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Biodegradation of Crude Oil Contaminated Soil in Sudan Using Sudanese Plant *Acacia seyal* Del

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Phytoremediation is an environmental technology in which plants are used for decontamination of organic and inorganic pollutants from soils and water. *Acacia seyal* Delile was tested for it is ability to degrade crude oil contaminated soil in four concentrations 0.5, 1, 1.5 and 2% (w/w) of crude oil. Plant parameters, degradation percentage, retention time and bacterial count were calculated. Results showed that at the end of the growth period of *A. seyal* Del no such adverse effect was observed on the shoot length and it is better than that of the control at the 2% concentration by the end of the growth period. This result highlights the ability of *A. seyal* Del to grow under stress of oil contamination. *A. seyal* root length has substantially increased at two and four month's interval and no significant difference was observed at these two intervals. *A. seyal* Del shoot biomass was significantly ($p<0.05$) negatively affected by crude oil at four month intervals, Root weight of *A. seyal* Del have decreased steadily. Degradation percentage was found to be in the range between 49-54%. Penadecane is the first compound appear in most cases and the retention time was between 13.7-32.9 min. Number of compounds detected in the rhizosphere those were 57, 70 and

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167 compound after two, four and six month of growth respectively. Viable count of the dominant bacteria showed that there is no significant different between concentrations (P=0.699), also there is no significant difference between intervals (P=0.08) in bacterial number. This study reveals the visibility of using tropical plant *Acacia seyal* in crude oil degradation.

Keywords: Phytoremediation; botany; Acacia seyal; Sudan.

1. INTRODUCTION

"The petroleum hydrocarbons are some of the most universally detected organic pollutants in the environment because of the high industrial use of petroleum products worldwide. The world petroleum consumption in 2001 was 77 million barrels per day, this scale of use results in a high potential for contamination from both accidental and fugitive releases. For example, in the United Kingdom alone there are estimated to be 120,000 contaminated petrol station sites with an associated remediation cost of £2.5 billion. The remediation of contaminated oil terminals and refinery sites will increase this figure. The US petroleum industry alone spent $0.8 billion dollars in 2001 on remediation" [1]. "The large scale and economic importance of this contamination has resulted in a significant effort in developing remediation technologies for its clean-up. Phytoremediation is one of the remediation technologies that had been developed to address this problem. Phytoremediation is advantageous when a low-cost solution is needed that can be easily applied to diffuse sources of contamination. This is typical of the large sites used by the petrochemical industry. Such sites are usually in places that do not have significant value for redevelopment (where economic pressures require a rapid clean up) so it is usually a matter of reducing liability. Phytoremediation can also be used to reduce the leaching of contaminants through soils, and hence, protect the groundwater where guidelines for contaminant levels are more stringent. This technology has also been used to prevent off-site migration of contaminants in groundwater. Finally, within the petrochemical industry there is recognition of bioremediation strategies such as land farming, so gaining acceptance for phytoremediation should be easier than in other commercial enterprises" [2].

Tiwari et al. [3] reported that "phytoremediation of a contaminated soil becomes a 'win–win' strategy if it is done with such plant species which are not only efficient in removing the pollutants but also have industrial application and generate large amount of biomass in form of food, fodder and biofuel. *A. seyal* Del have phytoremediation capability in soil and water medium. The biodiesel derived from the tree species is energy efficient and meets the requirement of international standards". Sudan is rich of indigenous normal flora and can be tentatively used in different environment in the field site in Sudan. The aims of this study were to use native plant species *A. seyal* to remediate soil pollution with crude oil. Therefore the main objectives of this study were to: Evaluate the effect of vegetation establishment on remediation of crude oil –contaminated soil , and determine the effect of the concentration of crude oil, in addition to analyze soil microorganisms.

2. MATERIALS AND METHODS

The role of the selected tree species and their associated microorganisms in cleaning up oil-contaminated soil was studied in a series of pot experiments and laboratory tests.

2.1 Selection of Plant Species and Collection of Seeds

A. seyal Delile which dominated the Savanna Zone. Seeds of this tree species were kindly donated by the Forest Research Center, Soba, Khartoum" Sudan."

