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Comparing the Effect of Kerosene Pollution on Forest and Industrial Soil Microbial Community

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ABSTRACT: Kerosene is the colorless liquid and slightly heavier than gasoline that specific odor removes after evaporation. Soil and underground water source are contaminated with different pollutants such as petroleum hydrocarbons. These pollutants have various negative environmental effects on soil and surrounding environment. The aim of this research is to understand the effect of kerosene pollution on two different soils. The two different collected soils include Industrial and Forest soil. Six microcosms were designed. Indeed, each soil has three microcosms: unpolluted microcosm, polluted microcosm, and polluted microcosm with nutrient (Nitrogen and Phosphor). Some factors were assayed in each microcosm during 120 day of experiment. These factors include total heterotrophic bacteria, total kerosene degrading bacteria, dehydrogenase enzyme, and kerosene biodegradation. The results of this study show that the highest quantity of heterotrophic bacteria is related to forest soil (6×10^9). The quantities of kerosene degrading bacteria significantly were lower than heterotrophic bacteria in all soil microcosms. The quantity of kerosene degrading bacteria have decrement pattern until 60^{th} day of experiment, but, after this day, these bacteria have increment pattern. The best dehydrogenase activity between different microcosms is related to polluted microcosm with kerosene except for farmland soil. The highest biodegradation of kerosene in all studied soil belongs to industrial microcosm (95%). Statistical analysis of the results shows that there is a significant correlation between MPN quantity of heterotrophic bacteria and other assayed factrs. Also, forest soil has significant difference with other soils. It may be possible to propose appropriate strategies for bioremediation of different studied soil types using the results obtained in this research.

Keywords: biodegradation, Kerosene, microcosm, pollution, soil.

INTRODUCTION

Petroleum hydrocarbons are widespread industrial pollutants that are released into the environment through crude oil transporting, storing, accidental leaking, petroleum refining, and wastewater irrigation. Ecological impacts of petroleum contamination on soil function are established. These pollutants alert composition and diversity of microbial community and also influence on the activity of microorganism and soil

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enzymes (Zanaroli *et al*., 2010; Nisha *et al*., 2013). On the other hand, soil microbial activities and diversities are sensitive biological and biochemical indicators for the assessment of soil perturbation (Bayat *et al*., 2016).

The behavior of pollutants in the environment is influenced primarily by the nature and amount of the contaminants present and the interaction between chemical, geochemical, and biological factors (Hong *et al*., 2005). Among biological factors, the diversity of microbial species and their metabolic capabilities constitute an important source of bio catalysis (Emtiazi *et al*., 2009). The structure and dynamics of the indigenous microbial communities are major characteristics influencing biodegradation (Hong *et al*., 2005). The degradation of complex pollutant mixtures such as petroleum requires a combination of different bacterial taxa that can degrade a broader spectrum of hydrocarbons than any single bacterial species alone (Hassanshahian *et al*., 2010). Several studies of contaminated sites have shown that the impact of contamination on bacterial communities is dependent on the previous pollution history (Hess *et al*., 1997). Few investigations have been undertaken to assess the impact of petroleum contamination on pristine microbial ecosystems (Mukherji and Vijay, 2002). Consequently, the impacts of petroleum contamination on pristine natural habitats are still poorly understood.

Kerosene, also known as lamp oil, is a combustible hydrocarbon liquid widely used as a fuel in industry and households. Kerosene is a thin, clear liquid formed from hydrocarbons obtained from the fractional distillation of petroleum between 150°C and 275°C, resulting in a mixture with a density of 0.78–0.81 g/cm^3 composed of carbon chains that typically contain between 6 and 16 carbon atoms per molecule (Hassanshahian *et al*., 2012a). It is miscible in petroleum solvents but immiscible in water. Regardless of crude oil source or processing history, kerosene's major components are branched and straight chain alkanes and naphthenes (cycloalkanes), which normally account for at least 70% by volume. Aromatic hydrocarbons in this boiling range, such as alkylbenzenes (single ring) and alkylnaphthalenes (double ring), do not normally exceed 25% by volume of kerosene streams. Olefins are usually not present at more than 5% by volume.

The aim of this research is to study the response of soil microbial community to kerosene contamination. In this study, two types of soil were selected: forest and industrial soil. The response of each soil to kerosene contamination was measured separately. To compare the effect of kerosene contamination with these soils, some microbial and biochemical factors were assayed.

