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Effect of Salinity on Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) of Heavy Crude Oil in Soil

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Abstract The spillage of crude oil in the soil damages the environment. Polycyclic aromatic hydrocarbons (PAHs) are one of the crude oil components that may be harmful for living organisms. PAHs can disappear from the environment by volatilization and biodegradation. The effect of different NaCl concentrations (0%–5%) on PAHs reduction from the heavy crude oil-contaminated soil was studied. Our results showed that increasing NaCl concentration in soil had decreasing effect on total crude oil and PAHs reduction. The biodegradation of total crude oil was higher in 0% NaCl (41%) while higher total PAHs reduction was observed in 1% NaCl (35%). The lower total crude oil and PAHs reduction were observed in 5% NaCl (12% and 8% respectively). Phenanthrene, anthracene and pyrene reduction were higher in 1% NaCl, while fluoranthene and chrysene reduction were higher in 0% NaCl.

Keywords Biodegradation · Crude oil · NaCl · PAHs · Soil

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic compounds which are slightly soluble in water. They are one of the crude oil components that are also formed by incomplete combustion of fossil fuels (Wang et al. 1999). Crude oil contains different kinds of aromatic hydrocarbons from two rings to more complex four and five-ring molecules. The spillage of crude oil in soil and water can damage the environment and the ecosystem. Some PAHs are toxic and may be mutagenic and carcinogenic for living organisms (Armstrong et al. 2004; Gibbs 1997; Hammond et al. 1976). The environmental fate of PAHs mainly depends on the number of rings in the molecule and environmental factors such as pH, temperature and salinity (Kanaly and Harayama 2000). PAHs are biodegraded by microorganisms present in soil, sewage and water.

The biodegradation of PAHs by microorganisms has been studied (Johnsen et al. 2005; Shuttleworth and Cerniglia 1995; Cerniglia 1992; Cerniglia 1984) and their biochemical pathway of biodegradation has been described. Some experiments have explained the mechanism of ring oxidation and also co-metabolism of these materials (Juhasz et al. 1997; Gibson et al. 1975) and other reports have shown that isolated bacteria from contaminated soil could use some PAHs as the sole carbon source (Kastner et al. 1994; Walter et al. 1991; Mueller et al. 1989). Some kinds of bacteria that use PAHs as their carbon source have been isolated from contaminated soil (Ahn et al. 1999; Trzesicka-Mlynarz and Ward 1995).

In this study the effect of different concentration of NaCl in biodegradation of PAHs was studied. Whilst there are some reports on biodegradation of PAH in soil, no reports have discussed the effect of salinity in PAHs biodegradation in soil.

Materials and Methods

Non-contaminated soil was obtained from a cultivation area near the city refinery. The cultivation area near the

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refinery may encounter risk of contamination by the products of refinery during oil transportation or pipe line leakage.

In the south zone of this area, there is a wide salty desert and a big salt lake. On windy days, the salty soil from the desert moves to the cultivation area by wind. As a result the salty soil covers the surface of the cultivation area and mixes with the cultivation soil. The salty area may be widening in the next decades.

The soil was air dried for a week and then sieved through 4 mm mesh. The soil texture was determined by hydrometer method (Table 1) and the organic matter was determined by Walkey and Black method (Robertson et al. 1999). Heavy crude oil (API = 20) (American Petroleum Institute Gravity) was obtained from Soroush oil field in the north of Persian Gulf. The oil was added to the soil in the final concentration of 2% (w/w) and mixed to make uniform contaminated soil. The soil was divided to equal parts; each part contained 1 kg of soil and was transferred to a 5-liter pail and remained intact for three weeks for aging.

Pail 1 was designated as control with no moisture, aeration and salt. Pail 2 was considered as non-aerated, with moisture, but no salt. Moisture, aeration and different NaCl (Merck) concentrations (0% to 5%) were added to pails 3 to 6 during the experiment. Each sample was prepared as three replicates.

For further study, 50 g of contaminated soil was kept at −20°C at time zero.