2.2 Sowing of Seeds and Transplanting of Seedlings

Seeds of the selected tree species were sown in sand soil contained in 50x70cm trays and watered daily for two months, before they were transplanted to polyethylene bags (29x20cm) containing clay soil contaminated with different crude oil concentrations. Clay soil were air dried, passed through 2 mm-screen sieve and mixed manually with electrical stirrer. The soil was divided into five lots and to each of the first four lots crude oil was added to make a concentration of either 0.5, 1.0, 1.5 or 2.0%. The fifth lot was left as a control in which seedlings of the selected tree species were raised in oil-free soil. The experiment was laid out in Complete

Random Desgin with three replicates. In all cases, seedlings were watered daily for eight months.

At two months intervals, seedlings in three out of the nine bags allocated for each tree species at specific concentration were uprooted and measurement such as shoot height, root length and fresh weight of shoot and root were determined. Petroleum hydrocarbon fractions expected to accumulate in tissues and organs of each plant species at each petroleum concentration were determined using GC-MS. Also, at these intervals, total petroleum hydrocarbon was determined in rhizospheric soil contaminated with different concentrations of oil. Moreover, samples of rhizospheric soils taken from underneath each plant species under study at each crude oil concentration were taken for microbial analysis. In each case data were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS19) [4].

2.3 Chemical and Microbial Soil Analysis

Harvesting of Plant species was conducted every two months, plastic container were opened, plants were air -dried after measurements, soil samples were taken for microbial analysis and stored at 4 ºC. Soil samples were also taken for Total Petroleum Hydrocarbon (TPH) extraction and determination.

2.3.1 Extraction and determination of TPH

After removing the roots, the soil in each container was homogenized and stored at room temperature until further processing. Total oil was determined according to the US Environmental Protection Agency (EPA) 1994. Fifteen grams of soil were transferred into paper extraction thimbles and placed into a Soxhlet apparatus containing dichloromethane for eight hours. The solvent was evaporated through rotary evaporator and the dry weight of the extract was determined. Percentage of total oil was calculated based on soil dry weight as follows:

$$\%\text{Total oil} = \frac{\text{Weight of residual oil} \times 100}{\text{Weight of the initial oil}}$$

2.3.1.1 Fractionation analysis of extracted oil

Dichloromethane solvent containing hydrocarbon was cleaned up according to Weisman [5]. Four mL n- Hexane was added to 1g silica gel 60A with pore size of 40-63µm mesh and with high purity. The samples were dissolved in more n-Hexane to 10 mL, stirred thoroughly and filtered through Whatman No 1 filter paper .The solvent was allowed to evaporate then one mL more n-Hexane was added and filtered again into GC-MS tube using 0.45 mm syringe filter for GC-MS analysis. Blank sample of crude oil was cleaned up using the same process [6].

Fraction analysis of the degraded hydrocarbon were performed using (HP) 5890 Gas Chromatography connected to Mass Spectroscopy devise according to EPA 8270 [7]. Chromatographic conditions were: column 25 m×0.2 mm×0.33 (-m) ULTRA 2; helium flow rate 1 ml min^{-1}, injector temperature 250ºC and detector temperature 280 ºC. The temperature program was set at: 40 ºC for 5 min, 4 ºC min^{-1} to 130 ºC hold for 2.2 min, increased by 12 ºC min^{-1} to 180 ºC, hold for 2.2 min, increased by 7 ºC min^{-1} to 300 ºC, hold 11:79 min. Qualitative analysis of samples was carried out by scanning the mass range between 35 and 550 amu, one run per sample. The interpretation of each spectrum was performed by comparison with the commercial NIST (National Institute of standard and Technology) database of spectra.

2.3.2 Isolation and bacterial count from oil contaminated soil

Starch Ammonium Agar was used for the isolation of potentially oil degrading microorganisms serial dilution method, one gram of oil- contaminated soil was transferred into nine ml of sterilized distilled water in a test tube. The contents were shaked vigorously to obtain the first dilution (10^{-1}). One mL of this soil water suspension was aseptically transferred to nine mL of sterilized distilled water to give the second dilution (10^{-2}). The third dilution was prepared in a similar way. One ml inoculum from each of dilutions 10^{-1}, 10^{-2} and 10^{-3} was aseptically transferred to a petri dish containing Starch Ammonium Agar for seven days. These media were prepared and used as suggested by Tepper et al. [8].

