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RESEARCH

THE ROLE OF PHYTOREMEDIATION IN REDUCTION OF ENVIRONMENTAL POLLUTION

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ABSTRACT

Petroleum hydrocarbons are among the most important environmental organic pollutants and their existence in nature has caused many concerns due to toxicity, carcinogenic and genetic modification. These pollutants can absorbed to surface of soil particles or organic particles in the soil and gradually increase their concentration and enter surface water along with surface currents. There are several chemical and physical methods to deal with oil pollution in soil, most of which are used less due to high costs and harmful side effects. Thus, biology techniques such as phytoremediation have attracted more attention in recent years. Phytoremediation is a new and emerging technology in which resistant plants are used to remove or reduce the concentration of organic and inorganic pollutants and hazardous substances from the environment. This technique contains a series of technologies using natural or transgenic plants for refining of organic and inorganic environmental pollution of soil, water and air. In fact, phytoremediation using human interference such as agricultural technology will create right conditions for growth and plant establishment and will increase the normal activities of cleaning. The greatest advantage of this method compared to other methods is its low cost and simplicity. Appropriate plant selection is very important in this method which depends on climatic conditions of area, type and rate of soil contamination. Khuzestan province is considered as one of the most important centers of Iranian oil with vast oil and gas reserves and massive amounts of pollutants are transferred to this province due to the fact that it is near Persian Gulf. Crude oil well No. 69 of Marun 3 Oilfield which was sprayed to soil as oil contaminant in this study was injected into gas chromatography device in Microliter after extraction from soil with Soxhlet and bitumen asphalten. Each of the normal alkanes was calculated after presenting the GC peak of areas under the curve. Results of analysis of variance showed that using NPK fertilizer and regular aeration during modification process coincided with the growth of heterotrophic bacteria degrading oil had a significant effect on oil decomposition on the level of five percent. 37% of oil decomposition was observed in the control soil which has been due to regular aeration of oil and creation of 25-30 ° C soil temperature and humidity suitable for bacterial activity.

KEY WORDS
Phytoremediation, Environmental Pollutants, Heterotrophic Bacteria, Oil Pollution, Khuzestan Province.

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INTRODUCTION

The relation of today's humans with environment has faced a crisis. This crisis in the environmental is due to irrational intervention and exploitation and utilitarian destruction and leads to harmful effects for humans and the environment. Use of phytoremediation in remediation of any contaminated system that plants can grow in it has been recently considered. Purified plant are a collections of plants which are used to remove organic materials, metals, pesticide residues and residues of radioactive substances from soil or waste. This Herbal Technology can be used both independently and in combination with other purification methods. According to this definition, a purified plant must accumulate have at least 100 mg / g (10% of dry weight), cadmium, arsenic and some rare metals, 1000 mg / g (0.1 percent dry weight) cobalt, copper, chromium, nickel, lead, and 10000 mg / g (1% of dry weight) of magnesium and nickel in its tissues. Phytoremediation using green plants engineering including Herbaceous and woody plants is used to remove pollutants from soil and water or reduce the risk of environmental pollutants such as heavy metals, trace elements, organic compounds and radioactive materials. Unlike water and air, soil contamination is not easily measurable in terms of chemical composition and a clean or pure soil cannot be defined. Thus, we have to study the potential problems of soil contamination in the context of the risks and possible damages in soil function.

In phytoremediation, plants are classified based on adsorption mechanism and pollution with heavy metals is reduced using chemical, physical and biological methods. Based on researches of Environmental Protection Agency Office of Water and soil pollution, soil decontamination is usually carried out with two methods of in place and out of place. In out of place method, the contaminated soil is shifted to another place and returned after decontamination. In another method which does not require shifting, biological absorption of pollutants decreases by making them organic. Green plants and their relation with soil microorganisms are used to reduce soil and groundwater contamination in technology of using plants as phytoremediation. This technology can be used to remove both organic and inorganic pollutants in the soil. The main objective of this research was feasibility and evaluation of potential of soil decontamination form cyanide using non-woody plants such as grasses and seeds. In addition, the rate of accumulated cyanide in the plant was evaluated in comparison with the amount of destroyed cyanide by plant or other environmental and climate factors. Results of this research can be used by industries, especially gold extraction mining industry for natural treatment of containing cyanide and cleaning soils contaminated with cyanide in addition to expanding knowledge of phytoremediation [1].

