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# Effect of Light Crude Oil-Contaminated Soil on Growth and Germination of *Festuca arundinacea*

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**Abstract:** In this study the effect of different concentrations of light crude oil (up to 10%) on the growth and germination of *Festuca arundinacea* (Tall fescue) was studied. Present results showed that the germination number and dry biomass of the plant decreased by increasing light crude oil concentration in the soil. The biomass was higher in 1% crude oil sample while it was lower in 10% crude oil sample. The length of leaves reduced in higher crude oil concentration in comparison with the control. Total and oil-degrading colony count of soil showed that the microbial population in 7 and 10% samples was higher than the control and low concentrations of crude oil (1 and 3% samples). The crude oil reduction in the vegetated and the non-vegetated samples was higher in 1% sample. All vegetated samples had higher crude oil reduction than non-vegetated samples. The higher reduction was occurred at 1% sample, while the lower reduction was seen at 10% sample.

**Key words:** Crude oil, phytoremediation, plant, soil, tall fescue

## INTRODUCTION

Crude oil consists of four main groups of hydrocarbons including alipahtics, aromatics, resin and asphaltine (Colwell and Walker, 1997). The leakage of crude oil in to soil damages the biological systems residing in the soil; including microorganisms and plants. Some fractions of crude oil are toxic for living organisms. However various microorganisms are able to use some crude oil fractions as sole carbon source and change these components to non-toxic materials such as $CO_2$ and $H_2O$ (Eweis *et al*., 1998). Phytoremediation is the use of plants and their associated microorganisms to remediate contaminated soil and water (Cunningham *et al*., 1996). Among the plants, grasses and legumes have higher potential on removal of oil from contaminated soil (Kim and Cho, 2006; Aprill and Sims, 1990; Gunther *et al*., 1996). Grass roots have maximum root surface area in the soil in comparison with other plants and can penetrate to depth of the soil (Aprill and Sims, 1990). The plant roots stimulate the bacteria, which enhance the biodegradation of petroleum hydrocarbons (Kaimi *et al*., 2006; Siciliano *et al*., 2003; Gunther *et al*., 1996). Oil contaminated soil can affect the growth and germination of plants (Adam and Duncan, 2002). The effect of contaminant on microorganisms and plants depends on the concentration and the kind of contamination (Boethling and Alexander, 1979). 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 the soil for many years (Walker *et al*., 1975). 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 (Atlas, 1975).

In this study different concentrations of light crude oil were added to the soil and the effect of contaminated soil on the growth and germination of a plant in grass family, *Festuca arundinacea* (Tall fescue), 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 sieved through 4 mm mesh. The texture of the soil was determined by hydrometer method (Robertson *et al*., 1999) and consisted of clay 54%, sand 16% and loam 30%. The organic matter was determined as 0.9% by Walkley-Black method (Robertson *et al*., 1999).

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The pH of the soil was determined 7.4 for the soil-distilled water slurry (1:5, w/vol) (Robertson *et al*., 1999).

**Soil preparation:** Light crude oil (API gravity = 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 the oil were well mixed to make homogenized contaminated soil and transferred to 1 L pots. Each sample consisted of 800 g of dry soil.

Chemical fertilizers were added to the soil before seeding. 75 mg $kg^{-1}$ nitrate ($NH_4NO_3$) and 30 mg $kg^{-1}$ of phosphate ($KH_2PO_4$) were added to all samples (Rosenberg *et al*., 1996).

Thirty seeds of Tall Fescue 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; the control samples did not receive seeds (non-vegetated).

Prior to planting (T = 0), 10 g of soil were removed from each sample and stored at -20°C for further preparations.

**Germination and biomass:** The experiments were done in laboratory and at room temperature (25-28°C). The number of germinations was counted 30 days after planting. The length of shoots was measured 60 days after planting. The number of green plant was counted at the end of experiment (120 days).

At the end of experiment (120 days), plants were removed from the soil and the roots washed with water to dislodge excess soil adhering to the roots. Roots and shoots were separated and dried at 50°C. Plant biomass was 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 the soil 60 days after planting. Determination of total colonies in the soil was done by *pure-plate* method with Nutrient agar as a medium. Determination of oil-degrading colonies was also done by the same method (Kirk *et al*., 2005) in Agar-agar as a medium with 1% sterilized light crude oil as sole carbon source.

**Crude oil extraction:** Crude oil extraction was done as Minai-Tehrani *et al*. (2006). For 48 h, 2 g of treated soil was dried in 50° then crushed well to make a homogenous soil. A total of 10 mL dichlromethane ($CH_2Cl_2$) (Aldrich) was added to the soil and shaken firmly to separate oil from the soil. The sample was centrifuged (3000 x g for 10 min) to precipitate the soil and the solvent phase was removed. The solvent extraction was repeated twice. The solvent vaporized during 24 h and the amount of the oil was measured by gravimetric method and its reduction compared with time zero (T = 0). Two samples from each replicate were taken for crude oil extraction.

