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ENHANCED BIOREMEDIATION OF ENGINE OIL POLLUTED SOILS BY NUTRIENT APPLICATION.

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ABSTRACT

Five experiments were done to study the effect of nutrient application to soils contaminated with engine oil on the stimulation of microbial activity and their biodegradation of engine oil. Adding a nutrient solution to soils taken at three different locations enhanced the microbial activity of the soil. This was shown through the increase of the first order reaction rate of the biodegradation process. The first order reaction rate increased for the three soils from zero to 0.014 (day $^{-1}$), from 0.0069 (day $^{-1}$) to 0.0139 (day $^{-1}$), and from 0.0211 (day $^{-1}$) to 0.0336 (day $^{-1}$), with the consequent decrease of the half life time from infinity to 48.8 days, 100 to 49 and from 32 to 20 for the three soils respectively.

Another experiment was done on the effect of adding more extra oil on the biodegradation of the engine oil. There was no effect in the earlier stages of degradation while it increased the biodegradation in later stages, probably due to the increase in count of oil bio-degraders from 5.7×10^6 to 2.0×10^8 and from 1.5×10^4 to 1.6×10^7 cfu g^{-1}, respectively after 3 weeks, during which about 56 % of engine oil was oxidized.

Four local different bacterial isolates were used to check for their ability in degrading the oil. There were large differences in the bacterial efficiency of bioremediation. The half life time decreased by about 60 %, 83 %, 13% for the first, second, third with comparison to the fourth strain which is least efficient. The half life time of the fourth one was 1.74 days.

The implications of this are discussed in the conclusions.

INTRODUCTION

Spent engine oil, which is also known as used mineral-based crankcase oil, is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain (ATSDR, 1997). Spent engine oil is a mixture of several different chemicals (Wang *et. al.*, 2000), including low and high molecular weight (C_{15}–C_{20}) aliphatic hydrocarbons, aromatic hydro-carbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products.

Large amounts of spent engine oil are liberated into the environment when the engine oil is changed and disposed into gutters, water drains, open vacant plots and farm lands, a common practice by motor mechanics and generator mechanics (Odjegba and Sadiq, 2002). In addition, the oil is also released into the environment from the exhaust system during engine use and due to engine leaks (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003).

The movement of these hydrocarbons through the soil and its fate is controlled by a myriad of physical, chemical and biological processes. The biodegradability of these compounds is greatly affected by their physical state and toxicity. In addition, the initial degradation of petroleum hydrocarbons often requires the action of oxygenase enzymes and so is dependent on the presence of molecular oxygen (Atlas, 1991). Aerobic conditions are therefore necessary for the initial breakdown of petroleum hydrocarbons. In subsequent steps, nitrate or sulphate may serve as a terminal electron acceptor but, oxygen is most commonly used.

Bioremediation is one of the most promising new technologies for cleaning up ground water and soil contamination because of its low cost and its potential for the complete destruction of pollutants. One important demand for the biotechnological concepts established at a dump site is the complete biodegradation of the pollutants (i.e. its conversion to H_2O+CO_2). Microorganisms require for its activity in soils a source of carbon and a source of energy and the availability of mineral nutrients, suitable temperature, pH, moisture etc.

Two engineered remediation approaches can be distinguished: (i) the stimulation of indigenous biodegrading organisms (biostimulation) and (ii) the introduction of organisms that manifest a desired activity which are not present at the contaminated site (bioaugmentation). The stimulation of the indigenous biodegrading microorganisms is the option chosen for and which could be done through securing the optimal conditions i.e. the availability of a carbon and energy source and all the chemical conditions needed for optimal activity of the indigenous microbial population i.e. nutrients, pH or aeration needed for optimal biodegrading activity of the resident micro-organisms.

The aim of the present study was to investigate the biodegradation of engine oil by the indigenous microorganisms present in the soil and the role of mineral nutrient application to enhance their bio-remedial activity. The effect of nutrients was quantified through a kinetic study of their effect on increasing the rate constants of the degradation reactions.

MATERIALS AND METHODS

Locations:

Two sites were chosen for the study;

1) The contaminated area is situated at Schlangenburg near Kronach (State of Bavaria, Germany). The area is an acid resin dump containing hazardous wastes including wasted mineral engine oil.

