

# THE INFLUENCE OF OIL ON THE TOTAL OF BACTERIA AMONG +SOIL DEPTHS

تأثير النفط على العدد الكلي للبكتيريا بين طبقات التربة المختلفة

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## Abstract

Strains of heterotrophic and hydrocarbon- utilizing bacteria were isolated from um-Qasir soil in Basrah .Aportion of expermental soil was treated with Regular Basrah crude oil .Another part of the soil was left untreated .One month after crude oil spreading ,adapted bacteria were isolated at different soil depths in the two plots .Bacteri genera *Pseudomonas, Micrococcus , Flavobacteriums , Vibrio, Bacillus , Staphylococcus , Aeromonas , Klebsiella , Actinomycetes , Acinetobacter , Artheobacter, and Corynebacterium* ,were identified. The biodegradation potential of the hydrocarbon- utilizing microorganisms isolated from polluted and unpolluted soils were similar. The high number of heterotrophic and hydrocarbon- utilizing bacteria in treated soil compaired with untreated ,There is high segnificant differences ( $p < 0.01$ ) in the total count of bacteria, were obtain among different depths, the highest bacterial count was isolated from the surface layer 0-20 cm in both treated and untreated soil.

## المستخلص:

عزلت أجناس من بكتيريا المكسرة للنفط وبكتيريا مختلطة التغذية من تربة أم قصر في البصرة ،تم معاملة جزء من التربة المستخدمة بالتجربة بنفط خام البصرة الاعتيادي ،بينما ترك الجزء الآخر بدون معاملة ،تم عزل وتشخيص الأجناس البكتيرية من أعماق مختلفة من التربة بعد شهر من المعاملة.

*Pseudomonas, Micrococcus , Flavobacteriums , Vibrio, Bacillus , Staphylococcus , Aeromonas , Klebsiella , Actinomycetes , Acinetobacter , Artheobacter, and Corynebacterium* ,

لوحظ تشابه في الأجناس من الأحياء المجهرية المستهلكة للهايدروكاربونات النفطية المعزولة من التربة الملوثة وغير الملوثة وجد أن عدد البكتيريا المكسرة للنفط وبكتيريا مختلطة التغذية أعلى في التربة الملوثة مقارنة بالتربة غير الملوثة ،كما وجد فروق عالية المعنوية في أعداد بكتيريا المكسرة للنفط وبكتيريا مختلطة التغذية بين أعماق التربة المختلفة، وان الطبقة العليا للتربة تحوي أعلى عدد من البكتيريا في التربة المعاملة و التربة غير المعاملة.

## Introduction :

Microorganism play an important role in degradation of organic compound in nature. The ability of microorganism include bacteria and fungi to degrade awide array of organic compound has created an explosion of interest in the use of it in polluted abatement and in mitigation environmental damage[1]. Microorganism have adapted to the wide array of natural

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and synthetic organic compounds emitted into the environment and possess enzymes that utilize some of the compounds as nutrients when petroleum hydrocarbons pollute various habitats. The effect on the indigenous microflora is twofold. Hydrocarbons may inhibit or cause the death of certain microorganisms. On the other hand, there will also be an increase in the number of certain chemoorganotrophic microorganisms, especially those capable of metabolizing the hydrocarbons [2]. In recent years, the presence of fuel and crude oil in the biosphere, including soils and aquatic ecosystems, has increased, which has affected terrestrial and aquatic life. The role of bacteria in the biodegradation of petroleum has been studied [3]. It has been reported that petroleum hydrocarbons (HC) can be metabolized by the indigenous microbial population of the soil. [4] Studied the indigenous microbial population in Basrah soil, including heterotrophic bacteria (HB) and hydrocarbon-utilizing bacteria (HUB), and the role of bacteria in the degradation of petroleum. In soil, it has been found that the microflora is diverse, and the major components were *Pseudomonas*, *Brevundimonas*, *Acinetobacter*, *Rhodococcus*, *Arthrobacter*, and *Corynebacterium* [4]. In contaminated soils, members of the same bacterial genera have already been found [5] and have also [6]. Moreover, the total of heterotrophic bacteria (THB) were isolated from different depths of unpolluted soil  $10^5$ - $10^8$  [5]. Some works stated that the biodegradation potential of strains isolated from contaminated environments was higher than those originating from non-contaminated soil [7&8]. The purpose of this study was to compare the total bacterial count among the depths of polluted and unpolluted soils.

