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# PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL USING TWO LOCAL VARIETIES OF CASTOR OIL PLANT (*Ricinus communis*) OF ASSAM

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## ABSTRACT

Accidental spillage of crude oil on soil makes the condition of the soil unsatisfactory for plant growth. Remediation of such soil using economic plants like castor (*Ricinus communis*) offers cost effective and eco-friendly method. This study aims to evaluate the growth response and remediation applicability of Castor oil plant *(Ricinus communis)* to crude oil contaminated soil. Here, soil contaminated with 1%, 3% and 5% (w/w) crude oil was used. The growth response of two different local varieties (green variety and red variety) of Castor was observed for a period of 180 days. Parameters taken into account for study of growth responses were plant height, stem girth, leaf area and days to flowering. Soil samples collected from the rhizosphere of the experimental plants were analyzed for total petroleum hydrocarbon (TPH) amount and an assessment of hydrocarbon utilizing bacteria (HUB) count was also done. It was observed that at 1% crude oil concentration, the plant shows more vigorous growth than that of the control (without crude oil), though at 3% and 5% plant exhibit reduction in the growth. GC-MS data analysis confirms the presence of some hydrocarbon compounds in the experimental plant roots. The amount of TPH was observed to be decreasing and the mean value of the HUB count was observed to be increasing along with time.

**KEY WORDS**: ***Ricinus communis***, **soil remediation, crude oil contamination, rhizodegradation.**

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# 1. INTRODUCTION

Crude oil in one form or another has been used since ancient times. In today's world crude oil plays a very important role across our society, influencing the economy, politics and technology. Crude oil is the most important source of hydrocarbon of our time[1]. Naturally, crude oil is found in certain areas of the world. For refining purpose it is needed to be transported from one place to another. A very obvious consequence of all the transportation activities is the accidental spill of the oil into both land and water[2] .The contamination of the environment (terrestrial and aquatic) by crude oil is referred to as oil pollution[3]. Among the North Eastern states of India, Assam has a unique position regarding exploration and production of crude oil. Crude oil pollution is one of the regular phenomenon in the oil drilling sites and in the areas through which the pipelines carry the crude oil[4]. Crude oil pollution has been reported to have deleterious effect on plant germination and seedling growth[5]. Presence of crude oil in the soil makes the condition of the soil unsatisfactory for plant growth[6] due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron and zinc[7]. Frequent crude-oil spillage in agricultural soils, and the resulting adverse effect on all forms of life, renders the soil (especially the biologically active surface layer) toxic and unproductive. The oil reduces the soil's fertility such that most of the essential nutrients are no longer available for plant and crop utilization[8]. Although several methods for reclamation of contaminated soil are available, phytoremediation is considered to be an alternative green technology for remediation of hydrocarbon contaminated soil. Phytoremediation is a method which utilizes plants to degrade, stabilize and/or remove soil contaminants. It is a cost effective, environmental friendly and carbon neutral approach .Studies conducted by Palmroth *et al.*(2002) shows that root exudates from plants help to degrade toxic organic chemicals and acts as substrates for soil microorganisms in the soil, which directly results in increased rate of biodegradation of the organic contaminants[9]. Various studies have shown that different types of plants are found to be useful for phytoremediation of hydrocarbon contaminated soil. Alfalfa and horse radish were found to reduce kerosene based jet fuel concentration in soil by 57-90% in 5 months[10]. Peng *et al.,* reported that *Mirabilis jalapa* L. removed 41.61-63.2% total petroleum hydrocarbon from contaminated soil[11]. Studies by Vwioko *et al.,* (2005) shows that *Ricinus communis* can grow in spent lubricating oil contaminated soil. The study also emphasizes the necessity to evaluate the performance of *Ricinus communis* in revegetation of cleaned up oil polluted soils[12]. A study conducted on six different species of plants including the castor plants shows that it has a very good tolerance to crude oil contaminants[13]. Castor plant (*Ricinus communis*) belongs to Euphorbiaceae family .It is a fast growing C3 plant, native to tropical Africa. It is a very important industrial crop as its oil is used for plant based industries for making eco-friendly paints and coatings used in chemical industry[14]. Castor is also found to be able to grow to in heavily polluted soil and also capable of metal ion accumulation and has fast growth rate[14]. In addition, castor is an industrial crop with multiple non-food uses and an excellent rotation and companion crop. It has an economic advantage as a cash crop in modern agriculture along with remediation of heavy metal contaminated soils [14,16]. India is the world's largest exporter of castor oil. Many studies show that oil obtained from various sources like soybean, sunflower ,corn ,palm etc can be used for biodiesel production .However, non edible sources like castor oil provide a better alternative source for biodiesel production in a more economic way .Castor oil is available at low cost. Reports suggest that castor can be grown on marginal lands, which are usually unsuitable for cultivation of food crops. All these features has made castor a very attractive biodiesel feed stock[17]. This study aims to evaluate the growth response and remediation applicability of Castor oil plant *(Ricinus communis)* to crude oil contaminated soil,.Castor plant is selected because of its characteristics as non edible plant which can grow in tropical areas and its commercial viability for biodiesel production.