Soil sampling was done at four sites by sleeve core drilling to a depth of 2m. The tube core of soil was divided into segments of defined depths. The ram-cores drillings were designated as follows: SB4 (0.50. 0.8, 1.0, 1.5 and 2.00 m), SB5 (0.25, 0.5 and 1.00 m), SB6 (0.25, 0.75, 0.85, 1.0 and 1.20m) and SB11 (0.40 and 0.80 m) [Fig. 1].

Soil samples were carefully transferred to sterile 500-ml brown glass bottles with screw caps avoiding any contamination, quickly transported to the laboratory, and either immediately analyzed or stored at 4 °C until analysis.

Table 1: the characteristics of the tested German soil

Sample Name	Altitude (m)	Depth (cm)	Description
SB4	-6.0	0- 50 50-90 90-200	Humus Fine sand Sand Loam + Gravel Mixture
SB5	-5.5	0-25 25-50 50-100	Humus Sand Sand and Loam
SB6	-0.95	50-85 85-100 100-120	Sand and Loam Sand Sand and stones
SB11	-1.17	40-80	Loamy Black soil

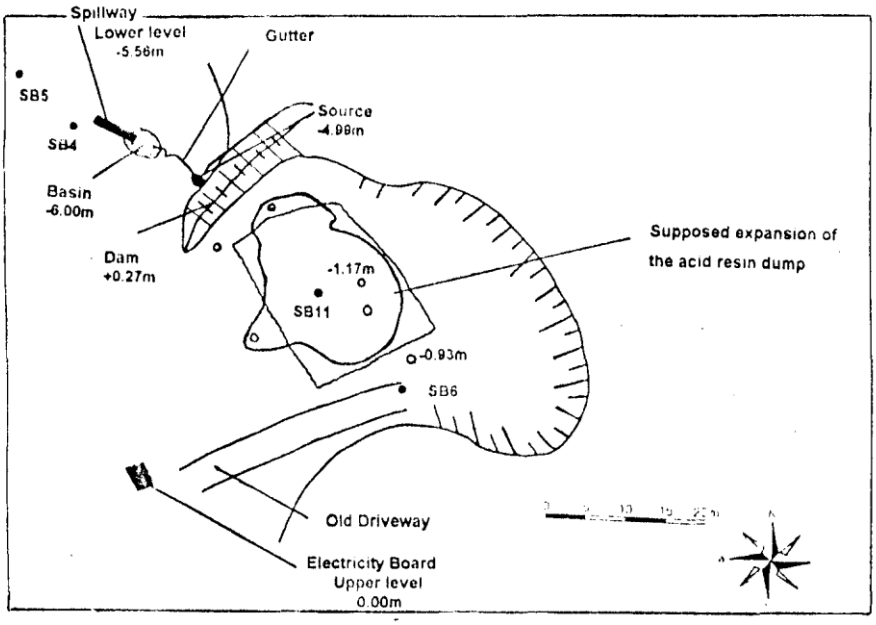

Fig.1: An overview of the contaminated area illustrating the distribution of the engine oil pollution and the position of the sampling sites (SB4, SB6, and SB11).

2) Egyptian soil contaminated with engine hydrocarbons (initial concentration of non-volatile oil was 42.9 g kg^{-1}) was obtained during 2005 from Warraq El-Hadar Island, Giza, Egypt. The oil contamination exists since about 16 years in an agricultural area. The soil had a pH of 7.3, EC of 1.05 dS/m and clay percentage of 31.72%, silt 22% and sand of 46.28 %. The texture was sandy clay loam.

Aliphatic mineral oil content in soils

Aliphatic mineral oils were determined, in the experiments done in Germany by using infrared spectroscopy (Bio-Rad, Excalibur Series, FTS 3000. Cambridge, MA 02139, USA) according to Deutsches Institut für Normung (DIN; 38409, part 18) [1981]. Soil pH was determined in 1 g soil (wet weight) suspended in 2.5 ml distilled water employing a glass electrode

in conjunction with a microprocessor pH-meter (model 763, Knick, Germany). The gravimetric water contents of soil were determined by Forster (1995).

