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Biodegradation of Crude Oil-contaminated Soil using Canned-food-industry Wastewater Sludge for Soil Application

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Abstract

The objective of this study was to evaluate the effects of crude oil (application doses of 0.5% and 5%) from hydrocarbon contamination on the removal of the Total Petroleum Hydrocarbons (TPH) from soil and to determine the removal of TPH at different temperatures (18°C and 28°C) during an incubation period of 240 days. The possible use of wastewater sludge as a biostimulating agent in crude oil-contaminated soils was also evaluated. The results of the 240 days of incubation indicated that the TPH removal percentages in crude oil-contaminated and sludge-treated soils at 18°C were 89% and 79%, for doses of 0.5 and 5%, respectively. Incubation at 28°C resulted in higher TPH removal with removal percentages of 83% (dose of 0.5%) and 91% (dose of 5%). The degradation of crude oil in contaminated soil treated with a 5% dose was significantly enhanced by the addition of wastewater sludge, whereas no apparent biostimulating effect on TPH removal was observed in the case of low-dose (0.5%) crude oil contamination.

Keywords: *biostimulation, crude oil, incubation, soil, petroleum hydrocarbons, wastewater sludge*

1. Introduction

Hydrocarbons are the primary components of crude oil. Crude petroleum, a complex volatile mixture of hydrocarbons with some sulfur, nitrogen, oxygen, and trace metals, is the most important source of energy in the world. Due to the increasing production of crude oil and the increasing probability of tanker accidents, petroleum compounds are one of the most common contaminants in soil (Banks *et al*., 2003). Soil microorganisms act a significant effect in the biodegradation of organic contents, such as crude oil and its products (Gianfreda and Rao, 2008). Soil contamination has been reported as highly threatening because contaminants have the capacity to influence the natural organisms that live in the soil and to destroy the food chain (Moreno *et al*., 2009). Soil remediation is known to be among the most expensive processes in the world. Thus, bioremediation is the most convenient approach in that it does not require high inputs and may remove contaminants in a shorter time (Erdoğan and Karaca, 2011).

Biostimulation refers to the supplement of substrates, vitamins, oxygen and other compounds that enhance the activity of microorganisms so that they can deteriorate petroleum hydrocarbons more rapidly.

Some of environmental factors known to limit biodegradation of soil containing petroleum hydrocarbons temperature fluctuation and nutrient availability are among the most significant ones (Coulon *et al*., 2005). Temperature influences the rate of biodegradation, besides the physical nature and chemical characterization of hydrocarbons (Rowland *et al*., 2000). Although hydrocarbon biodegradation can consist over a wide range of temperatures, the rate of biodegradation generally declines with the decreasing temperature. Das and Chandran (2011) noticed that highest degradation rates that usually occur in the range 30-40°C in soil environments. According to Venosa and Zhu (2003), ambient temperature of the environment affected both the composition of spilled oil and microbial activity.

The stimulation of microorganisms by the addition of nutrients introduces large quantities of carbon sources, which leads to rapid consumption of the present pools of important inorganic nutrients such as nitrogen and phosphorus (Sang-Hwan *et al*., 2007). The main benefits of biosolids contain their low cost (or no cost), slow exposure of the nutrients (similar to animal manures), and easy availability (McBride, 2003; Sarkar *et al*., 2005).

In this respect, wastewater sludge contains significant amounts of nutrients required by plants, including nitrogen, phosphorus,

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potassium, and micronutrients, making sludge an great fertiliser for use in agriculture and forestry.

This study aimed to utilise canned food industry Wastewater Sludge (WS) to enhance the biodegradation of crude oil in contaminated soil. To achieve the study objectives, canned food industry sludge was selected as the organic component for addition to individual 100 ton/ha crude-oil-contaminated soils.

This paper reports a laboratory study that evaluated the effects of WS, ageing time and temperature on the Total Petroleum Hydrocarbon (TPH) degradation efficiency in crude oil-contaminated soils, based on remediation approaches that consisted of biostimulation for 240 days. Soils contaminated with 0.5% and 5% crude oil were tested during the incubation period (at 30, 60, 90, 150 and 240 days).

2. Materials and Methods

2.1 Materials

An agricultural field in the Bursa-Balabancık village (latitude, 40° 15' 55.1" N; longitude, 28° 47' 07.55"E) was selected as soil sampling area. Soil samples were taken to a depth of 20 cm. The chemical characteristics of soil are listed in Table 1.

De-watered sludge samples were collected from a wastewater treatment facility with an activated sludge system of a canned food factory in Bursa (Turkey). Domestic wastewaters originated from the staff as well as process wastewaters were treated together in the treatment facility. The chemical properties of sludge samples are also given in Table 1.

