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Comparative study on bioremediation of oil contamination soils in Libya at Al-Sharara Oil field

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Abstract This paper summarizes the research findings of an extensive experiment conducted on the bioremediation of crude oil that collected from Al-Sharara field which is located in Murzuq Basin in the southern of Libya. This Field is operated by Spanish Repsol through joint venture 50% with Akakus Oil operations which is producing around 150,000 oil barrel per day. The degree of oil biodegradation was investigated by using gas chromatography analysis. This study focused on the process of reducing soil microcosm process, during the biological restore of oil contaminated soil under three different conditions. Biodegradation activity of hydrocarbons with nutrient (nitrogen and phosphorous containing compounds) and adding poison (mercuric acetate) were studied through the determination of optimal conditions which improve bioremediation process. Indeed, after four weeks, about 98% removal rate of total petroleum hydrocarbon (TPH) in soil treated by microcosm's technique were observed. The results indicated that the total area of chromatographic peaks represented the concentration of total hydrocarbons in different conditions. The results confirmed that the soil samples consisted mainly of n-alkanes C11 through C31. The ratio of these hydrocarbons were approximately lower than 80%, with intermediate branched chain hydrocarbon, along with cycloalkanes, aromatic compounds and other petroleum-based compounds within the experimental samples. The degradation of the treated samples with nitrogen and phosphorous indicated the TPH concentration decreased rapidly at the end of treatment, it is relatively high when compared to the control (untreated) and the poison system.
Keywords: Bioremediation; Soil; Hydrocarbon (TPH); Nutrient.

دراسة مقارنة حول المعالجة البيولوجية للتربة الملوثة بالنفط في ليبيا بحقل الشرارة النفطي

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الملخص تلخص هذه الورقة نتائج دراسة توصلت إليها تجارب مكثفة أجريت على المعالجة الحيوية لعينات من النفط الخام تم جمعها من حقل الشرارة النفطي الواقع في حوض مرزق جنوب ليبيا. يتم تشغيل هذا الحقل من قبل شركة ريبسول الإسبانية من خلال مشروع مشترك بنسبة 50 ٪ مع عمليات النفط لشركة اكاكوس التي تنتج حوالي 150،000 برميل يوميا. تم فحص درجة التحلل الحيوي للنفط الخام باستخدام تحليل كروماتوجرافيا الغاز. ركزت هذه الدراسة على عملية الحد من عملية تلوث التربة ، أثناء الاستعادة البيولوجية للتربة الملوثة بالزيت في ظل ثلاثة ظروف مختلفة. تمت دراسة نشاط التحلل الحيوي للهيدروكربونات مع المغذيات (مركبات تحتوي على النيتروجين والفوسفور) وإضافة مادة سمية (خلات الزئبق) من خلال تحديد الظروف المثلى التي تعمل على تحسين عملية المعالجة البيولوجية. في الواقع ، بعد أربعة أسابيع ، لوحظ معدل إزالة حوالي 98 ٪ من إجمالي الهيدروكربونات البترولية (TPH) في التربة التي عولجت بتقنية ميكروكوسم. أوضحت النتائج أن المساحة الكلية للقمم الكروماتوجرافية تمثل تركيز الهيدروكربونات الكلية في ظروف مختلفة. كما أكدت النتائج أن عينات التربة كانت تتكون أساساً من الالكانات وذلك في مدي من C11 الي C31. وقد كانت نسبة هذه الهيدروكربونات أقل من 80٪ تقريباً ، مع الهيدروكربونات المتسلسلة الوسيطة المتشعبة ، إلى جانب السيكلو ألكانات والمركبات العطرية والمركبات الأخرى التي المكونة للنفط ضمن العينات التجريبية. كما اشارت التجارب الي تدهور العينات المعالجة بالنيتروجين والفوسفور وأن تركيز TPH انخفض بسرعة في نهاية المعالجة ، وهذا كان مرتفع نسبياً عند مقارنته بالعينات الغير معالجة وكذلك العينات المعالجة بنظام المادة السمية.

كلمات مفتاحية: التربة، الهيدروكربون (TPH) ، العناصر الغذائية.