# 2. MATERIALS AND METHODS

### *2.1. Growth study*

Crude oil required for the growth experiments were collected from the R&D Department of Oil India Limited, Duliajan, Assam. For conducting the growth experiments of castor plant, seeds of two local varieties of castor were collected from the areas in and around Guwahati city. The viable seeds were selected by floatation method. Seeds were soaked in water for 12 hours for priming; the primed seeds were sowed in two separate pots for germination. Seeds germinated at about 7 to 10 days. The healthy seedlings of uniform sizes were selected and transferred to the experimental pots and their growth was observed for a period of 180 days. Experimental pots with three different concentrations of crude oil were prepared by mixing measured amount of composite soil with 1%, 3% and 5% (weight of oil/weight of soil) concentrations of crude oil ( A,B,C signify 1%, 3% and 5%percentage concentration of crude oil in case of red variety and E,F,G signify the same in case of green variety). The soil was mixed with crude oil manually. After mixing, the soil was transferred to polythene bags having perforations at the bottom, then it was allowed to stabilize for four days before transplanting the castor seedlings. The control set of pots were prepared without crude oil. Each set of treatment was replicated three times. The pots were watered every alternate day. The design of the experiment was completely randomized one. One way ANOVA was carried out using SPSS software. For observing growth behavior, the following parameters were taken into account: plant height, stem girth, leaf area, fresh and dry weight of the plants and initiation of flowering. Plant height was measured using a meter rule from the soil level to terminal bud. Stem girth was measured by wrapping a thin non elastic thread around the stem of the plant at soil level and its length was determined. Leaf area was determined by comparing the weight of the cut out traced area with standard paper of known weight to area ratio using the following relationship[12].

***LEAF AREA (specimen) = (SPECIMEN WT×STANDARD AREA) / STANDARD AREA WEIGHT***

For obtaining fresh weight the castor plants were weighed immediately after uprooting at the completion of the experimental period. For recording dry weight, the plants were oven dried at 65ºC until constant weight is obtained[18]. Initiation of flowering was observed from the days after plantation of the seedlings.

***2.2. Extraction of the root tissue***

After the whole experiment i.e. completion of 180 days, root tissue of the treated castor plants was extracted using Soxhlet apparatus in DCM (Dichloromethane) for 12 h. Then the extracts were analyzed using GC-MS for presence of crude oil originated hydrocarbon. This was done to determine if the root tissues of the crude oil treated plants absorbed crude oil or any of its fractions from the soil.

***2.3. Sampling***

***A. Microbial count***

The soil samples were collected from the rhizosphere of the experimental plants including the control plants at an interval of 30 days for assessment of the hydrocarbon degrading bacterial (HUB) counts. This was done by plating a serially diluted1g of soil on oil agar media fortified with 1% (v/v) crude oil and incubated at 30ºC for 72 h. The colonies on each plate were counted and recorded as colony forming unit per gram of soil (CFU/g).