METHODOLOGY

Sampling

Soil samples were collected from two different ecosystems: forest and industrial areas. Forest soil was collected from the Ghaem forest in Kerman province, Iran and industrial soil was collected from Gol Gohar mine in Sirjan, Kerman province, Iran. Sampling was done under sterile conditions. First, 10 cm of soil was removed and about 3 kg of soil was poured into sterile containers. Soil samples were transported to the laboratory on ice and kept at 4˚C until further study (Alef and Nanniper, 1995; Hassanshahian *et al*., 2012b).

Set-Up of the Microcosm Systems and Experimental Planning

Microcosm is small environmental laboratory that is designed for test conditions. Four different microcosms were performed in glass tanks (50 cm long, 10 cm deep, and 25 cm wide). Soils were sieved (<2mm) to remove large particles of shells and then 500 g of sieved soil were used for

each microcosms. Three microcosms' simulated natural and artificially polluted conditions were carried out for each type of soil. In particular, the first microcosm without kerosene contamination was used as control and is indicated as not polluted (FN, IN), the second microcosm was artificially contaminated with kerosene (10 g kg^{-1}) and is indicated as polluted (FP, IP), and in the third microcosm inorganic nutrient (nitrogen and phosphorus 5 g kg^{-1}) were added to soil and also contaminated with kerosene and indicated as polluted soil with nutrient (FNP, INP).

Microcosms were incubated in the dark at 25°C for 120 days. The water content of the microcosms was adjusted and maintained at 60% of its water holding capacity (WHC) during the whole incubation period. Aerobic condition was maintained by mixing of the microcosms' content every day. The soil samples were taken from each microcosm at five times, including Zero time, 30 days, 60 days, 90 days, and 120 days (Ives *et al*., 1996).

Enumeration of Heterotrophic and Kerosene Degrading Bacteria in the Soil Microcosm by Serial Dilution Method

Measurements of bacterial abundance within each type of soil in designed microcosm were performed by serial dilution procedure (Cappello *et al*., 2006). Heterotrophic bacteria in soils were estimated by spreading 100µL of 10-fold diluents on plates of Nutrient Agar medium (NA) and incubating at 30°C for 3 days. Also, kerosene degrading bacteria in soils were estimated by spreading 100µL of 10-fold diluents on plates of Bushnell Hass Agar medium (BHA) with kerosene and incubating at 30°C for 7 days. The results were expressed as $CFU \cdot g^{-1}$ (Robertson *et al*., 2011).

MPN of Heterotrophic and Kerosene Degrading Bacteria

Total heterotrophic and kerosene degrading bacteria in each microcosm were enumerated by a miniaturized Most Probable Number (MPN) method according to Brown and Braddock (1990). Nutrient Broth (NB) and Bushnell-Hass (BH) media were used for enumeration of total heterotrophic bacteria and kerosene degrading bacteria, respectively. Sterile kerosene (1%) was used as a selective growth substrate for the enumeration of kerosene degrading bacteria. Soil samples were diluted in a saline buffer solution that contained 0.1 % sodium pyrophosphate (pH 7.5). Tenfold serial dilution was performed in microplates that were inoculated by adding 20 µL of each dilution to 1 of the 12 row wells. Kerosene (1%) was applied to the samples as described above. The first row of each plate was served as sterile control. Microplates were incubated at 20±1°C for 15 days. MPN was carried out as triplicate. MPN counts were performed with the computer program MPN calculator (Wrenn and Venosa, 1996).

Measurement of Residual Kerosene in the Microcosm Soils

The kerosene removal assay in the soil of microcosms was carried out by dissolving the residual kerosene in the soils in dichloromethane (DCM) and reading the optical density of the oil extract against blank (distil water) at 420 nm (Rahman *et al*., 2004).

Dehydrogenase Activity in the Microcosm Soils

Dehydrogenase activity was determined by a colorimetric method using 2, 3, 5-triphenyltetrazolium chloride (TTC, Sigma 298-96-4) as substrate. Soil samples were incubated at 37°C for 24 hours and the reaction product the 1, 3, 5-triphenylformazan (TPF) was extracted by methanol and was quantified by spectrophotometer at 488 nm (Hassanshahian *et al*., 2014).

