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Biodegradation of used engine oil in a wastewater sludge-amended agricultural soil

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Abstract: Soil contamination by used engine oil is a common occurrence in most developing countries. This has been shown to have harmful effects on the environment and human beings at large. Used oils are considered to be hazardous waste materials. These are composed of toxic chemicals, such as heavy metals (which come from additives and wear and tear of engine parts), combustion products, light hydrocarbons, polar compounds, uninuclear and polynuclear aromatic compounds, resinous materials, and organometallic compounds. Some of these pollutants are carcinogenic in nature. The objective of this study was to evaluate the effects of used engine oil (doses of 0.5% and 5%) with hydrocarbon pollution on total petroleum hydrocarbon (TPH) removal from soil and determine the fate of TPHs 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 used engine oil-contaminated soils was also evaluated. The results of 240 days of incubation indicated that TPH removal percentages in used engine oil-contaminated+sludge amended soils at 18 °C were 68% and 66% for doses of 0.5% and 5%, respectively. Incubation at 28 °C resulted in higher TPH removal with values of 56% (dose of 0.5%) and 74% (dose of 5%). Based on the first-order kinetics model, the high dose (5%) of used engine oil-contaminated soil amended with wastewater sludge showed the highest biodegradation rate of 0.00562/day and half-life of 123.13 days at the end of the incubation period at 28 °C. These rates were significantly higher than those of the control soil (0.00366/day and 189.01 days).

Key words: Biostimulation, used engine oil, soil, total petroleum hydrocarbons, wastewater sludge

1. Introduction

Engine oil is used for lubrication requirements of various kinds of automotive and other engines. During these types of use, engine oil picks up a number of additional components from engine wear. These include heavy metals such as lead, chromium, and cadmium and other materials like naphthalene, chlorinated hydrocarbons, and sulfur. Fresh engine oil contains a higher percentage of volatile and water-soluble hydrocarbons that would be a concern for acute toxicity to organisms (Boonchan et al., 2000). Used engine oil contains metals and heavy polycyclic aromatic hydrocarbons that could contribute to chronic hazards including mutagenicity and carcinogenicity (Hagwell et al., 1992). As the usage of petroleum hydrocarbon products has increased, soil contamination with diesel and engine oils is becoming one of the major environmental problems. Therefore, the development of research to remediate soils contaminated with used engine oils, in particular bioremediation, provides an effective and efficient strategy to speed up the clean-up processes (Mandri and Lin, 2007). Addition of nutrients (biostimulation) is therefore needed as an effective approach to enhance the bioremediation process (Semple et al., 2006; Walworth et al., 2007).

Biostimulation is the addition of substrates, vitamins, oxygen, and other compounds that stimulate microorganism activity so that they can degrade the petroleum hydrocarbons faster.

Among the environmental factors known to limit biodegradation of soil containing petroleum hydrocarbons, temperature fluctuation and nutrient availability are among the most important ones (Coulon et al., 2005). Temperature affects the rate of biodegradation, as well as the physical nature and chemical composition of hydrocarbons (Rowland et al., 2000). Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. Das and Chandran (2011) reported that the highest degradation rates generally occur in the range of 30–40 °C in soil environments. According to Venosa and Zhu (2003), ambient temperature of the environment affected both the properties of spilled oil and the activity of microorganisms.

Stimulation of microorganisms by the addition of nutrients brought large quantities of carbon sources, which tend to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P (Sang-Hwan

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et al., 2007). Various nutrient sources such as inorganic fertilizer, urea, sawdust, compost, manure, and wastewater sludge have been used in biostimulation (Namkoong et al., 2002). The primary benefits of biosolids include their low cost (or no cost), slow release of the nutrients (similar to animal manures), and easy availability (McBride, 2003).

In this respect, wastewater sludge contains significant amounts of nutrients required by plants, including nitrogen, phosphorus, potassium, and micronutrients, making them an excellent fertilizer for use in agriculture and forestry.

This study aims to identify canned food industry wastewater sludge (WS) in enhancing the biodegradation of used engine oil in contaminated soil. To achieve the objective, canned food industry sludge (100 t/ha) was selected as the organic component to be added individually to the used engine oil contaminated soils.