***B. Total petroleum hydrocarbon of the experimental soil***

Soil samples were collected from the experimental pots for extraction of total petroleum hydrocarbon at an interval of 30 days, for the experimental period of 180 days to measure the degradation. It was done by suspending 10g of soil in 20 ml of DCM in a 250 mL capacity Erlenmeyer flask. After shaking for 1h on an orbital shaker the solvent oil mixture was filtered into a beaker of known weight. After complete evaporation of the solvent the new weight of the beaker containing the residual oil was recorded. Percentage biodegradation of used crude oil was determined by the following formula, [19]

**% Biodegradation = [Wt of oil (control) - Wt of oil (degraded)] / Wt of oil (control) × 100/1**

## 3. RESULTS AND DISCUSSION

***1. Growth study***

The castor plants grown in different concentrations of crude oil were monitored for the experimental period of 180 days. They were mainly observed for any changes in the appearance compared to that of the control plants. No plant deaths were recorded in case of 1% and 3% crude oil treated plants. However, some of the plants at 3% and 5% crude oil treatment showed signs of phytotoxicity such as yellowing of the leaves and stunted growth when compared to the control set of plants. These observations were found to be in line with the findings of Agamuthu *et al.*,(2010) and Vouillamoz & Mike (2009)[19,20]. In case of red variety castor plants mean plant height observed are depicted in the Fig 1**.** The highest values are observed at of 1% treatment. The lowest values were observed in 5% crude oil treated plants. The mean height of the 3% crude oil treated plants was found to be slightly smaller than that of control. The difference between mean height of control and 5% treatment was found to be statistically significant (p=0.05).The difference between mean height of 1% crude oil and 5% crude oil treatment were also found to be significant. The castor plants of green verity also showed a similar pattern of findings. The highest mean height was observed in 1% treatment. In this case also the difference between mean height of the plants at 1% and 5% treatment was found to be statistically significant. The critical differences calculated showed that response of the plants in case of both the varieties, in terms of height with respect to concentration of crude oil in soil can be graded as 1>0(control)>3>5**.** These results showed similarity with the findings of Vwioko *et al.*,(2005)[12]*.* Regarding the plant height, the mean differences between the control and 1% treatment were found to be non significant.

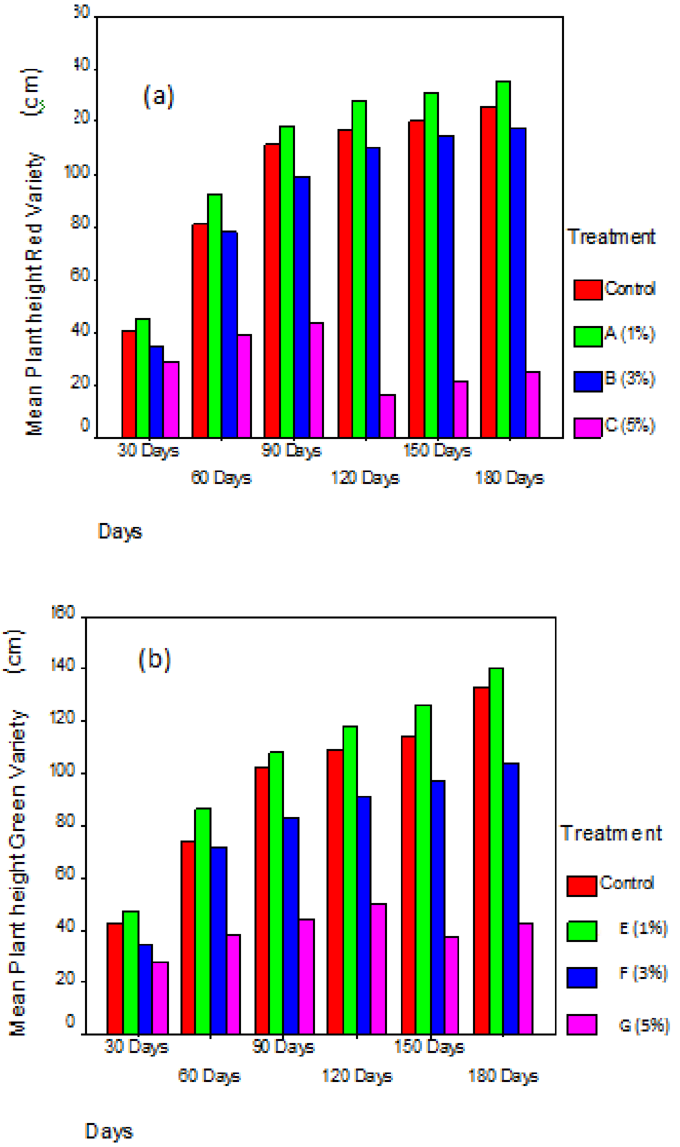
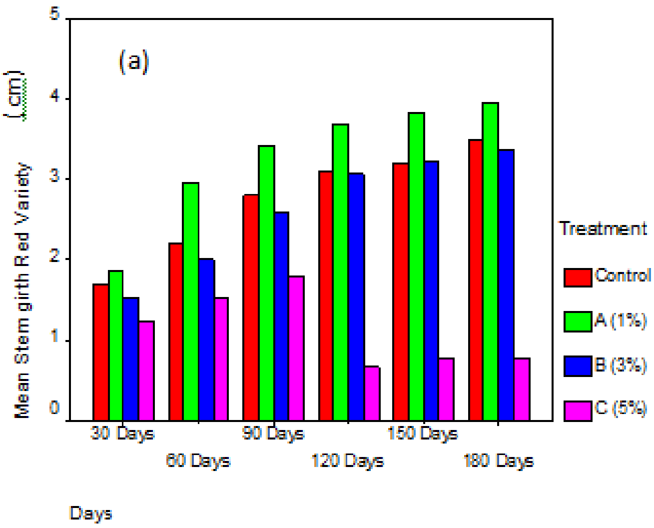



