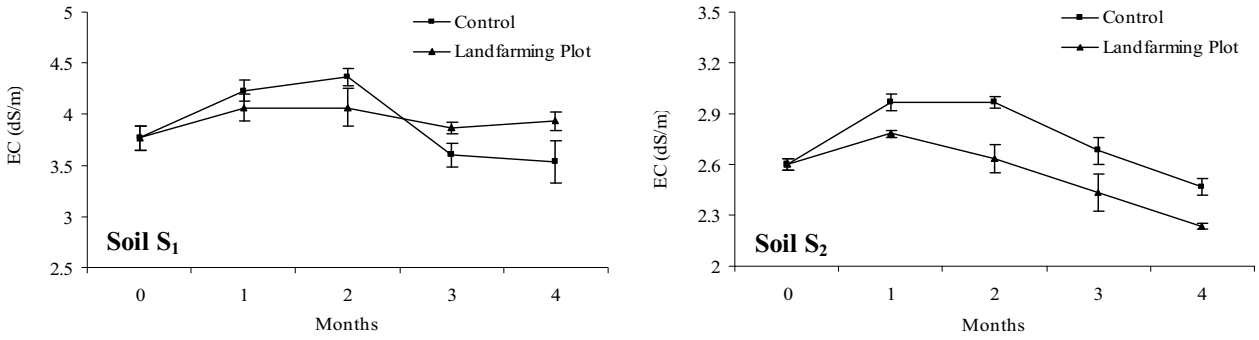

Figure 4. Electrical conductivity (EC) in the soils S_1 and S_2 during the landfarming experiment

3.5 Total Nitrogen in Soil

The effects of landfarming processes on total nitrogen contents (TNC) in the soils are shown in Figure 5. The results indicated that during most times of the experiment, the TNC in the landfarming plots was lower than that in the control plots. However, the TNC in the landfarming plots for soil S_1 at the end of the first and second months of the experimental period was higher than that in the control plots. Furthermore, no significant differences ($P<0.05$) were obtained for TNC at the end of the last month of the experiment between the landfarming and control plots. Reduction of TNC in the landfarming plots might be due to higher consumption of N-compounds by microorganisms in the soil [7].

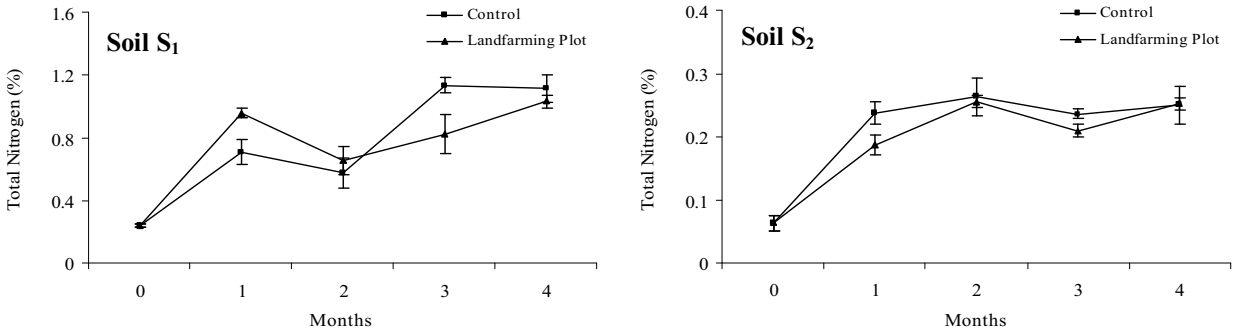

Figure 5. Total nitrogen content in soils S_1 and S_2 during the landfarming experiment

3.6 TPH degradation

Figure 6 shows the soil hydrocarbon contents as a function of time in the landfarming and control plots. Landfarming operations significantly ($P<0.05$) reduced the hydrocarbon contents as compared to the control in both soils S_1 and S_2. There were about 50 and 57% reductions in the TPH-concentration for soils S_1 and S_2 in the landfarming plots at the end of the experiment, respectively. Moreover, during the degradation process in the landfarming treatment, two clearly differentiated phases were observed: a first stage with a high velocity in which hydrocarbon degradation rate was maximal (36 and 39% of TPH-degradation in soils S_1 and S_2, respectively, took place during the first 2 months) and a second slower stage (lower than 13 and 17% of TPH-degradation in soils S_1 and S_2, respectively, took place during the last 2 months, from month 3 to 4). The first stage lasted 2 months, after which the degradation rate slowed down, demonstrating that, as the most easily biodegradable hydrocarbons are consumed, the microorganisms turn their attention to other fractions, such as the aromatic, condensed cyloalkanes, etc., that are degraded at different rates [8]. After these 2 months the degradation rate slowed down, probably, the remaining fractions were structurally more complex hydrocarbons and therefore less accessible, their recalcitrance and low bioavailability causing the activity of the microbial populations to drop.

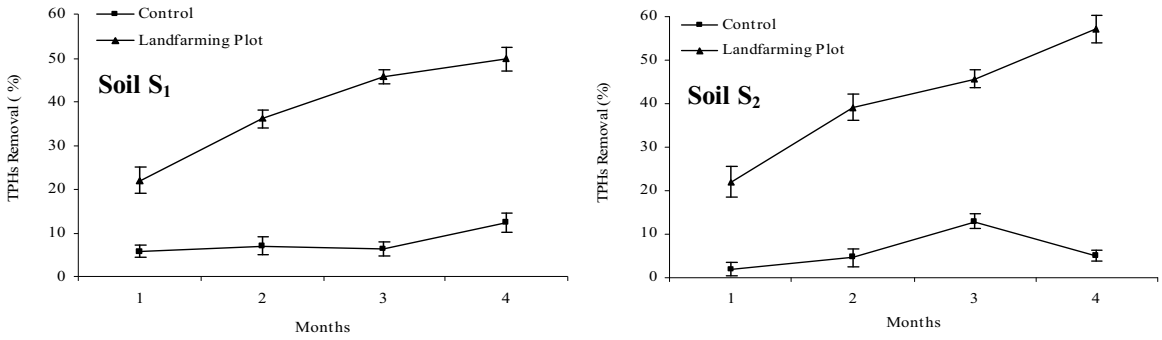

Figure 6. TPH-removal from soils S_1 and S_2 during the landfarming experiment

4. CONCLUSIONS

It is proposed that the bioremediation of investigated petroleum-contaminated soils using landfarming technique is possible. The biodegradation of total hydrocarbons has been established at 50 and 57% in 4 months for soils S_1 and S_2, respectively. However, TPH-concentration reduction in the landfarming plots during the first 2 months of the experimental period was more than the last 2 months. In general, parameters indicative of soil microbial activity measured during the process of hydrocarbon degradation in the landfarming plots showed higher values than the control plots. Plots with landfarming operations showed higher pH values than control. A diminishing trend for soil EC in both landfarming and control plots were found with time. As in the case of these results, it can be inferred that improving soil aeration and exposing new layers of soil to sunlight as a result of landfarming operations intensified the microbial activity and hence, facilitated degradation of petroleum hydrocarbons.

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