

Bioremediation of Petroleum Hydrocarbons Contaminated Soil using Bio piles System

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Abstract:

This study was focused on biotreatment of soil which polluted by petroleum compounds (Diesel) which caused serious environmental problems. One of the most effective and promising ways to treat diesel-contaminated soil is bioremediation. It is a choice that offers the potential to destroy harmful pollutants using biological activity.

Four bacterial strains were isolated from diesel contaminated soil samples. The isolates were identified by the Vitek 2 system, as *Sphingomonas paucimobilis*, *Pentaoae species*, *Staphylococcus aureus*, and *Enterobacter cloacae*. The potential of biological surfactant production was tested using the Sigma 703D stand-alone tensiometer showed that these isolates are biological surfactant producers. The better results of the surface tension reduction showed that these isolates are biological surfactant producers. The better results of the surface tension reduction test were obtained using the mixed bacterial culture which reduced the surface tension of the medium from 66mN/m to 33.89mN/m. For further evidence of the biodegradation effect of these isolates individually and as a mixed culture, which was supported by the use of Gas-Chromatography technology confirming the occurrence of biodegradation.

The capability of mixed bacterial culture was examined to remediate the diesel contaminated soil in bio piles system. Two pilot scale bio piles (25 kg soil each) were constructed containing soils contaminated with approximately 2140 mg/kg total petroleum hydrocarbons (TPH_s). Both systems were equipped with oxygen to provide aerobic conditions, incubated at ambient temperature and weekly sampling within 35 days (during summer season). Overall 75.71 % of the total petroleum hydrocarbons were removed from the amended soil and 33.18 % of the control soil at the end of study period. The study concluded that the *ex-situ* bioremediation (bio piles) is a good option for treating the soil contaminated with diesel as economical and environmentally friendly.

Key words: Biodegradation, Bio piles, Bioremediation, Diesel, Soil pollution

Introduction:

Oil pollution accidents has become a universal phenomenon and has caused serious environmental problems, such as the introduction of toxic compounds into food sources and changes in physical and chemical properties of the soil (1). Soil pollution with oil products is a permanent problem, and diesel is a medium distillate of petroleum hydrocarbons containing: normal alkanes, branched alkanes, olefins, and small concentrations of polycyclic aromatic compounds (2).

With increasing the interest in the conservation of the environment, biological

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treatments have been improved and developed to clean up soils contaminated with hazardous compounds and have become a valuable alternative to physical and chemical treatments (3). It can be defined as the conversion of toxic and chemically complex organic compounds into non-toxic and inorganic compounds, such as carbon dioxide and water along with the accumulation of microbial biomass through oxidation under aerobic conditions. Bioremediation is a successful procedure for cleaning up polluted sites by petroleum compounds because it is applicable to large areas, leads to the full removal of the contaminants, and cost-effective (4).

Remediation technologies can be divided into *ex-situ* and *in-situ* methods (5). Amongst *ex-situ* methods available to bioremediate soils, bio piles have been described as an effective way to remove hydrocarbons which exist in diesel (6). In

bio piles, contaminated soils are accumulating above ground, and then the biological processes are motivating through aeration followed by addition of water and nutrient besides controlling temperature and pH (7). Aeration is carried out by the air compressor which drives the oxygen through the perforated tubes that are placed throughout the pile. Snelgrove (2010) studied the degradation of diesel in two bio pile systems during 65 days, one bio pile containing soil contaminated with diesel (amended soil by NH_4NO_3) and the other containing diesel-contaminated soil without amendments (control). At the end of remediation period, for amended soil, 42% of diesel was degraded while for unamended soil 38% of diesel was degraded at the same time.

Among the main advantages associated with the use of the bio pile system are the following: possibility control of system conditions (pH, temperature, ventilation and moisture content) and continuous monitoring. This provides optimal conditions for treatment and contributes to support of bacterial activity, which results in reduced processing time (8). Compared to composting or land farming, the efficiency of mass transfer of water, nutrients and air in bio piles contributes a better contaminants removal strategy (9).

