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Research Article

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Phytoremediation effect of *Ricinus communis, Malva parviflora* and *Triticum repens* on crude oil contaminated soil

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ABSTRACT

Oil and its derivatives spills have been a major issue across decades and it is hard to biodegrade even though there are many techniques are being developed to clean up petroleum contaminated soil. Phytoremediation has long been applied as a treatment technology that is cost-effective, ecologically friendly and efficient for the decontamination of hydrocarbon pollution. In this study, four crude oil contaminated soil samples were collected from oil extraction fields in Libya. Three plants were chosen (Malva punilora, Ricinus communis and Triticum repens) on 0.5% and 1% crude oil contaminated soil .The chosen plant species were implanted individually in the contaminated soil pots. Soil sample (triplicate) was taken from each pot at zero time, after 15 days. After 30 days and after 45 days of experiment. Hexane was added to the soil samples, mixed, filtered and the absorbance was measured using spectrophotometer at 360 nm. The results were compared to the standard curve to calculate the crude oil concentration and percentage of removal. As a result the highest percentage of removal of 0.5 % crude oil contaminated soil was by Triticum repens (94%) after 30 days of experiment followed by Malva parviflora(88.5) and Ricinus communis(77 %). While in 1% crude oil contaminated soil pots experiment, the highest percentage of removal was by Malva parviflora (89%) after 30 days of experiment followed by Triticum repens (80%) and Ricinus communis (76 %). Triticum repens showed good results suggesting more field application.

Key words: Phytoremediation, crude oil, *Triticum repens*, *Malva parviflora*, *Ricinus communis*.

INTRODUCTION

Phytoremediation is a group of technologies that use plants for remediating soils, sludge, sediments and water contaminated with organic and inorganic contaminants. Plants have evolved a great diversity of genetic adaptations to handle the accumulated pollutants that occur in the environment. Growing and, in some cases, harvesting plants on a contaminated site as a remediation method is a passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination. Phytoremediation can be used to clean up metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, and landfill leachates[1]. This emerging technology promises effective, inexpensive, and less intrusive clean up and restoration of oil-contaminated environments [2].

Plants can decontaminate oil polluted sites by direct uptake of petroleum hydrocarbons into their tissues, direct biochemical transformation of petroleum hydrocarbons and by mycorrhizal fungi and the activity of soil microbial consortia [3].Plants that are resistant to crude oil toxicity such as black poplar and willows as well as miscanthus grass (elephant grass) have been found to be effective in the remediation of oil polluted soil[4]. In the marsh environment *Spartina patens*, *Sagittaria lancifolia*, *Spartina alterniflora* and *Juncus roemeriannus* are considered ecologically and economically important in phytoremediation [5].

Fossil fuels represent primary energy source in the global industry. This make a big threat on environmental pollution, and serious ecological damage. Fuel by-products and spills in sites where storage, transport, distribution, refining, consumption and the existing industries related fossil fuel can cause harm. About five million tons of crude oil and refined oil go into the ecosystem every year due to anthropogenic sources from oil spills[6]. Contamination of soil and water with hydrocarbons poses a major ecological and human health problem that needs an effective and affordable technological intervention. Many sites stay contaminated with no treatment in sight because it is very expensive to clean them with the available technologies [7].

With these aspects the present study was aimed to determine the percentage of crude oil removal by three plant species (*Ricinus communis*, *Malva parviflora* and *Triticum repens*).

EXPERIMENTAL SECTION

Apparatus
UV-Visible spectrophotometer 6505 UV/VIS. (JENWAY), magnetic stirrer, beaker (50 and 100 mL), adjustable pipettes (1-10, 10-100, and 100-1000 μL), sensitive balance, fume hood, autoclave and incubator were obtained from Faculty of Pharmacy, University of Tripoli. Organic solvent toluene was obtained from Merck, Germany.

Plant collection
Three plant species were selected and collected during winter season. Selection was based on its availability, resistance to the harsh environmental and soil conditions such as shortage of water and high temperature. These species were *Ricinus communis*, which known locally as castor, *Malva parviflora*or Malva and *Triticum repens* or what's known as Star).

Soil Preparation
According to the modified method of Ilyina et al. (2003)[8], soil was collected from Tripoli habitant area. In separate pots, 3kg of soil was added. There were two main groups of concentrations (0.5% and 1%) as shown in Chart (1), each contain four pots. The first potof each concentration of crude oil was used as a control (soil + crude oil), the second, third and fourth werefor the effect of plant phytoremediation(*Ricinus communis*, *Malva parviflora* and *Triticum repens* individually + crude oil). Crude oil (0.5% and 1%w/w) was added to the soil in thetwo groups of pots (respectively) and mixed thoroughly. Total pots numbers were 4 for each group.

