Article

Analysis of Total Petroleum Hydrocarbon Content in Contaminated Soil after Biodegradation Treatment: Tobruk Refinery (Marsa El Hariga Terminal), Libya

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Abstract

After oil-contaminated soil samples were collected from the affected area of an extensive oil spillage near the Tobruk refinery (El Hariga Terminal), a DOP-UNI biological product was used in this bioremediation field trail. DOP-UNI has the largest concentration of microorganisms, leading to the fastest rate of degradation in soils and water Gas. Chromatography with a flame ionization detector was used to test the collected samples. The overall petroleum hydrocarbon (TPH) concentrations in untreated soil samples ranged from 2700± 28 to 2800± 26 µg g-1, while the treated samples had concentrations ranging from 3.5± 0.1 to 210.2 ±54 µg g-1. This represents a 97 percent removal of total hydrocarbons from the polluted soil samples. Findings show that bioremediation is stronger and quicker in samples exposed to direct sunlight.

Keywords: Soil contamination; total petroleum hydrocarbon; bioremediation; linked health hazards.

1. Introduction

Since1957, Libya has been engaged in crude oil exploration and extraction [1], [2]. These activities have a significant effect on the country's social, political, and economic systems. Consequently, they contribute to the development of the country by creating employment, promoting industry, and providing critical infrastructure to the communities. However, as a result of industrial activities and pipeline leakage, oil spillages have been a concern in the oilfields and refineries region of Libya, where oil is extracted. The soil and groundwater around oil exploration and production zones, particularly in the oilfields and harbours area, are frequently contaminated.

Petroleum hydrocarbons, which make up crude oil, are divided into three classes. Alkanes (paraffin), alkenes (olefins), and aromatic compounds are all examples of these types of compounds [3]. The term of "Total Petroleum Hydrocarbon (TPH)" refers to a broad group of several hundred hydrocarbons. Petroleum products are made from crude oil, which can pollute the atmosphere. Since crude oil and other petroleum products contain so many different chemicals, measuring each one separately is impractical. Measurement of the total amount of TPH in environmental samples at a site is, however, useful. Hexane, naphthalene, and fluorine, as well as other petroleum products and fuel elements, may be present in TPH. TPH samples, on the other hand, are likely to contain either any or a combination of these chemicals. The volume and types of compounds released into the atmosphere by a petroleum hydrocarbon vary greatly depending on the substance spilled and how it weathered [3]. Because of this condition, determining the toxicity and mobility of worn petroleum products solely based on TPH results is difficult. However, depending on the hydrocarbon spectrum, the release situation, the location's characterization, and the land's intended usage, an examination of soil and/or groundwater may be used to estimate risk [4].

For instance, TPH has been detected in the vicinity of facilities handling refined petroleum oil in Lagos, Nigeria. TPH was calculated in three nearby locations (a mechanic workshop, a Nigerian Electricity Power Authority (NEPA) station, and a Petrol station, respectively [5]. The effects of these three stations were compared and contrasted. As a result, TPH concentrations in dust from gasoline stations selected residential areas, and high-traffic roads within the Tshwane metropolitan region in South Africa have been identified, with gasoline stations having higher TPH levels than the other two stations studied [6].

Oil spill treatment methods can be classified as physical/mechanical, chemical, or biological. Choosing the oil spill treatment method becomes an optimization task by minimizing both the environmental impact and cost of operation [7], [8].

1.1 Biodegradation

Biodegradation is defined as the transformation of a substance into new compounds through biochemical reactions or the actions of microorganisms such as bacteria [9]. A process by which microbial organisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment [10], [11].

Biodegradation is a biochemical reaction that is mediated by microorganisms. In general, an organic compound is oxidized (losses electrons) by an electron acceptor which in itself is reduced (gain electrons). Under aerobic conditions or toxic environmental conditions, oxygen acts as the electron acceptor. The oxidation of the organic compounds is coupled to the reduction of molecular oxygen, and this is termed aerobic respiration. When oxygen is not present (anaerobic conditions) microorganisms can use organic chemicals or inorganic anions as alternative electrons acceptors. Anaerobic biodegradation can occur under fermentative, denitrifying, iron-reducing, sulphate-reducing, or methanogenic conditions.