The aim of this study was to design a small scale bio piles system and to evaluate its efficiency to clean-up soil contaminated by diesel.

Materials and Methods:

Bacterial Strains and Inoculum Preparation

Bacterial cultures were isolated from soil contaminated with diesel at a depth of 0-15 cm (10) from Al Dura oil refinery in Baghdad. These degradable bacteria than isolated and identified using Vitek 2 system as *Sphingomonas paucimobilis*, *Pentocae species*, *Staphylococcus aureus*, and *Enterobacter cloacae*, they were cultivated under controlled conditions.

For the *ex-situ* soil remediation, a dense suspension of mixed culture was prepared as shown below: *Sphingomonas paucimobilis* was inoculated into sterile tube containing 5 ml of normal saline solution until a cell concentration of 2.1×10^8 CFU.ml⁻¹ (9 McFarland Standard) was reached. The 5 ml bacterial suspension was transferred into 100 ml of Bushnell-Haas Medium (BHM). [BHM containing (g/l): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0; K_2HPO_4 , 1.0; KH_2PO_4 , 1.0; FeCl_3 , 0.05; NH_4NO_3 , 1.0; CaCl_2 , 0.02; pH to 7 ± 0.2 which sterilized at 121°C for 15 min].

This was repeated using *Pentocae species* instead of *Sphingomonas paucimobilis* and so on. The mixed bacterial was prepared by mixing equal volumes of the culture of the above cell concentration for each isolate (11).

Measuring Biodegradation of Diesel

Measuring the biodegradation of diesel by isolating bacteria was done with the following ways:

• Determination of Surface Tension Reduction

The bacterial isolates were inoculated with 100 ml of Bushnell-Haas Medium containing 1.0 % (v/v) of diesel in 250 ml Erlenmeyer flask and incubated at 30 °C in a shaker incubator at 180 rpm for 8 days. A medium was prepared by adding 10 ml of culture to 20 ml of carbon tetrachloride (CCl_4) to separate hydrocarbons from the liquid culture. Medium poured in centrifuge tubes and centrifuged at 10000 rpm for 15 minutes at -5 °C to precipitate the cells. The upper free cells layer that containing the diesel was used for surface tension test using a Sigma 703D Du-Nouy-Ring tensiometer (4).

• The use of Gas- Chromatography Technique

The bacterial isolates and their mixed culture was suspended in 250 mL conical flasks, each containing 100mL of sterile Bushnell-Haas medium with 1.0 % (v/v) diesel as the sole carbon source. Flasks were incubated at 30°C in an incubator shaker at 180 rpm for 7 days. The diesel was extracted from Bushnell-Haas medium by liquid- liquid extraction method. The Chromatographic Gas device (GC-Shimadzu Japanese Company, Model 2010) was fused silica capillary column SE30 a 30m length, and equipped with flame ionization detector. The column temperature is programmed to rise from 100 °C to 300 °C and pressure 100 kPa. The detector temperature was 300 °C and injector temperature was maintained at 290 °C. The oven temperature was initially set at 100 °C and increased at rate of 3°C/ min to 300 °C, and pressure 100 kPa. Nitrogen was used as the carrier gas (12).

Preparation of Contaminated Soil

Twenty-five kilograms of the uncontaminated soil sample were collected at a depth of (0- 30) cm from Al-Jadriya city in Baghdad, where the geology of the soil there is similar to that of the refinery. Soil was air dried, passed through a 2-mm sieve and autoclaving at 121 C for 35min for two times. The prepared soil was mixed by a hand trowel and contaminated with 1% of diesel/ soil (v/w) [The choice of the percentage of soil contamination by diesel is based on previous work (13), in other words, any percentage above 1% will be harmful to the microorganisms while any percentage less than 1% does not contain the nutrients needed by the

microorganisms to grow and conduct the process of biodegradation], then thoroughly mixed to distribute diesel throughout the soil particles to achieve complete contamination. The contaminated soil was mixed with 1.5% sawdust to maintain moisture (14). For the nutrient addition, the soil was fertilized with nutritious salts solution $[(\text{NH}_4)_2\text{SO}_4$ and $\text{KH}_2\text{PO}_4]$ in order to provide the C: N: P ratio of approximately (100:5:1) (15). The treated soil was daily irrigated to maintain its moisture content within (50-60%) during the 35 days of experiment period (in summer season). Unamended contaminated soil (without additions) was used as the control.