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

2. Materials and methods

2.1. Materials

Soil samples were collected from the top 20 cm of an agricultural field located in Bursa Province, Balabancık village (40°15′55.1″N, 28°47′07.55″E).

The canned food industry wastewater sludge samples were obtained from a food company treatment plant in Bursa, Turkey. General characteristics of the canned food sludge and soil are presented in Table 1.

Used engine oil was collected from a car service center.

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

2.2. Incubation procedure

Soil samples were air-dried in the laboratory and sieved through 2-mm screens, and then portions of soil (40 g) were placed in cylindrical glass pots. The air-dried, sieved soil samples (40 g) were contaminated with 0.5% (w/w) and 5% (w/w) of used engine oil and thoroughly mixed. Oil application doses of 5% and 0.5% were chosen in this study in order to simulate major and minor oil contamination in soil, respectively.

Soil with used engine oil addition served as a control. After the oil addition, the wastewater sludge was thoroughly mixed with the soils at ratios equivalent to 100 t/ha (40 g kg^{-1}) on dry weight basis. The pots were incubated for 240 days in the dark at 28 ± 0.5 °C and 18 ± 0.5 °C. The moisture content in soil was maintained at 70% of field capacity and the content was tilled for aeration throughout the incubation period. Water loss by evaporation was compensated daily using distilled water to maintain soil water content. The experiment was planned with a completely randomized design and each treatment was performed in triplicate 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.

2.3. Determination of soil and wastewater sludge physical and chemical properties

Sample extracts were obtained by shaking the samples with distilled water at 1:5 (w/v). The electrical conductivity ($EC_{25°C}$) and the pH of the specimens were measured with conductivity and pH meters, respectively. The nitrate and ammonium nitrogen concentrations were determined by steam distillation with MgO and Devarda alloy in samples that were extracted with 2 M KCl (Keeney and Nelson, 1982). The Kjeldahl digestion method was used to measure the total nitrogen concentration (Bremner and Mulvaney, 1982). In addition, dichromate oxidation was used to measure the total organic carbon (Nelson and Sommer, 1982). A solution of 0.5 N $NaHCO_3$ was used to extract available P. Nitric acid-sulfuric acid digestion was performed in order to determine total P. PO_4^{-3}-P in extracts was measured according to the ascorbic acid method (American Public Health Association, 1998). The total concentrations of metals were determined after microwave digestion of the samples with HNO_3. Cr, Ni, Cu, and Zn were analyzed using an atomic absorption spectrophotometer (Isaac and Johnson, 1998).

2.4. Determination of total petroleum hydrocarbons in soil

The TPH concentration was determined by ISO 16703:2004 (International Organization for Standardization, 2004). Petroleum-contaminated soil, 20 g, was weighed and put into a glass extraction vessel with 40 mL of acetone. After briefly shaking by hand, 20 mL of RTW-standard solution was added. The specimens were extracted for 60 min by mechanical shaking. After the solid material settled, the supernatant was transferred into a separatory funnel. The acetone was removed by washing the organic phase twice by shaking thoroughly (5 min) with 100 mL of water. The organic layer was collected in a glass tube, and then sodium sulfate was added and 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.

The extracts were analyzed in random order within a single batch by gas chromatography, using an HP Agilent 7890A gas chromatograph (Agilent Technologies, www.agilent.com) equipped with a FID detector, an Agilent 7693 autosampler, and a low-bleed HP-5MS (Agilent part no: 19091S-433) capillary column (30 m × 0.25 mm i.d.) with a nominal film thickness of 0.25 µm. The 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 was 2 µL. Helium (2 mL min^{-1}) was used as the 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}, and was then held at 350 °C for 10 min. According to standard methodology (ISO 16703:2004), the amount of TPHs 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.

TPH data were fitted to a first-order kinetics model (Yeung et al., 1997) with the following equation:

$$y = ae^{-kt}$$

where

- y = the residual hydrocarbon content in soil (mg kg^{-1}),
- a = the initial hydrocarbon content in soil (mg kg^{-1}),
- k = the biodegradation rate constant (day^{-1}), and
- t = time (days).