## **Materials and methods**

### **Soil sampling**

All samples were taken from Um-Qasir city, during the summer season. Soil samples were collected from different depths (0-20, 20-40, 40-60, 60-80) cm in sterile sacs. Two fields were tested in this study, each one measuring (50\*50) cm. The first field was spread with 500 ml of crude oil, and the other control field was left untreated. After one month, experimental soil was taken aseptically at different depths.

### **Enumeration of heterotrophic bacteria (HB)**

One gram of soil sample was mixed with 9 ml of sterile normal saline (8.5g NaCl/1L distilled water –to refresh the bacteria). The suspension was left for 5-10 min. Decimal dilutions up to  $10^6$  of the sample were prepared, and 0.1 ml from the appropriate dilution was spread on to nutrient agar by a sterile spreader. The plates were inverted and incubated for 48 h at 37°C.

### **Enumeration of hydrocarbon-utilizing bacteria (HUB)**

One gram of soil sample was mixed with 9 ml of sterile normal saline. The suspension was left for 5-10 min. 1 ml from the sample was mixed in 10-fold aliquots with sterile normal saline and then serially diluted. 5 ml of the supernatant was filtered through a sterile 0.45 µm millipore filter, which was then placed on the oil agar surface. The Gunkel medium was prepared by adding 0.5 g of  $\text{NH}_4\text{Cl}$ , 0.5 g of  $\text{KH}_2\text{PO}_4$ , 0.1 g of  $\text{NaH}_2\text{PO}_4$ , 0.1 g of  $\text{NaCl}$ , and 15 g of Agar.

,1mm of crude oil to 1Liter of distilled water [9]. Triplicate plates were incubated in 37 C° for up 7-28days.

### Isolation of bacterial strain

strain of heterotrophic bacteria HB and hydrocarbon- utilizing bacteria HUB were isolated from the soils samples. Serial dilution of soil suspensions were plated on Gunkel medium, the plates were incubated at 20 ±1 °C for up to 7-28 days.. Bacteria were characterized with Gram coloration and morphology with physiological test [9].

### Result and discussion

**Heterotrophic bacteria HB and hydrocarbon- utilizing bacteria HUB were obtained from different depths on both the treated and un treated soil of Um Qaisr are presented as mean values in table (1&2).**

**Table 1: Bacterial counts (colony forming units gram soil )in the untreatment soil (cntrol plots)in differents depth of soil**

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Soil depth(Cm)	Total Heterotrophic bacteria	Total hydrocarbons utilizing bacteria	R1
0-20	7.4*10 <sup>7</sup>	2.1*10 <sup>5</sup>	0.28
20-40	3.5*10 <sup>7</sup>	1.5*10 <sup>5</sup>	0.42
40-60	1.5*10 <sup>6</sup>	4.1*10 <sup>4</sup>	2.73
60-80	0.5*10 <sup>6</sup>	2.3*10 <sup>4</sup>	4.6

Abbreviations: THB, total heterotrophic bacteria; HUB, hydrocarbon utilizing bacteria; R1=100\*(HUB/THB)

**Table1: Bacterial counts (colony forming units gram soil) in the treatment soil in**

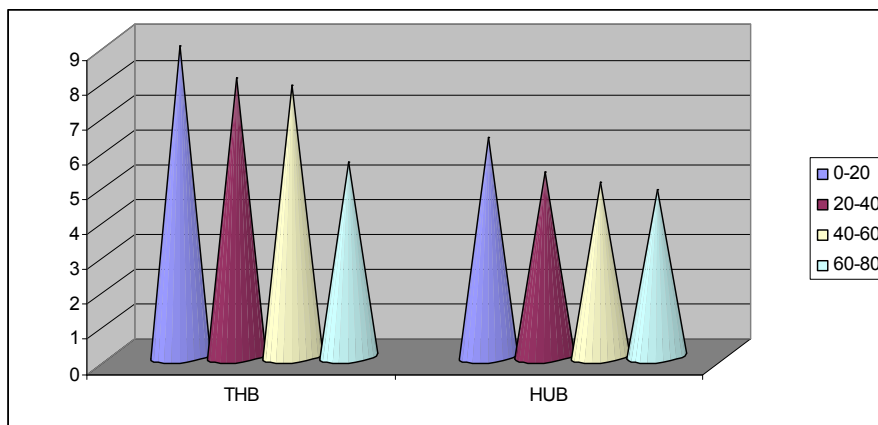
Soil depth(Cm)	Total Heterotrophic bacteria THB	Total hydrocarbons utilizing bacteria HUB	R1	R1	R!
0-20	80*10 <sup>7</sup>	70*10 <sup>5</sup>	.87	9.25	3
20-40	10*10 <sup>7</sup>	20*10 <sup>4</sup>	.19	3.46*	7.5
40-60	7.2*10 <sup>6</sup>	11*10 <sup>4</sup>	1.52	20.83	37.27
60-80	0.5*10 <sup>6</sup>	7*10 <sup>4</sup>	1.4	100	32.85