3. RESULTS

3.1 Effect on the Growth of *A. seyal* Del

The effect of crude oil on the growth of different part of *A. seyal* were observed and recorded as below:

3.1.1 Effect on shoots and root lengths

Shoot lengths of *A. seyal* seedlings grown at different oil concentrations are shown in Fig. 1 where it is evident that plant biomass was not negatively influenced by oil at four month interval. The plant height, at two month interval, was affected significantly at 1.00, 1.5and 2% oil concentrations. However, at the end of the growth period no such adverse effect was observed, instead the shoot length at 6 month interval was significantly higher than that of the control at 2% concentration. This result highlights the ability of *A. seyal* to grow under stress of oil contamination. Baker [9] and Merkl et al. [10] reported that oil contamination can stimulate growth of *Mimosa camporum* and *Staylosanthes capitata.* On the contrary, Merkl et al. [10] observed significant reductions in shoot length at 3% and 5% crude oil for most of the eight plant species studied in Venezuela.

Mean root length values of *A. seyal* Del seedlings grown in soil containing different concentrations of crude oil are shown in Fig. 2. In this figure it is clear that root length has substantially increased at four and six month intervals and no significant differences were observed within these two intervals. The root growth improved after the first two months, at for month interval the root length is better than the control and by the end of the growth period the root length at 2% concentration exceeded the control. It is also evident that at any particular interval a highly significant increase in root length values for seedlings grown in soil contaminated with 1.5% and 2.00% oil compared to those grown in oil free soil. In contrast to this, Muratova et al. [11] reported a significant inhibition in root development of ryegrass (*Lolium perenne* L.) when grown in oil -contaminated soil. Similar observation was also reported by Merkl et al. [10] for some legume species.

3.1.2 Effect on shoot and root fresh weight

A. seyal Del seedlings fresh weight at different intervals in different oil concentrations is shown in Fig. 3. The fresh weight of *A. seyal* Del was significantly ($P<0.05$) negatively affected by crude oil at four month interval. There were no significant differences in shoot fresh weight among the different levels of oil. It should be emphasized that *A. seyal* Delile have well tolerated crude oil stress at all levels tested during the first two months of growth. Similar reductions in shoot weight have been previously observed for species with tap root system compared to species with fibrous root system.

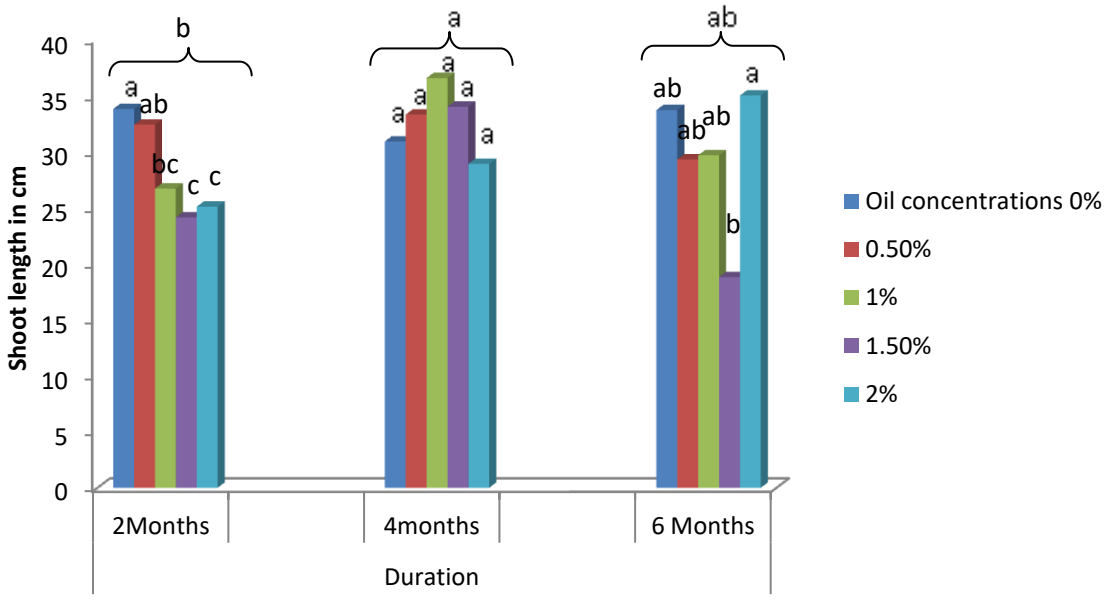

Fig. 1. Shoot length of *A. seyal* Del seedlings grown at different crude oil concentrations

a: there is no significant different between this concentration and others letters b and c

ab: there is no significant different between this (ab) concentration and (a)from side and (b) concentration from other side