Lee et al. (2002) stated that root developmental zone which is rhizosphere has more and more active microbial population compared to soil without root. Plants are able to release nutrients and fluids through the soil and transport oxygen to the root zone and Stimulate and enhance microbial activity damaging pollutants [2].

Schwab and Banks (1994) also noted the further reduction of total concentration of petroleum hydrocarbons in the presence of vegetation in comparison with the control without vegetation. Ferro et al (1999) also reported the significant reduction of Payrn, chrysene and fluoranthene in the presence of plant with permanent grass in their in greenhouse studies. Ayotamon et al (2006) concluded in the study about effects of phytoremediation of agricultural soil contaminated with crude oil in Nigeria that applying fertilizers will increase biodegradation of oil-contaminated soil and reduce hydrocarbon pollutants by 50 to 95 percent. The effect of applying fertilizer on stimulation of biological processes and increasing the biological treatment in Soils Polluted with crude oil were studied in this research [3].

METHODS

Firstly, agricultural soil was passed through 2 mm sieve after air-drying and then crude oil of well No. 69 of Marun oil field with is paraffin oil was sprayed on the soil to the ratio of 1 wt% in a way that soils were completely homogeneously contaminated by oil. All of the soils were accumulated after inoculation and were stored for 2 weeks for uniform distribution and adsorption on soil particles. Contaminated soils were treated in the next step. 3 fertilizers including urea fertilizer with 26% nitrogen, ammonium phosphate dioxide with 21% nitrogen and 46% phosphorous and potassium sulfate with 40% potassium were used in order to apply chemical fertilizer. NPK Chemical fertilizers were mixed in the ratio of 10: 10: 20 for N: P: K according to three fertilizer treatment levels. Nitrogen fertilizer was split into 2 phases and potash and phosphorus were given at the beginning of experiment. The treated soils were placed in greenhouse under controlled light and temperature conditions and phytoremediation process was studied there for 10 weeks [4]. Soil moisture was kept at field capacity (F. C) during this period in for activity of crude oil order to create condition degrading and soils were stirred 2 times a week to alleviate the lack of oxygen and create aerobic soil condition [5, 6]. CHROMAFIL CA / S 045 micron syringe filters were used in order to sterilize the crude oil.

MPN method was used in the next step is to count heterotrophic bacteria which is decomposition of hydrocarbons. In this method, 3 g of soil sample which has been collected at the right time is mixed with 10 ml of Bushnell Haas environment in order to create suspension after preparing the medium and ml of suspension is added to 9 ml of culture medium after half an hour and a series of diluted solution is prepared from 10-1 to 10-12 using this method. Resazurin reagent and then sterilized crude oil were added to each tube to the rates of respectively 90 microliters and 0.2 ml and prepared tubes were incubated for 2 weeks. After two weeks of regular evaluation of tubes, when changing of color form blue to pink was observed (which shows the heterotrophic bacterial growth), tubes were removed from the incubator and bacteria were counted based on MPN table [5].

In order to evaluate oil change, Oil present in the sample was initially extracted by Soxhlet and chloroform organic solvent and was injected into gas chromatography in microliter after asphalten. The initial temperature was 50 ° C and the final temperature was 320 ° C and temperature rise in it was 5 ° C per minute. Rock Evil and Kjeldahl method were used respectively in order to measure total organic carbon and total nitrogen content [7]. These experiments were carried out in form of completely randomized factorial statistical design with three replications. A total of 25 pots were used per treatment in this study. Soils of 20 pots were polluted by cyanide and soils of 5 pots were used as control group without contamination with cyanide. Clay loam and grain loam textures of soil was used. There were not plants in 6 pots contaminated with cyanide and the aim of experimenting these pots was evaluation of effect of irrigation and atmospheric factors on reduction of soil cyanide. Variables of pots were: the initial cyanide concentration added to the soil (C) at five levels of C1 to C5, Purification agents (M) with five levels of M1 to M5 and type of plant (P) with three levels of P1 to P5. Description of different levels of variables of initial cyanide concentration, purification factors and type of plant are mentioned in (Table 1).
Two plants including Fescue (lamb pasture grass) with endophytic and without endophytic and Sorghum were selected in this research after necessary studies on useful and available plants. These two plants have history of usage in phytoremediation of other pollutants and have good tolerance against unfavorable conditions [8].