**Figure 1**
***Mean plant height of Ricinus communis. in cm, (a).Red variety, (b).Green variety***

Plant mortality was recorded in case of both the varieties at 5% crude oil concentrations of soil. When stem girth of the castor plants were compared to the control plants, the lowest values were observed in plants at 5% crude oil treatment. When statistically analyzed the differences in mean values of stem girth of 5% treatment plants were found to be significant when compared to that of the 1% treatment as well as control plants. However when the mean values of control and 1% treatment were compared, the differences were found to be non significant. Both red and green varieties show similar patterns of findings. Fig 2 shows the pattern of stem girth of castor plant in both the varieties.



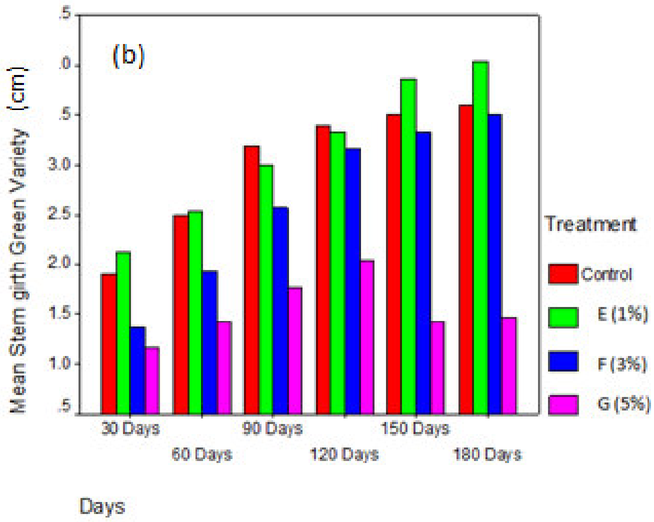




**Figure 2**
***Mean stem girth of Ricinus communis. in cm ,(a).Red variety, (b).Green variety***

The data regarding the leaf area shows that the crude oil concentration in soil (w/w) resulted in the decrease in leaf area. The highest mean value for leaf area was observed in the castor plants grown in soil at 1% crude oil concentrations (both green and red varieties) at 120 days after plantation. The mean values obtained at 3% crude oil concentrations as found to be higher than the control plants (Table 1)(Fig 3). In case of both the varieties the lowest values were obtained from the plants grown at 5% crude oil concentrations.

**Table 1(a)**
***Mean of Leaf Area of Ricinus communis in cm$^2$, Red variety***

| Mean area in cm$^2$ | | |
|---|---|---|
| | 60 days | 120days |
| control | 49.05 | 85.11 |
| A(1%) | 122.93 | 176.64 |
| B(3%) | 83.84 | 91.33 |
| C(5%) | 18.66 | 8.73 |

**Table 1(b)**
***Mean of Leaf Area of Ricinus communis. in cm$^2$, Green variety***

| | Mean area in cm² | |
|---|---|---|
| | 60days | 120days |
| control | 49.61 | 89.34 |
| E(1%) | 127.90 | 177.60 |
| F(3%) | 80.14 | 89.66 |
| G(5%) | 23.63 | 26.02 |