And this can be within the group and between major groups

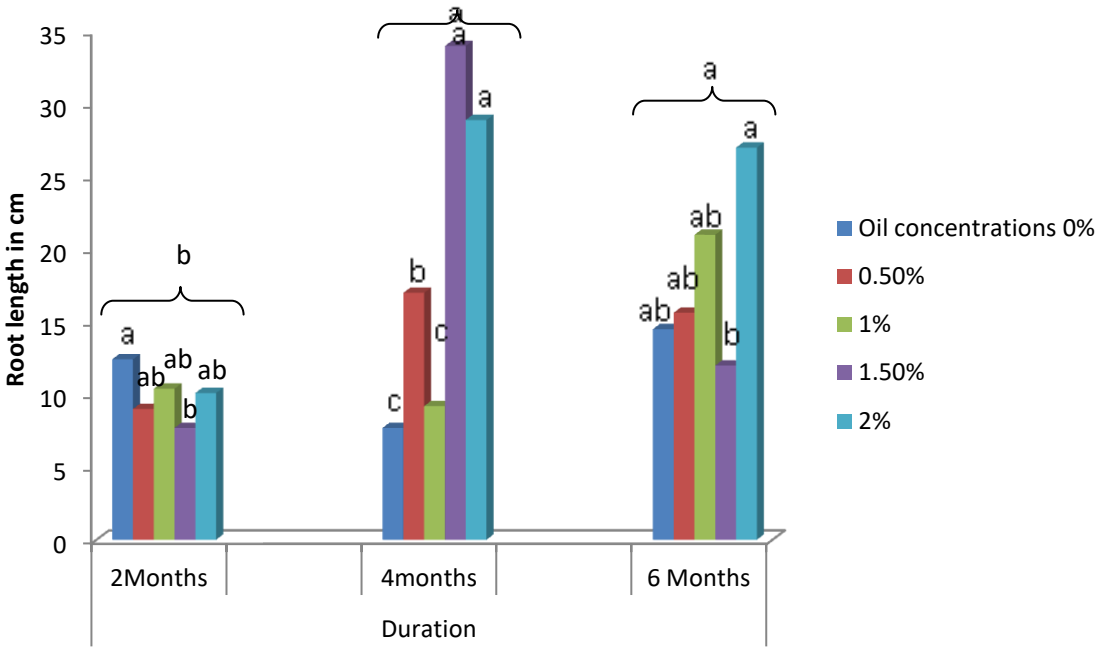

Fig. 2. Root length of *Acacia seyal* Del seedlings grown at different crude oil concentrations

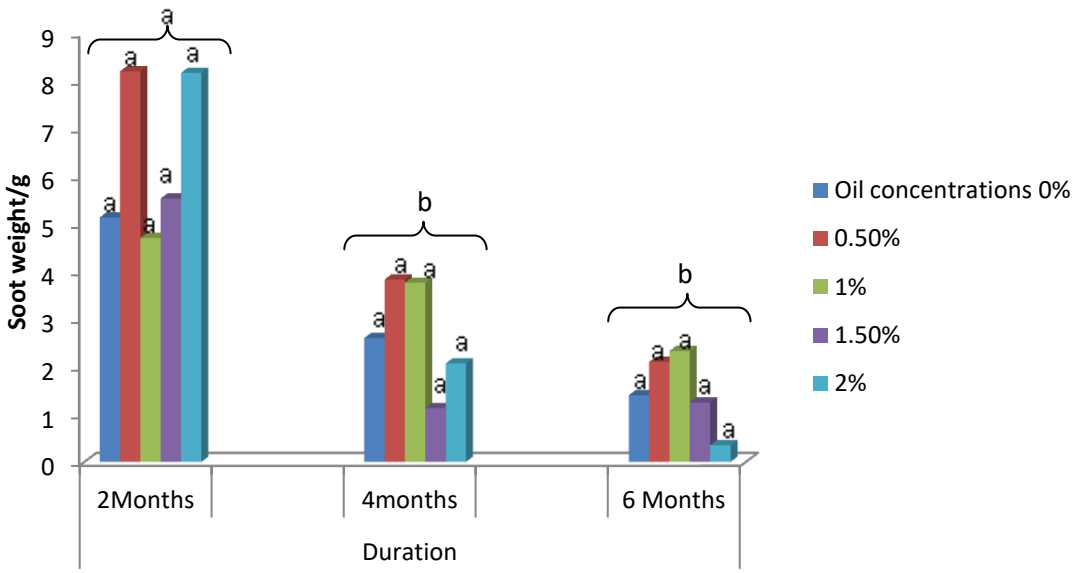

Fig. 3. Shoot weight of *A. seyal* Del seedlings grown at different crude oil concentrations.