Nutrient Media

The mineral nutrient medium proposed by Meyer and Schlegel (1983) was used in the biodegradation experiments. It consists of (g/l): $Na_2HPO_4.12H_2O$, 9; KH_2PO_4, 1.5; NH_4Cl, 1.5; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.02; F^{3+}- ammonium citrate, 0.001 plus 1 ml of trace element solution consisting of (mg/l): $ZnSO_4.7H_2O$, 100; $MnCl_2.4H_2O$, 30; H_3BO_3, 300; $CoCl_2.6H_2O$, 200; $CuCl_2.2H_2O$, 10; $NiCl_2.6H_2O$, 20; $Na_2MoO_4.2H_2O$, 30; Na_2SeO_3, 20. Unless otherwise stated, the final pH of the medium was 7.2 ± 0.1. As carbon and energy sources, either glucose (10 g/l) or aliphatic mineral oil (2 g/l) was used. The mineral oil was filtered through 0.2-µm-pore-size teflon filter (Sartarious) for sterilization and added to the auto-claved medium through a micropipete.

Nutrient agar composed of 5 g peptone, 3 g beef extract and 15 g agar/l of deionized water was used for the determination of total aerobic plate counts in engine oil- polluted soil samples.

Microbiological determinations of engine-oil-contaminated soil

Total and oil-degrading microbial counts: Total aerobic viable counts in soil were determined using the plate dilution technique on nutrient agar medium. Plates were incubated at 30 °C for 72 hr. Counts of oil-degrading bacteria were determined using oil-agar plate enumeration (Atlas and Bartha, 1973) on the solidified buffered mineral medium supplemented with engine oil (2 g/l) as a sole source of carbon and energy after incubation at 30 °C for 1 week.

Engine oil biodegradation assay:

1- Assay of oil biodegradation in Germany:

All experiments were conducted in 250-ml bottles with screw caps containing either 15 ml of the buffered mineral nutrient medium for experiments with pH 7 or 15 ml of distilled water. The bottles were autoclaved, allowed to cool down and about 6 g (wet weight) of soil were added. Sterilized NaOH was added to achieve soil neutrality. Sterilized aliphatic mineral oil (80mg/bottle) was added using micropipetes. Abiotic controls were the same as in the experiments except that the soil was autoclaved separately. Bottles were shaken at 20 °C in the dark and the mineral oil content in the bottles was determined at regular intervals according to DIN 38409 part 18. (1981). The extents of biodegradation of mineral oil were calculated by determining the percentage of the amount of mineral oil degraded (unrecovered) compared to the initial concentration.

2- Assay of oil biodegradation in Egypt:

The same was done except that the wasted engine oil (old oil) (80 mg/bottle) was added (when required) using a micropipette. Non-volatile lipophilic oil hydrocarbons were determined using the gravimetric method according to Deutsches Institut für Normung (DIN 38409, part 17, 1981).

To examine the biodegradation efficiency, four selected isolates were grown in 5 ml nutrient broth medium for 24 hours and transferred into Erlenmeyer flasks containing 200 ml portions of mineral nutrient medium received 2 g/l wasted engine oil (old oil). Flasks were incubated on a rotary

shaker at 100 rev min^{-1} and the oil content was determined at regular intervals.

In addition, the following treatments were applied:

1. 1 meter depth (SB4) soil + water versus 1 meter depth (SB4) soil +nutrient
2. 2 meter depth (SB4)) soil + water versus 2 meter depth (SB4) soil +nutrient media
3. SB (6) 0.75 m depth soil + water versus 0.75 meter depth (SB6) soil +nutrient media
4. A fourth experiment was done in Egypt on the polluted soil from Warrak El-Hadar to check the effect of adding extra engine oil to the soil to check for its effect on the microbial activity (co-metabolism). Soils without oil addition versus soil with extra oil addition were used. The average quantity added is 16.96 mg/g soil dry weight.
5. A fifth experiment was done to check for different microbial isolates (isolates 1 and 3, short rods; isolate 2, unicellular filamentous and isolate 4, spore-former) on biodegrading the engine oil.

Kinetics of Engine oil Biodegradation

A pollutant in the soil goes through a multi-stage process to yield derivative compounds till its complete degradation into CO_2 and H_2O. For such a consecutive reaction

$$\mathbf{A} \xrightarrow{k_1} \mathbf{B} \xrightarrow{k_2} \mathbf{C} \xrightarrow{k_3} \mathbf{D} \qquad \text{(eq.1)}$$

Where **A, B** and **C** and **D** represent the consecutive steps in the reaction. $\mathbf{k}_1$, $\mathbf{k}_2$ and $\mathbf{k}_3$ represent the kinetic rates of the irreversible reactions from A to B, B to C and C to D respectively.