1. Introduction

Pollution is the introduction of contaminants into an environment that causes harm to human health and other living organisms. Soil pollution comprises the pollution of soil with

materials[1,2], mostly chemicals that are out of place or present at concentrations higher than normal, which may have adverse effects on humans or other organisms. Huge oil spills during transportation and distribution, as well as spills during refining. Soil that is accidentally contaminated by petroleum fuel spills is classified as hazardous waste [3,4,5]. Cleaning up oil spills is not a small task, there are many methods used to clean up oil spills. An oil spill can be destroyed by fire, but burning often adds to the problems. Smoke, soot, fumes, and particulate matters from the burning oil can pollute the air over large areas, any materials such as chemicals, dust, and harmful gases in air and water are pollutants [6]. Petroleum hydrocarbons are commonly classified as environmental contaminants, though they are not usually classified as hazardous wastes [7,8]. Many petroleum products are used in modern society, including those that are fundamental to our lives (i.e; transportation fuels, heating and power-generating fuels, etc). The volume of crude oil or petroleum products that is used today dwarfs all other chemicals of environmental and health concern [9]. Due to the numbers of facilities, individuals, and processes and the various ways the products are stored and handled, environmental contamination is potentially widespread. Aromatic compounds are among the most prevalent and persistent pollutants in the environment [10,11]. Aromatics derived from industrial activities often have functional groups such as alkyls, halogens and nitro groups. Organic contaminants may enter the soil through many different pathways. Accidental spills, poorly designed hazardous waste facilities [12], and leaking underground storage tanks (LUSTs) [13]are all routes by which organic contaminants may enter soils. The magnitude of the problem is difficult to assess. One estimate is that there are >250 000 LUSTs at service stations across the United States [13] .Bioremediation is a process by which chemical substances are degraded by bacteria and other microorganisms. The use of these microorganisms has been successfully applied for the treatment of waste and wastewater in controlled systems. Several research studies have recently been performed to investigate the use of bioremediation for oil-spill cleanup in seawater, freshwater and terrestrial areas. The technique has been found to have a potential for broad applications in terrestrial and freshwater environments for treating soils and sediments contaminated with oil and other substances, as well as for coastal environments impacted by oil spills. Water is a more sensitive medium than soil and requires different remediation techniques. Spills to surface water are easier to clean up than spills to groundwater, for obvious reasons. It is not only much harder to see the extent of the contamination, but also to remove the source of the contamination as, for example, a leaking underground storage tank. The focus of this study is to determine the amount of degradation of petroleum hydrocarbons by the method of gas chromatography.

2. Materials and Methods

2.1 Sample collection

Soil samples were collected randomly from Al-sharara field from a depth of 10cm, Libya 2017. The soil samples were placed in a sterilized container and stored at room temperature. Soil samples were used to reflect a range of hydrocarbon concentrations and to represent various potential sources of contamination. All soil samples which were collected from the field location were mixed together. The petroleum products that contaminated the soil samples were extracted using a Soxhlet Extraction System. Chloroform was used for the extraction. Gas chromatography also was used to measure the degradation of hydrocarbons in soil.

2.2 Soil pH

Soil pH sample was determined using a pH meter (Jenway 3020) on soil suspensions in water. 25 grams of soil was stirred continuously with 50 ml of d-ionized water for 15 minutes and allowed to equilibrate for another 15 minutes. The pH meter was immersed in the supernatant and was rotated gently before recording pH. The pH was measured at room temperature equal 8.9 pH.

2.3 Experimental Set Up

The mixed soil samples which were collected from the locations were divided into three parts and used separately in the following experiments:

2.3.1 Untreated Samples (US)

These experiments will allow degradation of hydrocarbons in soil due to microbes present in soil itself.

2.1.2 Treated Samples (TS)

These experiments will allow degradation of hydrocarbons in soil due to microbes present in soil and having better conditions for biodegradation. The samples contain some nutrients (nitrogen and phosphorous containing compounds), moisture and aeration.

2.3.2 Poisoned Samples (PS)

These experiments do not allow and degradation due to microbes since all microbes have been killed by adding poison (mercuric acetate). Any decrease in concentration of hydrocarbons is only due to evaporation.