Root biomass of *A. seyal* Del have decreased steadily during the growth period (Fig. 4). No significant differences were observed in root weights at different levels at the two month and six month intervals. However, significant differences were observed for the four month interval. The significant reduction in root biomass under oil stress have been previously reported by Parrish et al. [12] for *Melilotus officinalis* and *lolium perenne*.

3.2 Chemical Analysis

3.2.1 Extracction and determination of total oil

Petroleum hydrocarbon residue was determined in soil samples collected after 180 days from underneath *A. seyal* Delile. seedlings and hydrocarbon degradation percentages was 54% comparing to the control 49%. Mathur et al. [13]

reported that "crude oil (Total petroleum hydrocarbons, TPH) was reduced by 30% in the rhizosphere soil of *Prosopis cineraria* plant and by 16.8% and 13.7 in the rhizosphere soil of *Acacia senegal* and *Acacia nilotica* plants respectively".

3.2.2 Fractionation analysis of extracted oil

Individual petroleum hydrocarbons were determined in soil contaminated with 0.5% crude oil and planted with *A. seyal* Delile seedlings. At zero time and then at intervals of two, four and six months of growth, samples were taken and analyzed by GC-MS, results are shown in Figs. 5 and 6.

3.2.3 Compounds detected in and around plant used for phytoremediation

Number of compounds detected in the rhizosphere of 0.5 % contaminated soil those were 57, 70,167 and 106 compound after two, four and six month of growth and at zero time respectively. Retension time of the first compound appear, number of compound detected in the crude oil residue, abundance of the compound, first compound appear and degradation percentage were recorded from GC-MS analysis in the three intervals two, four and six month as in Table 1.

3.3 Microbial Analysis

Viable counts of dominant bacteria in the rhizosheric oil contaminated soil were determined at different intervals using Starch Ammonium Agar (SAA) Fig. 7. Results showed there is no significant different between concentrations (P=0.699), also There is no significant difference between intervals (P=0.08). Muratova et al. [11] showed that "for alfalfa (*Medicago sativa*), the rhizosphere effect for PAH degraders was larger than that for heterotrophic bacteria and, thus, the legume facilitated a larger increase in the number of pollutant degraders than it did in the number of bacteria capable of utilizing other carbon sources".

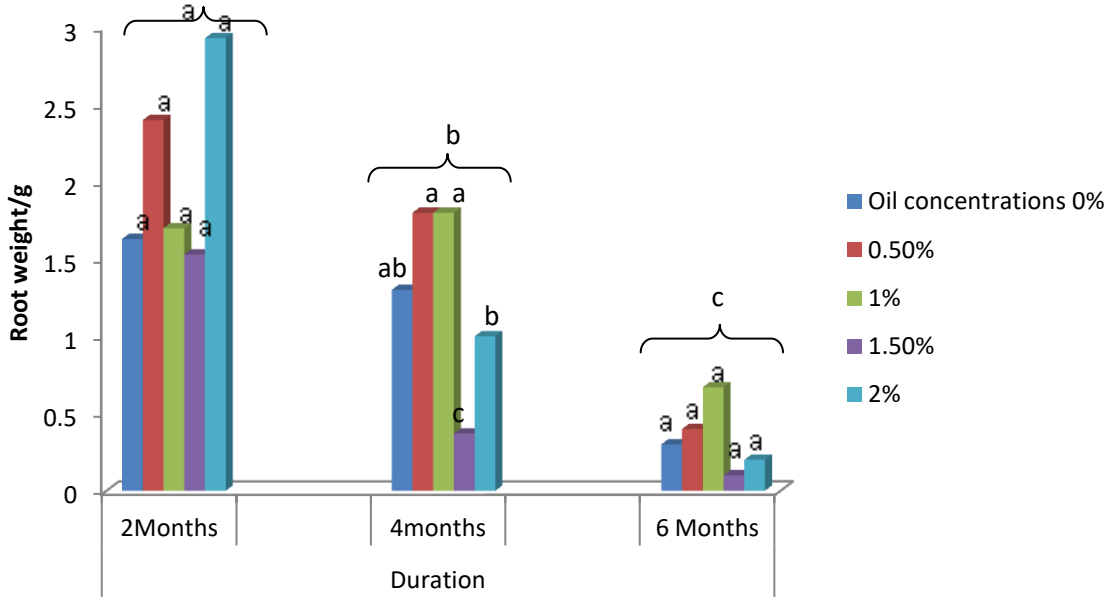

Fig. 4. Root weight of *A. seyal* Del seedlings grown at different crude oil concentrations