**different depths of soil.**

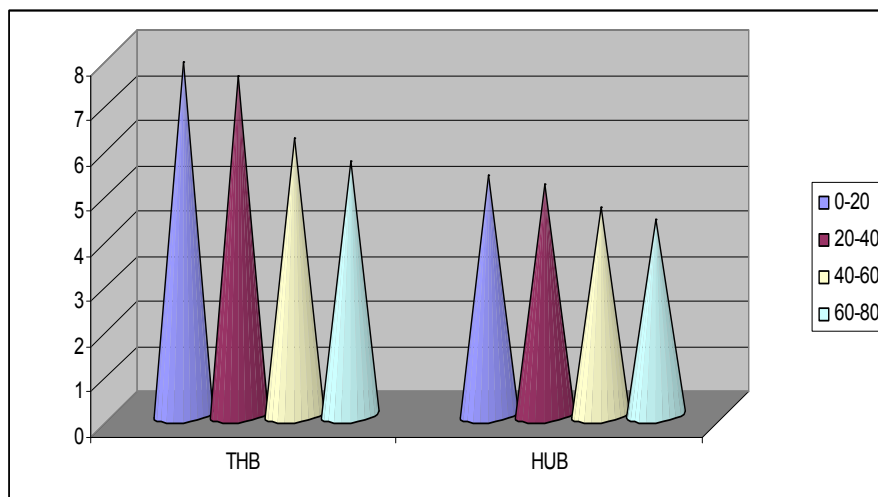
Abbreviations ;R1=100\*(HUB/THB).R2=100\*(THB in treated plot/THBin the control),R3=100\*(HUB in treated plot/ HUBin the control)

The highest bacterial count included HB and HUB is obtained from the 0-20 Cm in both treated and untreated soil layer followed by another layers ,it was clear when the depths of soil increase, the number of both HB and HUB was decrease significantly than when it was the surface of soil the depths of soil directly affect of the number of bacteria , this is in agreement with several studies.[6]Studied in France of polluted soil with fuel oil and observed,microbial population included bacteria and fungi has usually decrease with depth.[10].had reported that the introduction of hydrocarbons to environment induces rapaid increases in bacterial number in the surface.

High number of THB and HUB in 40-80 cm soil layer in treated plot, as a result of aselective migration of light hydrocarbons in these layers [11]



**Fig (1):Total viable count of the bacterial group (Total heterotrophic bacteriaTHB and hydrocarbons utlizing bacteriaHUB) in different depths of treatment soil.**