2.4 Preparation and Incubation of Hydrocarbon Contaminated Samples

We weighted (200g) from the soil sample and put it in the separator funnel and put it on (2g) the crude oil. The lower ends of the columns were closed with a plug and a closable drain spout.. After packing, water was added to the top of the column to adjust the moisture content of the soil to 50% untreated sample. About 5.714 mg Nitrogen as NH_4NO_3 and 26.32 mg phosphorous as KH_2PO_4 fertilizers were added to keep N and P concentration in soil maintained for biodegradation study. They were dissolved in water that was used for adjusting moisture content in the treated sample, tilling of the soil columns was performed by inserting the stainless steel wire into the soil column 15 times. This treatment was much less effective in earring the

soil than conventional tilling in the fields. 100 ppm mercuric acetate was used as biologically inactive poisoned control to differentiate losses from biodegradative losses. All types of sample put in Incubator 35 C for 24 hrs.

3. Analytical Method

For each point of analysis, hydrocarbon in the soil of 10 g was extracted. Crude oil were Soxhelet extracted (see Fig 1-A) for 6 hours by using choloroform[2]. Extracts of hydrocarbons were brought to volume by removing solvent using rotary evaporator (Fig 1-B). The analyzed for the content of residual hydrocarbons of samples were analyzed by gas chromatography, see Fig 2.

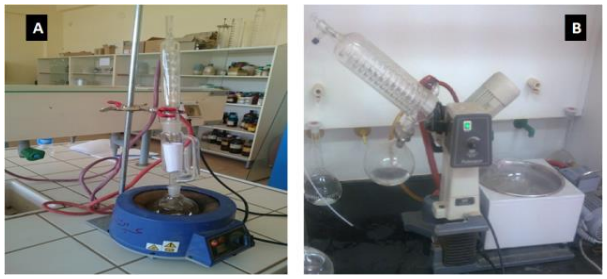

Fig. 1 show A) Soxhelet extracted system, B) Rotary evaporator

Fig. 2 shows Gas Chromatograph Analysis

4. Result and discussions

In this work, the degradation of hydrocarbons of selected fuels was studied. Remaining hydrocarbon concentrations in soil were determined quantitatively at different time intervals by using gas chromatographic technique. Nitrogen containing (NH_4NO_3) and phosphorous containing (KH_2PO_4) fertilizers were added to soil to keep their required concentrations. The soil was contaminated with hydrocarbons of fuels. Daily tilling to provide oxygen was performed by inserting the stainless steel wire into the soil and tilling eight times in each incubation sample. 100 ppm mercuric chloride was used as biologically inactive poisoned control to differentiate losses from biodegradative losses. The experimental results indicated that the total area of chromatographic peaks represented the concentration of total hydrocarbons in three different samples. The hydrocarbons concentrations under a variety of incubation conditions (untreated, bioremediation treated, poisoned) are given in Table 1. It can be seen that the reduction in total peak area as time increased for all samples was noted. This can be attributed to the evaporation of hydrocarbons with the increased in time.

Table 1. the hydrocarbons concentrations under a variety of incubation conditions

Time[Weeks]	Untreated Soil		Biorediation Treated Soil		Poisoned Soil	
	T PA	C T H g/kg og soil	T PA	C T H g/kg og soil	T PA	C T H g/kg og soil
0	88610343	10	88610343	10	88610343	10
1	79092314	8.93	72093766	8.14	84354397	9.52
2	70509141	7.96	53732253	6.06	78972461	8.91
3	62766205	7.08	47933880	5.41	72634325	8.20
4	54478046	6.15	36161427	4.08	66189231	7.47

T PA-Total Peak Area, **C T H** –Concentration of Total Hydrocarbons.

The depletion of fuel hydrocarbon concentration is under defined incubation conditions are shown in Fig. 3. In order to determine whether the presence of nitrogen and phosphorous within the mixture might limit the uptake of hydrocarbon of fuels, the degradation of this hydrocarbon, with and without nutrient supplementation, was expected. The results revealed that an initial concentration of hydrocarbon in oils 10g\kg was almost degraded within the experimental time (four weeks) of incubation in samples supplemented with nitrogen and phosphorous (~4.08 g\kg). The reduction in concentration of crude oil in the non-supplemented samples was noted. The value of degradation in these samples was reached 6.13 g\kg compared with that in the treated samples with nitrogen and phosphorous. The changes in degradation rate can be attributed to the presence of such nutrients. However, the degradation of hydrocarbon in poisonedsamples (~7.74 g\kg) through the experimental time was less than that in treated and un-treated samples with the nutrients. This reduction in the concentration of hydrocarbon is mainly due to the evaporation of hydrocarbon in oils. Also, the poisoned samples where the degradation due to microbes since all microbes

have been killed by adding poison (mercuric acetate).