For the kinetics of the reaction; at the initial moment, t=0 if we have *a* moles of substance A. At a moment $t \neq 0$ later, there remain a-x moles of substance A and correspondingly, there appear *x-y* moles of substance B and y-z moles of substance C. and Z moles of D. Since we do not the intermediate steps in the biodegradation reaction of the engine oil, we will be interested only in the first reaction. The equation expressing the behavior of the quantities *a-x,* as a function of time is given by (Panchenkov and Lebedev, 1976).

$$\mathbf{a - x = a\, e^{-k_1 t}} \qquad \text{(eq.2)}$$

We will determine the first order rate kinetics by taking the natural logarithm of the concentration of remaining un-degraded quantity of a pollutant, divided by its original concentration versus time, which should yield a straight line with a slope of k_1(the reaction rate constant)

$$-\ln \frac{\mathbf{a - x}}{\mathbf{a}} = \mathbf{k_1 t} \qquad \text{(eq.3)}$$

The value of that slope is a function of the environmental conditions and increases as the rate of reaction increases.

RESULTS AND DISCUSSION

Biodegradation of engine oil by indigenous soil microorganisms

Firstly, an experiment was designed to study the ability of indigenous soil microorganisms to consume engine oil, as a sole source of carbon. Following up the quantitative changes in engine oil content in the control (sterile) soils showed no changes in oil concentration throughout 42 days. This result indicates the absence of any abiotic factor that may cause any losses in the prevailing hydrocarbon. Accordingly, any decrease in oil content in the test soil will be attributed to the biological activity of the indigenous microflora capable of degrading engine oil and not due to any physico-chemical factor.

The obtained data showed that the concentration of non-volatile oil in the German soil reached 42.85 g/kg soil d. wt. Soil pH was considered by Hambrick *et. al.* (1980) to have a direct influence on petroleum biodegradation. Estimation of soil reaction in the present study revealed that the tested soil was neutral (pH 7.4). With respect to soil moisture which influences microbial growth and metabolism, the gravimetric water content was about 31 %.

Microbiological characteristics of contaminated soil

In the present study, the soil harbored a relatively dense population of total microorganisms (> 10^6 cfu g^{-1}) which exceed the minimum number, 10^3 cfu $g^{-1,}$ required to bioremediate the soil (Venkateswaran and Harayama, 1995). Prevalence of engine-oil-utilizing microorganisms in the soil was tested. This group of microorganisms could be detected (1.5 x10^4 cfu g^{-1}) representing ca. 0.3 % of the total microbial population. Atlas (1981) claimed that the population levels of hydrocarbon utilizers and their proportions within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons.

Table (2) shows the results of the first three experiments. The results of the first experiment show, for all the values for the soil +distilled water higher values of the oil concentration than the soil +distilled water except for the last one. Every reading represents a result of a separate bottle which has been used for determination and taken out from experiment. This is the dispersion of the results. The results show a decrease of concentration with time. Plotting the natural logarithm of the concentration of the remaining oil versus time gives the figure.2 with the resulting slope as the slope of the best fit shown on the graph and in table 3

The effect of nutrients on kinetics of engine oil Biodegradation

There was an increase of 50% in the value of the time constants of the second experiment (i.e. the ratio of (K_n/ K_w)) where K_n is the value of the first order constant when adding nutrients and K_w is the value of the first order constants for soil +distilled water. For 0.75 SB6, with no oil, the first order rate constant was 0.0211 which is quite high, probably due to the soil containing nutrients already.

The half life time was determined as

$$t_{0.5} = (\ln 2)/k$$

Table 2: The results of the first three experiments.