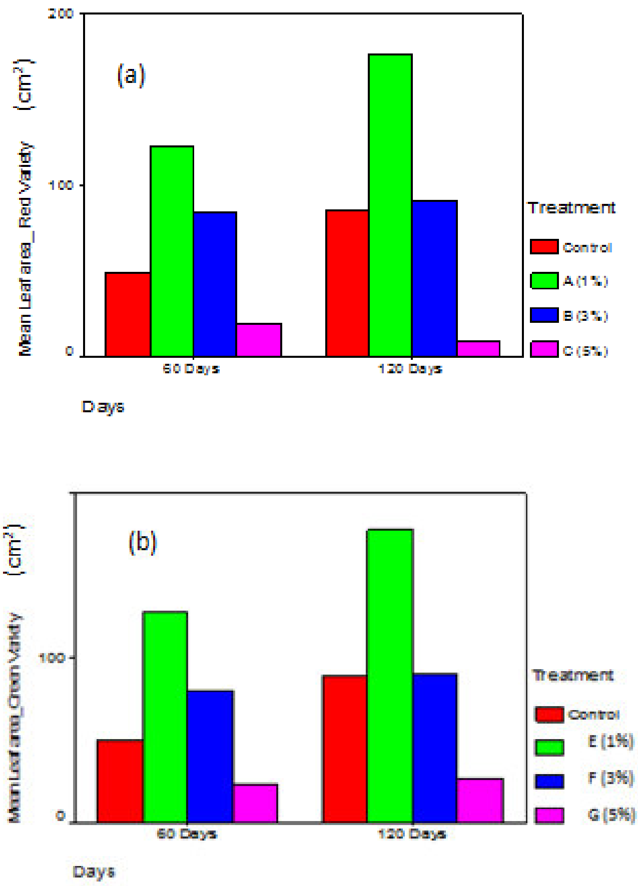



**Figure 3**
***Mean leaf area of Ricinus communis.***
***(a).Red variety, (b).Green variety.***

Adverse effect of crude oil content in soil also result s in reduction in biomass production. In the present study the results shows that at 3% and 5% crude oil concentrations biomass produced were less than the control. Table 2(a), 2(b) shows the data of biomass production. During the experimental period, flowering in the plants were first observed in the plants at 1% crude oil treatment. The plant grown at 3% treatment and control, flowers were observed later than 1% crude oil treatment. The castor plants grown in 5% treatment failed to produce flowers. These results were similar for both the green and red varieties.

**Table 2(a)**
***Mean of Biomass of Ricinus communis. in grams, Red variety***

| | Fresh wt(g) | Dry wt(g) |
|---|---|---|
| control | 138.00 | 43.26 |
| A(1%) | 149.84 | 60.88 |
| B(3%) | 78.75 | 25.47 |
| C(5%) | 11.19 | 3.20 |

**Table 2(b)**
***Mean of Biomass, of Ricinus communis. in grams, Green variety***

| | Fresh wt (g) | Dry wt (g) |
|---|---|---|
| Control | 142.73 | 47.31 |
| E(1%) | 163.25 | 53.95 |
| F(3%) | 80.47 | 26.08 |
| G(5%) | 23.53 | 6.50 |