Table 1. The Physicochemical Properties of the Soil and Wastewater Sludge

Parameters	Values	
	Sludge	Soil
pH (1:5)	6.97	7.76
EC, mS/cm (1:5)	5.04	0.23
Solid matter, %	16.4	-
Organic C , %	33.50	1.70
Total N, %	3.50	0.12
C/N ratios	9.57	14.17
NH_4-N, mg/kg dry weight	201.93	24.1
NO_3-N, mg/ kg dry weight	171.64	24.1
Total P, %	0.50	0.17
Available PO_4-P, mg/ kg dry weight	386.11	20.69
Exchangeable heavy metals (mg/kg dry weight):		
Zn	122.8	<2
Cu	27.55	<2
Ni	11.20	<2
Cr	0.11	<2
Cd	0.09	<2
Pb	1.79	<2
Total heavy metals (mg/kg dry weight):		
Zn	334.2	65.02
Cu	53.50	15.34
Ni	58.29	128.0
Cr	48.20	98.69
Cd	3.50	0.21
Pb	11.66	trace

Light crude oil was obtained from İzmir, Aliağa refinery and has a specific gravity of 0.86 (60 F/60 F), gravity of 33.4 API and viscosity of 10.20 cs (70 F), total sulphur of 1.79% (wt), vanadium of 20.50 ppm, nickel of 4.40 ppm, and total nitrogen of 0.0980% (wt) (Dindar *et al.*, 2015).

2.2 Incubation Procedure

The soil samples were air-dried in the laboratory and sieved through 2 mm screens. Then, 40 g soil was placed in cylindrical glass pots. Soil samples were contaminated with 0.5% (w/w) or 5% (w/w) of crude oil and thoroughly mixed. Oil application doses of 5% and 0.5% were choosen in this study in order to simulate major and minor oil contamination of soil, respectively. Soil with only the addition of crude oil served as a control. After the addition of oil, wastewater sludge was applied to soil pots with a dose of 100 t/ha (40 g kg^{-1}) based on dry weight. The pots were incubated for 240 days in the dark at 28±0.5°C and 18±0.5°C. The annual local temperature range between 15°C-28°C in Bursa city. In order to stimulate typical temperature values for Bursa city, 18°C and 28°C were prefered in the incubation study.

The moisture content in the soil was maintained at 70% of field capacity, and the content was tilled for aeration throughout the incubation period. Various amounts of distilled water was added daily to soil pots in order to *keep soil moisture* levels fairly consistent. The experiment was planned with a completely randomised design and each treatment was performed in triplicated to give a total of 120 experimental units at the start of the incubation. At each sampling time (30., 60., 90., 150. and 240 days) three sets of soil pots were removed and the TPH concentrations were determined (Dindar *et al*., 2015).

2.3 Determination of Soil and Wastewater Sludge Physical and Chemical Properties

Soil and wastewater sludge samples were prepared and analysed following the same procedures. The electrical conductivity ($EC_{25°C}$) and pH of the samples were measured in sample extracts which were obtained by shaking the samples with distilled water at 1:5 (w/v) with a conductivity meter and pHmeter, respectively (Rhoades, 1982; Mc Lean, 1982).

Samples were extracted with a solution of KCl (2M) for the determination of nitrate and ammonium nitrogen. The concentrations were determined by steam distillation method (automatic distillation system-Velp) using MgO and Devarda alloy (Keeney and Nelson, 1982). The Kjeldahl digestion method was used to measure the total nitrogen concentration (Bremner and Mulvaney, 1982). In addition, total organic carbon concentrations in samples were analysed using dichromate oxidation method (Nelson and Sommer, 1982). A solution of 0.5 N $NaHCO_3$ was used to extract available P. Nitric acid-sulphuric acid digestion was performed in order to determine total P. PO_4^{-3}-P in extracts was measured according to ascorbic acid method (Anonymous, 1998).

For the determination of total concentrations of metals, samples were microwave digested with HNO_3. Cr, Ni, Cu and Zn were analysed using an atomic adsorption spectrophotometer (Isaac and Johnson, 1998).

2.4 Determination of the Total Petroleum Hydrocarbons in Soil

The TPH concentration was analyzed according to the method of ISO 16703:2004. Twenty grams of petroleum-contaminated soil was placed in a glass extraction vessel containing 40 ml of acetone. After shaking the vessel, 20 ml of RTW (retention time window)-standard solution was added. Soil samples were shaked for 60 min via mechanical shaking. After the solid part of the extract settled, the supernatant was transferred into a separatory funnel. In order to remove acetone, organic phase washed with distilled water. The organic layer was taken in a glass tube. After the addition of sodium sulphate, 10 ml of extract was transferred to a clean-up column filled with Florisil. An aliquot of the purified extract was placed into a GC-vial and analysed via GC-FID.