1 meter depth (SB4)					
soil + distilled water			soil + nutrients		
Time in days	Oil concentration	ln(c/c0)	Time in days	Oil concentration	ln(c/c0)
0.083	28.74	0.00000	0.083	33.53	
3	34	0.00000	3	37.06	0.00000
7	35.55	0.04458	9	35.87	-0.03264
17	30.64	-0.10405	17	30.71	-0.18795
24	32.67	-0.03990	24	33.48	-0.10159
31	36.53	0.07177	37	17.82	-0.73222
41	33.16	-0.02502			
2 meter depth (SB4)					
soil + distilled water			soil + nutrients		
Time in days	Oil concentration	Ln(c/c0)	Time in days	Oil concentration	Ln(c/c0)
0	10.46		0.083	12.23	
6	11.77	0.00000	3	12.34	0
13	10.77	-0.08879	9	10.06	-0.2042789
20	10.3	-0.13341	17	8.55	-0.3669147
27	9.41	-0.22378	24	8.26	-0.4014214
37	8.61	-0.31263	37	7.82	-0.4561615
43	9.31	-0.23446	44	7.06	-0.558401
SB(6) 0.75 m					
soil + distilled water			soil + nutrients		
time	Oil concentration	ln(c/c0)	time in days	Oil concentration	Ln(c/c0)
0	11.3	0.00000	0	11.3	0.00000
6	10.4	-0.08300	6	11.16	-0.01247
15	10.2	-0.10242	15	8.42	-0.29419
23	6.1	-0.61651	23	4.94	-0.82744
37	5.1	-0.79556	37	3.01	-1.32286

There was a 50% decrease in the half life time from 100 to 49 days in the second experiment and 30% decrease from 32 to 20 days in the third experiment.

A time period equal to four times the time constant is required to decrease the pollutant concentration to 6% of its initial concentration. So, this decrease in the half life is quite significant in reducing the time period we have to wait for soil bioremediation.

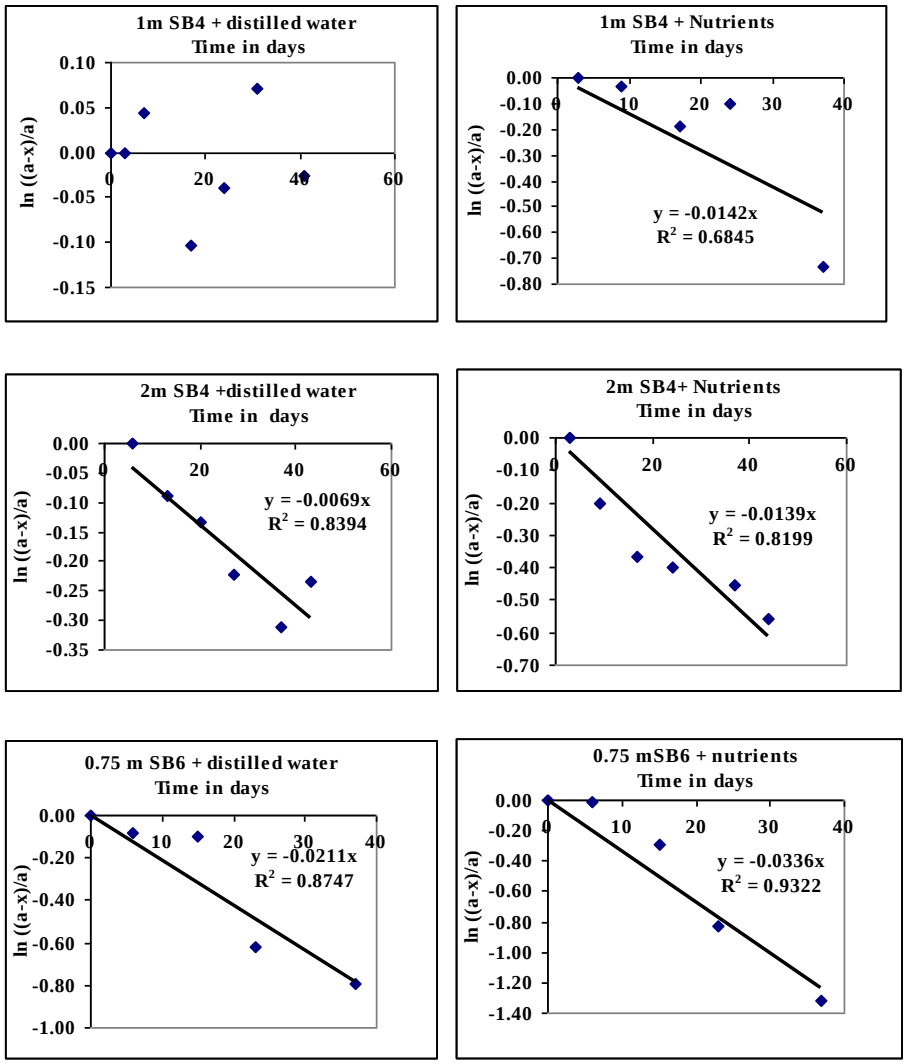

Fig. (2): The effect of soil nutrients on the biodegradation rate constants of engine oil

Table 3: the effect of nutrient addition on first order rate constants and half life time.