Experimental Design of Bio pile Tanks

Two rectangular stainless steel (316 L) tanks pilot-scale bio pile were used, as shown in Fig.1. The dimensions of the tank are 52 cm in length, 33 cm wide and 52 cm deep. The soil was filled up to 15 cm from the bottom of the tank shown in Fig.2 (6). In order to provide aerobic conditions, an air compressor was connected to the tank. The air flow was controlled by valves and it was supplied at the bottom of the tank using stainless steel perforated tubes that passed along the tank. These tubes inside the tank are 60 cm long and 0.5cm out-diameter with perforations 3 mm diameter, spaced 50 mm apart. To prevent soil from entering the ventilation pipes, they were designed so that the holes were directed downward (16).



Figure.1The pilot-scale stainless steel tanks of Bio pile system

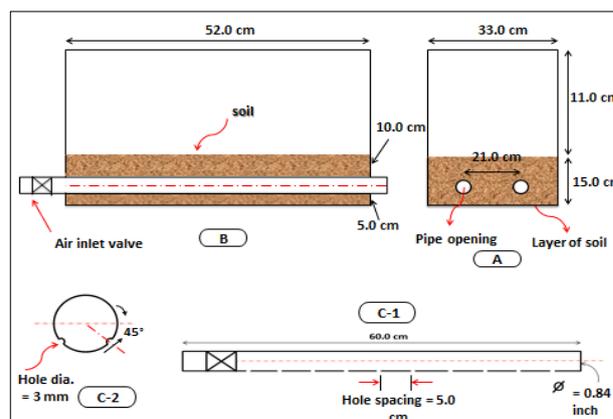


Figure.2 The dimensions of the stainless steel Bio pile system A: front view B: side view C-1: air supply tube C-2: cross section of air supply tube

Measuring the Physio-Chemical Properties of Soil:

Physio-chemical analysis of the soil: water holding capacity, soil nitrogen, phosphorus, potassium (NPK), and total organic content were determined according to the method of Motsara and Roy (2008) (9). The pH was determined by taking 10 g of soil and placed in 20 ml distilled water. The suspension was mixed well and left for 10 minutes. Then the measurement was done using pH electrode. Particle size distribution of soil was determined by hydrometer method.

Monitoring the Bioremediation Experiment:

• Physicochemical and Microbiological Analysis of Soil

The pH was determined using a pH meter by suspending 2.5g of each soil sample into 20 ml of distilled water and mixing well. Soil temperature was measured using a digital thermometer (17). To ensure that soil moisture had not changed significantly and the supplied air had not dried soil so it was necessary to measure the moisture level of soil using a soil moisture probe. The number of bacteria utilizing diesel in soil was obtained using pour plate method (10).

• Total hydrocarbons Analysis by Extraction Method

Twenty-five grams of soil sample was taken from each tanks and blended with an equal amount of anhydrous sodium sulfate (Na_2SO_4). The mixture was placed in a Whitman cellulose extraction thimble. The diesel remaining in this sample was extracted with 250 ml of dichloromethane (DCM) for two hours at a rate of 4 cycles/ hour using the Soxhlet system (14). The amount of residual diesel was determined by weight to quantify the amount of degraded diesel over time as follows;

weight of residual diesel =
weight of baker containing extracted diesel –
weight of empty beaker ... (1)

$$\text{weight of degraded diesel } \left(\frac{\text{mg}}{\text{kg}}\right) = \frac{\text{weight of residual diesel (gm)} \times 1000}{\frac{\text{weight of soil sample (gm)}}{1000}} \dots (2)$$

Results and Discussion:

• Determination of Surface Tension Reduction

The results demonstrated that all isolates and the mixed culture were able to produce biological surfactants in Bushnell-Haas medium containing diesel as the only carbon source. The greatest surface tension reduction of the medium was achieved with the mixed culture which reached 33.89 mN/m compared to the control 66 mN/m. While the other data of the surface tension obtained from each isolate were various and some of them still high and ranged from 27.18 mN/m to 55.62 mN/m, as shown in Table1.