The extracts were analyzed within a single batch by gas chromatography, using an HP Agilent 7890A gas chromatograph (Agilent Technologies, www.agilent.com) equipped with a FID detector and a capillary column (30 m × 0.25 mm i.d.) with a nominal film thickness of 0.25 μm. Splitless injection method was used with a deactivated, splitless inlet liner with adsorbent material and taper (Agilent Technologies, P/N 5183-4711). The injection temperature was 350°C and injection volume 2 μl. Helium (2 ml min^{-1}) was used as carrier gas. The final GC oven program started at 35°C, was held for 1.5 min, then increased to 60°C at 5°C min^{-1}, then increased to 350°C at 15°C min^{-1} an then held at 350°C for 10 min. The amount of Total Petroleum Hydrocarbons (TPH) was then determined as a sum parameter of resolved and unresolved components eluted from the GC capillary column between the retention times of "*n*-decane and *n*-tetracontane." (Dindar *et al.*, 2015).

2.5 First-order Kinetics

In order to estimate the biodegradation rate of hydrocarbons in soil, the experimentally obtained data were fitted to a first-order kinetics model (Yeung *et al.*, 1997) with

$$y = ae^{-kt} \quad (1)$$

where

a = The initial hydrocarbon content in the soil (mg kg^{-1}),
k = The biodegradation rate constant (d^{-1}), and
t = Time (d)
y = The residual hydrocarbon content in the soil (mg kg^{-1}),

In order to estimate the half-life of hydrocarbons in soil, the model reported by Yeung *et al.* (1997) was used:

$$\text{Half life} = \text{In}(2)/k \quad (2)$$

According to this model, the degradation rate of hydrocarbons is positively correlated with the amount of hydrocarbons in the soil (Agamuthu *et al.*, 2013).

Table 2. Variation of TPH Concentration Soil Contaminated with Crude Oil

Variation	SS	df	$F_{istatistic}$
0.5% crude oil			
Temperature	3602465	1	91.88*
Incubation time	4372204	5	111.52*
Treatment	256173	1	6.53
Treatment x incubation time	482091	5	12.30*
Temperature x treatment	846	1	0.02
Temperature x incubation time	1138551	5	29.04*
Temperature x incubation time x treatment	2197267	5	56.04
Error			48
5% crude oil			
Temperature	617922112	1	797.25*
Incubation time	207376432	5	267.56*
Treatment	1474062208	1	1901.86*
Treatment x incubation time	29753598	5	38.39*
Temperature x treatment	54743672	1	70.63*
Temperature x incubation time	29761734	5	38.40*
Temperature x incubation time x treatment	40106680	5	51.75*
Error			48

*$p<0.05$

2.6 Statistical Analysis

STATISTICA 6.0 software was used for performing all statistical calculations. ANOVA was used to test the effect of the temperature, treatment and the incubation time (Table 2). The effects of incubation time and treatment were further tested on TPH removal with two-way ANOVA for each temperature. When significant effects were indicated by ANOVA, post hoc comparisons were performed using a Tukey's HSD test.

3. Results and Discussion

3.1 Biodegradation of Crude Oil

The variation of TPH concentration in soil samples contaminated with crude oil and incubated at 18°C for varying amounts of time is shown in Fig. 1(a). For soil samples contaminated with 0.5% crude oil, the concentration of TPH decreased with time. The TPH concentration fell from the initial level of 4700 mg/kg to 900 mg/kg after the incubation period of 240 days. These results are in agreement with previous experiments (Plohl *et al.*, 2001; Coulon *et al.*, 2005; Abioye *et al.*, 2010). An examination of the TPH concentration of the samples contaminated with crude oil and treated with wastewater sludge showed that the accelerated biodegradation of the crude oil by the wastewater sludge began on the 90th day of the incubation process. On the 90th day, the TPH level for the soil samples contaminated with crude oil was 1700 mg/kg,

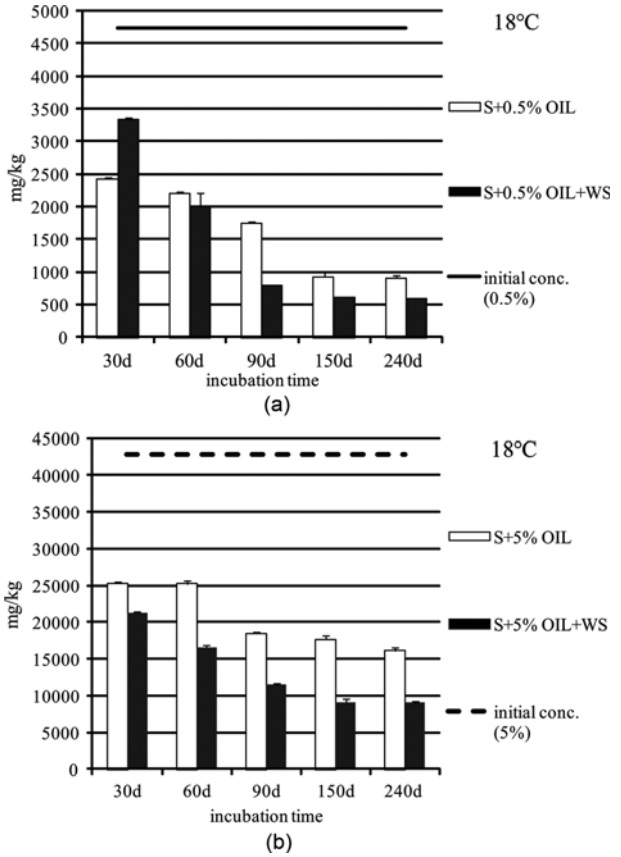

Fig. 1. Changes in TPH Levels in Soil Contaminated with Crude Oil and Canned Food Industry Sludge-treated Soil During the Incubation Period at 18°C: (a) 0.5% Dose of Oil, (b) 5% Dose of Oil