Soil of each pot was infected with a known concentration of potassium cyanide (KCL). Planting of all pots was done on a specified day. Previously amplified plants were used for planting of fescue and seed was used for sorghum. Plants were irrigated simultaneously with about 760 cubic centimeters of water per irrigation and with the same frequency. All plants in pots were taken out of soil after 98 days along with their roots and were stored in sealed envelope after drying and turning into powder. Soil in the pot was also crushed and mixed after drying so that it becomes thoroughly uniform and was then stored in sealed envelope [9].

Samples were distilled in order to measure total cyanide in soil and plant samples in accordance with the procedures of standard method and then colorimetric method was used for measuring cyanide using spectrophotometer [10]. Measured Cyanide in the soil was in terms of milligrams of cyanide per kilogram of soil (mg CN / kg soil) and in terms of milligrams of cyanide per kg of dry plant (mg CN / kg dry biomass) in plant. The accuracy of measuring of used device (spectrophotometer) to test the cyanide was 0.001.

Table 1: statistical summary of data related to measuring cyanide existing in soil and plant after removal of plant

Statistical parameters	Existing cyanide (mg/kg)		Ratio of existing cyanide compared to the initial value	
	soil	plant	soil	plant
Number of absorptions	60	36	60	36
Maximum (100%)	7.71	14.34	99.2	78.41
Median (50%)	1.49	0.09	49.58	0.80
Minimum (0%)	0.01	0.01	2.9	0.01
Range	7.30	14.34	96.3	78.4
average	2.21	1.89	48.84	18.04
SD	2.16	3.45	27.47	26.55

Statistical analysis of data collected in this study was done using statistical software such as SAS, SPSS and Excel. Methods of analysis of variance (ANOVA, Analysis of Variance) were used to evaluate the significance effect of variables as well as their interaction on soil cyanide removal and efficiency of cyanide accumulation in plants [10].

RESULTS

This experiment was done in form of split plot design with three replications in completely randomized design. The main factor of fertilization treatment was with 3 levels of 0, 1 and 2 tons and the factor of time included 2 levels of 5 and 10 weeks as secondary factor. SAS software was used for data analysis. Mean comparison for significant factors was done using Duncan method.

As it can be observed in table 1, cyanide removal efficiency from soil has had many changes in a way that the highest amount of cyanide in soil is 31.7 and its lowest amount is 0.008 and its average is 2.212 Mg per kg of soil. The highest soil cyanide reduction is 99.2 percent and its lowest rate is 2.9 percent and its average is 48.4 percent. Also the maximum amount of cyanide accumulation in plant is 14.34 and its lowest amount is 0.001 and its average is 1.89 Mg per kg dry plant. The highest, lowest and average percentage of cyanide accumulation in plant is respectively 78.405, 0.0045 and 18.04.

Effects of two variables of initial soil's cyanide concentration with four levels of C1 to C4 and purification agents with five levels of M1 to M5 were on cyanide removal efficiency were evaluated in factorial model of cyanide removal and results analysis of variance of related data are shown in table 2. This statistical analysis was conducted at confidence level of 99% (1% significance level). F calculated for effect each variable on soil cyanide removal rate must be used with its critical F value and if the calculated F is smaller than the critical F, that variable has no significant effect on the cyanide removal efficiency and the observed difference is due to random errors. It can be observed in (Table 2) that both variables of initial cyanide concentration and "purification agents" as well as their interactions have had significant impact cyanide reduction efficiency. It also shows that Interaction between the initial concentration × purification agents is also significant but it is t less effective than main variables in the soil cyanide removal efficiency changes.