Table 1. Compounds detected at the rhizosphere of *Acacia seyal* Del after 60,120 and 180 days

Species	R.T (min)	No. Compounds	Abundance	First compound	Area %	Occurance percentage	Degradation %
1T5A	32.903	57	1600000	Pentadecane	0.98	96	60
2T5A	32.891	70	1800000	pentadecane	0.52	98	33.3
3T5A	13.798	167	4500000	Octane	0.13	91	20
1T5F	32.903	32	1000000	Pentadecane	1.32	96	20

Species	R.T (min)	No. Compounds	Abundance	First compound	Area %	Occurance percentage	Degradation %
2T5F	32.897	30	630000	Pentadecane	2.35	96	73.3
3T5F	19.551	38	680000	Benzene 1,2,3,4,5 tetramethyl	2.85	94	47
1T20A	32.903	72	2200000	Pentadecane	0.16	96	50
2T20A	32.891	99	3600000	Pentadecane	0.33	98	63.3
3T20A	15.068	168	6000000	Decane	0.12	95	27
1T20F	30.420	30	800000		0.65	0	90
2T20F	32.891	69	4400000	Pentadecane	0.18	97	73.3
3T20F	15.515	220	6800000	Decane	0.17	95	60

(1- after 2 months 2 four months 3- 6 months-Talih is Arabic name-5,20g(0.5%,2.0%) the concentration of crude oil-A around the root-F far from the root)