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

## RESULTS

**Germination and biomass:** Figure 1 shows the number of germinations in the vegetated samples 30 days after planting. In 0% sample the number of germinations was higher than the other samples while it was lower in 10% sample. There was a sudden decrease in number of germinations in 10% sample in comparison with the other contaminated samples.

The dry biomass of roots and shoots was measured at end of the experiment (Fig. 1). The separation of roots from the soil showed that the distribution of roots in the soil has 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 lower roots biomass was seen in 10% sample. The roots distribution was poor in high crude oil concentrations (7 and 10%). The total dry biomass

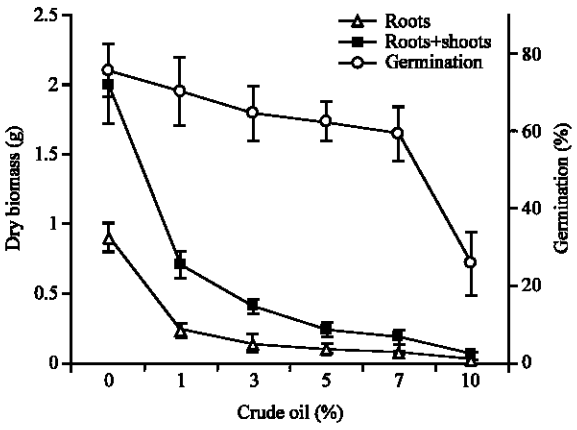


Fig. 1: The number of germination in different concentrations of crude oil after 30 days of seeding and total dry biomass (shoots+roots) and dry biomass of roots after 120 days of planting. Average values given ±Standard Deviation (±SD), $p<0.05$

(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 with the control (0%) sample.

The length of leaves reduced by increasing crude oil concentration, the shorter leaves was observed in 10% sample, while the tallest was seen in 0% sample (Fig. 2). The number of green plant at the end of experiment was lower in 10% followed by 7% samples, while the higher was observed in 0% followed by 1% samples (Fig. 2).

**Colony count:** Total colony count was determined in the vegetated and the non-vegetated soil (Fig. 3A). In the vegetated samples, the higher microbial population was observed in 7 and 10% samples, the lower was observed in the control (0%) sample. Increasing the crude oil concentration increased total microbial population in the vegetated samples. In the non-vegetated samples, the higher microbial population was observed in 7% sample, while the lower was seen at 0% sample. In all the vegetated samples the total colonies were higher than their equal concentration of crude oil in the non-vegetated samples.

Counting for the oil-degrading colonies in the vegetated samples showed that the higher microbial population was also observed in 7 and 10% samples and the lower was seen in 0% sample (Fig. 3B). In the non-vegetated samples the higher count for oil-degrading colonies was observed in 7% sample while it was lower in 0% sample. In all the vegetated samples the oil degrading colonies were also higher than their equal concentrations of crude oil in the non-vegetated samples.

**Crude oil reduction:** The crude oil reduction in the vegetated and the non-vegetated contaminated soil was measured and compared during 120 days (Fig. 4). In the first 30 days the rate of reduction was higher than the second 30 days in the 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% in both vegetated and non-vegetated samples. Increasing crude oil concentration decreased the reduction of crude oil in both vegetated and non-vegetated samples. In all the contaminated vegetated soils, the reduction of the crude oil was higher than the non-vegetated soils. In the higher concentrations (7 and 10%) the difference of crude oil reduction between the vegetated and the non-vegetated samples was not significant, while the reduction was significant between the vegetated and the non-vegetated samples in concentrations up to 5%.

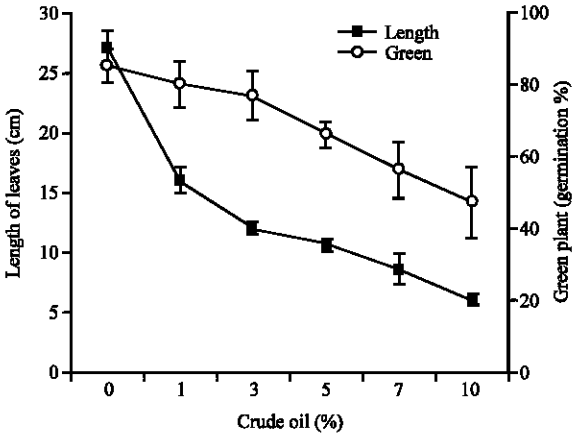


Fig. 2: The length of leaves was measured 60 days after planting; the green plant was counted at the end of experiment (120 days)