Table.1 Surface tension reduction by hydrocarbon-degrading bacterial isolates

Bacterial Isolates	Surface tension (m N/m) at concentration 1.0 % of diesel
<i>E. cloacae</i>	53.94
<i>S. aureus</i>	55.62
<i>S. paucimobilis</i>	39.15
<i>Pentoeae species</i>	27.18
Mixed bacterial culture	33.89
Surface tension of the control = 66 mN/m	

These results correspond to the findings of Cerqueira *et al.* (2011) (18) who reported that a microbial consortium that included (*B. cereus*, *P. aeruginosa*, *B. cibi*, *S. acidaminiphila* and *B. megaterium*) was reduced the surface tension of the medium containing oily sludge from 60.4 mN/m to 36.6 mN/m, however, the other surface tension values as a result of the singular action for the isolates ranged from 41mN/m to 42.6mN/m. In which *S. aureus* was the weakest among the isolates in the ability to reduce surface tension.

The reduction in surface tension of the medium depends on the type of biological surfactants produced by bacterial isolates as well as the critical micelle concentration (CMC). The micelles are large molecules which when aggregated the surface tension begins to decrease. The production of biological surfactants was enhancing the biodegradation of petroleum

hydrocarbons by increasing their solubility and increasing the surface area of the hydrocarbons substrates thus making them more bioavailable for microorganisms (19).

• Biodegradation of Diesel Hydrocarbons

Using Gas Chromatography (GC) technology, the analyzing for control sample demonstrated that diesel has 13 chemical materials (C9 – C21) [in general, they represent the straight chain hydrocarbons or normal alkanes] as shown in Fig.3. After exposure of diesel at concentration 1 % (v/v) to *E.cloacae*, *S.aureus*, *S.paucimobilis*, and *Pentoeae sp.* individually after 7 days of the experiment the low molecular weight compounds were degraded extensively where some ingredients of diesel, especially C9 - C10 were totally lost while C11 - C20 were reduced indicating that biodegradation of the hydrocarbons components has happened as shown in figures from 4 to 7.

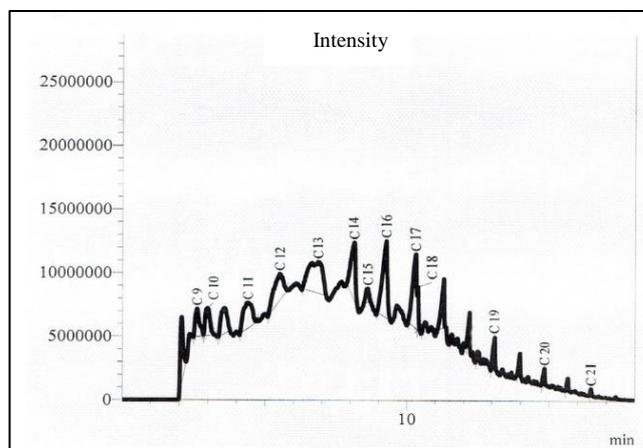


Figure.3 Chemical compounds present in the control sample of diesel

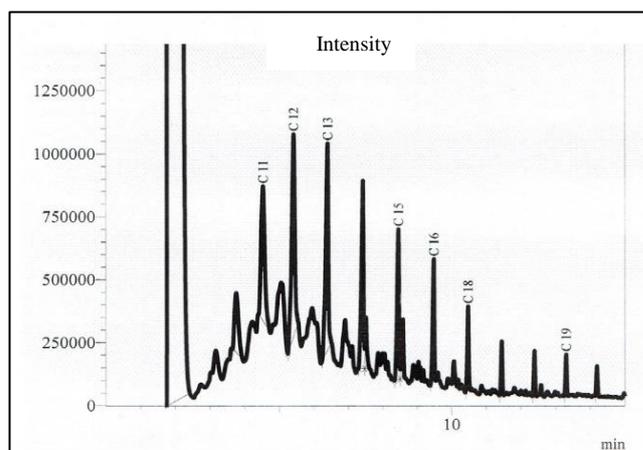


Figure.4 Residual chemical compounds in degraded diesel at concentration 1 % using *E. cloacae* after 7 days