	Soil + distilled water kw	Half life time (days)	Soil + Nutrients Kn	Half life Time (days)	kn/kw
1 SB4	0.0	∞	0.0142	48.81	∞
2 SB4	0.0069	100.46	0.0139	49.87	2.01
0.75 SB6	0.0211	32.85	0.0336	20.63	1.59

The effect of adding more Extra oil (Giza soil):

Concerning the effect of adding extra engine oil, Mineral oil addition did not have an effect in the first weeks while in the last two weeks, it increased

the k value from 1.26 week^{-1} to 2.92 week^{-1}. Both of these experiments have mineral nutrients added to the soil. The effect of adding mineral oil could be due to an increase in the log of total count of oil degraders in the final stages of decomposition as shown in table 4.a versus 4.b.

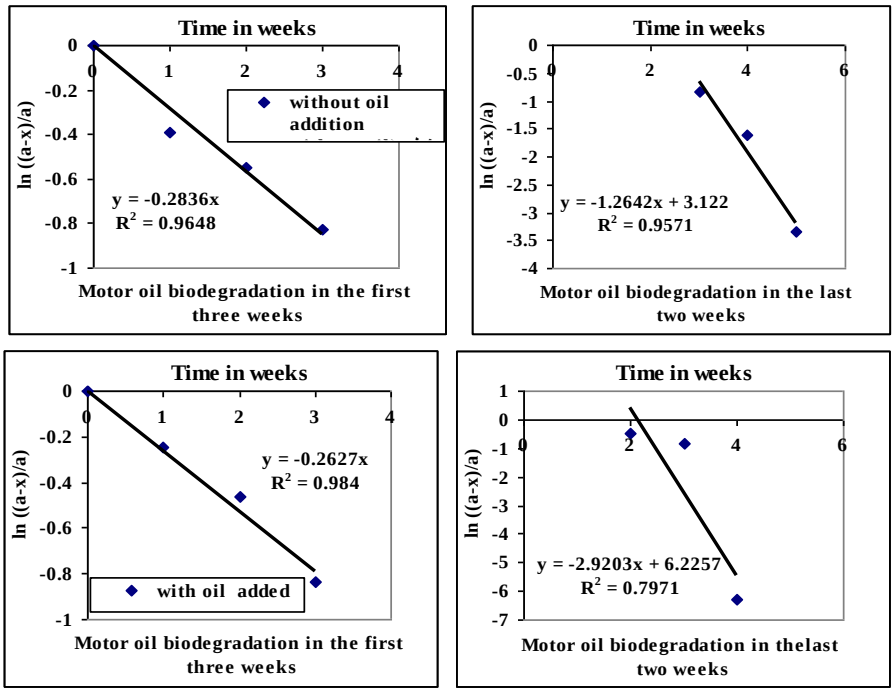

Fig. 3. The effect of adding extra engine oil to an already polluted soil.

Table 4 a: the effect of no engine oil adding to a soil

without oil addition				
Time in weeks	**oil mg/g soil dry weight**	**Log total count**	**log oil degraders**	**pH**
0	42.9	6.76	4.18	7.4
1	29	7.37	6.86	7
2	24.7	7.92	6.96	7
3	18.8	8.23	6.98	6.47
4	8.44	7.94	6.95	6.44
5	1.5	7.26	6.38	6.3
6	0	7.29	6.36	6.41

Table 4 b: the effect of engine oil added to a soil.

with oil added				
Time in weeks	**oil mg/g soil dry weight**	**Log total count**	**log oil degraders**	**pH**
0	54.8	6.76	4.18	7.4
1	42.9	7.37	6.8	7
2	34.4	8.28	6.96	6.7
3	23.8	8.35	7.15	6.58
4	0.1	7.92	6.72	6.51
5	0	8.15	7.09	6.46
6	0	7.4	6.7	6.26

The bacterial population of total and oil-utilizing microorganisms increased from 5.7 x 10^6 to 2.0 x 10^8 and from 1.5 x 10^4 to 1.6 x 10^7 cfu g^{-1}, respectively after 3 weeks, during which about 56 % of engine oil was oxidized. The addition of old engine oil to the contaminated soil in the present study resulted in a complete biodegradation of oil after 27 days table 4b. The maximum biodegradation activity was 3.4 g oil d^{-1} kg^{-1} soil dry weight.