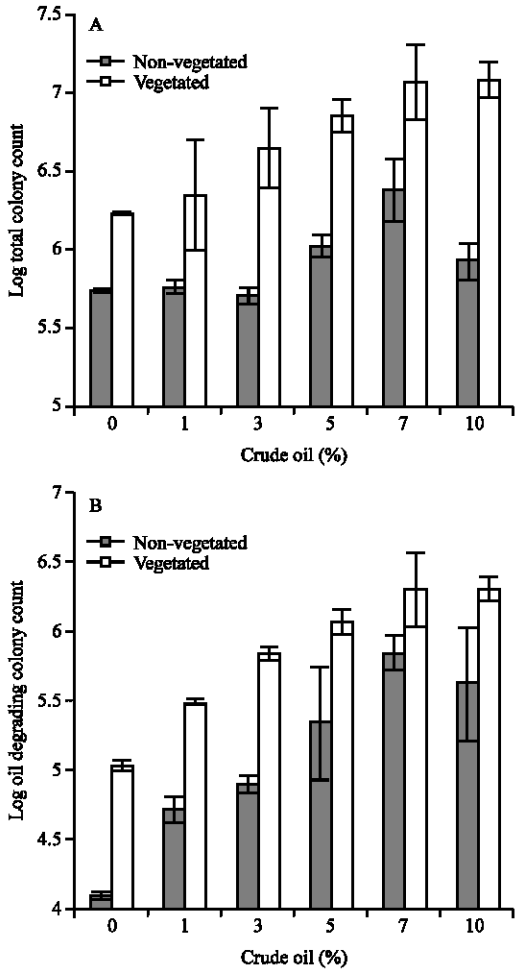


Fig. 3: Total colony count (CFU $g^{-1}$ soil) after 2 months of planting (A). Oil-degrading colony count (CFU $g^{-1}$ soil) after 2 months of planting (B). (±SD, n = 3, $p<0.05$)