For each 1000 mg of crude oil about 150 mg of nitrate (NH_4NO_3) and 30 mg of phosphate (KH_2PO_4) were added to all pails (Rosenberg and Ron 1996). Determination of soil pH (pH = 7.4) was performed by dissolving 1 g of soil in 5 mL of distilled water. The mixture was stirred well and allowed to stand for 30 minutes. The slurry was used for pH determination (Robertson et al. 1999).

After determination of the water holding capacity (field capacity) of the soil, the moisture of the soil was adjusted at 30% by adding distilled water to all samples (except pail 1).

Table 1 The soil characteristics used in this experiment

Soil parameters	
Clay	33%
Silt	55%
Sand	12%
Organic matter	3.1%
Calcium carbonate	28%
pH	7.3
Total N	0.02%
Total P	5 ppm

The soil water content was measured using the gravimetric method during the experiment (Robertson et al. 1999).

Induced aeration was done by mixing the wet soil every other day in all pails except pails number 1 and 2. All the pails were incubated at room temperature (25–28°C) during the experiment.

Extraction of crude oil from soil was conducted according to the method used by Minai-Tehrani and Herfatmanesh (2007). Two grams of soil was mixed with 10 mL of CH_2Cl_2 (Aldrich) and shaken firmly. The sample was centrifuged (3000*g* for 10 min) to precipitate the soil, and the solvent phase was removed. This solvent extraction was repeated twice. The solvent was vaporized during 24 h and the amount of oil was measured using the gravimetric method and its reduction was compared with the time zero sample. Two samples from each replicate were taken for crude oil extraction and further preparations.

To ensure that the soil used for experiments was not oil-contaminated at the starting time, the above mentioned method was used for detection of any oil contamination. No contamination was observed in the soil at the starting time.

After the oil was extracted using the mentioned method, the extract residue was dissolved in 5 mL n-hexane (Merck) and filtered. The sample was loaded to a 1 × 25 cm column filled with 20 cm Silica Gel and 5 cm Na_2SO_4 (Merck). The column was pre-washed with n-hexane. 30 mL of n-hexane was used as mobile phase to release aliphatic fractions and then 30 mL of n-hexane/dichlromethane (1:1, v/v) was used to release aromatic fractions. The aromatic fractions were collected and the solvent was evaporated. The residue was weighed to determine the amount of total aromatic fractions in each sample. The residue was dissolved in 5 mL acetonitrile (Fluka HPLC grade) and 20 μL of the solution was injected in the HPLC column (Shimadzu HPLC system equipped with C18 column), with water/acetonitrile (1:2, v/v) as mobile phase and a flow rate of 1 mL/min (the column was equipped by UV detector at 254 nm). Some PAHs such as phenanthrene, anthracene, fluoranthene, pyrene and chrysene were prepared as standard (Supelco mix PAH standards) and injected in the HPLC column. The region of their exit from the column was used to localize them in the main graphs.

The total peak area of each compound in the graphs was used to determine the reduction of PAHs and was compared with time zero.

Determination of the total colony number in soil was done using the *pure-plate* method (Cappuccino and Sherman 1996) every two months and compared with time zero. From each sample, 1 g of soil was dissolved in 9 mL of sterilized NaCl solution (9 g/L) and serial dilutions were prepared for each sample. Diluted samples were transferred

to nutrient agar (Merck) plates. The plates were incubated at 30°C for 48 hours and counted for number of colonies.

Results were expressed as mean ± standard deviation (±SD) and the analysis of variances and statistically significant difference ($p < 0.05$) was performed by one-way Anova Test. The comparison of the means to obtain the significant difference was done using the Tukey test.

Results and Discussion

This study has attempted to investigate the effect of salinity on reducing the PAHs level by biodegradation. The total colony count showed that in the presence of 5% NaCl the bacterial population was reduced to its minimum value in comparison with other samples (Fig. 1). There was significant difference between 5% and other contaminated soil for microbial population. The difference was not significant between the 0% and 1% samples. The colony reduction was also observed in the control sample that had a high total colony at time zero because of the cultivation content of the soil.