Data Analysis Methods

The results of all factors examined in each

microcosm during different times of incubation with three replications were entered into SPSS software and the statistical analysis of the relationship between different factors was analyzed. Duncan test was used to test the significant level of 0.05%.

RESULTS

The Quantity of Heterotrophic Bacteria in Soil Microcosms

The quantities of heterotrophic bacteria in two studied microcosms in different conditions were measured by serial dilution method. The results are presented in Figure 1. As shown in this figure, the quantity of heterotrophic bacteria have different patterns in these two soil types as the increase in the quantity of these bacteria in industrial soil takes place on the 60th day of incubation whereas this increment is observed on the 90th day for forest soil microcosms. Although, the quantity of heterotrophic bacteria in forest soil (2×10^{9}) was higher than industrial soil (5×10^{8}).

The results of most probable number (MPN) of heterotrophic bacteria in the study's microcosms are shown in Figure 1. It can be concluded from this figure that a reduction pattern on heterotrophic bacteria had been observed in industrial soil microcosms till 30th day, but after that, the quantity of heterotrophic bacteria has been increasing. Although, in forest soil microcosms this reduction takes place until the 60th day. The maximum MPN value of heterotrophic bacteria in forest and industrial soil was 1×10^{12} and 8×10^{11} (cell/g), respectively.