The model estimated the biodegradation rate and half-life of hydrocarbons in soil relative to treatments applied. Half-life was then calculated using the model of Yeung et al. (1997) as:

$$\text{Half-life} = \ln(2)/k$$

This model was based on the assumption that the degradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in the soil (Agamuthu et al., 2013).

2.5. Statistical analysis

All statistical calculations were performed using STATISTICA 6.0 software. ANOVA was performed to test the effect of the contaminant dose, treatment and incubation time (Table 2).

Table 2. Variation of TPH concentration soil contaminated with used engine oil.

Variation	$F_{statistic}$	P	MS	Deg. of freedom
0.5% used engine oil				
Temperature	0.18	n.s.	56,653	1
Incubation time	26.53	<0.05	8,549,404	5
Treatment	19.98	<0.05	6,437,942	1
Treatment × incubation time	4.90	n.s.	1,579,560	5
Temperature × treatment	18.82	<0.05	6,065,047	1
Temperature × incubation time	11.47	<0.05	3,696,015	5
Temperature × incubation time × treatment	3.91	<0.05	125,885	5
Error				48
5% used engine oil				
Temperature	11.15	<0.05	165,539E3	1
Incubation time	35.01	<0.05	519,674E3	5
Treatment	84.74	<0.05	125,778E4	1
Treatment × incubation time	1.86	n.s.	276,691E2	5
Temperature × treatment	2.13	n.s.	316,035E2	1
Temperature × incubation time	5.12	<0.05	759,362E2	5
Temperature × incubation time × treatment	4.74	<0.05	703,369E2	5
Error				48

n.s.: not significant.

The effects of incubation time and treatment on TPH removal were further tested with two-way ANOVA for each temperature. When significant effects were indicated by ANOVA, post hoc analysis was performed using Tukey's HSD multiple comparison test.

3. Result and discussion

3.1. Biodegradation of used engine oil

Used motor oil may contain minute quantities of gasoline, additives (detergents, dispersants, oxidation inhibitors, rust inhibitors, viscosity improvers), nitrogen and sulfur compounds, a broad range of aromatic and aliphatic hydrocarbons with chain lengths ranging from C15 to C50, and metals such as lead (Pb, 40 ppm), zinc (Zn, 650 ppm), calcium (Ca, 1200 ppm), barium (Ba, <5 ppm), and magnesium (Mg, 65 ppm). These contaminants arise from normal wear of engine components and from heating and oxidation of lubricating oil during engine operation. The typical viscosity value of used engine oil has been stated as 80 mm^2/s at 40 °C (Hewstone, 1994). Used oil may contain higher percentages of polycyclic aromatic hydrocarbons and additives compared to fresh oil (Dominguez-Rosado and Pichtel, 2004).

The variations in TPH concentration levels for soil samples contaminated with used engine oil and incubated at 18 °C for varying amounts of time are shown Figure 1a. For soil samples contaminated with 0.5% used engine oil, the concentration of TPH levels is observed to decrease over time. The TPH concentration fell from the initial level of 5400 mg/kg to 1700 mg/kg after an incubation period of 240 days.

An examination of the TPH concentration of the samples contaminated with used engine oil and treated with wastewater sludge showed that the accelerated biodegradation of the used engine oil by the wastewater sludge began on the 60th day of the incubation process. Amendment of soil contaminated with used engine oil with wastewater sludge positively enhanced the rate of biodegradation during 60–150 days ($P < 0.05$). This result may be due to differences in the nutrients, and particularly N and P, in the wastewater sludge that stimulate indigenous microorganisms. The addition of N and P to oil-polluted

