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Potential of Alfalfa for Use in Phytoremediation of Soil Polluted with Total Petroleum Hydrocarbons

Abstract- *Remediation technology is a promising technique decrease pollutant like hydrocarbons from the environment. An experimental work was made at green house of University of Technology in order to study the effect of crude oil on the vegetate growth and to measure the decrement which happened on shoot height, germination rate and the reduction of total petroleum hydrocarbon (TPH), which result, by this phytoremediation technique. The samples of soil were measured for TPH reduction and removal by Horiba model (oil content analyzer) OCMA–350. Five doses were used in this experiment (0 control, 10x10^3, 30 x10^3, 50 x10^3, 75 x10^3) (mg crude oil / kg soil). The polluted soil used in this study appeared to be a harmful environment for alfalfa plants, leading to serious adverse effects on alfalfa germination and growth. Seed germination is known to be a sensitive process affected by environmental factors like the presence of soil pollutants.*

Keywords*: Soil, pollution; TPH, phytoremediation, alfalfa*

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1. Introduction

Petroleum pollution of soil is extensive and well known as a global environmental problem [1]. Soil pollution with petroleum and petroleum-based hydrocarbons has made a dangerous to the human health and to the environment [2]. Exploration and exploitation of crude oil are one of several ways that soil is polluted with petroleum and have left trace of woes in their route with a great amount of harm to the biological community and issues to human life [3]. Some of the troubles that linked with crude oil exploration and exploitation include agricultural degradation, destruction and land of aquatic habitats, environmental large-scale pollution, among others. Most problems of crude oil contamination came because of mechanical failure, accidental discharge, improper disposal, loading offloading and sabotage [3]. Soil serves not just as a supporting medium for plant development; it is additionally serves as a territory for a few creatures. Its quality goes far to manage functioning and community structure. Efforts are made to recover or clean up contaminated soils. Different remediation technology was used for cleaning up contamination as efforts to increase attention for developing and implementing innovative technology. Remediation Techniques are those treatments that made for restore a contaminated or polluted water and soil [4].

Remediation of crude oil from soil can enhance the status of soil through the change of the supplement status and in addition, the natural matter substance (organic matter content) and can likewise lessen the phytotoxicity of soil [5]. Crude oil can be clean up by different processes such like physical, chemical and biological [6]. However, because of physical and chemical treatment are expensive methods of remediation, biological processes currently being more preferable than other remediation methods [7]. The biological technique, which is used for cleaning up the soil, is called phytoremediation. Research evidences show that many plants have the capability to remediate crude oil that which polluted soils [8-9-10].

2. Materials and Methods

Seeds were germinated and seedlings grown in plastic PVC pot (25 cm length, 25 cm height) filled with 7 Kg of sandy clay. Soil, which was taken from area near Tigers River. Pots were put in greenhouse and irrigated with tap water twice a week. The experiment was performed from March to June 2016 and diurnal temperatures varied between 20-40°C. Each pots have A hole at the bottom was made for the drainage of excessive water from the pots. Eight pots were ready to seed the selected plant. The pots were divided the following treatments:

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T1: Unpolluted soil (control) which in turn have two division seeded and without seeded with the selective plant.
T2: Polluted soil with crude oil (10000 mg/Kg which in turn have two division seeded and without with the selective plant
T3: Polluted soil with crude oil (30000 mg/Kg which in turn have two division seeded and without with the selective plant.
T4: Polluted soil with crude oil (50000 mg/Kg which in turn have two division seeded and without with the selective plant.
T5: Polluted soil with crude oil (75000 mg/Kg which in turn have two division seeded and without with the selective plan.
Control soil was used to show the degradation of crude oil by microorganisms without the plant effect and compare it with planted pots to see the effect of plant on degradation petroleum hydrocarbon pollution have the impact on plant growth, which was discover by this comparison. Alfalfa plant (Medicago sativa) was chosen for this study. Account the number of developed vegetates or the upper layer of soil density monitoring was made to see the germination rate in the first weeks. The shoot height was monitored and measured. In addition, permeability test was made to see the effect of crude oil on soil permeability, which effect on plant ability to get water from the soil. Core sampler (inside D = 10 mm) was used to take soil samples from the 5cm depth of the container in each month of study. For TPH measurement, soil samples were dried by air putting it at normal room temperature and sieved by a 2 mm sieve. There are various procedure, which will accurately measure TPH in soil. This method is provided as a simple procedure for Horiba model OCMA – 350 oil concentration monitoring and analysis. Samples of 5g of soil were taken putted in 40 ml clean vial. 1g of anhydrous sodium sulphate, Na_2SO_4 was added. To dry the soil samples .30 ml of solvent (infrared spectrometer grade of Horiba S-316) was add to the vials. An extraction of oil in soil to oil in solvent was performed by shaking the vials vigorously for one or more minutes. After the shaking, the vials were placed in its upright position and wait for one min at least to allow setting of soil particles. Whatman 40 filter paper was putted in glass funnel. 2gm of conditioned silica gel were added above the filter paper. The solvent / extract were filtered through the filter into clean beaker. Pipette about 6 ml of extract into the OCMA-350 cell. Place the cell into the OCMA -350 and measured.
Constant head permeability experiment was done to the soil specimens to demonstrate the effect of crude oil on soil permeability, which may effect on the plant growth. Dry weight was measured by dried one gram of plant sample in the oven and after 24 hours the samples was taken out and measured by sensitive balance.