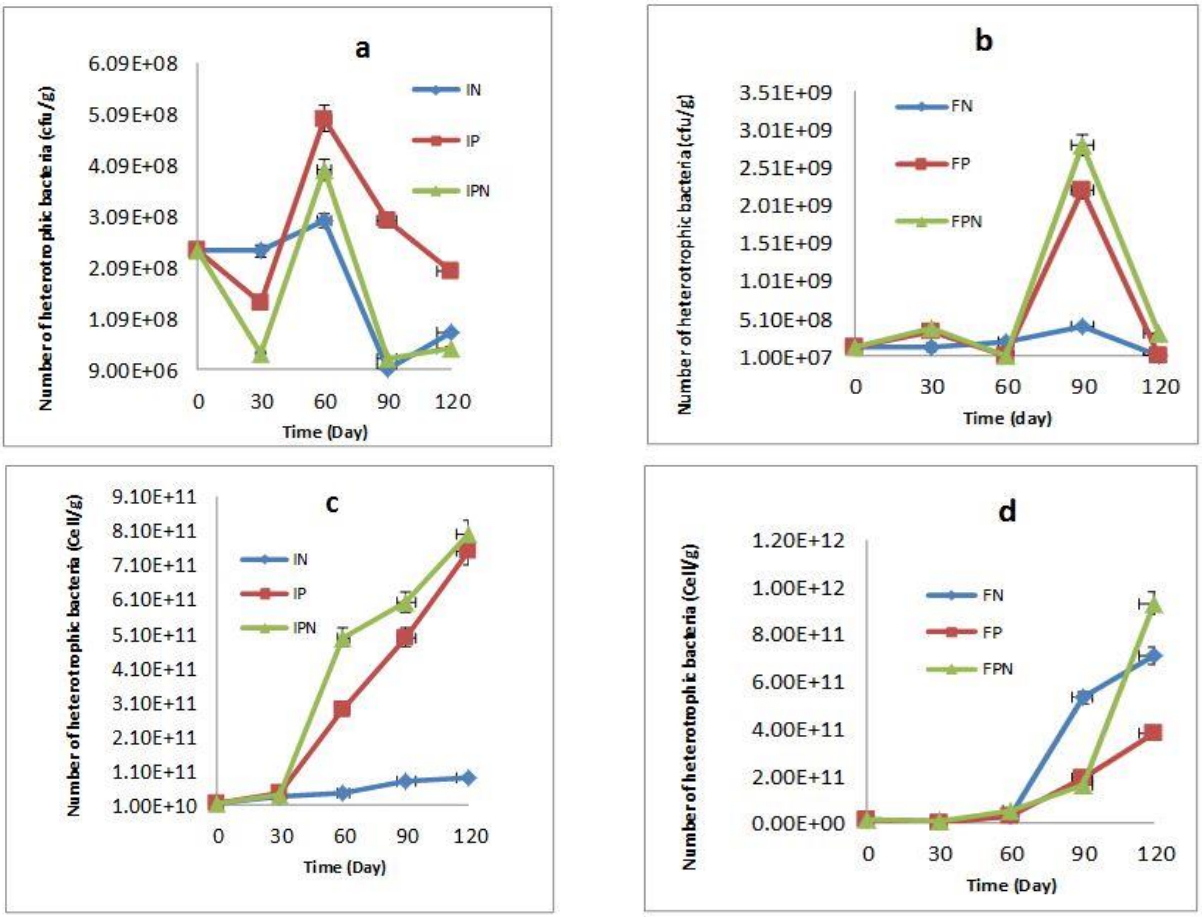

Fig. 1. Quantification of heterotrophic bacteria in two soil types by CFU and MPN methods; a) CFU of heterotrophic bacteria in industrial soil, b) CFU of heterotrophic bacteria in forest soil, c) MPN of heterotrophic bacteria in industrial soil, d) MPN of heterotrophic bacteria in forest soil

The Quantity of Kerosene Degrading Bacteria in Soil Microcosms

Kerosene degrading bacteria were counted in Bushnell Hass medium with kerosene as the only carbon and energy source. The results of this enumeration are illustrated in Figure 2. According to this figure, the number of kerosene degrading bacteria in soil microcosms had reduced until the 60th day of treatment, but after that, the number of kerosene degrading bacteria has been increasing in all microcosms except on uncontaminated microcosm (FN and IN). Generally, the number of kerosene degrading bacteria in all soils is dramatically less than the number of heterotrophic bacteria in soils. Of course, this result is also expectable because kerosene degrading bacteria only use kerosene but heterotrophic bacteria can use other carbonic resources as well. The highest quantity of kerosene degrading bacteria in forest and industrial soil was 1×10^5 and 9×10^4 (CFU/g) respectively.

Kerosene degrading bacteria were quantified in MPN method using kerosene as the only carbon source and turbidity as positive MPN indicator. The results are shown in Figure 2. As shown in this figure, the quantity of kerosene degrading bacteria is slowly increasing. This pattern can be attributed to the toxic effect of kerosene and bacteria adapted after passing the time. The other noticeable point which can be understood from this figure is that the number of kerosene degrading bacteria in contaminated microcosm with nutrient (FNP and INP) was higher than other microcosms (FN, FP and IN, IP). The highest MPN values of kerosene degrading bacteria in forest and industrial soil were 1×10^5 and 8×10^5 (cell/g) respectively.