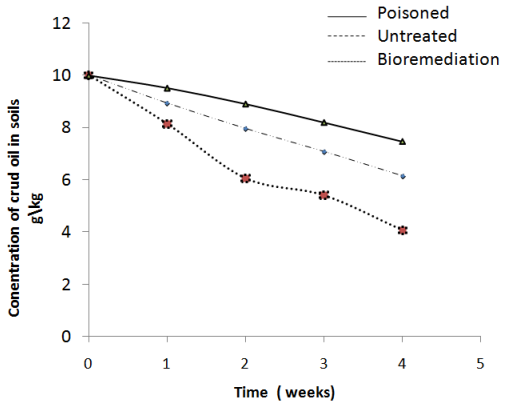

Fig. 3 biodegradation of hydrocarbon in three different mediums

The experimental result obtained from the chart of gas chromatography showed the total peak area for the concentration of crude oil vs the time for untreated, treated and poisoned soil. In the first week for the blank sample in all type of soil, the same concentration was 10 g of oil mixed in 1 kg of soil. The GC/MS analysis was performed to identify the presence of hydrocarbon petroleum in the untreated soil sample. The results confirmed that the soil samples consisted mainly of n-alkanes C11 through C31. The ratio of these hydrocarbons were approximately lower than 80%, with intermediate branched chain hydrocarbon, along with cycloalkanes, aromatic compounds and other petroleum-based compounds. Gas chromatographic analysis of the control untreated soil indicated that the TPH (Total Petroleum Hydrocarbons) removal of crude oil (1.0% w/v) was higher in the middle-chain compounds, see Fig 4. The control samples, which had no bacteria added, showed losses of the total petroleum hydrocarbon to a certain extent but these losses were mainly in the shorter fraction and were significantly ($p<0.05$). During the experiment, it was also observed that the transformation of different molecular weight compounds are varied between the two types of treatment. Based on the total peak area, the addition of Nitrogen containing (NH_4NO_3) and phosphorous containing (KH_2PO_4) enhanced degradation of middle and long chain compounds compared with the control system that was not supplemented with any microbial species. In addition, these components are worked as activation to microorganism to eat hydrocarbons and result carbon dioxide and water gently in the environment. Other degradation in the untreated sample have observed the presence of natural microorganism. The poisoned sample have the smallest degradation because add the mercuric chloride to stop any action of microorganism. Also, other factor may play a role in the degradation process such as the temperature parameter which is effected in all type of samples at incubator at 35 °C.

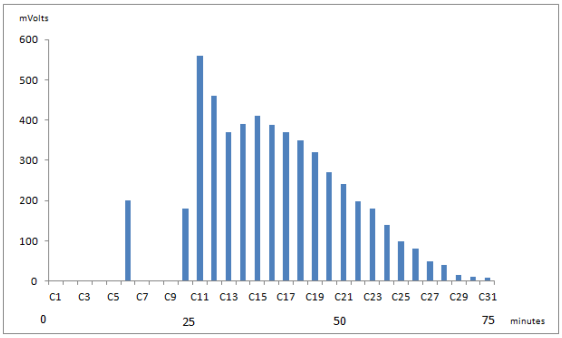

Fig. 4 gas chromatography analysis for the control system

5. Conclusions

The finding from this work can be summarized as the following;

1- The reduction in total peak area as time increased for all samples was noted. This can be attributed to the evaporation of hydrocarbons with the increased in time.
2- The results revealed that an initial concentration of hydrocarbon in oils was almost degraded within the experimental time (four weeks) of incubation in samples supplemented with nitrogen and phosphorous.
3- The value of degradation in the untreated samples was highly reached compared with that in the treated samples with nitrogen and phosphorous. The changes in degradation rate can be attributed to the presence of such nutrients.
4- The degradation of hydrocarbon in poisonedsamples through the experimental time was less than that in treated and un-treated samples with the nutrients. This reduction in the concentration of hydrocarbon is mainly due to the evaporation of hydrocarbon in oils. Also, the poisoned samples where the degradation due to microbes since all microbes have been killed by adding poison (mercuric acetate).

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