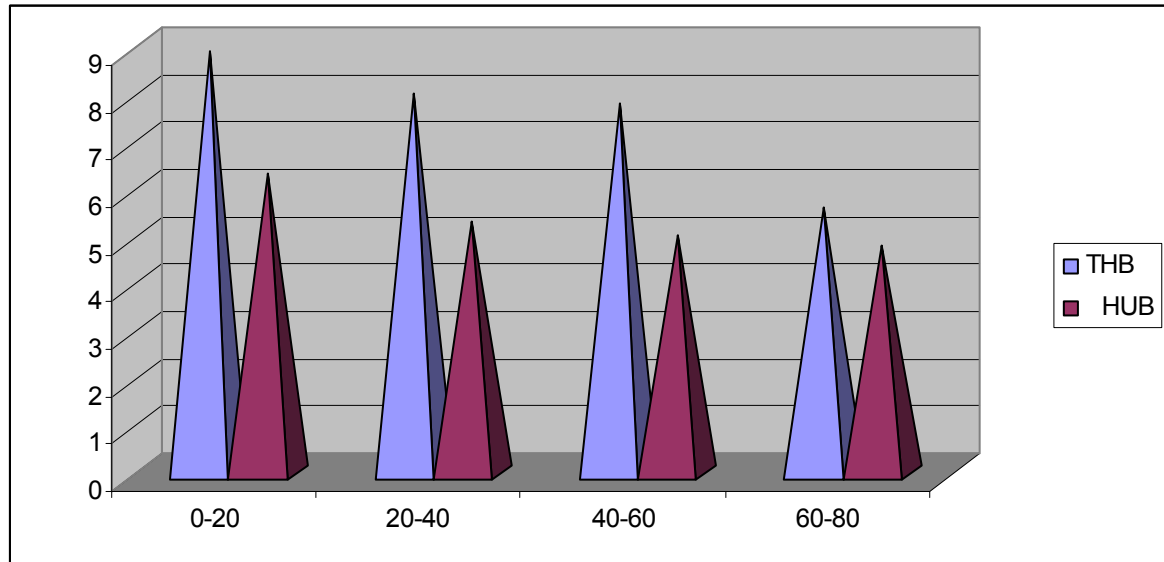


**Fig (2):Total viable count of the bacterial group (Total heterotrophic bacteriaTHB and hydrocarbons utlizing bacteriaHUB) in different depths ofun treatment soil.**

R1 represent the percentage ratio of HUB/THB, it was increase with increase the depths of soil. This due to the fact the adaptation level of HUB had reached a high rate of degradation activity when relatively large quantities of oil have been spilled on the surface

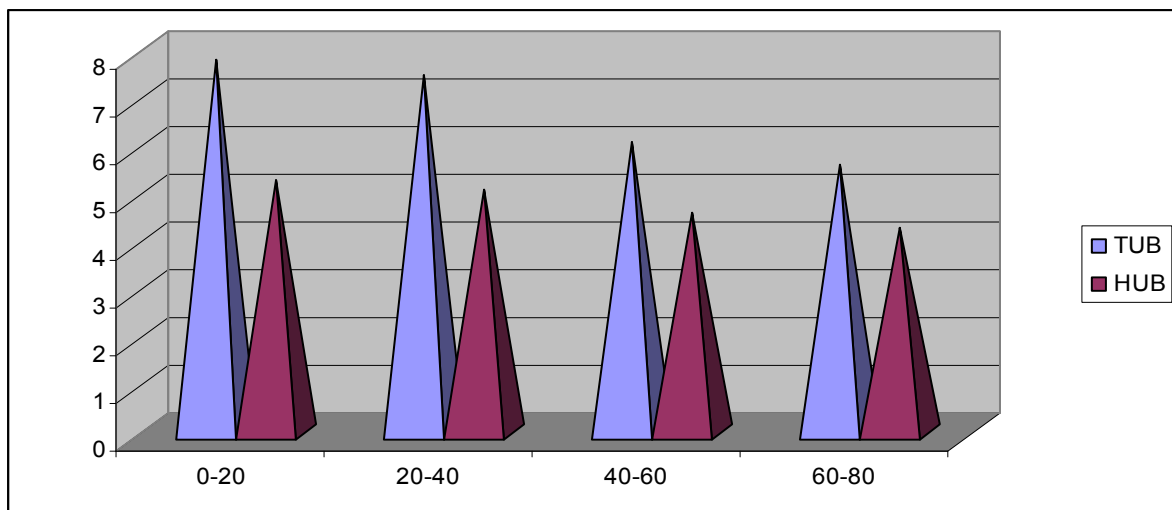
compartment with followed layers. The same result was observed in R2,R3 it were increase with increase the depths of soil.