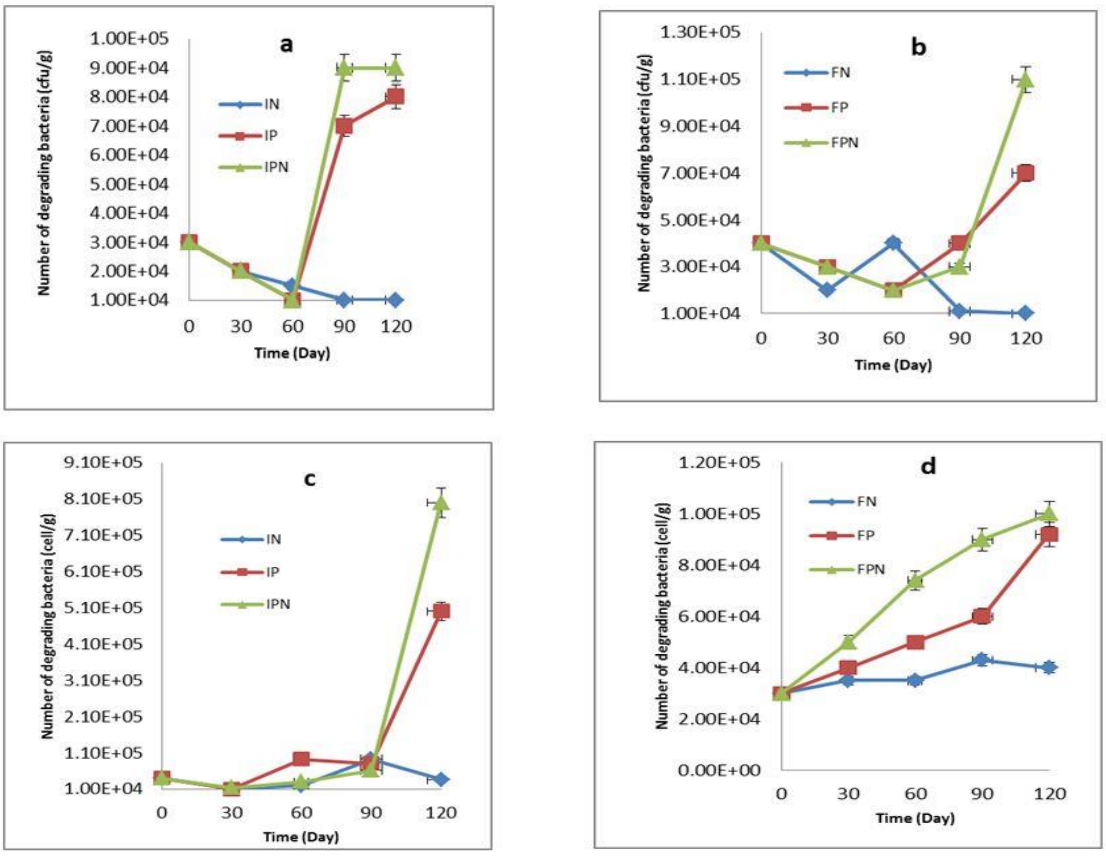

Fig. 2. Quantification of degrading bacteria in two types of soil by CFU and MPN methods, a) CFU of degrading bacteria in industrial soil, b) CFU of degrading bacteria in forest soil, c) MPN of degrading bacteria in industrial soil, d) MPN of degrading bacteria in forest soil

Dehydrogenase Activity in the Study's Microcosms

Evaluating the activity of some enzymes in microcosms can provide suitable information about bacteria and microbial community. In this study, the activity of dehydrogenase enzyme was investigated. This enzyme is a suitable indicator for the overall intensity of microbial metabolism. As it can be seen in Figure 3, in industrial soil microcosms, this enzyme has increased its activity until 30th day of experiment and after this time the activity of enzyme has dramatically decreased. However, in the forest soil microcosms, the reduction in enzyme activity is seen on the 90th day of incubation. Among three different kinds of microcosm, polluted microcosm to oil and with addition of nutrient (FPN and IPN) has the highest dehydrogenase enzyme activity.

Kerosene Degradation in the Study's Microcosms

One of the important indicators in studying soil's microcosms is the rate of eliminating kerosene during the time of incubation. The percentage of kerosene degradation was calculated for each soil microcosm separately. The results are shown in Figure 4. This figure confirms that by passing the incubation time, the rate of kerosene degradation in all microcosms has increasing pattern. Thus, the lowest percentage of degradation was on the first day of experiment (T_0) and the highest kerosene degradation took place on the last day of experiment (120th day). But this pattern and the rate of degrading are not equal in all microcosms. The highest percentage of kerosene degradation is related to polluted microcosms with nutrition (FNP, INP).