All pots were left under a shade, at temperature ranging from 10-20 °C, protected from rain and humidity maintained everyday by spraying with distilled sterilized water to avoid dryness. Samples were analyzed at zero time, after 15 days, 30 days and 45 days. The analysis was to determine the percentage of degradation of hydrocarbon.

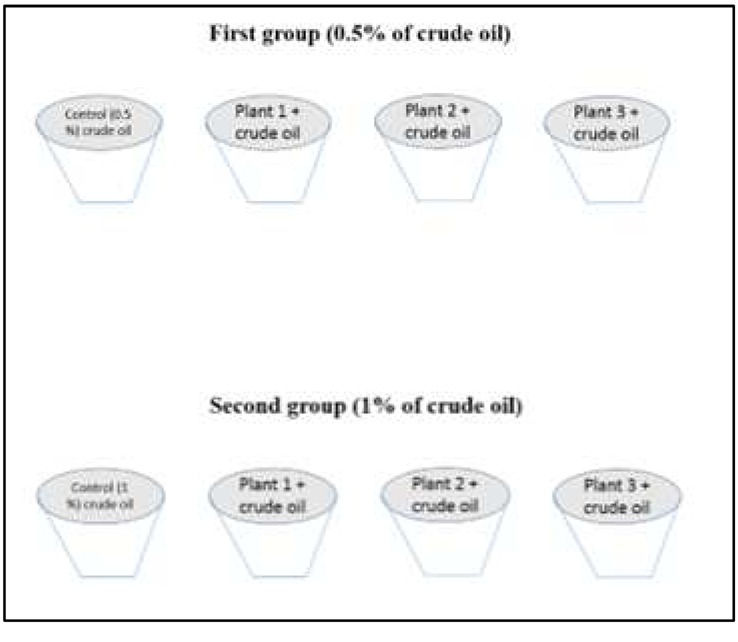

Chart 1: Experimental design for the effect of phytoremediation at 0.5% and 1% crude oil contaminated soil

Measurement of Total Extractable Hydrocarbon Content
At zero time, 1 g (triplicates) of soil in control pots (contain 0.5%and 1% w/w of crude oil) was weighed and transferred to dry, clean test tubes. Into this was added 10 mL of hexane, shook well and allowed to settle down for 30 min. The hexane-oil extracts were placed in cuvette wells and its absorbance was determined using spectrophotometer at 360 nm wavelength for crude oil. The wave length was chosen after screening of several dilutions of crude oil in the spectrophotometer. The best absorbance was at 360 nm. Then, all pots were sampled in triplicate by the same way mentioned above at 15 days, 30 days and 45 days period.

Absorbance was converted to concentration by comparing it with standard curve. A calibration curve was obtained by measuring absorbance of dilute standard solution of crude oil. Total hydrocarbon content was calculated after reading the absorbance of extracts from the spectrophotometer, exploiting from calibration curve and multiplying by an appropriate dilution factor (Osuji et al., 2006).

Calculation of percentage of removal of crude oil from soil
The percentage of degradation of crude oil was calculated by comparing the concentration results of the test with those of the control using the formula:

$$\text{Percentage of removal of crude oil samples} = \frac{(\text{Concentration of control} - \text{Concentration of test})}{\text{Concentration of control}} x\ 100$$

RESULTS AND DISCUSSION

The percentage of crude oil removal from soil
A triplicates of soil in all pots in two groups (0.5% and 1% w/w of crude oil) was added 10 mL of hexane. The absorbance of hexane-oil extracts was determined using spectrophotometer at 360 nm wavelength for crude oil. The sampling was at zero time, 15 days, 30 days and 45 days period.

After conversion of absorbance to concentration by comparing it with standard curve, total hydrocarbon content was calculated.

The result of percentage of removals of 0.5 % crude oil contaminated soil group of pots affected by the chosen plant species(*Ricinus communis*, *Malva parviflora* and *Triticum repens*) were plot in figure 1. As the result showed, the percentage of removal of 0.5 % crude oil concentration, *Triticum repens* or Star and *Malva punilora* shown to be the most effective, reaching 94% of crude oil removal followed by *Ricinus communis* (77%). The crude oil percentage of removal increased with time during the experiment for the three chosen plants.