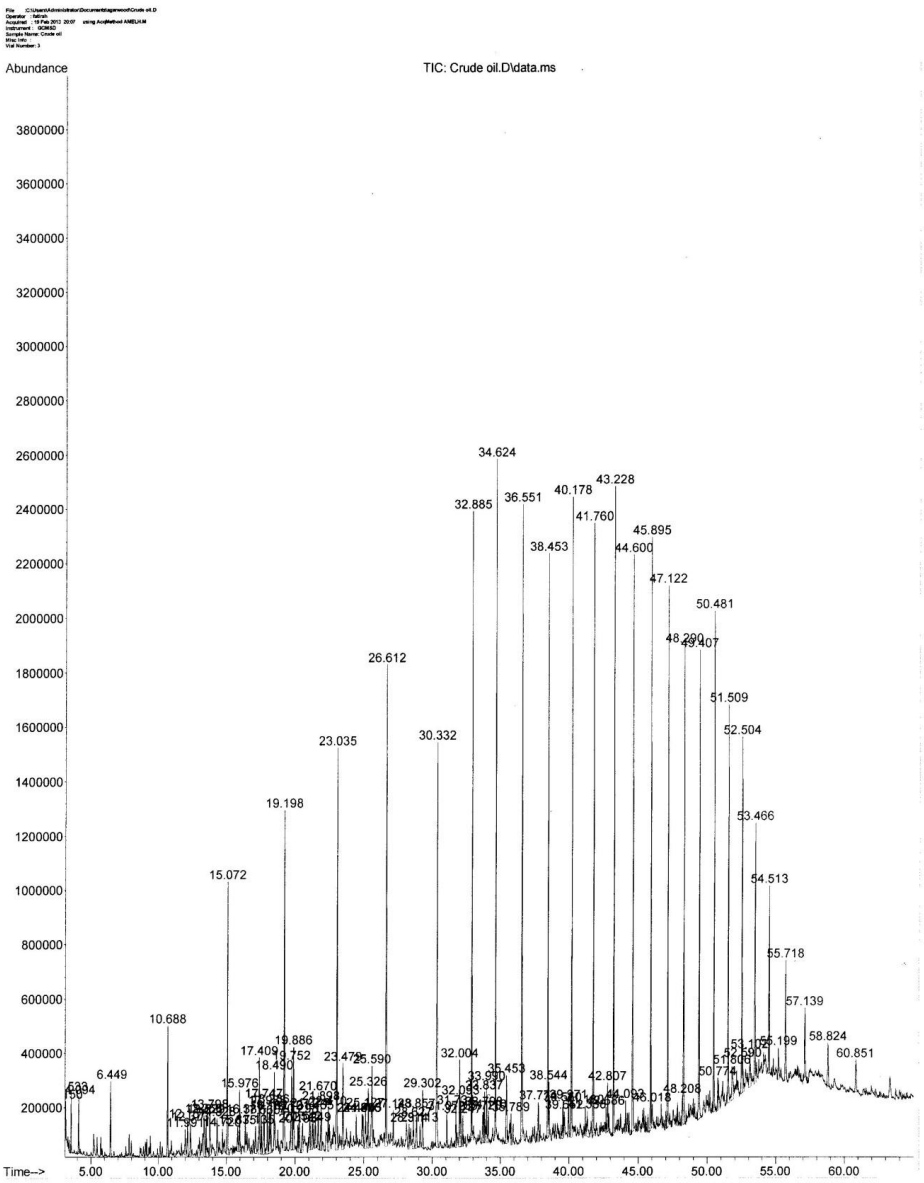

Fig. 5. GC-MS chromatograph of crude oil at zero time: (Clear file attached)

