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# The Effect of Light Crude Oil-Contaminated Soil on the Growth and Germination of *Sorghum bicolor*

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## ABSTRACT

Light crude oil has volatile components that can be toxic to living organisms when spilled to the soil and water. The use of plants for treatment of crude oil-contaminated soil has been of particular interest in environmental cleansing. Some plants have demonstrated to have a good capacity for biodegrading oil in soil. In this study the growth and germination of *Sorghum bicolor* was studied in the presence of different concentrations of light crude oil (1-10%) in the soil. Root and shoot biomass, leaf length and germination percentage decreased as crude oil concentration increased. Light crude oil delayed germination and affected the normal growth of *S. bicolor*, inducing chlorosis in plants. The measurement of total petroleum hydrocarbons (TPHs) of soil at the end of treatment (45 days) showed that TPH was more reduced in the 1% sample than in the 7 and 10% samples. *S. bicolor* can be a good plant for phytoremediation of oil-contaminated soil when the concentration of crude oil is lower than 3% in the soil.

**Keywords:** petroleum, phytoremediation, plant, pollution

## INTRODUCTION

Crude oil and its byproducts are utilized by human being for transportation, industrial production and home heating. Leakage of crude oil into soil during extraction, refining and transportation can cause environmental damage and mostly is toxic to the flora and fauna in soil. There are some techniques for removing or decreasing the oil from contaminated sites including chemical, physical and biological methods, among which, the biological method is cheaper and more efficient.

Bioremediation, as a method of detoxification which uses bacteria and fungi, is a potent way of removing oil from contaminated soil and water. Microorganisms are able of biodegrading the crude oil hydrocarbons into less toxic products than the parent compounds (Eweis *et al*. 1998).

Phytoremediation is on site use of plants and their associated microorganisms to remediate contaminated soil and water (Cunningham *et al*. 1996). It can be used to clean up heavy metals, pesticides, organic solvents, crude oil, and other contaminants that leak to the soil. Various plant species have been identified with potential of facilitating the phytoremediation of oil contaminated soils (Banks *et al*. 2003; Merkl *et al*. 2004; Brandt *et al*. 2006; Tanee and Akonye 2009). Grasses and legumes are proved to have high potential in this regard (Aprill and Sims 1990; Gunther *et al*. 1996; Reilley *et al*. 1996). Grasses have vast root surface area compared with other plant species which can penetrate and spread in soil to cooperate with microorganisms in remediation process (Aprill and Sims 1990). The plant roots stimulate the bacteria, which enhance the biodegradation of petroleum hydrocarbons (Gunther *et al*. 1996; Muratova *et al*. 2003).

In this study *Sorghum bicolor* was planted in different concentrations of light crude oil-contaminated soil. *Sorghum* was chosen for this experiment because its root system is like the other grasses, which are commonly used for bioremediation. The effect of crude oil on germination and growth was studied. Furthermore, the amount of crude oil reduction in the soil was also determined.

## MATERIALS AND METHODS

### Chemicals

All the chemicals used for this experiment were reagent grade and obtained from Merck (Darmstadt, Germany).

### Mixing soil with oil

The soil was obtained from a cultivated area in north of Tehran refinery. Light crude oil (American Petroleum Institute (API) gravity = 40) was obtained from oil processing factory of Sarkan in west of Iran and in different concentrations (1, 3, 5, 7 and 10%, w/w) was added to 500 g of the soil. Mixing the soil with oil was done according to Minai-Tehrani and Herfatmanesh (2007). In brief, the soil was transferred in the pail and appropriate amount of oil was added to the soil. Lid of the pail was fastened tightly and the pail was shaken firmly by hand. Shaking continued until homogeneity was achieved. To confirm the homogeneity of contamination in the soil, two samples from the pail were then taken from different parts of the soil and analyzed for the oil contamination with the method that will be explained in the crude oil extraction.

### Plant growth conditions

Fifty seeds of *S. bicolor*, cv. 'KFS3' (Pegah) were planted in each pail (seed of *S. bicolor* was obtained from the Research Institute of Forests and Rangelands (R.I.F.R) of Iran). For each concentration we considered three replicates. 5 g of animal manure was added to each sample as fertilizer and tap water was used to moisten the samples. Light for the growth of plants was obtained from the sun. Samples were positioned behind the glass windows of laboratory and received natural solar energy during the experiment. The temperature and humidity were about 30°C and 45%, respectively.