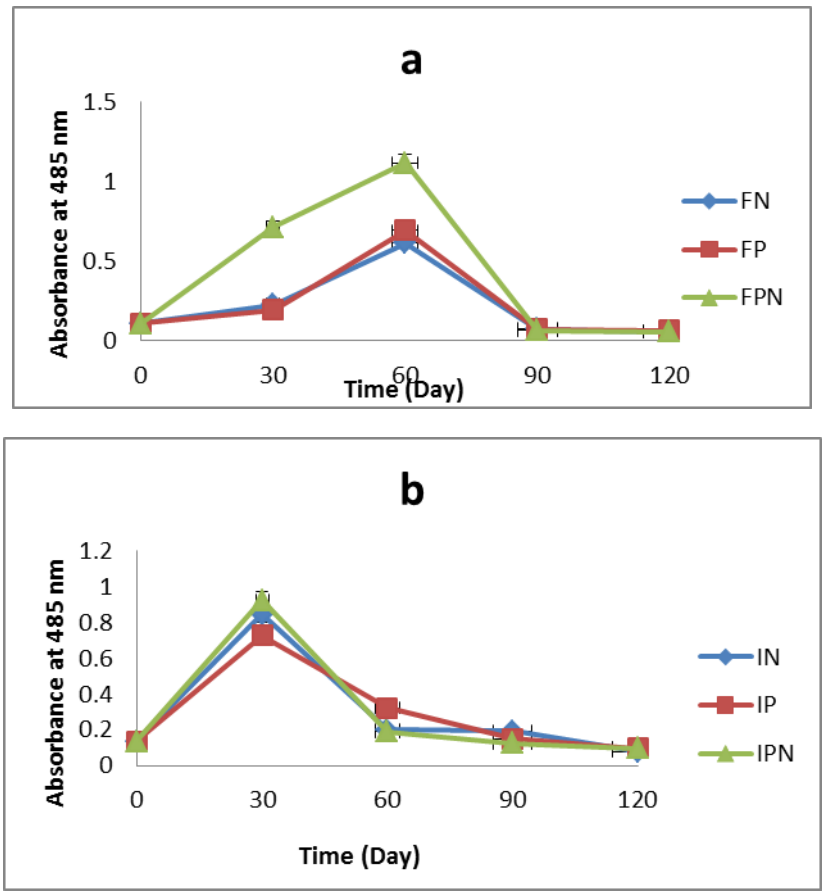

Fig. 3. The activity of dehydrogenase enzyme in designed soil microcosms a) Forest soil, b) Industrial soil

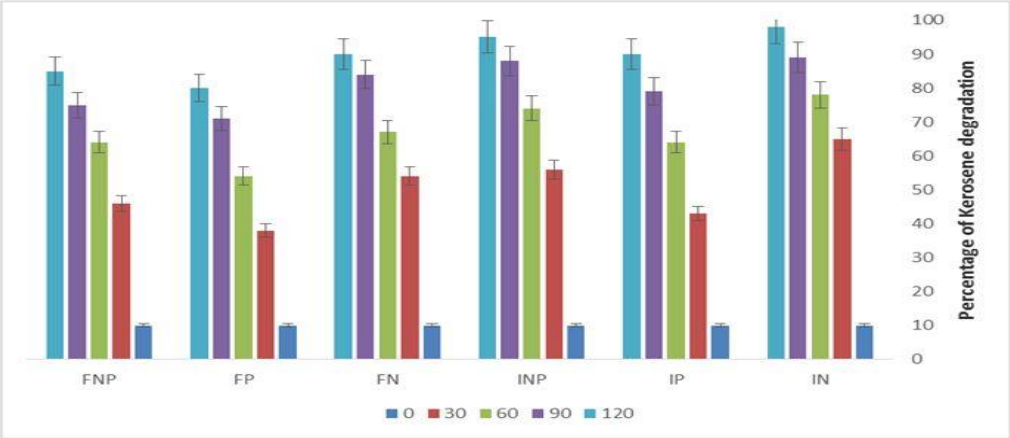

Fig. 4. The Percentage of kerosene degradation in soil microcosm's during 120 days of incubation

Statistical Analysis of Data

Each studied factor in all soil microcosms such as total numbers of heterotrophic bacteria, total number of degrading bacteria, enzyme activity, and percentage of kerosene degradation was statistically analyzed in SPSS software with Duncan test in confidence level of 0.05 percent. The results of this analysis are shown in Table 1. As shown in this table, there is a significant relationship between total numbers of heterotrophic bacteria (MPN) with other investigated factors. The other study's factors are not significantly different.

The effect of incubation times on evaluated factors and especially the percentage of kerosene degradation have been shown in Table 2. This table shows that times of 90 and 120 days of experiment are significantly different with other times (0, 30, and 60). This means that the evaluated factors in these two times are significantly different from other sampling times. This result confirmed that the main changes in microbial community take place after 60 days of oil pollution.

Table 1. Statistical analysis of all assayed factors in the mesocosms and relationship between these factors

Factor Assay Duncan$_{a,b}$	N	Subset	
		1	2
Enzyme	2.89E+10	2.12E-01	
Degradation	2.89E+10	4.67E+01	
CFU Degrader	2.89E+10	3.50E+05	
MPN Degrader	2.89E+10	6.41E+04	
CFU Heterotroph	2.89E+10	6.61E+08	
MPN Heterotroph	2.89E+10		1.73E+11
Sig.	P>0.05		