Table 2: Analysis and Variance of reduction percentage of cyanide in the soil

	Source of change	Degree of freedom	Sum of squares	Average squares	Calculated F	Critical F	P value
Model		19	39958.00	2103.05	18.47	2.73	<0.0001
	Purification agents	4	23218.0	5804.55	50.98	3.83	<0.0001
	initial cyanide concentration	3	10383.2	3461.07	30.4	4.31	<0.0001
	Purification x concentration	12	6356.6	529.72	4.65	2.66	<0.0001
Error		40	4554.36	113.86			
Total		59	44512.37				

We can compare different data averages related to different modes of variable in Fig.1. In different concentration of initial l soil's cyanide, the maximum efficiency of cyanide reduction has occurred in C1 concentration with the mean of 70.13% and its minimum rate has been in C4 concentration with average of 34.30 percent.

Also among different purification factors, the maximum removal efficiency of cyanide is in M3 (sorghum) which is equal to 76.742 percent and its minimum rate has been in M5 (without plants and irrigation) which has been equal to 16.085. These results show that Pots with plants had better cyanide removal efficiency than the unplanted pots and sorghum has played as better role in phytoremediation of cyanide-polluted soils compared to Fescue which is due to cyanogenic feature of sorghum.

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In factorial model of soil cyanide accumulation in plants, effects of tow variables including initial soil's cyanide concentration with four levels of C1 to C4 and type of plants with three levels of P1 to P3 were evaluated on percentage of soil cyanide accumulation in plants and only changes of type of plant made significant changes in the efficiency of cyanide accumulation in plants and changes of Cyanide concentration have no significant effect on the percentage of cyanide accumulation in plants. In addition, interaction between the percentages of the initial concentration of cyanide accumulation in plants does not have such difference at the level of 1%. Thus it was concluded that the increasing amount of initial soil cyanide concentration will not increase percentage of cyanide accumulation in plants. It can be observed that percentage of cyanide accumulation in plants has increased a little at low level of initial soil's contamination cyanide in a way that highest percentage of cyanide accumulation in plants has been in C3 concentration. It seems that C3 concentration has been the threshold of tolerance of plants and by increasing soil pollution more than C3 concentration cyanide accumulation in plant efficiency has reduced due to the incidence of cyanide toxicity of the plants. It has been observed in practice that Fescue dried and died slowly in C4 concentration. This observation again confirmed the fact that phytoremediation is an appropriate method for contaminated soils with low concentration. Also it can be observed with comparison of mean percentage of cyanide accumulation in different plants that the highest amount of accumulation has been in sorghum with 52.952 percent and its lowest amount has been related to fescue without endophyte with 0.448 percent. In pots with Sorghum, sorghum has had the main role in removal of soil cyanide with the highest concentration of cyanide but concentration of cyanide in plant is not significant in pots with fescue.