### Plant growth and biomass

The germination number was counted 20 days after planting. Germination was defined as the radicle protruding from the seed coat.

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The length of the leaves was measured on the 30th day after planting. To determine the biomass of the plants, they were removed from the soil at the end of the experiment (45 days) and the roots were washed with water to remove excess soil adhering to them. Roots and shoots were separated and dried at 50°C for 7 days. The plant biomass was reported as dry weight for roots and shoots.

## Crude oil extraction

Extraction of crude oil was conducted according to the method used by Minai-Tehrani and Herfatmanesh (2007). For 48 h, 1 g of the treated soil was dried at 50°C and then crushed well to make a homogenous soil. After that, 5 ml of dichlromethane ($CH_2Cl_2$) (Aldrich) was added to the soil and the mixture was shaken firmly to separate the oil from the soil. The sample was centrifuged (3000 × *g* for 10 min) to precipitate the soil, and the solvent phase was separated. The solvent extraction process was repeated twice. The solvent vaporized during 24 h and the amount of oil was measured by gravimetric method and its reduction compared with start time. Three samples from each replicate were taken for the crude oil extraction.

## Total colony count

After 30 days, determination of the number of colonies in the soil was done by pure-plate method and compared with start time. 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 plates containing nutrient agar. The plates were incubated at 30°C for 48 h and then the number of colonies was counted (Minoui and Minai-Tehrani 2009).

## Statistical analysis

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. To obtain significant differences, means were compared by Tukey's test. The statistical results were analyzed by GraphPad Prism 5 program.

# RESULTS AND DISCUSSION

## Soil bacterial count

Total colony count was determined for the soil (**Fig. 1**). The highest number of microbial population was observed in 7 and 10% samples, and the lowest number was observed in the control group (0%). Just as increasing the crude oil concentration so increased total microbial population in the samples. Counting the oil-degrading bacteria in the samples showed that the highest number of these microbial populations was in 7 and 10% samples and the lowest was in 0% sample (**Fig. 2**). There was significant difference between all the contaminated samples and the control one.

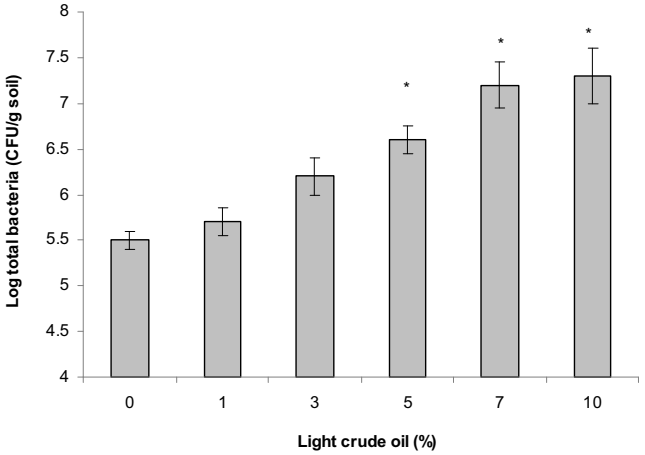


**Fig. 1 Total colony count in the control group and contaminated sample**. Average values given ± standard deviation (± SD). Asterisks indicate significant difference between the control and treated samples ($P < 0.05$) after one-way ANOVA according to Tukey's test (n = 3).

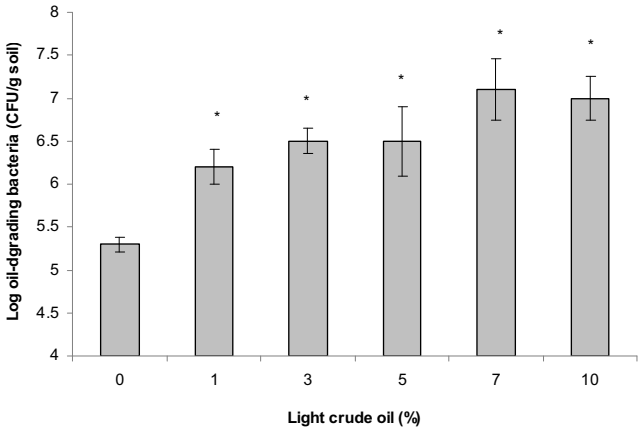


**Fig. 2 Oil degrading bacteria colony count**. There was significant difference between all contaminated sample and the control group. Average values given ± standard deviation (± SD). Asterisks indicate significant difference between the control and treated samples ($P < 0.05$) after one-way ANOVA according to Tukey's test (n = 3).