Highly significant differences ( $p < 0.01$ ) between bacteria count of heterotrophic bacteria and hydrocarbons utilizing bacteria in different depths of treatment soil, these values are shown in Fig (3)



**Fig (3):.Significant difference between bacteria count of heterotrophic bacteria and hydrocarbons utilizing bacteria in different depths of treatment soil**

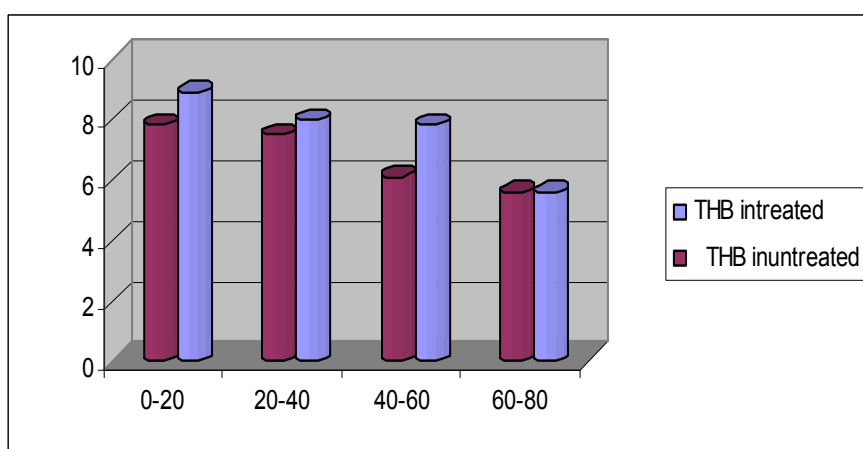
The same result was observed in differences of numbers of HB and HUB among different depths of untreated soil, these results are shown in Fig. (4).



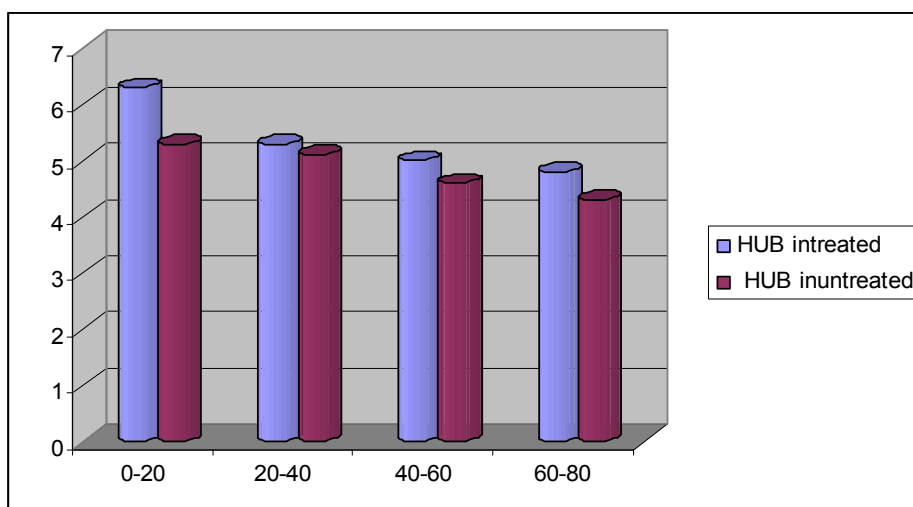
**Fig (4):.Significant difference between bacteria count of heterotrophic bacteria and hydrocarbons utilizing bacteria in different depths of un treatment soil**

The differences in the number of Hband HUB in both treated and untreated soil was decrease with increase the deph, aerobic microbial population has usually decrease with the depth[12].In this study the various bacterial isolates recovered from unpolluted soil can be categorized into *Pseudomonas Micrococcus,Flavobacterium,Vibrio, Bacillus, Staphyloccus,Aeromonas,Klebbsiella,Actinomyces,Acinetobacter,Arthrobacter,and Corynebacterium*. Member of the same bacterial genera have already found in contaminated soil[13]and this was in agreement with aprevious study where isolated members of bacterial population from soil[12].

One month after the spreading of crude oil on experimental soil,an increase in numbers of THB and HUB was observed, in treated soil compared with un treated soil in different depths (Fig 5&6)



**Fig (5):Total viable count of heterotrophic bacteria in different depths of treatment and untreatment soil.**



**Fig (6):Total viable count of hydrocarbons utlizing bacteriaHUB in different depths of treatment and untreatment soil**

This indicated that the spreading of oil caused an adaptation of the microbial population, when fuel oil hydrocarbons were spread onto soil surface, hydrocarbons interacted with the different soil compartment through abiotic and biological processes, and led to the stimulation of hydrocarbons degraders. This was in agreement with a previous study [14]

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