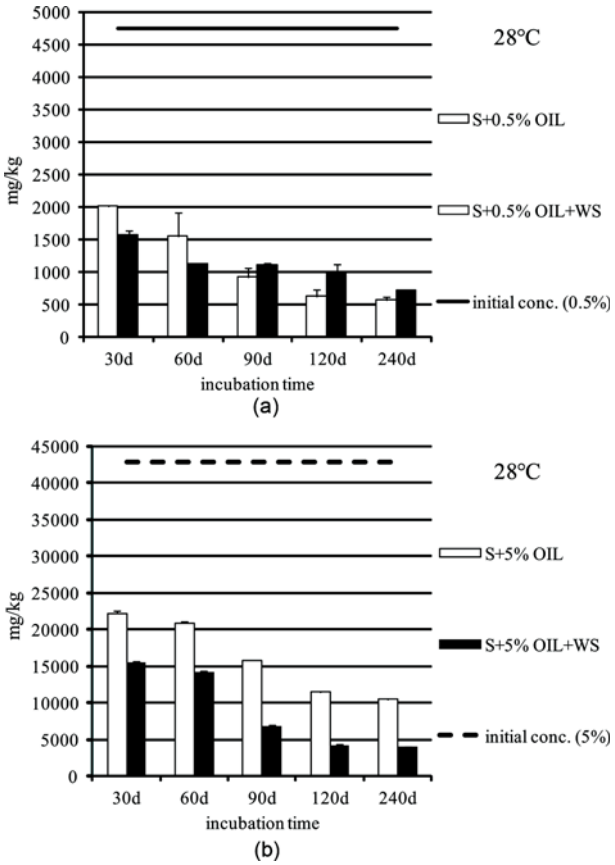

Fig. 2. Changes in TPH Levels in Soil Contaminated with Crude Oil and Canned Food Industry Sludge-treated Soil During the Incubation Period at 28°C: (a) 0.5% Dose of Oil, (b) 5% Dose of Oil

whereas the level for samples contaminated with crude oil and treated with wastewater sludge was 800 mg/kg.

This result may be due to differences in the nutrients, particularly nitrogen and phosphorus, in the wastewater sludge that stimulate indigenous microorganisms. The supplementation of nitrogen and phosphorus to oil-polluted soil has been indicated to enhance the biodegradation of the petroleum in soil (Ijah and Safiyanu, 1997; Abioye *et al*., 2009; Dindar *et al.*, 2015). For the soil samples contaminated with 5% crude oil, the TPH concentration decreased markedly in accordance with the duration of incubation. As shown in Fig. 1(b), wastewater sludge treatment of the soil samples accelerated the degradation of crude oil. The TPH concentration for the soil samples contaminated with 5% crude oil and treated with wastewater sludge was lower than that of contaminated soil samples that were not treated with wastewater sludge for all incubation periods ($p<0.05$). The supplementation of a carbon source as a nutrient in contaminated soil is known to improve the rate of contaminant degradation by stimulating the growth of microorganisms responsible for biodegradation of the contaminant (Xu and Obbard, 2003). For highly contaminated soil samples, the effects of wastewater sludge on TPH concentration were more distinct. This effect was observable from the 90th day of incubation onwards. The TPH level at the end of the incubation period was 9000 mg/kg.

The changes in TPH concentration with incubation period for the soil samples contaminated with crude oil and incubated at 28°C are given in Fig. 2. For the soil samples contaminated with 0.5% crude oil, the initial level of 4700 mg/kg decreased to 600 mg/kg by 240 days of incubation (Fig. 2(a)). This result proposed that the mesophilic temperature alone has a large effect on the biodegradation in the contaminated soil if oxygen concentration and soil moisture content are proved at adequate levels (Hesnawia and Mogadam, 2013). The positive effect of the wastewater sludge was observed within the first 60 days. For other incubation periods, the wastewater sludge had negatively effect. Higher mineralization of wastewater sludge was expected the incubation conditions at 28°C and probable toxic mineralization products may inhibit decomposition of crude oil.