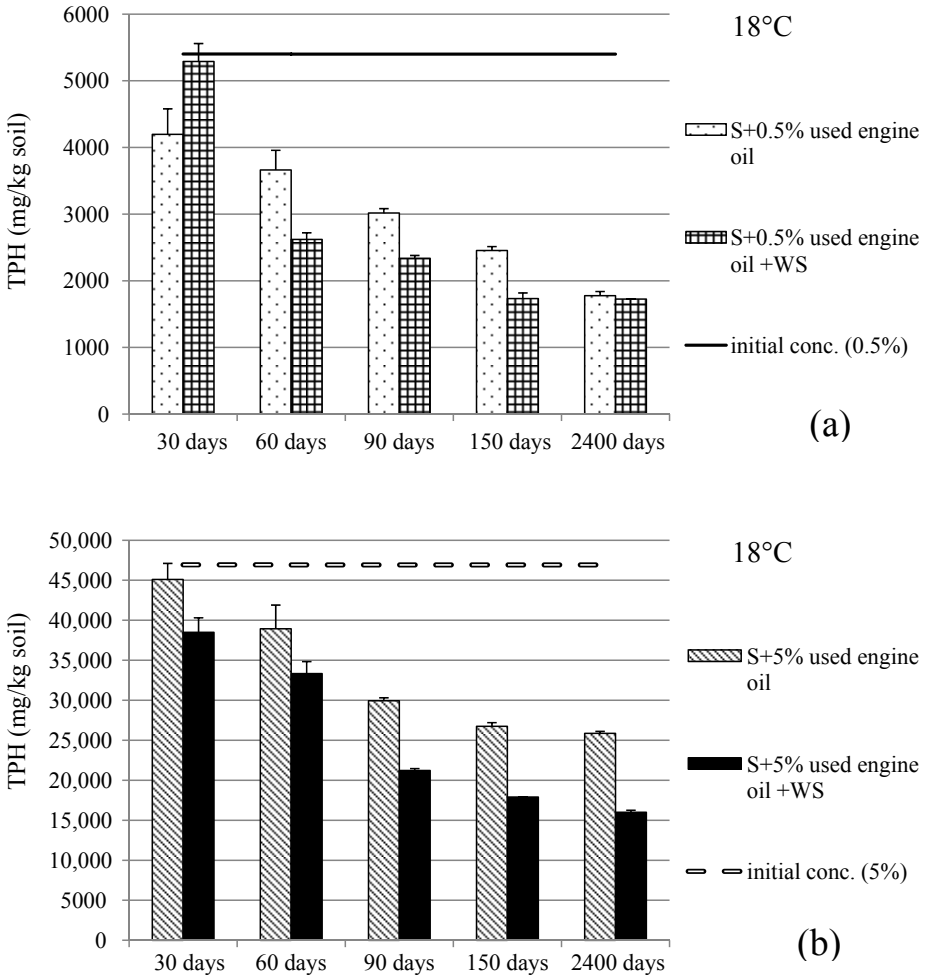

Figure 1. Changes in TPH levels in used engine oil-contaminated and canned food industry sludge-amended soil during the incubation period at 18 °C.

soil has been shown to accelerate the biodegradation of the petroleum in soil (Ijah and Safiyanu, 1997; Abioye et al., 2009).

For the soil samples contaminated with 5% used engine oil, the TPH concentration decreased markedly in accordance with the duration of incubation. As shown in Figure 1b, wastewater sludge treatment of the soil samples accelerated the degradation of used engine oil. The TPH concentration for the soil samples contaminated with 5% used engine 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 ($P < 0.05$). Hur and Park (2003) reported that the addition of sewage sludge was very effective for the degradation of diesel engine oil contaminated soil.

On the 90th day, the TPH level for the soil samples contaminated with used engine oil was 30,000 mg/kg, whereas the level for samples contaminated with used engine oil and treated with wastewater sludge was 21,000 mg/kg. According to Aleer et al. (2011), biodegradation of waste engine oil in test soils showed that by week 8, the levels of TPH in biostimulated samples fell below 10,000 mg/kg.

The changes in TPH concentration with incubation period for the soil samples contaminated with used engine oil and incubated at 28 °C are given in Figure 2. For the soil samples contaminated with 0.5% used engine oil, the initial level of 5400 mg/kg decreased to 1285 mg/kg by 240 days of incubation (Figure 2a). The positive effect of the wastewater sludge was observed within the first 90 days. Positive effects of nutrient amendment on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated by various authors (Margesin and Schinner, 2001; Riffaldi et al., 2006; Agamuthu et al., 2013).