The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of “catalyst” present. In this context, the amount of “catalyst” present represents the number of organisms able to metabolize the contaminant as well as the number of the enzyme(s) produced by each cell.

1.2 Soil respirometry

Soil respirometry provides a measure of oxygen (O_2) consumption or carbon dioxide (CO_2) production in soils and is an indication of net aerobic biological activity in situ. The determination is typically made by measuring the consumption of oxygen by contaminated soils over time and comparing the rate to that observed with soils from a nearby region that is not contaminated. Increased O_2 use, measured as lower O_2 levels, is taken as an indication of increased respiratory activity that is potentially due to the metabolism of contaminant(s) [12].

Another technique in the biodegradation of petroleum and other hydrocarbons is in situ. This biodegradation technique has been discovered to be a more reliable, cost-effective, and environmentally acceptable method of degrading or eliminating hydrocarbon toxicity. Many laboratory studies have relied on the isolation of different bacterial genera strains from petroleum-contaminated soils and their subsequent growth in the presence of petroleum and aromatic hydrocarbons. The bacteria were then enriched in special media before being used to break down petroleum and hydrocarbons [13]. Hydrocarbon compounds are known to be degraded by a wide range of species under several environmental conditions. Pseudomonas is effective at degrading hydrocarbons. Candida has been found to be more effective at degrading C10-C16. Alkanes and fatty acids, and Rhodococcus are well known for degrading fatty acids.

In 2016, a DOP-UNI biological product was used to treat an oil spill on land near the Tubrok refinery. DOP-UNI has the largest concentration of microorganisms, resulting in the fastest rate of degradation in soils and water. Three types of microorganisms make up the universal "DOP-UNI." Each type of organism consumes a different part of the hydrocarbon chain. Candida, for example, eats the fastest light fractions, resulting in a visual effect 7-10 days after a biological substance is deposited (changes the color of the spill). Other bacteria (Rhodococcus, Pseudomonas) begin to feed later and the impact will last longer (1-3 months), but it will be visible when vegetation starts to grow on the spill site. For longer periods, these bacteria "eat" the most complex chains of hydrocarbons [14].

Tobruk refinery (Marsa El Hariga terminal) on the coast of north-eastern Libya, has experienced several spills of crude oil and petroleum products, resulting in substantial contamination of soil and groundwater. Biodegradation treatment has been applied to mitigate the negative effects of oil spills on the environment, Figure 1 presented some location of the contaminated soil.

The main objective of this study is to determine the levels of TPH content in soil samples inside and around the Tobruk refinery (Marsa El Hariga Terminal) after biodegradation treatment to reduce the environmental impact and associated health hazards of TPH and to protect communities that are currently cultivating the land and using groundwater for domestic activities.

Figure 1. Contaminated areas around the AGOCO Company's El Hariga refinery in Tobruk, Libya.

2. Materials and method

Several steps were taken to collect samples and prepare them for laboratory final analysis.

2.1 Samples collection and purification

The contaminated areas had previously been subjected to bacterial biodegradation treatment to transform and reduce the THP in the soil. As shown in Figure 2, one crude oil contaminated soil sample (untreated) (C1), one uncontaminated soil sample from the same experimental area (C2), and four contaminated soil samples treated with biodegradation (1T, 2T, 3T, and 4T) were collected from various points in the biodegradation field trial experiments.

Soil samples were taken with an auger at depths ranging from 0 to 10 cm and 10 to 25 cm. The soil samples were homogenized in clean plastic containers that had been washed after processing. The homogenized part of the soil samples was transferred to a clean amber bottle and preserved at 4.0 °C for later use. A 15 g of soil sample was placed in an amber glass container. To extract the moisture, anhydrous sodium sulphate (Na_2SO_4) was mixed into the glass bottle containing the soil sample. The soil sample was treated with 300 g/ml of the surrogate (1-chlorooctadecane) normal. To extract the solvent, 30 ml of dichloromethane (DCM) was applied to the sample, which was then sealed tightly and transferred to a mechanical shaker [15].