Although the pH in the two treatments showed slight decreases (from 7.4 to 6.3) during the six weeks of incubation, microbial degradation continued to increase during the period. The decrease in the pH was observed to be directly related to the decrease in substrate (engine oil) concentration table 4.a and 4. b. This is believed to be due to the production of microbial acidic metabolites which resulted in decrease in the pH. Similar observations were reported by Atagana *et. al*. (2003) during study the of creosote biodegradation in contaminated soil.

Biodegradation of engine oil by some isolated bacteria (Giza Soil)

Figure (4) shows the efficiency of nutrient liquid cultures of four representative isolates (isolates 1 and 3, short rods; isolate 2, unicellular filamentous and isolate 4, spore-former) using engine oil as the sole carbon source. Engine oil was consumed to an extent close to 100 % (isolates 1, 2 and 4) and 70.7 % (isolate 3) after 7 days. These results indicate that engine oil was used by the bacterial isolates, since the bacterial population increased from 2.0 x 103 to 2.6 x 107 cfu ml^{-1}.

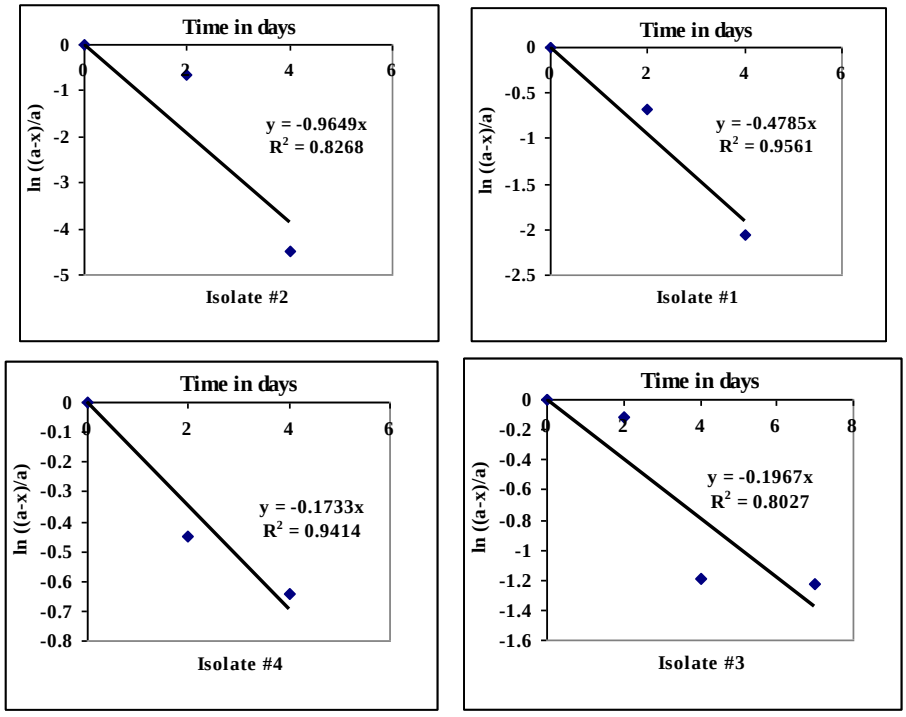

Fig. 4 The efficiency of different strains in decomposing engine oil.

The maximum engine oil biodegradation rates (calculated from the maximum slopes of the time courses obtained for the biodegradation) in the liquid cultures were 87.5,106.5,115 or 270 mg $l^{-1}d^{-1}$ for isolates 1, 2, 3 or 4, respectively.

From the graphs, it seems that isolate 2 had the highest efficiency in biodegrading the engine-oil in soil. It had a first order rate constant of 0.9649 day^{-1} versus 0.4785 day^{-1} for isolate # 1. Isolate 3 and 4 had similar efficiencies.

	k	half life time (days)	Ratio 0f half life times
Isolate # 1	0.4785	0.63	0.36
Isolate # 2	0.9649	0.31	0.17
Isolate # 3	0.1967	1.53	0.87
Isolate # 4	0.1733	1.74	-

The half life time decreased by about 60 %, 83 %, 13% for the first, second, third with comparison to the fourth strain which is least efficient. A selection of the strain, active in bioremediation could be easily implemented in ex-situ bioremediation schemes. In the In-situ schemes of bioremediation, an inoculation of the soils with the most efficient strains and a study of the most favorable soil condition for different strains (e.g. pH) could be implemented to stimulate the most efficient strains and reduce the bioremediation time.