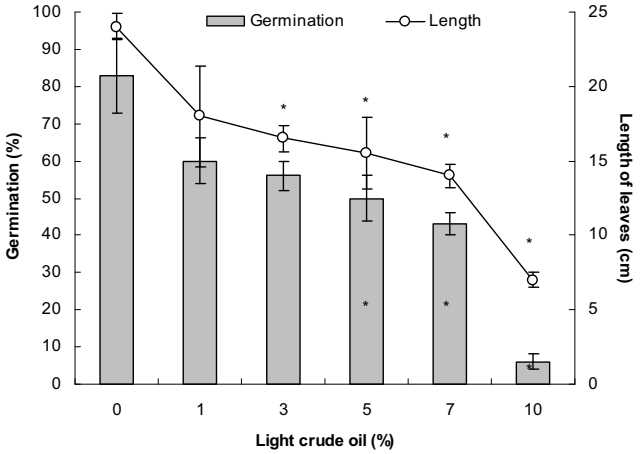


**Fig. 3 Germination in samples was counted after 20 days and the length of leaves was measured 30 days after planting**. Average values given ± standard deviation (± SD). Asterisks indicate significant difference between the control and treated samples ($P < 0.05$) after one-way ANOVA according to Tukey's test (n = 3).

## Germination and length of the leaves

In the control group number of germinations was higher than the other samples and it was the lowest in 10% sample. There was a sudden decrease in number of germinations in 10% sample comparing with the other contaminated samples. **Fig. 3** shows the number of germinations in the samples 20 days after planting.

The length of the leaves was measured at the 30th day after planting. The length of the leaves in the control group was higher than other samples (**Fig. 3**). There were significant differences in the length of leaves between control group and the samples with higher than 1% of crude oil. The shorter leaves were observed at 10 and 7% samples, while the tallest leaves were seen in the control group.

## Biomass

The dry biomass of roots and shoots was measured at end of the experiment (**Fig. 4**). Separation of the roots from the soil showed that the distribution of roots in the soil has decreased by increasing the crude oil concentration. A higher root biomass was observed in the control group, in which the roots were well-distributed in the soil. The lower

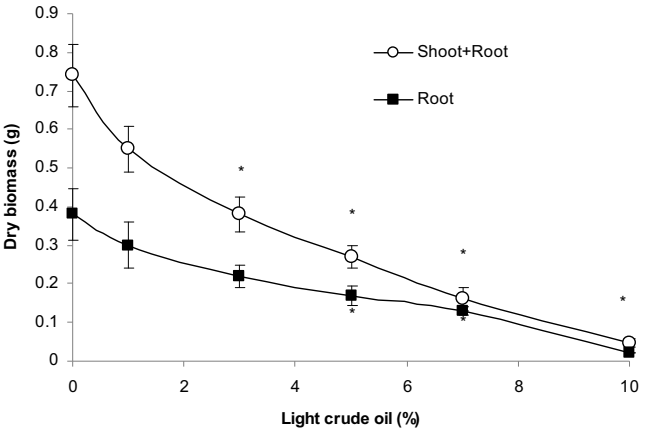


**Fig. 4 Total dry biomass (roots + shoots) and dry biomass of roots, 45 days after planting**. Average values given ± standard deviation (± SD). Asterisks indicate significant difference between the control and treated samples ($P < 0.05$) after one-way ANOVA according to Tukey's test (n = 3).