The variation over time in the soil samples contaminated with 5% crude oil exhibited a negative trend, as illustrated in Fig. 2(b). The wastewater sludge treatment accelerated the biodegradation of crude oil for all incubation periods. This effect was especially apparent on day 120 of incubation. At the end of incubation, the TPH level for the samples treated with wastewater sludge was 4800 mg/kg, whereas the level for soil that was not treated with wastewater sludge was 10000 mg/kg.

Comparing the levels among different incubation temperatures showed that the rate of TPH decrease was greater at 28°C. Walworth *et al.* (2001) reported that hydrocarbon degradation is increased at higher temperatures.

Analysing the variance confirmed that the temperature and incubation period significantly affected the TPH concentration. The treatment of soil with wastewater sludge had a significant effect on TPH biodegradation for samples contaminated with high doses. The results of repeated measures of ANOVA indicated that no significant effect was found due to wastewater sludge treatment of samples contaminated with low doses of crude oil (Table 1).

The biodegradation rates expressed as percentages of TPH at 18°C and 28°C over time for the soil samples contaminated with various doses of crude oil are shown in Table 3. An examination of the biodegradation rates at 18°C shows that the TPH biodegradation rate of the soil samples contaminated with a low dose of crude oil was 81% at the end of the incubation period. For the contaminated soil samples stimulated with wastewater sludge, the biodegradation rate increased to 89%. The TPH biodegradation rate of the soil samples contaminated with a high dose of crude oil was 62%, which increased to 80% after treatment with wastewater sludge. The highest rate of TPH biodegradation was observed in the soil contaminated with a lower dose of crude oil and treated with wastewater sludge. The lowest rate of biodegradation was observed in the untreated soil samples contaminated with a higher dose of crude oil.

At 28°C, the biodegradation rate for the soil samples contaminated with a low dose of crude oil was 89%, whereas treatment with wastewater sludge did not enhanced this ratio. For soil contaminated with a high dose of crude oil, the TPH biodegradation rate was 75%, which increased to 90% after treatment with wastewater sludge. The lowest biodegradation rate was observed for the soil contaminated with a high dose of crude oil, and treatment with wastewater sludge was also the most effective for the soil contaminated with a high dose of crude oil.

These results suggest that nutrient (N, P, etc.) availability is a limiting factor in the bioremediation of contaminated soil during wastewater treatment. Moreover, these results agree with reports by Coulon *et al.* (2005) and Perfumo *et al.* (2007).

3.2 Biodegradation Rate Constant and Half-life of Crude Oil

A first-order kinetics model (Yeung *et al.*, 1997) was used to assess the rate of biodegradation of crude oil in soil that was treated with wastewater sludge. Kinetic analyses are key for understanding biodegradation processes and bioremediation speed measurements and for developing efficient clean-up methodologies for a crude oil-contaminated environments. The biological half-life is the time required for one-half the amount of a substance to degrade. The understanding of biodegradation half-lives is important for many applications, such as chemical screening (Aronson *et al.*, 2006) environmental fate modelling (Sinkkonen and Paasivirta, 2000) and describing the transformation of contaminants (Matthies *et al*., 2008; Dimitrov *et al.*, 2007).

Table 4 and Table 5 show the biodegradation rate constants (k) and half lifes ($t_{1/2}$) for the soil contaminated with crude oil and treated with wastewater sludge for 240 days.

According to Table 4, low-dose (0.5%) crude oil-contaminated soil treated with wastewater sludge exhibited the highest

Table 3. Biodegradation Percentage of TPH in Soil Contaminated with Crude Oil and Treated with Canned Food Industry Wastewater Sludge at 18°C and 28°C

	% TPH removal				
18°C	30 days	60 days	90 days	150 days	240 days
S+0.5% OIL	48.77846	53.37466	63.0378	80.29652	80.87392
S+5% OIL	41.05149	41.20516	56.84321	58.85208	62.44062
S+0.5% OIL+WS	29.47075	57.69398	83.10454	87.13326	87.28828
S+5% OIL+WS	50.25432	61.46524	72.93689	78.64909	78.68637
28°C					
S+0.5% OIL	57.54171	67.30729	80.50644	86.82154	88.12904
S+5% OIL	48.26962	51.31829	63.25945	73.41267	75.61678
S+0.5% OIL+WS	66.72967	76.20971	76.59937	78.83168	84.77973
S+5% OIL+WS	63.77004	66.99092	83.93472	90.21785	90.58931

Table 4. Biodegradation Rates and Half Lifes of Hydrocarbon in Crude Oil-polluted Soil Treated with Wastewater Sludge During the Incubation Period (28°C)

28°C	Biodegradation constant, k/day					Half life ($t_{1/2}$) (days)				
Treatment	**30d**	**60d**	**90d**	**150d**	**240d**	**30d**	**60d**	**90d**	**150d**	**240d**
Soil+0.5% oil	0.0285	0.0186	0.0181	0.0135	0.0088	24.32	37.27	38.29	74.07	78.77
Soil+5% oil	0.0219	0.0119	0.0111	0.0088	0.0058	31.65	58.25	62.45	78.77	119.48
Soil+0.5% oil+WS	0.0366	0.0239	0.0160	0.0103	0.0078	18.94	29.00	43.32	67.30	88.87
Soil+5% oil+WS	0.0338	0.0184	0.0203	0.0154	0.0098	20.51	37.67	34.15	45.01	70.73