Conclusion

It seems that enhancing the bioremediation of the engine oil polluted soil could be done through a manipulation of the water added to the soil by enriching it with the required nutrients for microbial growth. There is no need to add high purity chemicals to the soil. Use could be made of water streams which are high in its mineral content (due to pollution loads) except for heavy metals and augment it with missing nutrients. So, polluted water could be use in bioremediation of engine oil polluted soils. Use is made then of the nutrient pollutants in the water by the bacteria which will biodegrade the organic pollutants in soil and two aims are achieved.

To prove the point, incubation experiment in which nutrients solution was added to a polluted soil with engine oil. The experiments show an increase in the first order rate constants expressing the effect of microbes in degrading the pollutant compounds. In the present study, evidence was provided for the existence of microbial communities capable of degrading petroleum products. The findings of engine oil biodegradation experimentation conclude that the indigenous oil-utilizing microorganisms proved to be able to start an efficient bioremediation process through their active metabolism. The reduction in engine oil concentration achieved over such a short incubation period of 6 weeks was high and of significant consideration in future bioremediation programs. So, an understanding of the factors that influence the biodegradation of pollutants is necessary to effectively model, manage and remediate contaminated sites

The importance of the bacterial strains is their selection for ex-situ bioremediation which cuts the time required or the size of reactors used to bioremediate the soil. An in-situ treatment could be made more efficient

through inoculation of the soil with the most efficient strain and securing the most favorable condition for its activity.

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حث الهدم الحيوى للتربة الملوثة بزيت المحركات بواسطة إضافة المغذيات المعدنية

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لقد تم إجراء خمس تجارب لدراسة تأثير إضافة المغذيات المعدنية لأراضى ملوثة بزيت المحركات على تحفيز النشاط الميكروبى و هدمهم الحيوى لزيت المحركات. لقد شجعت إضافة محلول مغذى إلى تربة مأخوذة من ثلاث مواقع مختلفة من النشاط الميكروبى. ولقد ظهر ذلك من خلال زيادة معدل تفاعل الدرجة الأولى لعمليات الهدم الحيوى. فلقد زاد معدل تفاعل الدرجة الأولى للثلاث أنواع من الأراضي من صفر إلى 014, (يوم $^{-1}$), من0069, (يوم $^{-1}$) إلى 0139, (يوم $^{-1}$) و من 0211, (يوم $^{-1}$) الى0336, (يوم $^{-1}$) بانخفاض ناتج فى قيمة فترة نصف العمر من ما لانهاية إلى 48.8يوم ومن 100 يوم إلى 49 يوم ومن 32 يوم إلى 20 يوم للثلاث أنواع من التربة على وجه الترتيب.

ولقد تم إجراء تجربة على تأثير إضافة مزيد من زيت المحركات على تحلل زيت المحركات. لم يكن هناك تأثير فى المراحل الأولى من التحلل الحيوى بينما زاد الهدم الحيوى فى المراحل المتأخرة وغالبا يكون ذلك راجعا إلى زيادة عدد محللات الزيت الحيوية من $5.7 * 10^{6}$ إلى $2.0 * 10^{8}$ ومن$1.5 *10^{4}$ إلى $1.6*10^{7}$ على وجه الترتيب بعد ثلاث أسابيع, التى خلالها تم هدم 56% من الزيت.

لقد تم استخدام أربع معزولات مختلفة محلية من البكتريا لفحص قدرتهم على هدم الزيت. ولقد كانت هناك فروق واسعة فى الكفاءة الميكروبية للهدم الحيوى. فلقد تناقصت فترة نصف العمر بنحو 60% ,83% و 13% للسلالة الأولى و الثانية و الثالثة بالمقارنة بالسلالة الرابعة التى كانت اقلهم كفاءة. ولقد كانت فترة نصف العمر للسلالة الرابعة 1.74 يوم.

ولقد نوقشت عواقب ذلك فى الأستنتاجات.

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