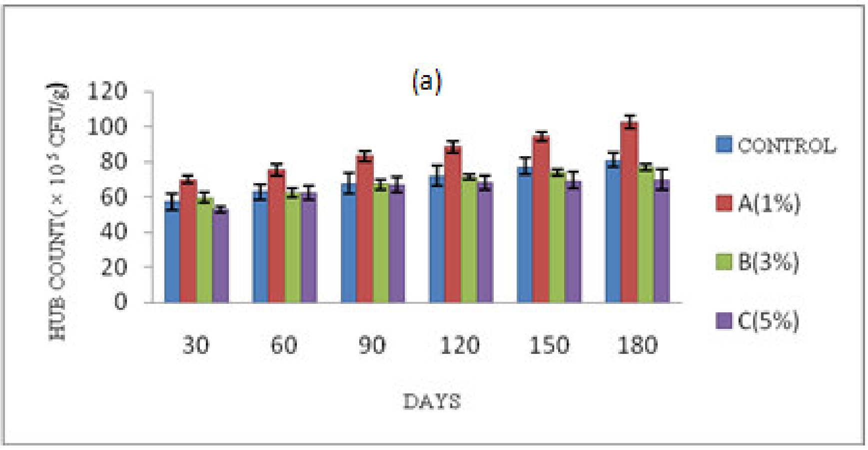
From the analysis of the growth data, which include plant height, stem girth, leaf area and biomass production, it is clear that at 1% crude oil (w/w) concentration in soil, the castor plants showed more vigorous growth than that of the control set of plants. But at higher crude oil concentrations of 3% and 5% (w/w),the castor plants showed signs of phytotoxicity and a reduction in the growth when compared to the control and 1%treatment. These results are in conformity with the findings of Vwioko *et al*. (2005) whose study on castor plants grown in spent lubricating oil showed that at1% concentrations SLO has stimulating effect on the growth of the plants[12]. Anoliefo *et al*. (2000) also reported a similar effect at 1% SLO on the growth rate of *Solanum melogena.* But this effect on growth was not observed for the other plants species like *Capsicum annum, Solanum lycopersicon, Abelmosclus esculentum* and *Solanum incanum.* This growth stimulating effect of crude oil at 1%(w/w) concentrations may be because of the fact that the rhizosphere micro flora of the castor plant can degrade crude oil at lower concentrations, thereby increasing the organic matter of the soil which in turn stimulate the growth of the castor plants. But crude oil at higher concentrations of 3% and5% level resulted in altered soil physical properties which have a negative impact on aeration and water holding capacity of soil[22]. Because of which, the castor plants at 3%and 5% crude oil concentrations showed reduction in growth. McCown *et al*., (1972) stated that the disruption of the soil physical properties by crude oil with anaerobic and hydrophobic condition was found largely responsible for reduction in plant growth[23].

### *3.2 Uptake of crude oil by castor plant*

The castor plants of each treatment after 180 days were Soxhlet extracted to determine if there was any phytoaccumulation of hydrocarbons in the roots. GC-MS analysis of the extracts detects the presence of trace fraction of petroleum hydrocarbon in the roots. These trace amounts does not signify any phytoaccumulation. This observation is in the line with the findings of Chaineau *et al*., (1995), who did not observe any uptake of hydrocarbon by the maize roots[24]. Phytoremediation studies using *Jatropha curcus* also shows that no phytoaccumulation of hydrocarbon in the roots of the experimental plants[19]. But again this is in sharp contrast with the results of Palmroth *et al*., (2002). According to his findings phytoaccumulation of diesel fuel occurred in grass roots.

### *3.3. A. Hydrocarbon utilizing bacterial counts*

Figure 4 shows the Hydrocarbon Utilizing Bacterial (HUB) counts in the rhizosphere soil of the experimental plants. The HUB count was found to be highest at 1% crude oil treated soil and lowest at 5% crude oil treated soil. HUB count is found to be directly proportionate to the plant growth parameters in this case.



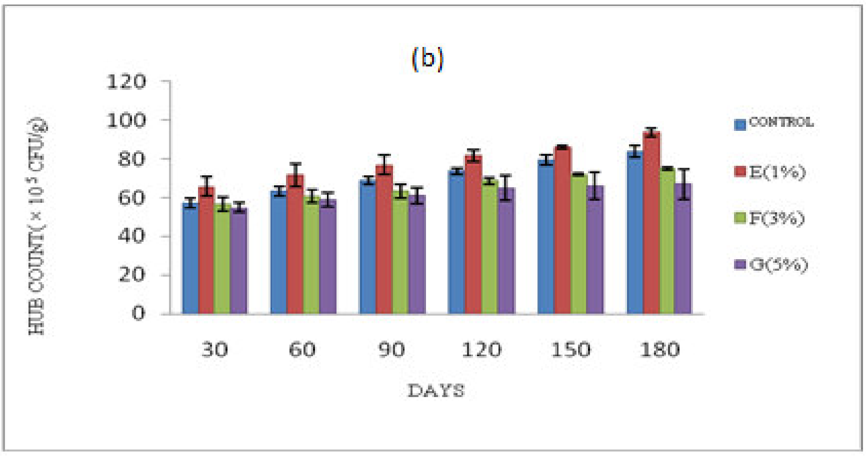




**Figure 4**
***Hydrocarbon utilizing bacterial (HUB) count in rhizosphere soil of Ricinus communis (a).Red variety, (b).Green variety***

Studies conducted by Jing *et al*., (2008) showed that the microbial population of the rhizosphere soil increased three orders of magnitude compared to the unplanted soil[25]. Various other authors also reported from the previous studies that the number of soil microorganisms increases in presence plant species. Because the plant rhizosphere provides optimal conditions for bacterial growth[26, 27, 28, 29]