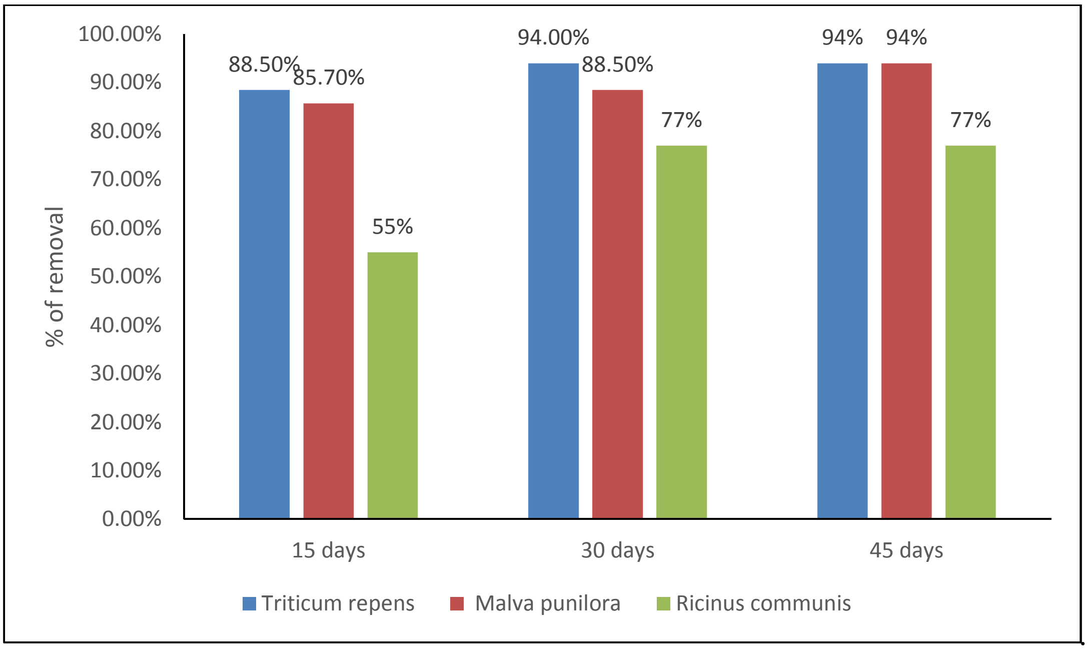

Figure 1: Percentage of removal of 0.5 % crude oil contaminated soil by *Ricinus communis*, *Malva parviflora* and *Triticum repens* at 15 days, 30 days and 45 days of experiment

The result of percentage of removals of 1 % crude oil contaminated soil group of pots affected by the same chosen plant species showed that same above pattern was obtained but with less percentages (Figure 2).