The population of microorganisms in 3% NaCl and the non-aerated sample was nearly the same after four months and the difference was not significant between these two samples. The non-aerated sample was used to compare the effect of moisture on oil reduction with the control that had no moisture during experiment. The reduction of microbial population in all samples, suggests that the presence of crude oil or NaCl in the soil is responsible for the reduction of the population of microorganisms. The higher reduction of microbial population in 5% NaCl in comparison with the 0% NaCl samples, suggests that the main factor that has decreased the number of colonies is the presence of NaCl. Some reports have indicated the increase of microbial population in the presence of oil contaminated soil (Minai-Tehrani et al. 2007). The significant reduction of colonies in the control sample suggests that moisture is also an important factor for the microorganisms' growth.

Figure 2 shows the reduction of the heavy crude oil and total PAHs in the presence of NaCl in the contaminated soil. The crude oil reduction was higher in 0% NaCl (40%), and it was lower in 5% NaCl (13%). Increasing NaCl concentrations decreased crude oil reduction. In the non-aerated sample the reduction of crude oil (15%) was close to 3% NaCl (16%). The rate of PAHs reduction was almost in accordance with crude oil reduction. The PAHs reduction was higher in 1% NaCl (35%) and it was lower in 5% NaCl (8%). In the non-aerated sample the reduction of PAHs (12%) was lower than 3% NaCl sample (17%). The significant difference was observed in the reduction of both crude oil and PAHs between 5% NaCl sample and other contaminated samples except the non-aerated sample. The reduction (crude oil and PAHs) was not significant between the 0% sample and 1% sample, while considerable reduction was observed between the 3% sample and the two other more dilute samples (0% and 1% samples). Our results show that increasing NaCl concentration decreases crude oil and PAHs biodegradation. The highest inhibition of biodegradation occurs in the 5% NaCl due to low population of microorganisms. The effect of high NaCl concentration on reducing biodegradation of crude oil and its components has been reported in liquid medium (Diaz et al. 2002; Mille et al. 1991; Ward and Brock 1978), which is in accordance with our results in the soil.

Weathering phenomena (such as volatilization and photooxidation) and biodegradation are the factors which can reduce the PAHs content of soil after crude oil

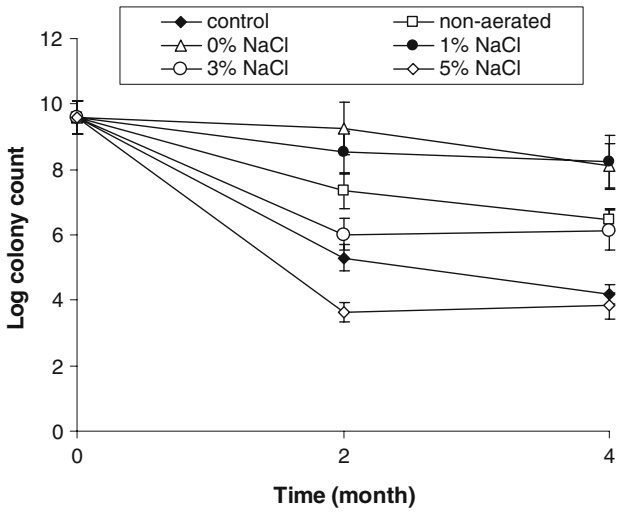


Fig. 1 Log of colony count (CFU/g soil) in soil during 4 months. Average values given ± Standard deviation (±SD)