***3.3. B. Loss of Total Petroleum Hydrocarbon (TPH) in the experimental soil***

As shown in Figure 5 the percent loss of total petroleum hydrocarbon at the end of 180 days is highest in case of soil at 1% crude oil concentrations (when compared to that of the concentration of the crude oil before treatment i.e. before plantation of the castor seedling). The lowest values were observed at 5% concentrations, which clearly show that biomass production is directly proportionate to the percent degradation of the crude oil from the soil. These results are similar for both red and green varieties. Previous studies have shown that degradation of petroleum hydrocarbon in planted soil was more than unplanted soil. This was because of the fact that the degradation of petroleum hydrocarbon in the planted soil was stimulated by plant roots[25]. Muratova et al., (2008) in their studies reported that up to 52% loss of TPH during three years of rye cultivation[30].

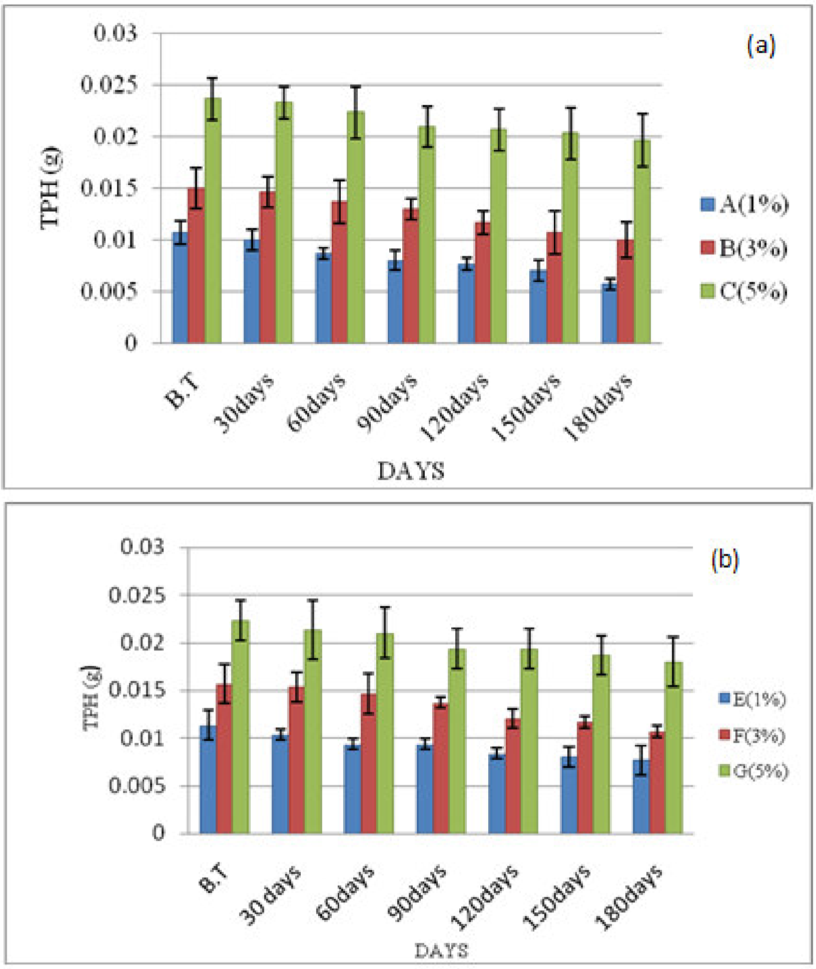



**Figure 5**
***Loss of total petroleum hydrocarbon in rhizosphere soil of Ricinus communis***
***(a) Red variety, (b).Green variety.(B.T – Before Treatment)***

## 4. CONCLUSION

It may be concluded that the two local varieties of the castor plant (*Ricinus communis)* which were considered for our study has the potential to withstand a minimum degree (1% and 3% w/w) of exposure to the crude oil in soil. However, no significant amount of petroleum hydrocarbon was detected in the root tissue of the plant. But it is found that the rhizosphere of the plant supports the growth of hydrocarbon degrading bacteria. This suggests that oil loss from the soil might be due to rhizodegradation. This may provide us an alternative method for removing crude oil contaminants from the polluted soil while promoting seed production which definitely has great economic importance.

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