Table 5. Biodegradation Rates and Half Lifes of Hydrocarbon in Crude Oil-polluted Soil Treated with Wastewater Sludge During the Incubation Period (18°C)

18°C	Biodegradation constant, k/day					Half life ($t_{1/2}$) (days)				
Treatment	**30d**	**60d**	**90d**	**150d**	**240d**	**30d**	**60d**	**90d**	**150d**	**240d**
Soil+0.5% oil	0.0220	0.0127	0.0115	0.0110	0.0068	31.51	54.58	63.02	63.01	100.43
Soil+5% oil	0.0176	0.0088	0.0093	0.0059	0.0041	39.38	78.77	74.52	117.48	169.02
Soil+0.5% oil+WS	0.0116	0.0143	0.0190	0.0140	0.0086	59.75	48.47	36.48	49.57	80.59
Soil+5% oil+WS	0.0230	0.0160	0.0145	0.0102	0.0064	30.14	43.32	47.81	67.96	108.31

biodegradation rate of 0.0366/day and a half life of 18.94 days, whereas the biodegradation rate and half life for the control soil were 0.0285/day and 24.32 days, respectively, for 30 days. At the end of the incubation period, low-dose (0.5%) crude oil-contaminated soil treated with wastewater sludge exhibited a biodegradation rate of 0.0088/day and a half life of 78.77 days.

High dose (5%) crude oil-contaminated soil treated with wastewater sludge showed the highest biodegradation rate of 0.0338/day and a half life of 20.51 days, whereas the biodegradation rate and half life for the control soil were 0.0219/day and 31.65 days, respectively, for 30 days. At the end of the incubation period, high dose (5%) crude oil contaminated-soil treated with wastewater sludge showed a biodegradation rate of 0.0098/day and a half life of 70.73 days.

The high biodegradation rate determined in crude oil-contaminated soil treated with wastewater sludge may be due its high nitrogen and phosphorus contents and its limiting effects on the microbial activity compared to control soil (Lee *et al.*, 2003).

According to Table 5, biodegradation of the crude oil at 18°C resulted in lower biodegradation constants and higher half lives. Low-dose (0.5%) crude oil-contaminated soil treated with wastewater sludge showed the highest biodegradation rate of 0.0116/day and a half life of 59.75 days, whereas the biodegradation rate and half life for the control soil were 0.0220/day and 31.51 days, respectively, for 30 days. At the end of the incubation period, low-dose (0.5%) crude oil-contaminated soil treated with wastewater sludge exhibited a biodegradation rate of 0.0086/day and a half life of 80.59 days.

High-dose (5%) crude oil-contaminated soil treated with wastewater sludge showed the highest biodegradation rate of 0.0230/day and a half life of 30.14 days, whereas, the biodegradation rate and half life for the control soil were 0.0176/day and 39.38 days, respectively, for 30 days. At the end of the incubation period, high-dose (5%) crude oil-contaminated soil treated with wastewater sludge showed a biodegradation rate of 0.0064/day and a half life of 108.31 days.

In a previously reported study, the addition of 10% (w/w) wastewater sludge to soil contaminated with 10% (w/w) lubricating oil and incubated at 30°C for 98 days was examined. The results showed that at the end of the incubation period, the biodegradation rate for the samples treated with the wastewater sludge was 0.1490 per day, with a half life of 4.65 days (Agamuthu *et al*., 2013).

4. Conclusions

Bioremediation is a method used to degrade petrol-based hydrocarbon contaminants via biological processes, without causing damage to the physical or chemical qualities of the soil. One technique adopted in this approach is biostimulation, which involves adding nutrients such as nitrogen or phosphorous to the soil to enhance the microbial activity in the soil.

1. In examining the results of this study, the effects of wastewater sludge from the canned food industry for biostimulation on the TPH concentration in soil contaminated with crude oil can be summarised as follows:
2. The greatest amount of TPH biodegradation took place at a temperature of 28°C.
3. At both temperatures, the majority of the TPH biodegradation occurred between the 3rd and 5th months of the incubation period.
4. Crude oil-contaminated soil treated with wastewater sludge exhibits greater oil biodegradability compared to untreated soil. By the end of the 240th day of the incubation period, the use of wastewater sludge had triggered TPH biodegradation in the soil samples contaminated with crude oil at a rate of 6% to 18%.

Based on the first order kinetics model, high doses of (5%) crude oil-contaminated soil treated with wastewater sludge showed a maximum biodegradation rate of 0.0098/day and a half life of 70.73 days at the end of the incubation period at 28°C. These values were significantly higher than those of the control soil (0.0058/day and 119.48 days, respectively).