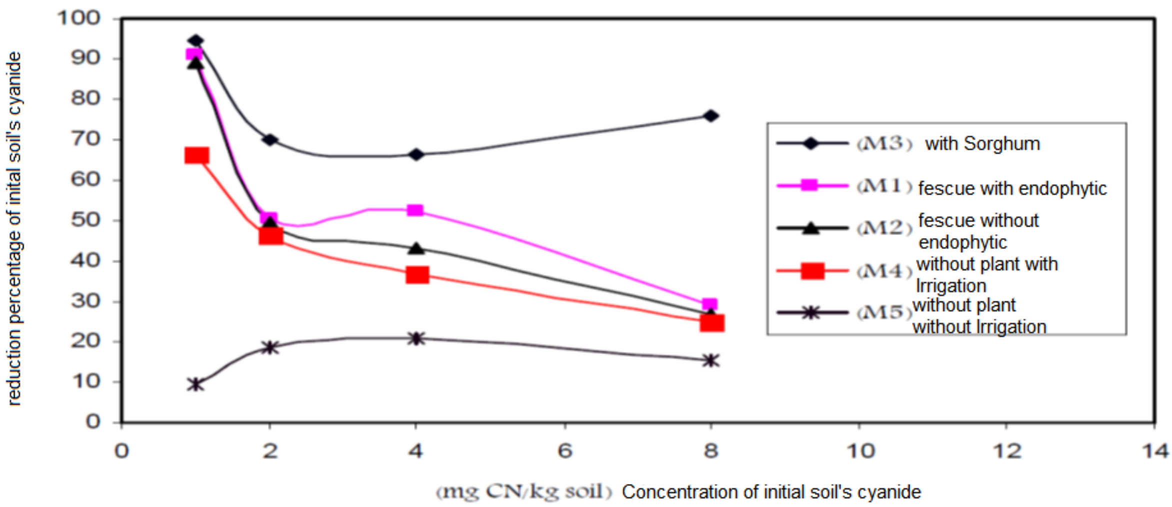

Fig. 1: Reduction percentage of soil cyanide in different forms of purification agents for initial cyanide concentration in soil

CONCLUSION

Results of analysis of variance showed that application of NPK fertilizer and regular aeration during the process of coincides reforming with increasing growth of heterotrophic bacteria have had significant effect as the level of five percent. 37 percent of oil decomposition was also observed in control soil which has been due to regular soil aeration and temperature conditions ((25-30°C) and suitable humidity for bacterial activity. The effect of time factor on oil decomposition was significant at 1% probability. It can be observed in (fig.3) that oil decomposition has slowed down with time and oil decomposition is more in fifth week compared to tenth week. This is entirely consistent with the growth of heterotrophic bacteria and highest bacterial growth has occurred in the fifth week. The activity of bacteria and oil decomposition has been probably more in first 5 weeks of treatment due to presence of rapid decomposition normal alkanes the abundance of mineral nutrition but over time, petroleum compounds which are difficult to decomposition, have long-chain and residual nitrogen deficiency remain and bacterial growth and thus oil decomposition reduce [11]. Oil decomposition increases with an increase in microbial population in the soil and this increase is faster during the first month of Treatment and this process decreases with time. Oil decomposition has statically significant difference in the samples treated with samples not treated at level of 1 percent. There is also significant difference between the average soil decomposition in the both time extraction of soil (5 and 10 weeks) and average oil decomposition in soil is higher in five weeks compared to ten weeks (Fig.2).

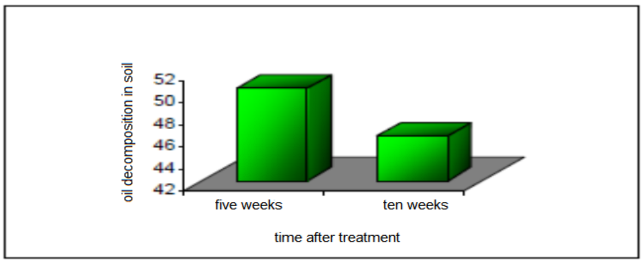

Fig. 2: Comparison of the average effect of oil decomposition in soil

Analysis of variance shows significant difference results between effects of different treatments with NPK fertilize compared to C / N of soil. Ration of C / N in the samples without treatment is higher than treated samples (Fig. 3) because the lack of mineral nutrients in control sample limits growth of hydrocarbon degrading bacteria and oil decomposition and C / N ratio rises. C / N ratio was about 3 times bigger in control samples compared to the treated samples.

