Study of growth and germination of Medicago sativa (Alfalfa) in light crude oil-contaminated soil

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Abstract: In oil producing countries, crude oil is one of the main organic pollutants of soil and water. Light crude oil has volatile components that can be toxic to living organisms of the soil. In this study the effect of different concentrations of light crude oil (0% to 10%) on the growth and germination of Medicago sativa (Alfalfa) was studied. Our results showed that the germination number and the number of leaves in plant decreased by increasing light crude oil concentration in the soil. Total dry biomass of plant was higher in control (0%) sample while it was lower in 10% sample. Total colony and oil-degrading colony counts in soil showed that in all vegetated samples, the microbial population was higher than non-vegetated samples. In vegetated samples, the total microbial population in 7% samples was higher than control and also higher than low concentrations of crude oil (1% and 3% samples). In all vegetated samples the reduction of crude oil was higher than non-vegetated samples. The higher reduction was occurred in 1% sample, while the lower reduction was observed in 10% sample.

Keywords: Crude oil, germination, phyto remediation, soil.

INTRODUCTION

In oil producing countries, crude oil is one of the most important contaminant of soil. Large amounts of soil have been contaminated with petroleum near the oil refineries and oil storage tanks. Contaminated soil can damage environment by affecting the plants and microorganisms of the soil. The effect of contaminant on microorganisms and plants depends on the concentration and the kind of contamination[1]. Heavy crude oil has higher resin and asphaltine than light crude oil, these compounds do not well biodegrade by microorganisms and plants and remain in soil for many years[2]. On the other hand some gaseous and volatile hydrocarbons are higher in light crude oil than heavy crude oil. These compounds are toxic for biological systems of soil[3]. The effect of crude oil and its components on germination and growth of some plants has been studied[4,5]. However some plants are able to remediate the organic pollutant from the soil. Phytoremediation is the use of plants and their associated microorganisms to remediate contaminated soil and water[6]. Among the plants, grasses and legumes have higher potential on removal of oil from contaminated soil[6,10]. Grass roots have maximum root surface area in the soil in comparison to other plants[11]. The plant roots stimulate the bacteria in rhizosphere area, which enhance the biodegradation of petroleum hydrocarbons[12]. Legumes are able to fix nitrogen and do not compete with microorganisms for limited supplies of available nitrogen at oil contaminated soils[13]. In this study the effect of different concentrations of light crude oil on growth and germination of a legume, Medicago sativa (Alfalfa), was studied and the reduction of light crude oil in the soil in the presence of plant was investigated.

MATERIALS AND METHODS

Soil analysis: The soil was obtained from Sarkan zoon, near the oil processing factory of Sarkan in the west of Iran. The soil was dried in room temperature and then was sieved through 2 mm mesh. The texture of soil was determined by hydrometer method[11] and it consisted of clay 54%, sand 16% and loam 30%. The organic matter was determined as 0.9 % by Walkley-Black method[11]. The pH of the soil was determined 7.4 for the soil-distilled water slurry (1:5, w/vol)

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Soil preparation: Light crude oil (API= 40) was also obtained from oil processing factory of Sarkan and added to the dry soil with concentrations of 0, 1, 3, 5, 7 and 10 % (w/w). The soil and oil was well mixed to make homogenized contaminated soil, and then transferred to the 1 liter pots. Each sample consisted of 800 g of dry soil.

Chemical fertilizers were added to the soil before seeding. 75 mg/kg nitrate (NH₄NO₃) and 30 mg/kg of phosphate (KH₂PO₄) were added to all samples[12].

20 seeds of Alfalfa were planted in each sample. All vegetated samples were prepared as three replicates. The control samples for each concentration were also prepared as three replicates which did not receive seeds (non-vegetated).

Germination and Biomass: The number of germinations was counted 30 days after planting and indicated as germination number. The number of leaves was counted 60 days after planting and also the number of green plant was counted at the end of experiment.

The plant biomass was collected at the end (120 days) of experiment and dried at room temperature and reported as total dry weight for roots and shoots.

Colony Count: The colony count was done for determination of total colonies and also for oil-degrading colonies of soil 60 days after planting. Determination of total colonies in soil was done by pure-plate method with nutrient agar as medium[13]. Determination of oil-degrading colonies was also done by the same method in agar-agar with 1% sterilized light crude oil as sole carbon source[14].

Crude oil extraction: Crude oil extraction was conducted according to the method used by Minai-Tehraniet al[15]. For 48 hours, 1 g of treated soil was dried in 50°C then crushed well to make a homogenous soil. 10 ml of CH₂Cl₂ (Aldrich) was added to soil and shaken firmly to separate oil from soil. The sample was centrifuged (3000 × g for 10 minutes) to precipitate the soil, and the solvent phase was removed. The solvent extraction was repeated twice. The solvent vaporized during 24 hours and the amount of oil was measured by gravimetric method and its reduction compared with time zero. Two samples from each replicate were taken for crude oil extraction.

Statistical analysis: Results were expressed as the mean ± Standard deviation (±SD) and statistically significant difference (p<0.05) was performed by Student t-test.

RESULTS AND DISCUSSIONS

Germination, Growth and Biomass: The number of germinations in the vegetated samples was higher in 0% sample and it was lower in 10% sample (Fig 1). There was a sudden reduction in number of germinations above 5% concentration of crude oil.

Figure 1 also shows the dry biomass of roots and shoots 120 days after seeding. The separation of roots from the soil showed that the distribution of roots in the soil decreased by increasing the crude oil concentration. The higher roots biomass was observed in 0% sample, in which the roots were well distributed in the soil. The roots and shoots in 7% sample were very low and there was no root in 10% germinated sample.