The overall evaluation of the study indicated that application of food industry wastewater sludge and temperature enhanced TPH removal from oil-contaminated soil. Wastewater sludge contain nutrients and organic matter that can provide soil microbial activity and are widely used as soil improvers.

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References

Abioye, O. P., Abdul Aziz, A., and Agamuthu, P. (2009). "Stimulated biodegradation of used lubricating oil in soil using organic wastes."

Malaysian Journal of Science, Vol. 28, No. 2, pp. 127-133.

Abioye, O. P., Abdul Aziz, A., and Agamuthu, P. (2010). "Enhanced biodegradation of used engine oil in soil amended with organic wastes." *Water Air and Soil Contamination*, Vol. 209, No. 1, pp. 173-179, DOI: 10.1007/s11270-009-0189-3.

Agamuthu, P., Tan, Y. S., and Fauziah, S. H. (2013). "Bioremediation of hydrocarbon contaminated soil using selected organic wastes." *Procedia Environmental Sciences*, Vol. 18, pp. 694-702, DOI: 10.1016/j.proenv.2013.04.094.

Anonymous (1998). *Standard Methods for the Examination of Water and Wastewater*, APHA-AWWA-WPCF Copyright by American Public Health Association, Washington, 1269p.

Aronson Boethling, D. R., Howard, P., and Stiteler, W. (2006). "Estimating biodegradation half-lives for use in chemical screening." *Chemosphere,* Vol. 63, No. 11, pp. 1953-1960, DOI: 10.1016/j.chemosphere.2005.09.044.

Banks, M. K., Schwab, P., Liu, B., Kulakow, P. A., Smith, J. S., and Kim, R. (2003). "The effects of plants on the degradation and toxicity of petroleum contaminants in soil: A field assessment." *Adv. Biochem. Eng. Biotechnol*., Vol. 78, No. 9, pp. 75-96, DOI: 10.1007/3-540-45991-X_3.

Bremner, J. M. and Mulvaney, C. S. (1982). *Nitrogen-Total. Methods of Soil Analysis*, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No:9 (2nd ed.) ASA-SSSA. Madison, Wisconsin. USA, 595-622.

Coulon, F., Pelletier, E., Gouhant, L., and Delille, D. (2005). "Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil." *Chemosphere*, Vol. 58, No. 10, pp. 1439-1448, DOI: 10.1016/j.chemosphere.2004.10.007.

Das, N. and Chandran, P. (2011). "Microbial degradation of petroleum hydrocarbon contaminants: An overview." *Biotechnology Research International,* Article ID 941810, 13.

Dimitrov, S., Pavlov, T., Nedelcheva, D., Reuschenbach, P., Silvani, M., Bias, R., Comber, M., Low, L., Lee, C., Parkerton, T., and Mekenyan, O. A. (2007). "Kinetic model for predicting biodegradation SAR QSAR." *Environ. Res.*, Vol. 18, Nos. 5-6, pp. 443-457.

Dindar, E., Topaç Şağban, F. O., and Başkaya, H. S. (2015). "Variations of soil enzyme activities in petroleum-hydrocarbon contaminated soil." *International Biodeterioration& Biodegradation*, Vol. 105, pp. 268-275, DOI: 10.1016/j.ibiod.2015.09.011.

Erdoğan, E. and Karaca, A. (2011). "Bioremediation of crude oil polluted soil." *Asian Journal of Biotechnology*, Vol. 3, No. 3, pp. 206-213, DOI: 10.3923/ajbkr.2011.206.213.

Gianfreda, L. and Rao, M. A. (2008). "Interactions between xenobiotics and microbial and enzymatic soil activity." *Critical Reviews in Environmental Science and Technology*, Vol. 38, No. 4, pp. 269-310, DOI: 10.1080/10643380701413526.

Hesnawia, R. M. and Mogadam, F. S. (2013). "Bioremediation of libyan crude oil-contaminated soil under mesophilic and thermophilic conditions." *APCBEE Procedia*, Vol. 5, pp. 82-87, DOI: 10.1016/j.apcbee.2013.05.015.

Ijah, U. J. J. and Safiyanu, H. (1997). "Microbial degradation of Escravos light crude oil in soil amended with chicken dropping and NPK fertilizer." *10th Annual Conference of Biotechnology Society of Nigeria*, 2nd -5thApril 1997.

Isaac, A. R. and Johnson, W. C. (1998). *Elemental determination by inductively coupled plasma atomic spectrometry*, In: Karla, Y.P. (ed) handbook of reference methods for plant analysis, CRC Pres, Washington, D.C., pp. 165-170.

ISO 16703 (2004). Soil quality-Determination of content of hydrocarbon in the range C_{10}-C_{40} by gas chromatography.

Keeney, D. R. and Nelson, D. W. (1982). *Nitrogen-Inorganic Forms*, Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No:9 (2nd ed.) ASA-SSSA. Madison, Wisconsin. USA, pp. 643-693.