The mechanical shaker was used to agitate the soil sample for 4 to 6 hours at room temperature. The sample was allowed to settle for about an hour after shaking and agitation and then filtered through 110 mm filter paper into a clean beaker. By evaporating the filtrate overnight in a fume cupboard, it was reduced to around 1.0 ml.

A glass column was used to clean up the samples. Glass cotton was introduced into the column as part of the column planning. Silica gel was dissolved in DCM to make a slurry, which was then poured into the column. Pentane was applied to the column after anhydrous Na_2SO_4 was added. After preparation of the column, the concentrated sample extract was mixed with cyclohexane in a conical flask and transferred into the prepared column. Pentane was used to elute the sample extract, and the eluted sample was collected in a conical flask below the column. More pentane was added to the column to further elute the sample. The column was rinsed with DCM after elution. The eluted sample was left in a fume cupboard overnight at room temperature to allow for evaporation [15]. Step 1 and step 2 of Figure 3 show the methodology of samples purification and separation.

Figure 2. The study area for TPH biodegradation treatment and the location of soil samples (TPH bioremediation experiments).

Table 1. Characteristics of soil, paraffin, and sand samples were taken from the TPH biodegradation field trial experiment.

Soil samples ID	Sample details
C1	Contaminated soil sample from the same experimental site with crude oil TPH spillage, without bacterial treatment (control)
1T	Contaminated soil sample with TPH spillage, treated with bacteria, exposed to sunlight for 3 weeks during the experiment.
2T	Contaminated soil sample with TPH spillage, treated with bacteria, unexposed to sunlight for three weeks during the experiment
3T	Contaminated soil sample with paraffin spillage, treated with bacteria for three weeks during the experiment
4T	Contaminated sand sample with paraffin spillage, treated with bacteria for three weeks during experiment
C2	Uncontaminated soil sample, taken from the same experimental site

2.2 Samples analysis

The separation and detection of compounds in soil samples were carried out using Agilent 6890N Gas Chromatograph - Flame Ionization Detector (GC-FID) instrument [16]. 3.0 µ l of the concentrated sample was injected into the GC vial after it was eluted from the column. Before taking the sample for analysis, the blank DCM was inserted into the GC micro-syringe six times to clean the syringe. The sample was then rinsed out of the micro-syringe. Then the sample was pumped into the column for the separation of compounds in the sample. The compounds were separated and then passed through a flame ionization detector (FID), which detects the compounds in the sample. In µg g-1 units, the volume of TPH, was resolved at a specific chromatogram. Step 3 of Figure 3 shows the methodology of sample analysis.

Step 1: Samples purification

15.0 g of soil sample was placed in an amber glass container.

To extract the moisture, Na_2SO_4 was mixed to the soil sample

The sample was treated with 300 g/ml of surrogate normal (1-chlorooctadecane)

To extract the solvent, 30 ml of dichloromethane (DCM) was added to the sample

A mechanical shaker was used to agitate the sample for 4 to 6 hours at room temperature.

The sample was allowed to settle for about an hour, and then filtered through 110 mm filter paper into a clean beaker.

For evaporating step, the filtrate sample was left overnight in a fume cupboard

Step 2: Separation method

Glass cotton was introduced into the column as part of the column

Silica gel was dissolved in DCM to make slurry, which was then poured into the column

Pentane was applied to the column after anhydrous Na_2SO_4 was added. After preparation of the column

the extracted sample from step 1 was mixed with cyclohexane in a conical flask and transferred into prepared column

Pentane was used to elute the sample extract, and the eluted sample was collected in a conical flask below the column

More pentane was added to the column to further elute the sample. The column was rinsed with DCM after elution

The eluted sample was left in a fume cupboard overnight at room temperature to allow for evaporation