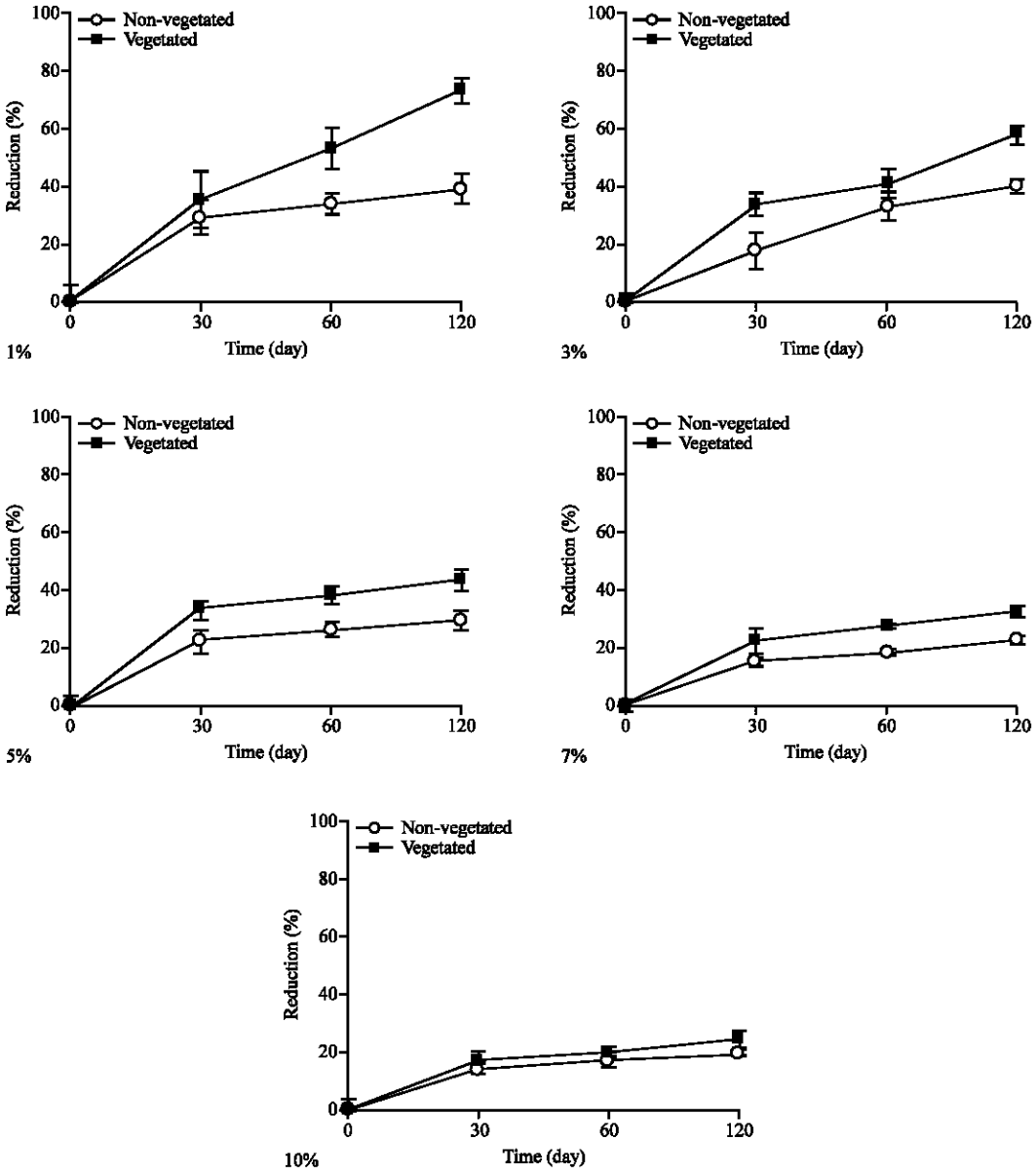


Fig. 4: Reduction of crude oil during 120 days in different concentrations of light crude oil-contaminated soils. (±SD, n = 3, $p<0.05$)

## DISCUSSION

This study focused on the effects of light crude oil-contaminated soil on the growth and germination of *Festuca arundinacea* (Tall fescue).

The germination of this plant in 10% light crude oil-contaminated soil suggests that the plant could tolerate high concentration of crude oil (10%) in the soil. The reduction of biomass and the length of leaves in high concentration of light crude oil (7 and 10%) suggest that the toxic compounds of crude oil in the soil could reduce the number of germination and affect the normal growing of the roots and the shoots in contaminated vegetated samples. The distribution of fibrous roots of the plant in contaminated soil mainly decreased in comparison with control. The germination of Tall fescue in 5% TPH contaminated soil was reported to reduce about 70% of control (Huang *et al*., 2005). Exposure of the plants to tolerable concentrations of petroleum can cause the chlorosis of the leaves, plant dehydration, stunted growth and also death (Udo and Fayemi, 1975).

At the end of experiment the number of green plants decreased in high concentrations of light crude oil (5-10%) which was accompanied by chlorosis and dehydration. This was in accordance with other studies that have reported the effect of oil in growth and germination of plants (Merkl *et al*., 2004; Merkl *et al*., 2005).

In this experiment, for the first time, the number of total bacteria and oil degrading bacteria was compared in different concentration of light crude oil. Our results showed that in the presence of high concentration of crude oil (10 and 7%), the microbial population and oil degrading bacteria, increased in comparison with the control and lower concentrations of crude oil (1 and 3%). This phenomenon might be due to presence of high concentration of crude oil in the soil that prevented the fast evaporation of water from the soil, thus the contaminated soils were always wet in microenvironment area of the soil. In contrast, in the control and lower concentrations of crude oil, the soil might lose the moisture due to fast evaporation of water. Consequently, in the soil with higher concentrations of crude oil, the presence of water in microenvironment could also prevent the diffusion of non-polar toxic materials of crude oil into the microenvironment which could help the bacteria to be vital, but the absence of sufficient oxygen reduces the crude oil degradation by bacteria in the samples with high crude oil concentration. The oily shield may prevent freely diffusion of oxygen to microenvironment area in the soil. Oxygen plays important role in crude oil and its component biodegradation (Von Wedel *et al*., 1998; Jamison *et al*., 1975).

Present results also showed that in all the vegetated samples the microbial populations were higher than the non-vegetated samples, suggesting that the presence of roots in the soil could increase the microbial population in comparison with non-vegetated samples. Previous reports have also indicated the increase of microbial number in the vegetated contaminated soil (Gunther *et al*., 1996; Anderson *et al*., 1993).

The comparison of crude oil reduction in the vegetated and the non-vegetated soil showed that in all the vegetated samples the crude oil reduction was higher than the non-vegetated samples. It has been shown that the planted contaminated soil had higher efficiency for reduction of oil than unplanted soil (Reilley *et al*., 1996; Pradhan *et al*., 1998). The high reduction of crude oil in the 1 and 3% vegetated samples suggests that the oil reduction has enhanced in the presence of well distributed plant roots and the plant roots might play important role in removal of crude oil and its component. The nearly equal reduction of crude oil in the vegetated and the non-vegetated of 7 and 10% samples suggests that the presence of plant roots in reduction of crude oil were important for oil reduction. The roots were poor distributed in the soil with 7 and 10% crude oil concentrations.

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