For other incubation periods (during 150–240 days), higher TPH values were recorded for sludge amended soil pots. It was stated that the amendment of wastewater

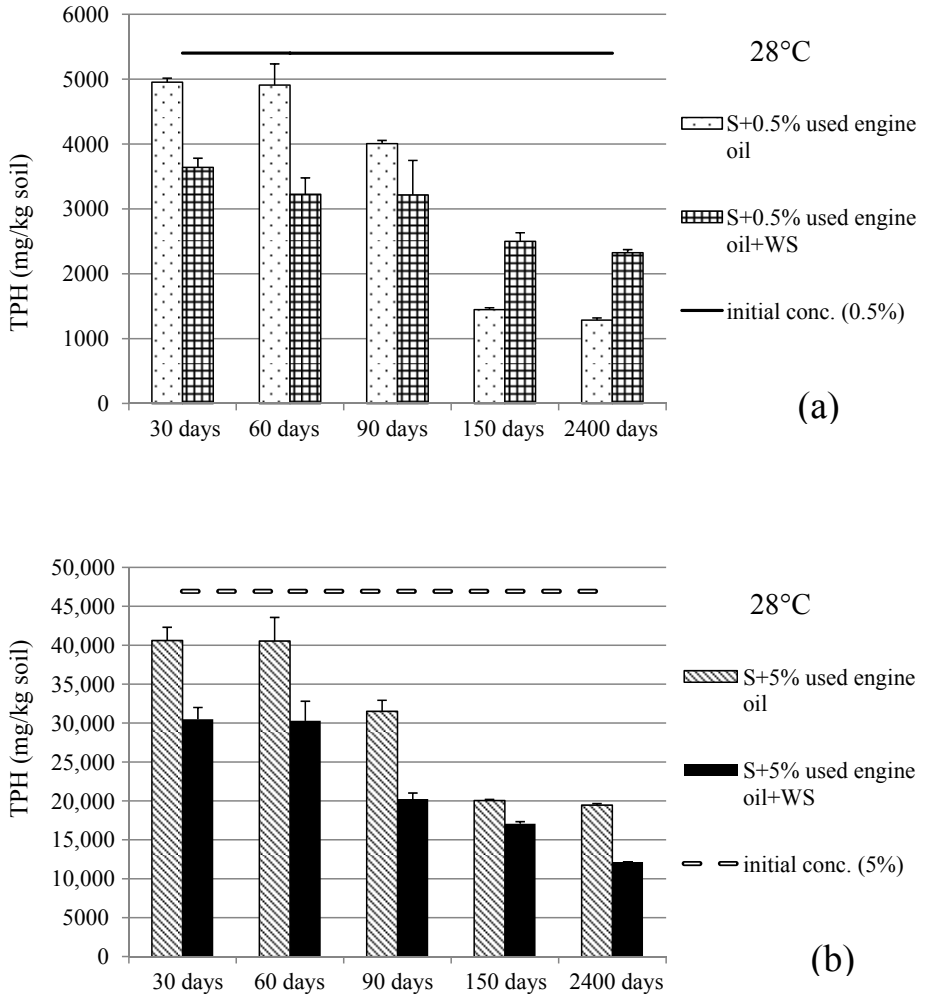

Figure 2. Changes in TPH levels in used engine oil-contaminated and canned food industry sludge-amended soil during the incubation period at 28 °C.

sludge due to nitrogen content can have deleterious effects (Bento et al., 2005; Walworth et al., 2007). Wastewater sludge mineralization may inhibit decomposition of less biodegradable compounds (Chaillan et al., 2006).

In addition, Sarkar et al. (2005) found that the microbial population in the fertilizer-amended soils dropped appreciably, suggesting a toxic effect due to fertilizer-induced acidity and/or NH_3 overdosing.

The variation over time in the soil samples contaminated with 5% used engine oil exhibited a decreasing trend within 150 days of incubation, as illustrated in Figure 2b. The wastewater sludge treatment accelerated the biodegradation of used engine oil for all incubation periods. This effect was especially apparent on the first 90 days of incubation. At the end of incubation, the TPH level for the samples treated with wastewater sludge was 12,150 mg/kg, whereas the level for soil that was not treated with wastewater sludge was 19,450 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.