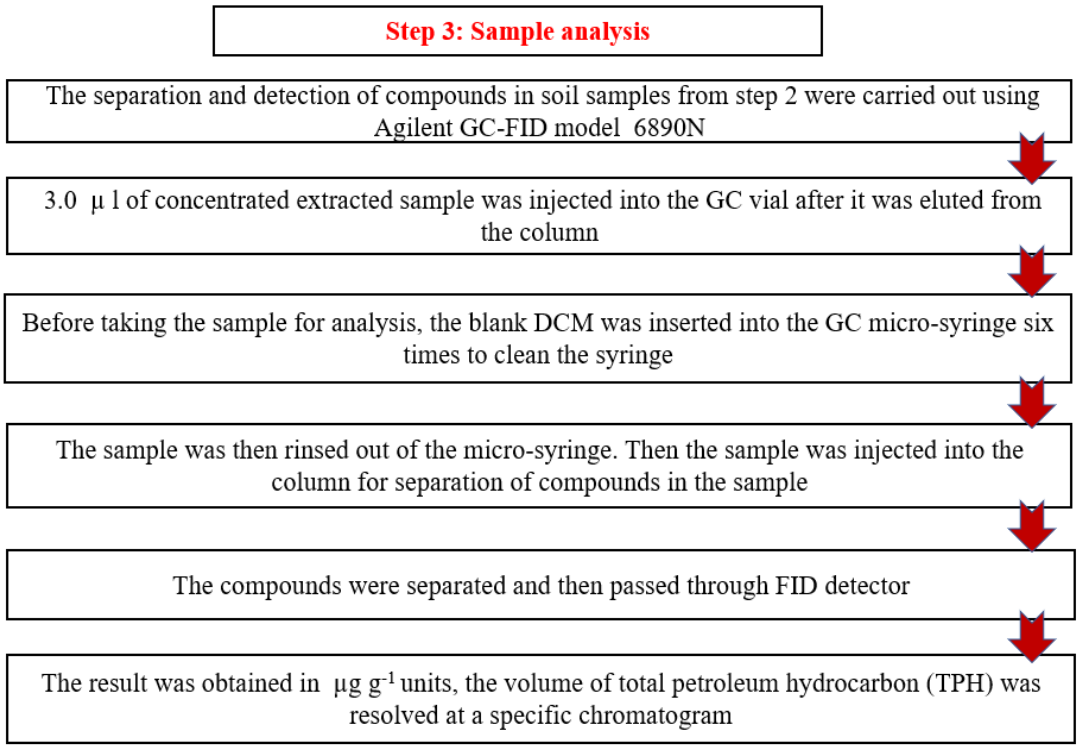

Figure 3. Methodology flow chart of sample preparation and analysis.

3. Results and discussion

Microbial activities have biodegraded crude oil TPH that had been left in the soil for a long time. The percentage of biodegraded TPH by field trial bioremediation experiments conducted between 24/07/2016 and 14/08/2016 was reported between 93.27% and 99.84%, respectively. This high percentage of biodegraded TPH indicates that TPH removal from polluted soil is extremely effective**.** The average results from this analysis are shown in Figures 4 and 5 and Tables 2 and 3 below.

Figure 4 and Table 2 show the mean TPH concentrations of soil samples collected from various bioremediation experiments and control soil samples. The results of the analysis show that as depth increases, the mean TPH concentrations in the soil from bioremediation field experiments (experiments 1T, 2T, 3T, and 4T) decrease. However, when TPH concentrations in control polluted soil samples (C1) were compared to TPH concentrations in bioremediation field experiments (Figure 3 and Table 2), it was discovered that TPH levels decreased from an average of 2800±26µgg^{-1} in controlled soil (C1) to 108.7±15.2µgg^{-1} in treated soil samples (bioremediation field experiments). This suggests that biodegradation of TPH in polluted soil samples (1T, 2T, 3T, and 4T) results in a significant reduction in hydrocarbon content as compared to control contaminated soil samples (C1), with an average rate degradation percentage of 96 percent. Also, the outcome of TPH biodegradation by bacteria in field trial experiment No. 1 (treated contaminated soil and exposed to sunlight)) is higher than the result of field trial experiment No. 2 (treated polluted soil and unexposed to sunlight).