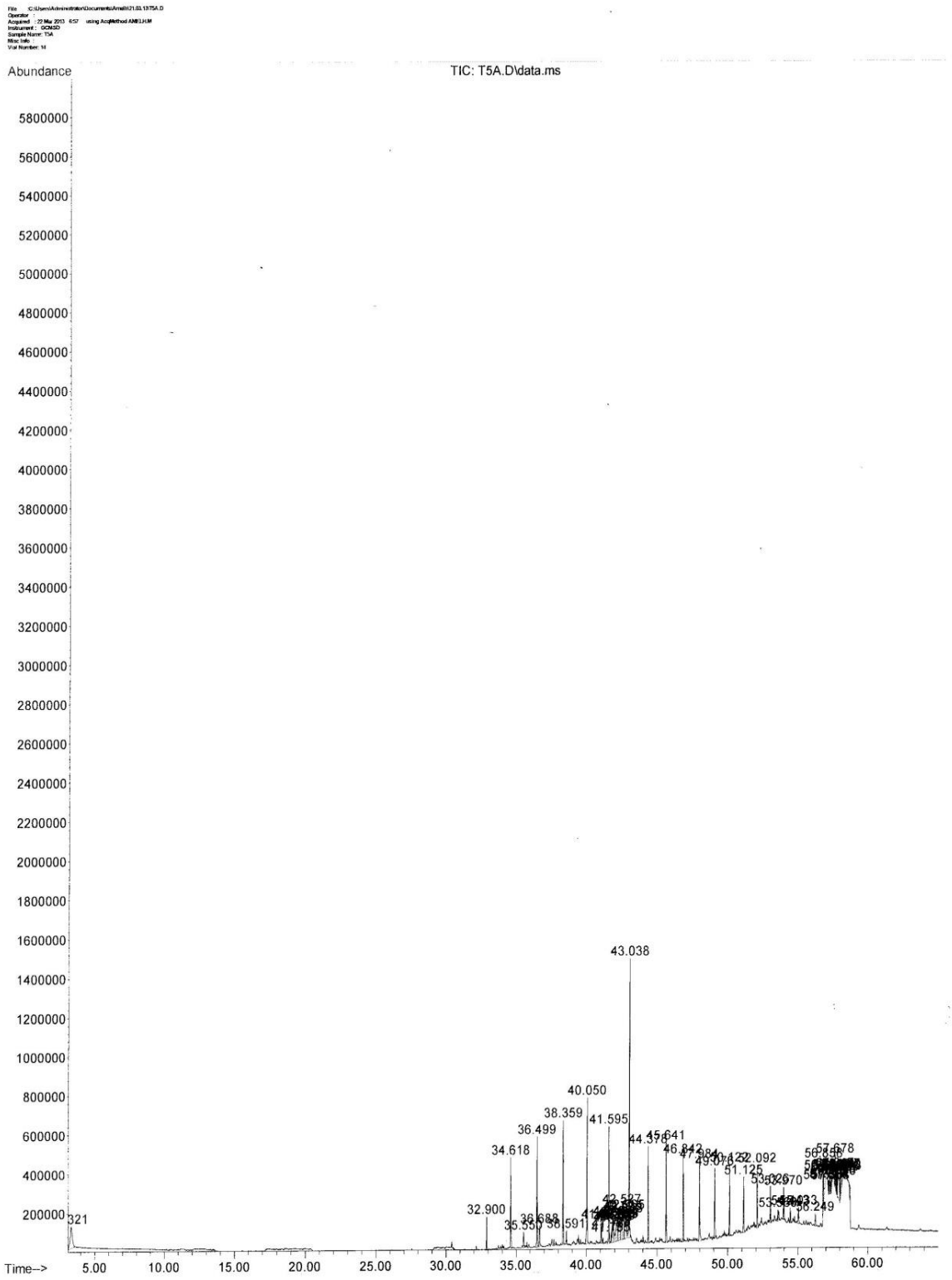

Fig. 6. GC-MS chromatograph of *Acacia seyal* in 0.5% oil after two months

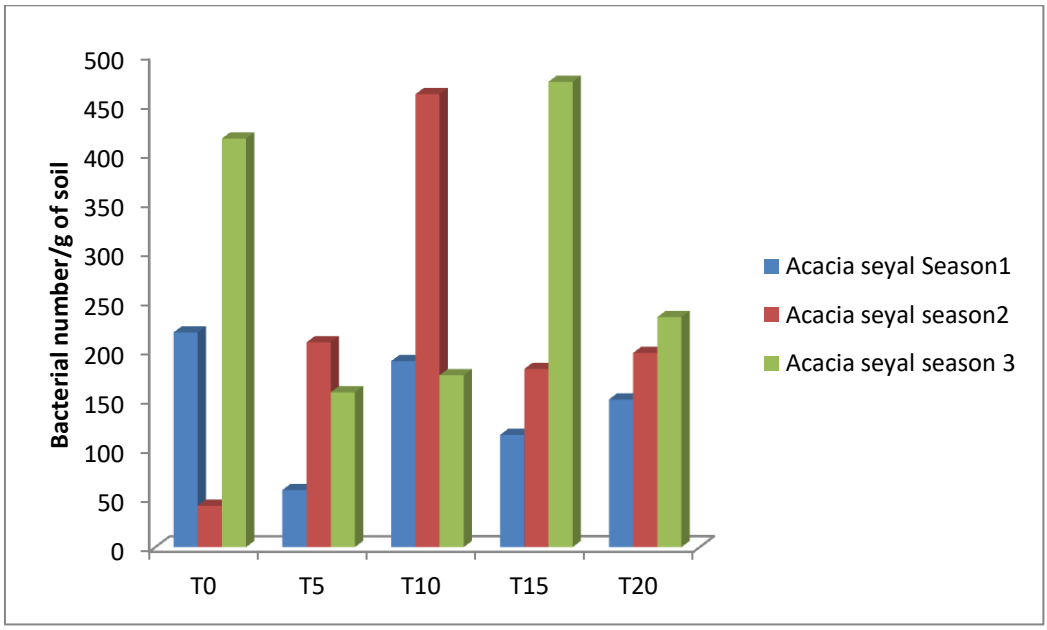

Fig. 7. Number of colony forming units per gram of *A. seyal* rhizospheric soil in SAA medium at three sampling intervals

4. DISCUSSION

At the end of the growth period of *A. seyal* Del no such adverse effect was observed on the shoot length and it is better than that of the control by the end of the growth period .This result highlight the ability of *A. seyal* Del to grow under stress of oil contamination. Merkl et al. [10] the species *Mimosa camporum* showed a small but significant increase of shoot growth at both oil concentrations compared to the control. *A. seyal* Del root length has substantially increased at two and four month's interval and no significant difference was observed at these two intervals. In contrast to this, Merkl et al. [10] A significant reduction of root growth in contaminated soil 3% and 5% was found for all legumes except for *Desmodium glabrum* and *Mimosa camporum* in Phytoremediation study in Venezuela in eight species. *A. seyal* Del shoot biomass was significantly ($p<0.05$) negatively affected by crude oil at four month intervals. Agbogidi et al. (2007) reported plant height, fresh biomass of the test plants *Tectona grandis* and *Gmelina arborea* were significantly ($P\geq0.05$) affected at higher levels of oil treatment (10 and 15%). Root weight of *A. seyal* Delile have decreased steadily by the end of the six month interval. Reduction in Root biomass under oil stress has been previously reported by Unterbrunner et al. (2007) in their phytoremediation study indicated the biomass production of all plants was reduced by up to >60% because of crude oil phytotoxicity except for poplar (*Populus nigra maximowiczii*).

5. CONCLUSION

This result highlights the ability of *A. seyal* Del to grow under stress of oil contamination. The excellent growth of this plant indicates the benefit of phytoremedation and vegetation establishment on the degradation of crude oil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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