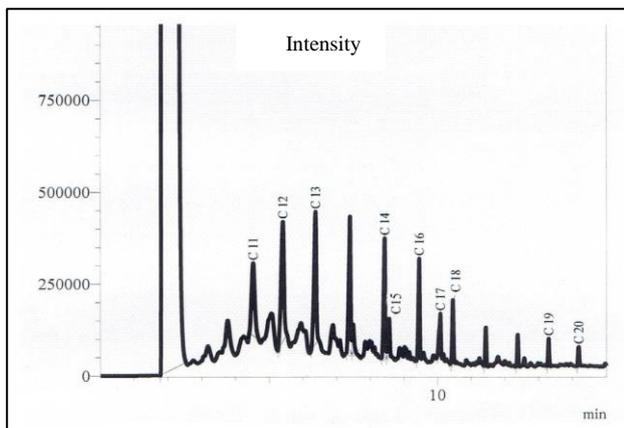


Figure.5 Residual chemical compounds in degraded diesel at concentration 1% using *S.aureus* after 7 days

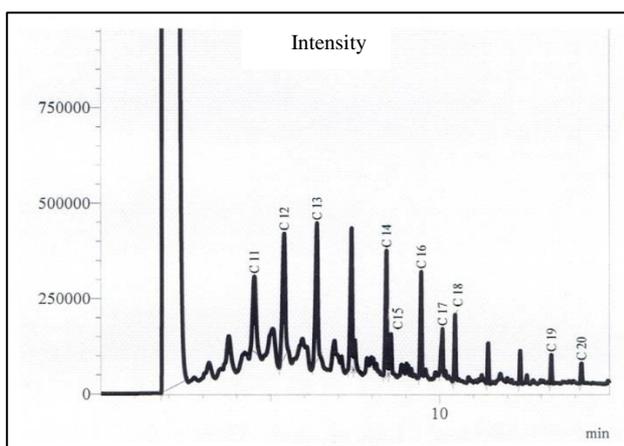


Figure.6 Residual chemical compounds in degraded diesel at concentration 1% using *S.paucimobilis* after 7 days

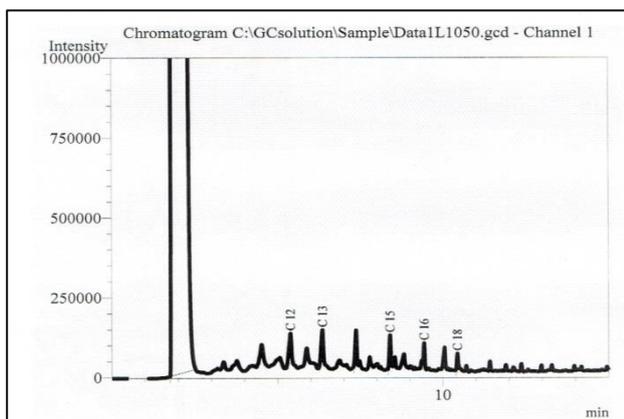


Figure.7 Residual chemical compounds in degraded diesel at concentration 1% using *Pentoeae species* after 7 days

The difference in the peaks appearance of diesel components can be attributed to the varying capabilities of bacteria in dealing with the hydrocarbons compounds (20). The potential of isolates may vary in the process of diesel metabolism because of the differences in their enzymatic degradation systems. Where some

isolates have a strong affinity with the hydrocarbon components, some may be long-delayed due to the inhibitory components of diesel prior to the initiation of biodegradation (21). Despite that the four isolates capable of degrading diesel, the visual checking of CG proven that the degradation rate of a mixed culture were higher than for any of the individual isolates as shown in Fig.8. Individual organisms may metabolize only a limited range of diesel substrates; therefore it is likely that assemblages of different bacterial species with broader enzymatic capabilities have a greater effect to degrade complex diesel compounds (22). The results denote that these bacteria have the ability to attack all hydrocarbons components of diesel when being mixed culture.