Table 2. Compression of average levels for TPH in the treated soil profile at various points (µg g^{-1} ± 1 SD, n=3)*.

Sample ID	Average conc. (µg g^{-1})* at depth of 0.0 – 10 cm	%RSD	average conc. (µg g^{-1})* at depth of 10 – 25 cm	%RSD
C1 (untreated contaminated soil)	2800 ± 26	0.93	2700 ± 28	1.04
1T (treated soil, exposed to sunlight)	100 ± 3.5	3.50	75.6 ± 2.6	3.44
2T (treated soil, unexposed to sunlight)	210.2 ± 5.4	2.57	105.3 ± 2.8	2.66

3T (treated soil with paraffin, with bacteria treatment)	5.25 ± 0.2	3.81	3.5 ± 0.1	2.85
4T (treated sand sample with paraffin, with bacteria treatment	150 ± 4.5	3.00	220 ± 7.3	3.32
C2 (uncontaminated soil)	1.50 ± 0.05	4.17	1.30 ± 0.03	2.73

* Average TPH levels in treated soil profiles compressed at different points (µg g-1± 1 SD, n=3).
* Results are given as mean ± one standard deviation (n = 3).

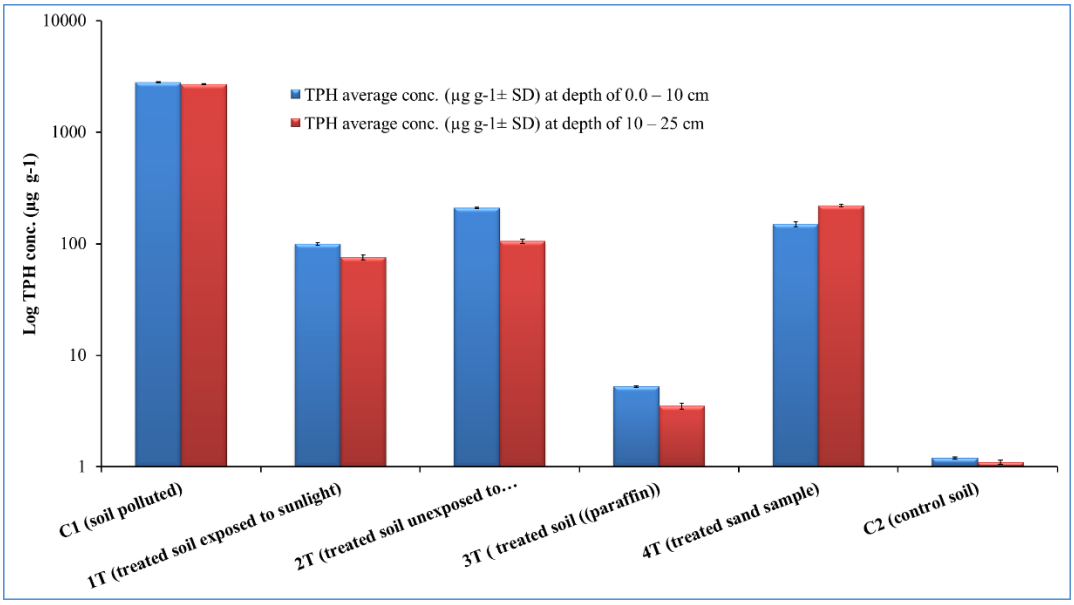

Figure 4. TPH concentrations (g g-1) in four bioremediation field samples and two control soil samples (n =6) at depths of 0-10 cm and 10-25 cm from the soil surface. The error bars reflect a difference of less than one standard deviation (SD) between replicates (n = 2).

Table 3. The percentage rate of biodegraded TPH in soil samples (n-4) by field trial bioremediation experiments compared polluted and uncontaminated soil samples as controls.