3. Results

Figure 1 shows the effect of crude oil on soil permeability. High concentration of crude oil would cause a reduction in permeability up to 2.7 cm/S at T5 treatment.

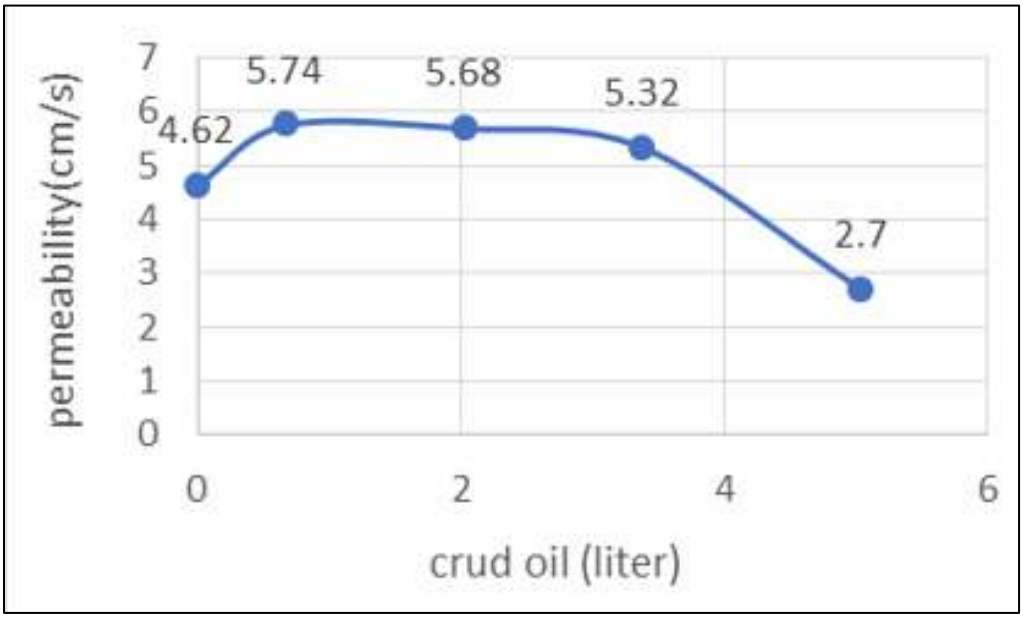

Figure 1: Crude oil effect on the soil permeability

Table 1 describes the number of seeds which would be affected by the presence of the crude oil pollution in soil this showed clearly from the number of seeding in T2 (8) seed and T3 (4) seed Per pot at in a comparative with T1 which recorded (17) seed per pot. AT the end of the study, no germination was observed for T4 and T5. It has been shown that the crude oil would significantly affect the plant leaf which would be (35, 10, 3) leaf per plant for the T1, T2 and T3 respectively. A great reduction of the leave area in T2, which reach to half from the uncontaminated surface area. Petroleum crude oil extremely decrease the plant dry weight in a comparative with the untreated control with decreasing of 100% for the plant dry weights at T4 and T5. Plant shoot height would greatly decreased to 83.7 % at T3.

Table 1: Development parameters of 30 seeds of alfalfa plants after four months

Concentration of crude oil mg/kg	**Growth parameter**				
	Number of seed per pot	Plant height (cm)	Number of leaves per plant	Leaf surface area (cm^2/leaf)	Dry weight plant
T1	17	18.5	35	1.2	1.143
T2	8	15	10	0.6	0.5
T3	4	3	3	0.4	0.3
T4	0	-		-	-
T5	0	-	-	-	-

The reduction of TPH concentration was shown in Table 2. The effect of the vegetated plant on petroleum hydrocarbon elimination at specific period was monitored. The seeded plant caused petroleum hydrocarbon dispersion.

Table 2: Efficiency of crude oil removal for four months

Crude oil concentration mg/kg	**Efficiency for five months %**
T2	31.8
T3	27.2
T4	32.2
T5	8.42

4. Discussion

The polluted soil used in this study appeared to be a harmful environment for alfalfa plants, leading to serious adverse effects on alfalfa germination and growth. Seed germination is known to be a sensitive process affected by environmental factors like the presence of soil pollutants [11].
Group of chemical compounds, which are polyaromatic compound (PAHs), has been shown to have indirect secondary effects including disruption on plant -water-air relationships [12]. These results are backed by those obtained by the previous study [13] which found that alfalfa seeds germinated in soil contaminated with up to a 50g crude oil/kg soil (5% w/w) and that was near to the result of this research has showed before. Alfalfa plants could not stand oil concentration more than $50x10^3$ and $75x10^3$.
Likewise, oil coating the seed may prevent oxygen and water uptake and oil-penetrating seed coats may result in the embryo death [14]. A study [15] has also found a reduction of biomass plant species. Plant height and shoot biomass were good indicators of plant health. In addition, the effect of crude oil on permeability of the soil may cause a leak of water in the soil layers, which affect directly on plant growth.

5. Conclusion

Alfalfa survival was severely affected in the contaminated soil and premature plant death was manifest. As shown in table1, mortality rates constantly increased over time, reaching absolute plant mortality at 120 days of experiment Therefore, these deleterious processes may explain the observed reduction in alfalfa germination rates in the present contaminated soil.

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