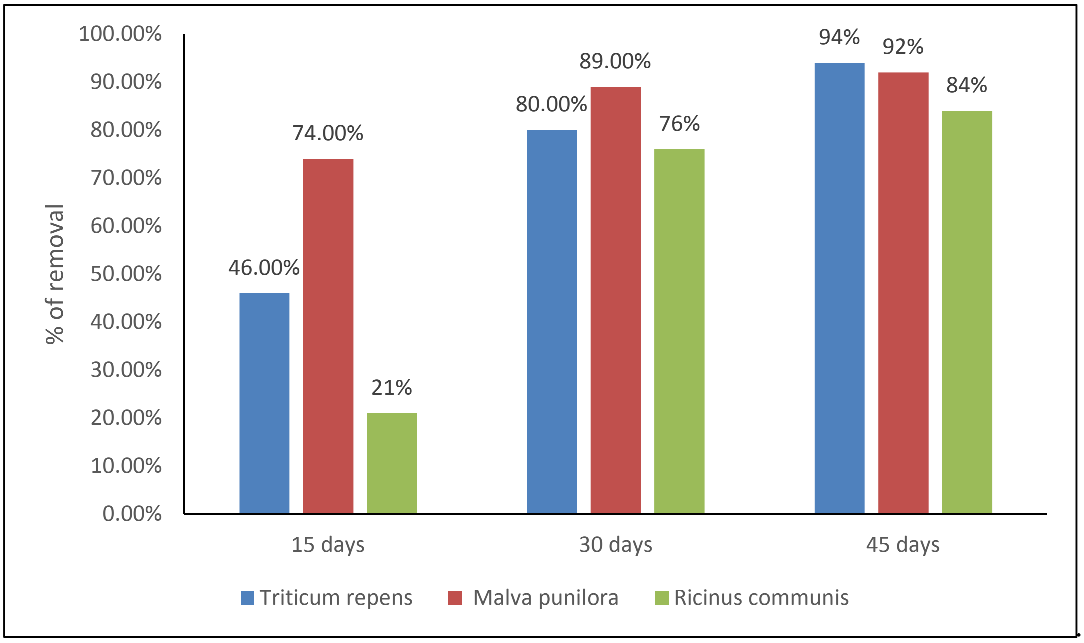

Figure 2: Percentage of removal of 1 % crude oil contaminated soil by *Ricinus communis, Malva parviflora* and *Triticum repens* at 15 days, 30 days and 45 days of experiment.

From the results, the three plant species *Ricinus communis*, *Malva parviflora* and *Triticum repens* showed to be effective in removal of 0.5 % of crude oil contaminated soil. This can be explained that the group of techniques that the plant use for phytoremediation process (phyto-accumulation, phytostabilization, rhizofiltration, phytovolatilization, phytodegradation and rhizodegradation) were effective in crude oil removal. The time was crucial in this process showing that the plant species were adapting with the new conditions in time of experiment and the highest percentage of removal was obtained at the end of the experiment.

When crude oil concentration increased by double, the plant couldn't adapt by the same level of crude oil removal at the same time of experiment, which indicate that the plant species in this study need more time to degrade 1 % of crude oil compared to 0.5 %. This may be taken in consideration in field study. As consistent with the previous report by Basumatary et al [9] which was conducted to evaluate the efficacy of *Cyperus rotundus* (nut grass), that could be effective in phytoremediation of crude oil contaminated soil. Total oil and grease (TOG) degradation and microbial numbers were analyzed at different intervals i.e. 60,120 and 180 days in different percentages of crude oil contaminated soil. In presence of crude oil, TOG content in soil, *C. rotundus* could decrease up to 50.01% in vegetated pots during 180 days proving the efficacy of this plant species for use in phytoremediation.

This can be explained as the dissipation of petroleum contaminants in the rhizosphere is likely the result of enhanced microbial degradation. Plant roots may encourage rhizosphere microbial activity through exudation of nutrients and by providing channels for increased water flow and gas diffusion. Phytoremediation of crude oil in soil was examined in other study using carefully selected plant species monitored over specific plant growth stages. Four sorghum (*Sorghum bicolor* L.) genotypes. Soil contaminated with crude oil. All vegetated treatments were associated with higher remediation efficiency, resulting in significantly lower total petroleum hydrocarbon concentrations than non-vegetated controls [10].

Carpet grass (*Axonopus compressus*) was also examined in the phytoremediation management of oil impacted soil in Ubeji and Alesa Eleme communities of Niger Delta region of Nigeria. The study employed an experimental research design that involved the use of *Axonopus compressus* in the management of oil-impacted soil sites of Ubeji and Alesa Eleme. The experiment lasted for four months. Axonopus sp. was used for the treatment of the crude oil impacted sites. Laboratory analysis of the soil samples was conducted to determine the effect of phytoremediation on hydrocarbon. The study revealed that the use of Axonopus sp. resulted in 66% loss of hydrocarbon from crude oil-impacted soils of Ubeji and Alesa Eleme. The implication of the findings is that Axonopus sp. has the tenacity to phytoremediate hydrocarbon concentration in soil effectively in any geographical region of the world [11].

Sang-Hawn et al.(2007) [12]concluded that hydrocarbon degrading bacterial populations increased rapidly during the first four weeks of 14 weeks testing period. They proposed this finding that it may be considered as an indicator for the feasibility of oil-contaminated soils degradation. However, with increasing of time, due to the oil-resistant components with high chain and within less remaining nutrients, the bacteria growth and oil degradation decreased[13]. Van Gestel et al. (2001)[14]reported a significant increment of the oil-polluted soil degradation in bacteria population.

CONCLUSION

We conclude that, among the three selected plants, *Triticum repens* showed the highest crude oil degradability or percentage of removal followed by *Malva parviflora*and *Ricinus communis* during the period of experiment. As the concentration of crude oil in soil increased the ability of plant phytoremediation decreased. All chosen plants showed ability to degrade crude oil. These phytoremediation abilities were tested under the laboratory condition but not at the field. Hence, field study of the above mentioned plants on higher crude oil concentration could be useful in practical phytoremediation approaches and reduction of the risk from crude oil to human health.

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