Sample ID	Field bioremediations experiment for TPH removal from soil % efficiency (%)
C1 (untreated contaminated soil)	0.00
1T (treated soil, exposed to sunlight)	96.81
2T (treated soil, unexposed to sunlight)	94.27
3T (treated soil, with paraffin)	99.84
4T (treated sand, with paraffin)	93.27
C2 (uncontaminated soil)	99.96

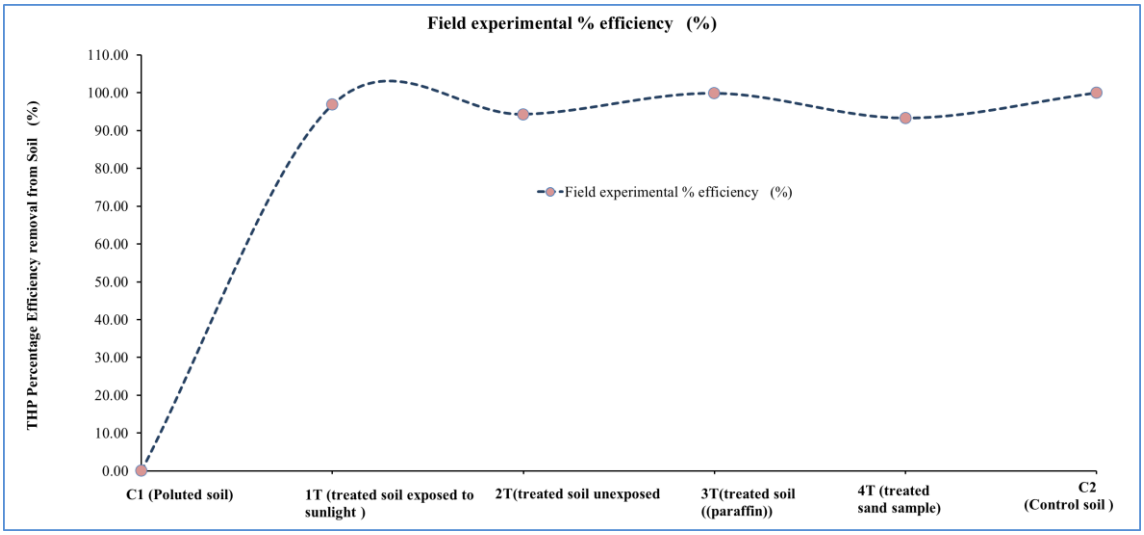

Figure 5. The percentage rate (TPH %) of biodegraded TPH in soil samples (n=4) using field trial bioremediation experiments with polluted and uncontaminated soil samples as control.

Finally, the small areas of land that were targeted for the TPH bioremediation field experiment were treated with bacteria for three weeks to remove TPH contamination. This is to ensure that any residual TPH is removed during the three-week field experiment. These plots of land were plowed and seeded with barley seeds, and they were irrigated with water. The results show evidence of biological transmutation of hydrocarbon waste to environmentally friendly products, as seen in Figure 6, where Barley grew well, indicating that the primary bioremediation field experiment was successful.

Figure 6. The cultivated soil with barley seeds after treatment.

4. Conclusion

According to the findings of this study microbial activity, such as the use of bacteria, is effective in the treatment of contaminated soil. As shown by laboratory study of soil samples collected from field trial sites at various depths of the soil, TPH has been absorbed and removed. The Total Petroleum hydrocarbons in the polluted soil have been reduced by 97 percent on average. Similarly, the best results were obtained by exposing soil samples

to direct sunlight for around three weeks, which resulted in the biodegraded total hydrocarbons being obtained faster and more effective in cleaning up soil contaminated with TPH. Therefore, this field trial experiment could be scaled up to large-scale contaminated soil surface areas exposed to direct sunlight. This technique increases the rate of biodegradation of total hydrocarbons in soil, as well as the effectiveness of bacteria-causing activity, and help bio-degradable total hydrocarbon in polluted soil. Furthermore, to minimize harming the surrounding environment, as well as affecting society's health, from the locations near the leaks and crude oil pollutants, this treatment is of great help.

Conflicts of Interest: The authors declare no conflict of interest.

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