Analyzing the variance confirmed that the temperature and incubation period significantly affected the TPH concentration. The results of ANOVA indicated that no significant effect was found due to temperature of samples contaminated with low doses of used engine oil. The treatment of soil with wastewater sludge had a significant effect on TPH biodegradation for samples contaminated with all doses (Table 1). Wastewater sludge addition indicated an enhancing effect on TPH biodegradation for soils contaminated with the high dose of used engine oil. On the hand, negative effects or no effect with respect to TPH removal was observed in case of the low level of oil pollution, especially for 28 °C.

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 used engine oil are shown in Figures 3 and 4.

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 used engine oil was 66% at the end of the incubation period. For the contaminated soil samples stimulated with wastewater sludge, the biodegradation rate had no increased. Some studies reveal that nitrogen and phosphorus correction may have no effect on the decontamination (Seklemova 2001), or even may represent an inhibitory effect in the biodegradation process by excessive addition (Mariano et al., 2007). Nitrogen, when added as ammonium salts, can be toxic to microorganisms due to the ammonia generation in the soil, which can be lethal in high concentrations, and some sources of phosphorus (phosphate and orthophosphate) may present diverse effects on the biodegradation depending on their toxicity and solubility (Trindade et al., 2002). Marchal et al. (2003) reported that many studies performed with soil microcosms had incomplete degradation of diesel engine oil. The partial TPH biodegradation obtained in their work (57.8%) in 55 days of treatment is in agreement with the results obtained by Bento et al. (2003), who achieved in 84 days a TPH removal of approximately 75% with similar bioremediation strategies.

The TPH biodegradation rate of the soil samples contaminated with a high dose of used engine oil was 45%, which increased to 66% after treatment with wastewater sludge.

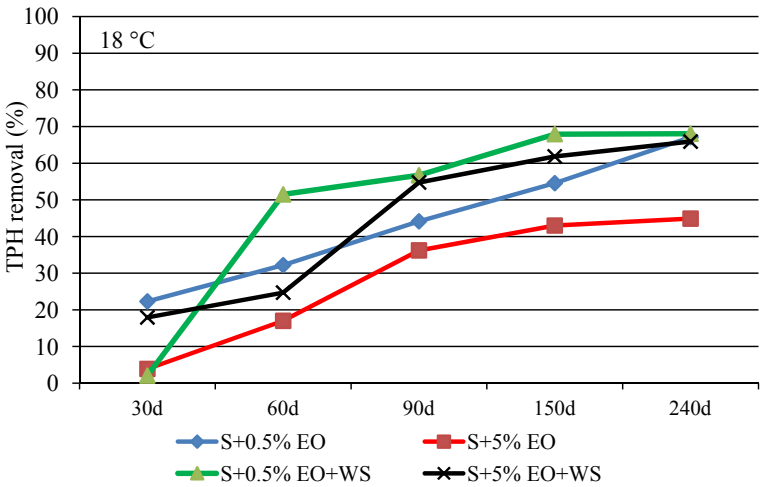

Figure 3. Percentage biodegradation of TPH in soil contaminated with used engine oil and amended with canned food industry wastewater sludge at 18 °C.

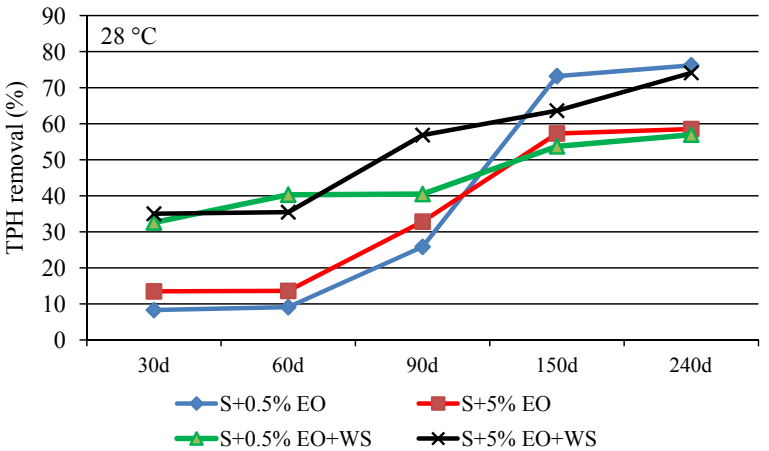

Figure 4. Percentage biodegradation of TPH in soil contaminated with used engine oil and amended with canned food industry wastewater sludge at 28 °C.