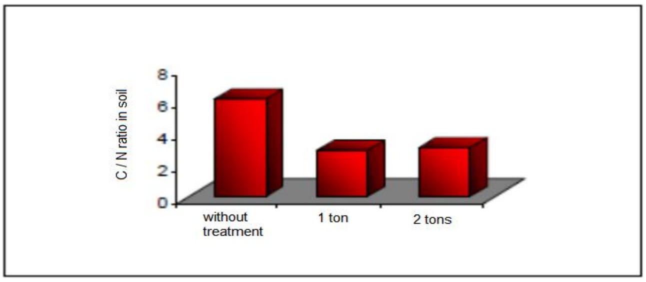

Fig. 3: Comparison of the average effect of chemical fertilizer treatments to C / N in soil

C / N ratio decreased with the passage of time of the treatment but this ratio is lower in fifth week compared to the tenth week because there are enough food available for bacteria in the fifth week but mineral deficiencies reduces over time and limit bacterial growth and oil decomposition. Average ratio of C / N in soil has statistically significant difference in treated samples and control group. This C / N ratio is higher than in the control samples compared to treated samples. It was observed in evaluation comparing the average effect of time on ratio of C / N in the soil that there is a significant difference at 1% probability in the soil at both decomposition times and C / N ratio is higher in 10th week compared to 5th week.

Results of Analysis of variance showed the significant effect of chemical fertilize on the growth of heterotrophic bacteria degrading petroleum in hydrocarbons contaminated soils at the level of five percent probability. There is a significant difference between the treated samples with control samples in terms of bacteria growth (figure 4). The growth of heterotrophic oil degrading bacteria has significantly increased due to the effect of treatment of mineral nutrients containing nitrogen, phosphorus, and potassium which increased oil decomposition and decreased C / N ratio. In fact, a major aspect of phytoremediation operation involves the use of nutrients such as nitrogen and phosphorus fertilizer, aeration use of hydrocarbon degrading microorganisms in contaminated areas [12].

In the present research, bacterial growth and oil decomposition are high at the fifth week due to normal presence of alkanes and environmental conditions and good nutrition but only aromatics and asphalts reaming in tenth week and Nutrient deficiency occurs which will reduce phytoremediation process. The average growth of bacteria in treated samples has statistically significant difference with control samples and bacterial growth is lower in treated samples compared to non- treated samples (figure 4). Evaluation of comparison of the mean effect of time on bacterial growth in the soil confirms that the growth of bacteria decreases with time.

Crude oil of well No. 69 of Marun Oilfield which was sprayed on soils in this study was injected into gas chromatography device in Microliter after extraction from soil with Soxhlet and bitumen asphalten. Each of the normal alkanes was calculated after presenting the GC peak of areas under the curve. Priest and titanium were used as internal standards [10]. In order to facilitate consideration of changes in normal alkanes, they were divided into six categories follows based on the proximity of their chemical composition: <C13, C13-C16, C17-C21, C22-C25, C26-C29, C29-C36.

As it can be observed, C13-C16 category has formed about 12% of the total crude oil and largest volume of normal alkanes and C26-29 category has formed the lowest volume with only 4% of crude oil. According to composition of crude oil which includes gasoline (C4-C10), kerosene (C11-C12), diesel (C13-C20), oils (C21-C40) and residues <C40, crude gasoline oil in this sample is 23% and non-cyclic isoprenoids of Priest and Phytane are respectively 1.34% and 1.55% of total oil.

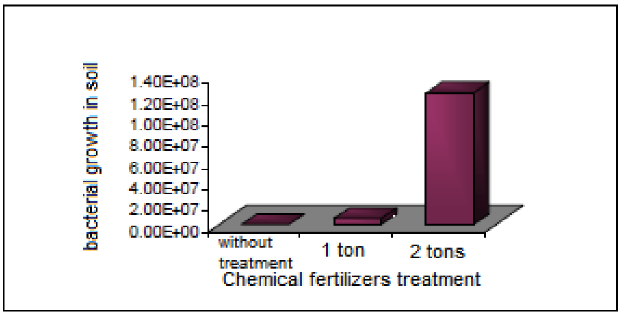

Fig. 4: Comparison of the average effect of chemical fertilizer treatments on bacterial growth in soil