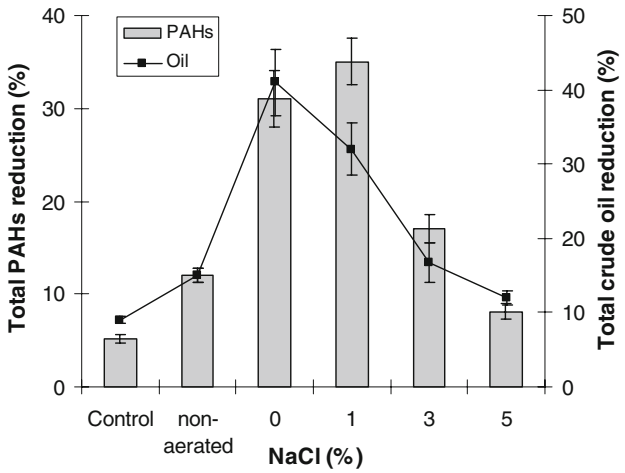
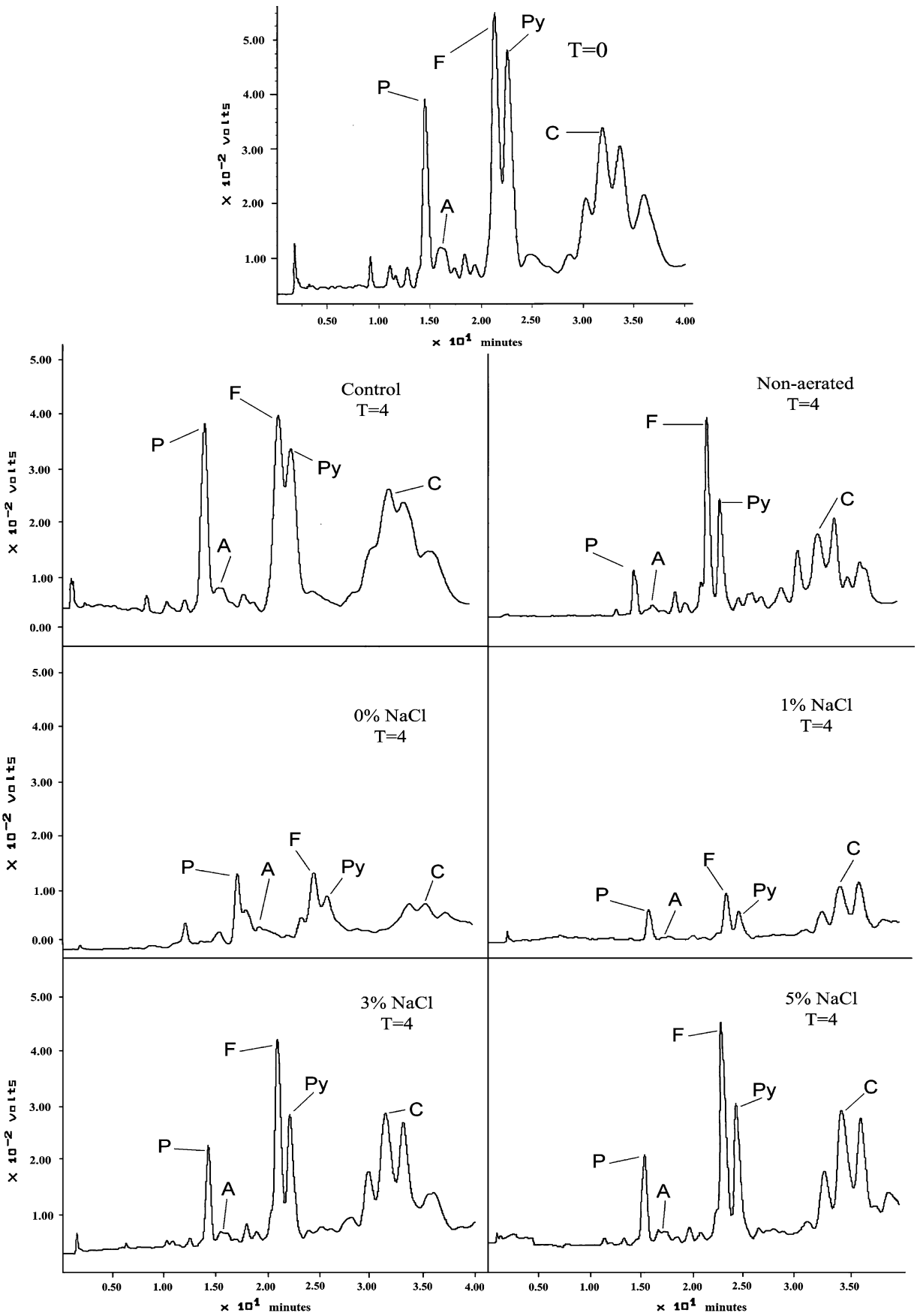


Fig. 2 The reduction of crude oil and total PAHs in soil, (±SD)