After a total incubation period of 240 days at 28 °C, the biodegradation rate for the soil samples contaminated with a low dose of used engine oil was 74%, whereas treatment with wastewater sludge decreased this rate (57%). This is in contrast to the findings of Abioye et al. (2010), who showed that amendment of engine oil-contaminated soils with organic matter led to between 22.8% and 54.03% net loss of TPH compared to the naturally attenuated control samples.

For soil contaminated with a high dose of used engine oil, the TPH biodegradation rate was 58%, which increased to 74% after treatment with wastewater sludge. Abioye et al. (2012) reported 92%, 84%, and 79% biodegradation in soil contaminated with 5% used lubricating oil and amended with brewery spent grain, banana skin, and spent mushroom compost within the period of 84 days. The differences in these results might be due to different compositions of used engine oil utilized for the studies, or differences in the organic wastes used.

The lowest biodegradation rate was observed for the soil contaminated with a low dose of used engine oil and treated with wastewater sludge.

3.2. Biodegradation rate constant and half-life of used engine oil

A first-order kinetics model (Yeung et al., 1997) was used to determine the rate of biodegradation of crude oil in soil amended with wastewater sludge. Kinetic analysis is a key factor for understanding the biodegradation process, bioremediation speed measurements, and development of efficient clean-up for a crude oil-contaminated environment.

Tables 3 and 4 show the biodegradation rate constant (k) and half-life ($t_{1/2}$) for the soil contaminated with used engine oil amended with wastewater sludge during 240 days of study.

According to Table 3, the low dose (0.5%) of used engine oil-contaminated soil amended with wastewater sludge showed the highest biodegradation rate of 0.01430/day and half-life of 48.25 days, whereas the biodegradation rate and half-life of control soil were 0.00288/day and 240.24 days for 30 days. At the end of the incubation period, the low dose (0.5%) of used engine oil-contaminated soil amended with wastewater sludge showed a biodegradation rate of 0.00351/day and half-life of 197.39 days.

The high dose (5%) of used engine oil-contaminated soil amended with wastewater sludge showed the highest biodegradation rate of 0.01310/day and 52.72 half-life days, whereas the biodegradation rate and half-life of control soil were 0.00482/day and 143.78 days for 30 days. At the end of incubation period, the high dose (5%) of used engine oil-contaminated soil amended with wastewater sludge showed a biodegradation rate of 0.00562/day and half-life of 123.13 days.

The high biodegradation rate recorded in used engine oil-contaminated soil amended with wastewater sludge

Table 3. Biodegradation rate and half-life of hydrocarbon in used engine oil (EO)-polluted soil amended with wastewater sludge during the incubation period (28 °C).

28 °C	Biodegradation constant, k/day					Half-life ($t_{1/2}$) (days)				
Treatment	30 days	60 days	90 days	150 days	240 days	30 days	60 days	90 days	150 days	240 days
Soil+0.5% EO	0.00288	0.00158	0.00332	0.00878	0.00598	240.24	436.27	208.72	78.94	115.88
Soil+5% EO	0.00482	0.00243	0.00442	0.00567	0.00366	143.78	284.43	156.79	122.23	189.01
Soil+0.5% EO+WS	0.01430	0.00860	0.00576	0.00513	0.00351	48.25	80.56	120.25	135.02	197.39
Soil+5% EO+WS	0.01310	0.00725	0.00933	0.00674	0.00562	52.72	95.61	74.23	102.85	123.13

Table 4. Biodegradation rate and half-life of hydrocarbon in used engine oil (EO)-polluted soil amended with wastewater sludge during the incubation period (18 °C).