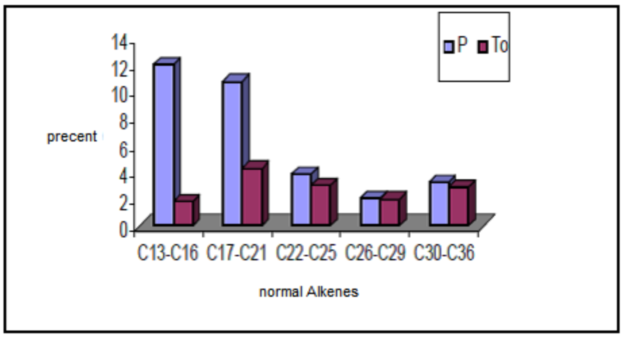

Fig. 5: Comparing the percentage of normal alkanes in the control sample (T0) with primary crude oil

Fig. 6 compares the effect of chemical fertilizer treatment in both 5 and 10 weeks on normal alkane's oil changes with the control samples. This figure shows that approximately 80% of normal alkanes C13-C16 are decomposed after the fifth week which shows the extent of consumption of hydrocarbons by bacteria. Extensive use of this category is due to short carbon chain and the abundance of mineral nutrition and environmental conditions at this stage but C17-C21 and C22-C25 categories have only had decomposition for 35-38 percent which shows Low consumption of medium-chain paraffins by studied bacteria due to low decomposition of odd normal alkanes in these two categories. These normal alkanes with terrestrial origin (horsetail) are decomposed in a small amount by oil degrading bacteria. The fourth and fifth categories which are respectively C26-C 29 and C30-C36 have been decomposed by 60% compared to the control samples.

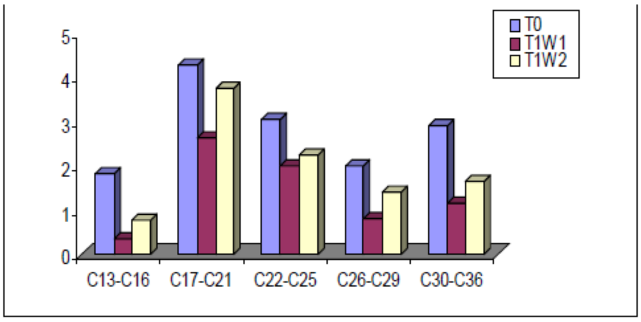

Fig. 6: Comparing the percentage of normal alkanes in the treated samples with one ton of chemical fertilizer in the fifth week (T1W1) and in the tenth week (T1W2) with the control sample (T0)

CONFLICT OF INTEREST

There is no conflict of interest.

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REFERENCES

[1] Geller A, Michels J, Track T, Gehrke U, and Sell D. [2002] Grundlagen derbiologischen Bodensanierung.
[2] Lee M., and Ward CH. [2002] Environmental and biological methods for the restoration of contaminated aquifers. Environ. Toxicol. Chem, 4:743-750.
[3] Ayotamuno M, J Kogbara RB. Ogaji SOT, and Probert SD. [2006] Bioremediation of a Crude – Oil Polluted Agricultural – Soil at Port Harcort, Nigeria. Applied Energy 85:1249-1257.
[4] Kim SH, Lee S, Kim DY, and Kim JG. [2007] Degradation characteristic of waste lubricants under different nutrient codition. Jaurnal of Hazardous Materials 143:65-72.
[5] ARamsay M, Swannell PJ, Duke W, A.Sh, and Hill TR. [2000]. Effect of bioremediation on the microbial community in oiled mangrove sediments. Marine Pollution Bulletin 41(7-12):413-419.
[6] www.magiran.com
[7] Ho Y, Jackson M, Yang Y, Mueller JG, and Pritchard PH. [2000] Ization of uoranthene and pyrene-degrading bacteria isolated. PAH- contaminated soil and sediments and comparison of several sph- ingomona ssp, J Ind Microbial, 2:100-112.
[8] www.civilica.com
[9] www.sid.ir
[10] Journal of Science and Technology of Agriculture and Natural Resources Soil and Water Sciences, 14(53) autumn [2010].
[11] Kim SJ, Choi DH, Sim DS, and Oh YS. [2005] Evaluation of bioremediation effetiveness on crude oil contaminated sand. Chemosphere, 59:845-852.
[12] Journal of Science and Technology of Agriculture and Natural Resources Soil and Water Sciences 11(42), winter [2007].