T=0
Control
T=4
Non-aerated
T=4
0% NaCl
T=4
1% NaCl
T=4
3% NaCl
T=4
5% NaCl
T=4
P
A
F
Py
C
× 10⁻² volts
× 10¹ minutes

◀ **Fig. 3** HPLC pattern of samples in start time (T = 0) and after 4 months (T = 4). (P = Phenanthrene, A = Anthracene, F = Fluoranthene, Py = Pyrene, C = Chrysene)

contamination (Moore and Ramamoorthy1984). In the non-aerated sample that received the moisture, the reduction of total crude oil and PAHs was higher than control, suggesting that the moisture plays an important role in biodegradation of contamination in soil.

Figure 3 presents HPLC patterns of different samples. In the non-aerated sample, the reduction of PAHs was higher than in the control. In samples with different concentration of NaCl (0% to 5%) the rate of reduction of PAHs was increased by decreasing the concentration of NaCl in the samples. In the 0% and 1% NaCl samples, the HPLC pattern showed clear reduction of PAHs in comparison to the time zero sample, while this pattern showed minor reduction of PAHs in the 5% NaCl sample.

Figure 4 shows the reduction of some PAHs from crude oil in soil. The higher reduction occurred in the 1% NaCl and 0% samples. The reduction of pyrene, phenanthrene and anthracene was higher in 1% NaCl while the reduction of fluoranthene and chrysene were higher in 0% NaCl. However the reduction of these PAHs was not significant between the 0% and 1% samples. In the non-aerated sample the reduction of phenanthrene (47%) was lower than in the 5% NaCl (48%) sample, while the reduction of anthracene, fluoranthene and chrysene in the non-aerated sample was higher than in the 5% NaCl sample. A significant difference was observed in the reduction of all these PAHs between the 1% NaCl and 3% and 5% NaCl samples, while there was no significant reduction between the 3% and 5% samples. The high reduction of phenathrene, anthracene, fluoranthene, pyrene and chrysene occurred in 0% and 1% NaCl concentrations, suggesting that the biodegradation of PAHs has not been decreased in the presence of 1% NaCl. The low

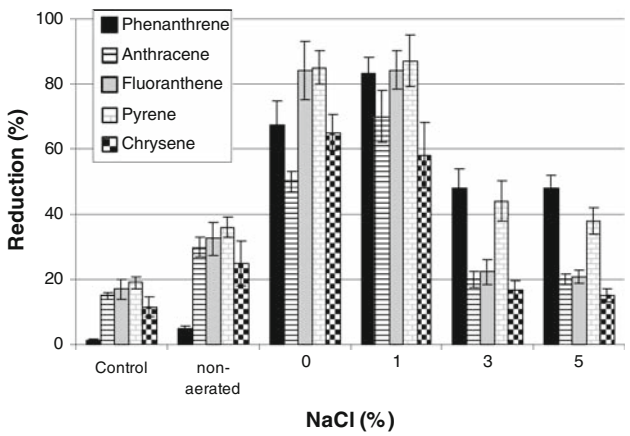


Fig. 4 The reduction of phenanthrene, anthracene, fluoranthene, pyrene, chrysene in soil. (±SD)

reduction of these PAHs in the soil with 3% and 5% NaCl suggests that soil with a salt content higher than 1% can decrease the biodegradation of PAHs. Shiaris (1989) has reported a positive correlation between salinity and the rate of mineralization of phenanthrene in estuarine sediments.

The higher reduction of phenanthrene in comparison to other PAHs in the 3% and 5% samples suggests that the presence of high NaCl concentration in the soil has a lower affect on reduction of phenanthrene. This suggests that volatilization might have an important role in phenanthrene reduction.

In conclusion, our results show that in the oil-contaminated soil which receive salts by the wind and which has the risk of oil contamination, the biodegradation of crude oil and PAHs does not change in the presence of 1% NaCl. However the presence of NaCl higher than 1% can decrease the oil and PAHs reduction significantly. The presence of less than 1% NaCl, does not seem to have substantial effect on biodegradation of oil and PAHs in the soil.

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