Table 2. The effect of incubation time on measured factors in soil microcosms

Duncan $_{a,b}$	Average of samples	Subset		
Day	N	Level		
		1	2	3
0 Day	2.04E+10	2.50E+09		
30 Day	2.04E+10	3.96E+09		
60 Day	2.04E+10	4.09E+09		
90 Day	2.04E+10		3.45E+10	
120 Day	2.0E+10			5.71E+10
Sig.	P>0.05			

DISCUSSION

The results of enumeration of heterotrophic and degrading bacteria in industrial soil microcosm show that the quantity of heterotrophic bacteria in this soil is lower than forest soil microcosms but kerosene degrading bacteria have highest quantity in the industrial soil microcosms. These results confirm that, whereas the organic carbon in the industrial soil is high, the quantity of heterotrophic bacteria is low and it means that petroleum products cause the disappearance of sensitive bacteria and only degrading bacteria are selected and prevalent to total microbial community (Del Arco *et al*., 2001; Barathi and Vasudevan, 2001; Shukor *et al*., 2013).

The response of industrial soil microcosms to kerosene contamination was as follows: kerosene contamination has the low effect in this microcosm compared to forest microcosm. Two results confirm this interpretation; (a) the quantity of heterotrophic bacteria increase after the 60^{th} day of experiment, whereas this increase in other microcosms takes place on the 90^{th} day of incubation and (b) the number of kerosene degrading bacteria in the industrial microcosm was higher than forest microcosm. On the other hand, chronic pollution that is present in industrial soil cause enrichment and selection of degrading bacteria and for this reason the kerosene contamination does not have sever effect on this soil type (Delille and Coulon, 2008; Hassanshahian, 2014).

Some researchers also studied the effect of kerosene contamination on soil. For example, Li et al (2007) designed 110 days experiment to understand the effect of different concentrations of kerosene on industrial soil microcosms. Their results show that when 1000 mg/kg of kerosene enter the soil the growth of aerobic bacteria is stimulated and the activity of some enzymes such as dehydrogenase, orease, and poly phenol oxidase is increased.

Delille and Coulon (2008), by simulation of kerosene contamination on industrial soil in Belgium, show that kerosene degrading bacteria dramatically increase after kerosene contamination. Also, they conclude that after 90 days of contamination some biological and chemical properties of soil have changed.

Forest ecosystems are very rich in organic carbon. Forest soil humus is high because the plant material and the activity of rhizosphere microbes enhance the humus of this soil. In this research, the highest quantity of heterotrophic bacteria was reported in these soil microcosms that confirmed the abundance of organic carbon in this soil and it is used by all soil microbial community (Tebyanian *et al*, 2013).

The response of forest soil microcosms to kerosene contamination was interesting. The quantity of heterotrophic bacteria dramatically decreased after kerosene pollution. Kerosene degrading bacteria have lower quantity compared to industrial soil microcosms. These results establish that the adaptation of forest soil microbial community takes place more slowly compared to industrial soil. On the other hand, microbial community of forest soil was more sensitive than to kerosene contamination industrial soil (Radwan *et al*., 2005; Hassanshahian *et al*., 2013).

Some researchers have reported the effect of hydrocarbons pollution on forest soil that their results are in agreement with our results in this research. For example, Amadi *et al*. (1996) studied some properties of forest soil and microbial factors in Nigeria rainy forests after 17 years of crude oil contamination. Their results show that organic carbon decrease after oil contamination and the concentration of heavy metals was increased. Their results confirmed that crude oil contamination cause dramatic reduction in microbial diversity of forest soil. In another study performed by Riffaldi *et al*. (2006), a positive relationship

between the remaining of kerosene concentration and quantity of organic carbon in forest soil with activity of deydrogenase and lipase enzyme was reported.

CONCLUSION

Soil pollution by kerosene is an unavoidable case on the earth but the important issue is the accurate recognition of these effects and attempt to minimize them. In total, the results of this research showed that forest soil is a more sensitive soil to kerosene pollution compared to industrial soil. Statistical analysis of these results showed that appropriate time for soil return to normal condition is 90 days which is significantly different from other times. Using obtained results of this research, based on the type of soil, suitable strategies can be recommended for their survival. The novelty of this work is that until now there is not a comprehensive research on the effect of an oil product (such as kerosene) on soil. There are some studies in this field, but they use crude oil as source of pollutant and do not use crude oil products.

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