Lee, K., Park, J. W., and Ahn, I. S. (2003). "Effect of additional carbon source on naphthalene biodegradation by Pseudomonas putida G7." *Journal of Hazardous Materials,* Vol. 105, Nos. 1-3, pp. 157-167, DOI: 10.1016/j.jhazmat.2003.08.005.

Matthies, M., Witt, J., and Klasmeier, J. (2008). "Determination of soil biodegradation half lives from simulation testing under aerobic laboratory conditions: A kinetic model approach." *Environ. Poll*., Vol. 156, pp. 99-105, DOI: 10.1016/j.envpol.2007.12.040.

McBride, M. B. (2003). "Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks?." *Advances in Environmental Research,* Vol. 8, No. 1, pp. 5-19, DOI: 10.1016/S1093-0191(02)00141-7.

McLean, E. O. (1982). *Soil pH and lime requirement*, In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, second ed. Am. Soc. Agron., Madison, WI, pp. 199-224.

Moreno, J. L., Bastida, F., Ros, M., Hernández, T., and García, C. (2009). "Soil organic carbon buffers heavy metal contamination on semiarid soils: Effects of different metal threshold levels on soil microbial activity." *European Journal of Soil Biology*, Vol. 45, No. 3, pp. 220-228, DOI: 10.1016/j.ejsobi.2009.02.004.

Nelson, D. W. an Sommers, L. E. (1982). *Total Carbon, Organic Carbon and Organic Matter*, Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No:9 (2nd ed.) ASA-SSSA. Madison, Wisconsin, USA, pp. 539-577.

Okoh, A. I. (2006). "Biodegradation alternative in the cleanup of petroleum hydrocarbon contaminants." *Biotechnology and Molecular Biology Review*, Vol. 1, No. 2, pp. 38-50.

Perfumo, A., Banat, I. M., Marchant, R., and Vezzulli, L. (2007). "Thermally enhanced approaches for bioremediation of hydrocarbon-contaminated soils." *Chemosphere*, Vol. 66, No. 1, pp. 179-184, DOI: 10.1016/j.chemosphere.2006.05.006.

Plohl, K., Leskovsek, H., and Bricelj, M. (2001). "Biological degradation of motor oil in water." *Acta Chim. Slov.,* Vol. 49, pp. 279-289.

Rhoades, J. D. (1982). *Cation exchange capacity*, In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.). "Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, second ed." *Am. Soc. Agron., Madison,* WI, pp. 149-158.

Rowland, A. P, Lindley, D. K., Hall, G. H., Rossal, M. J., Wilson, D. R., Benhan, D. G., Harrison, A. F., and Daniels, R. E. (2000). "Effects of beach and sand properties,temperature and rainfall on the degradation rates of oil buried in oil/beach sand mixtures." *Environmental Contamination*, Vol. 109, No. 1, pp. 109-118, DOI: 10.1016/S0269-7491(99)00224-9.

Sang-Hwan, I., Seokho, I., Dae Yaeon, K., and Jeong-gyu, K. (2007). "Degradation characteristics of waste lubricants under different nutrient condition." *J. Hazard. Mater.*, Vol. 143, Nos. 1-2, pp. 65-72, DOI: 10.1016/j.jhazmat.2006.08.059.

Sarkar, D., Ferguson, M., Datta, R., and Birnbaum, S. (2005). "Bioremediation of petroleum hydrocarbons in contaminated soils: Comparison of biosolids addition, carbon supplementation, and monitored natural attenuation." *Environmental Pollution*, Vol. 136, No. 1, pp. 187-195.

Sinkkonen, S. and Paasivirta, J. (2000). "Degradation half-times of PCDDs, PCDFs and PCBs for environmental fate modeling." *Chemosphere*,

Vol. 40, Nos. 9-11, pp. 943-949, DOI: 10.1016/S0045-6535(99)00337-9.

Venosa, A. D. and Zhu, X. (2003). "Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands." *Spill Science and Technology Bulletin*, Vol. 8, No. 2, pp. 163-178, DOI: 10.1016/S1353-2561(03)00019-7.

Walworth, J., Braddock, J., and Woolard, C. (2001). "Nutrient and temperature interactions in bioremediation of cryic soils." *Cold Regions Science and Technology*, Vol. 32, Nos. 2-3, pp. 85-91, DOI: 10.1016/S0165-232X(00)00020-3.

Xu, R. and Obbard, J. (2003). "Effect of nutrient amendments on indigenous hydrocarbon biodegradation in oil contaminated beach sediments." *Journal of Environmental Quality*, Vol. 32, No. 4, pp. 1234-1243, DOI: 10.2134/jeq2003.1234.

Yeung, P. Y., Johnson, R. L., and Xu, J. G. (1997). "Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration." *Journal of Environmental Quality*, Vol. 26, No. 6, pp. 1511-1576, DOI: 10.2134/jeq1997.00472425002600060009x.