![Fig. 1](image.png)

Fig. 1: The number of germination in different concentrations of crude oil after 30 days of seeding and total dry biomass (shoots + roots) and dry biomass of roots after 120 days of planting. Average values given ± Standard deviation (±SD), p<0.05.
Fig. 2: The number of leaves in vegetated samples after 30 days of seeding, and number of green plants after 120 days (end of experiment) of planting.

The total dry biomass (roots + shoots) was also high in 0% while it was low in 7% and 10% samples. A sudden decrease in total dry biomass was observed in 1% sample in comparison to 0% sample. Figure 2 shows the relationship between the concentration of crude oil in soil and the number leaves. The presence of high concentrations of crude oil in soil reduces the number of leaves, the lower numbers were observed in 10% followed by 7% sample. The number of green plants was also shown in figure 2. There was no green plant in 10% at end of experiment. The number of green plants was also reduce in 7% sample.

**Colony count:** Total colony count was determined in vegetated and non-vegetated soil (Fig 2A). In vegetated samples, the higher microbial population was observed in 5% and 7% samples and the lower was observed in control (0%). Increasing crude oil concentration increased the total microbial population in vegetated samples. In non-vegetated samples, the higher microbial population was also observed in 7% sample, while the lower was seen at 3% sample. In all vegetated samples the total colonies were higher than their equal concentrations of crude oil in non-vegetated samples. Counting for oil-degrading colonies in vegetated samples showed that the higher microbial population was also observed in 7% and 5% samples and the lower was seen in 0% sample (Fig 2). In non-vegetated samples the higher count for oil-degrading colonies was observed in 7% while it was lower in 0% sample. In all vegetated samples the colonies were also higher than their equal concentrations of crude oil in non-vegetated samples.

**Crude oil Reduction:** Figure 3 shows the reduction of crude oil in vegetated and non-vegetated contaminated soil during 120 days. In first 30 days the rate of reduction was higher than 60 and 120 days in both vegetated and non-vegetated soil. At the end of experiment (120 days) the higher reduction was observed in 1% vegetated sample and the lower was seen in 10%. No significant difference was observed in vegetated and non-vegetated samples in 10% sample. Increasing crude oil concentration decreased the reduction of crude oil in both vegetated and non-vegetated samples. In all contaminated vegetated soils, the reduction of crude oil was higher than non-vegetated soils, except 10% sample. Significant difference in reduction between vegetated and non-vegetated samples was observed in concentrations up to 5%.

This study assesses mainly the effect of light crude oil-contaminated soil on growth and germination of Medicago sativa (Alfalfa). There are few reports about
the effect of crude oil and its products on germination and growth of Alfalfa. The effects of fertilizers in germination of alfalfa in the presence of crude oil have been reported previously\cite{16}.

Low germination of Alfalfa in 10% light crude oil-contaminated soil showed that the toxicity of light crude oil has decreased the number of germinations in 10% sample distinctly in comparison to the control, suggesting that the toxic materials of crude oil could inhibit the germination of plant partially. No germination has been reported in maize in 10.6% crude oil\cite{17}. The number of leaves in plant was also decreased in high concentrations of crude oil, suggesting that the plant has not grown well in 10% and 7% samples in comparison with control. The sudden reduction of biomass in 1% and other contaminated samples in comparison with control suggests that despite good germinations of the plant in contaminated samples up to 5%, the toxicity of crude oil has prevented the roots and the shoots of vegetated samples to grow well in comparison with control (0% sample). This phenomenon was significant in 7% and 10% sample. The distribution of roots in contaminated soil mainly decreased in comparison with control. No green plant was observed in 10% sample at the end of experiment, and there was significant reduction of green plant in 7% sample, suggesting that the toxic effect of crude oil has been caused early chlorosis in plants. Exposure of plants to tolerable concentrations of petroleum can cause chlorosis of leaves, plant dehyrdation, stunted growth and death\cite{17}. Total colony count and hydrocarbon degrading colony count showed that despite the high concentrations of crude oil in 5%, 7% and 10% samples the population of microorganisms was higher than the control and also higher than low concentrations of crude oil (1% and 3% samples). This showed that the microbial population has been increased in higher crude oil concentrations, suggesting that the presence of crude oil in soil could prevent the fast evaporation of water from the soil, so the contaminated soils were nearly always wet in microenvironment of the soil, while in control and lower concentrations of crude oil, the soil loses its moisture due to fast evaporation of water. Soil moisture is one of the most important elements for growth and reproduction of microorganisms.

In all vegetated samples the microbial population was higher than non-vegetated samples suggesting that in rhizosphere area, the microbial population of the soil has been increased. Previous reports also indicated the increase of microbial number in vegetated contaminated soil\cite{9, 14, 18, 19}.

The reduction of crude oil in vegetated and non-vegetated soil showed that in all vegetated samples the crude oil reduction was higher than the non-vegetated samples. In all vegetated samples the slope of reduction in first 30 days was high, but it decreased distinctly in 7% and 10% during 60 and 120 days of planting while
the slope of reduction did not decreased in 1% and 3% samples, suggesting that in the first 30 days the reduction of crude oil may be due to both evaporation and biodegradation of crude oil in soil. After 30 days evaporation played minor role in reduction and the main factors for oil reduction were phytoremediation and biodegradation which were lower in 7% and 10% samples.

In conclusion, light crude oil either in high or low concentrations (1%-10%) consist of toxic compounds that can prevent normal growth and germination of Alfalfa in soil.

REFERENCES