18 °C	Biodegradation constant, k/day					Half-life ($t_{1/2}$) (days)				
Treatment	30 days	60 days	90 days	150 days	240 days	30 days	60 days	90 days	150 days	240 days
Soil+0.5% EO	0.00840	0.00647	0.00647	0.00525	0.00463	82.47	107.04	107.04	131.83	149.65
Soil+5% EO	0.00131	0.00310	0.00499	0.00374	0.00248	527.11	223.08	138.78	185.02	279.24
Soil+0.5% EO+WS	0.00688	0.0121	0.00930	0.00758	0.00475	1006.6	57.49	74.52	91.45	145.87
Soil+5% EO+WS	0.00659	0.00472	0.00881	0.00642	0.00448	105.16	146.6	78.64	107.92	154.60

might be due its high N and P contents and its buffering effects on the microbial flora in the crude oil-contaminated soil compared to control soil (Lee et al., 2003).

According to Table 4, biodegradation of used engine oil at 18 °C resulted in lower biodegradation constants and higher half-lives in general. The low dose (0.5%) of used engine oil-contaminated soil amended with wastewater sludge showed the highest biodegradation rate of 0.0121/day and half-life of 57.49 days, whereas the biodegradation rates and half-life of control soil were 0.00647/day and 107.04 days for 60 days. At the end of the incubation period, the low dose (0.5%) of used engine oil-contaminated soil amended with wastewater sludge showed a biodegradation rate of 0.00475/day and half-life of 145.87 days.

The high dose (5%) of used engine oil-contaminated soil amended with wastewater sludge showed the highest biodegradation rate of 0.00881/day and 78.64 half-life days, whereas the biodegradation rates and half-life of control soil were 0.00499/day and 138.78 days for 90 days. At the end of the incubation period, the high dose (5%) of used engine oil-contaminated soil amended with wastewater sludge showed a biodegradation rate of 0.00448/day and half-life of 154.60 days.

The results show a relationship between the rate of biodegradation and concentration of used engine oil in the contaminated soil. According to the results, higher biodegradation rates were recorded in soil contaminated with 0.5% used engine oil at 18 °C. This high biodegradation rate could be attributed to an increase in the activity of soil microorganisms at this oil pollution level (Adesodun and Mbagwu, 2008; Abioye et al., 2012). Liu et al. (2011) reported that a successful stimulation of the indigenous communities was achieved with nutrient addition.

For 28 °C, higher biodegradation rates were found in soil contaminated with 5% used engine oil during 30–90 days. In the later incubation periods, higher biodegradation rates were recorded in soil contaminated with 0.5% used engine oil. Bossert and Bartha (1984) stated that sensitivity of soil microflora to petroleum hydrocarbons is a factor of quantity and quality of oil spilled.

3.3. Conclusion

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 used engine oil can be summarized as follows:

- The results showed high biodegradation of used engine oil by the end of 240 days for contaminated soil compared to the initial concentration of used engine oil for both of temperatures.
- At 28 °C, the TPH concentrations in soil contaminated with high (5%) and low (0.5%) doses of used engine oil were reduced from approximately 46,920 mg/kg to 12,150 mg/kg and from 5400 mg/kg to 2325 mg/kg, respectively.
- At 18 °C, the TPH concentrations in soil contaminated with high (5%) and low (0.5%) doses of used engine oil were reduced from approximately 46,920 mg/kg to 16,000 mg/kg and from 5400 mg/kg to 1700 mg/kg, respectively.
- At both temperatures, the majority of the TPH biodegradation occurred between the 90th and 150th days of the incubation period.
- The greatest amount of TPH biodegradation took place at a temperature of 28 °C.
- The results showed high biodegradation of used engine oil by the end of 240 days for soil treated with wastewater sludge compared to the initial concentration of used engine oil for both of temperatures.
- Used engine oil-contaminated soil (5%) 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 a high dose of used engine oil at a rate of 28% to 111%.
- In some incubation periods, wastewater sludge either had no effect, or negative effects on oil biodegradation in case of low level oil pollution.

Consequently, the possibility of using wastewater sludge in soils contaminated with oil (petroleum hydrocarbons) should initially be evaluated by incubation studies to estimate the appropriate rate of sludge application dose and oil pollution level.

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