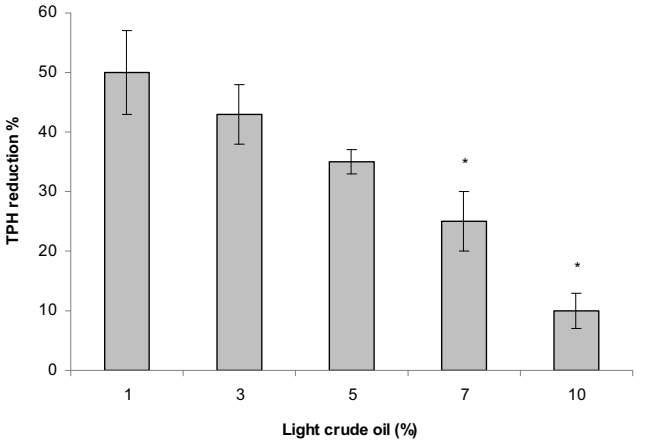


**Fig. 5 Reduction of total petroleum hydrocarbons (TPH) in contaminated samples**. Average values given ± standard deviation (± SD). Asterisks indicate significant difference between the control and treated samples ($P < 0.05$) after one-way ANOVA according to Tukey's test (n = 3).

root biomass was seen in 10% sample. Distribution of the roots was poor in high crude oil concentrations (7 and 10%). There were significant differences in root biomass between the control group and the samples with higher than 3% crude oil. The total dry biomass (roots + shoots) was also high in the control group while it was low in 7 and 10% samples. A meaningful difference was observed in dry total biomass between control group and the samples with higher than 1% crude oil.

### Crude oil reduction

The reduction of total petroleum hydrocarbons (TPHs) from the soil was measured at the end of experiment in the contaminated samples. The highest reduction was observed in 1% sample and the lowest was seen in 10% sample (**Fig. 5**). The reduction of TPH was considerable between 1% and the samples with higher crude oil content (7 and 10%).

## DISCUSSION

This study focused on the effect of light crude oil on the growth of *S. bicolor* as well as the phytoremediation of crude oil. Many reports indicate the use of grasses and legumes as the right choice of phytoremediation of oil-contaminated soil (Aprill and Sims 1990; Gunther *et al*. 1996; Reilley *et al*. 1996; Kirk *et al*. 2005; Minai-Tehrani 2008). *S. bicolor* was chosen because of the few published reports concerning the ability of this plant in phytoremediation. Schwab *et al.* (1995) reported the significant mineralization of phenanthrene in soil planted to sorghum. Sorghum has some characteristics that make it outstanding in removing the oil from contaminated soil. Its roots, like those of other grasses, are widely distributed and have the maximum surface area in soil. Our results showed that light crude oil could damage the plant growth. Germination delayed and the number of germination reduced in the contaminated soil. Leaves in the treated plants were shorter than leaves in the control group and early chlorosis was observed in treated plants comparing with the control group. Total biomass and root biomass measurement showed reduction in contaminated samples. Increasing crude oil concentration in the soil decreased the root biomass and total biomass which was accompanied by shortening the length of leaves and roots. In a previous report the effect of heavy crude oil on the growth of *Poa trivialis* indicated decrease of germination which occurred in above 3% of contamination and the early chlorosis was observed in contaminated samples (Minai-Tehrani 2008). Another report also indicated that light crude oil could affect germination of *Festuca arundinacea* with concentrations above 5% (Minai-Teharni *et al*. 2007). Our results showed that germination was affected in samples with contaminations higher than 1%. Increasing crude oil concentration in soil was accompanied by expansion in total and oil degrading bacteria numbers. As the previous reports also mentioned, the total and oil degrading bacteria numbers increased by higher concentration of oil in the soil (Minai-Teharni 2008; Minai-Tehrani *et al*. 2009).

TPH reduction was observed in all samples which proposed that roots of sorghum were able to induce a suitable condition for soil microorganism to remove the oil from contaminated soil. The reduction of root biomass in 7 and 10% sample means that in these oil concentrations the reduction of TPH has reached its minimum level, while the reduction of TPH in 1% sample was about 50%. Another report also indicated that in soil planted by *Festuca* the reduction of TPH was about 55% (Minai-Tehrani *et al*. 2007). This result suggests that the root system of sorghum has the same efficiency to reduce the oil from contaminated soil comparing with other grass roots.

In conclusion, our finding showed that light crude oil could affect the growth of *S. bicolor* either in low or high concentrations of oil. However, sorghum can be considered as a good option in phytoremediation of crude oil in soil when the concentration of crude oil is less than 3%.

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