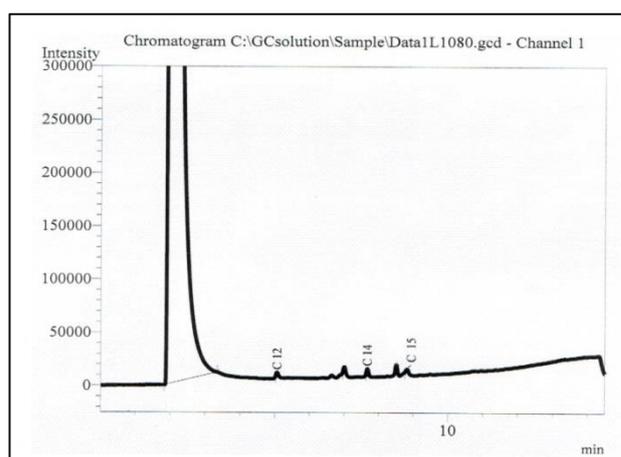


Figure.8 Residual chemical compounds in degraded diesel at concentration 1% using mixed culture after 21 days

Physical and Chemical Properties of the Soil

The results of the soil characterization are presented in Table 2. The basic pH of the soil sample 7.4 was within the pH range preferred for biological treatment. The soil contained a low percentage of organic matter 1.2%. The nutrient level in the soil was 51mg nitrogen /kg, 9.2 mg phosphate /kg, and 153 mg potassium/kg. Soil texture is the primary determinant of water holding capacity. The higher the percentage of clay and silt was the greater the soil's ability to retain water and nutrients. The small particles of clay and silt have a larger surface area than large sand particles. This large surface area allows more water to be trapped.

Table. 2 Physical and chemical properties of the used soil

Characteristics	Values
Nutrients	Concentration (mg/kg soil)
Total nitrogen (mg N/ kg)	51
Phosphate (mg P/ kg)	9.2
Potassium (mg K/ kg)	153
Soil texture	12% sand, 20% clay, 68% silt Silty clay soil
CaCO ₃	22.3 %
Soil pH	7.4
Water holding capacity	16.85 %

Ex-situ Experiment Bioremediation (Bio piles)

Bio pile system was used to simulate bioremediation treatments through 35 days period. Two stainless steel tanks, one tank containing an amended soil: (contaminated soil with the addition of nutrients and bacterial inoculum), and the second tank was prepared for unamended soil as a control. Soil contaminated with diesel initially was contained 2140 mg TPH/kg at zero days for both amended and unamended soil (control). The reduction of total petroleum hydrocarbons (TPHs) was rapid within the first 7 days for amended soil and reached 1524 mg TPH/kg when compared with that of the control soil 2000 mg TPH/kg. At the end of treatment period, the reduction of TPHs reached to 520 mg TPH/kg for amended soil while reached to 1430 mg TPH/kg for the control as shown in Table 3.

Table.3 Bioremediation of a soil contaminated with diesel during 35 days

Time (days)	Total petroleum hydrocarbons (mg TPH/kg soil)	
	Amended soil	Unamended soil (control)
0 th	2140	2140
7 th	1524	2000
14 th	1256	1800
21 th	1228	1650
28 th	1200	1545
35 th	520	1430

As apparent from the results, the addition of mixed bacterial culture and nutrients has an advantageous effect on the bioremediation process. Biostimulation accelerated TPHs degradation because it plays an important role in supplementing nutrient constantly. It is important to add nutrients especially nitrogen source because nitrogen is a key building block of proteins and nucleic acids (23). Moreover, the addition of mixed bacterial culture represents the actual behavior of microorganisms in the

environment contaminated with hydrocarbons. Since, in nature, the bioremediation process depends on the cooperative metabolic activities of organisms (18).The biodegradation rate in the bio pile systems increased with time. After the first 7 days of remediation, the degradation rate of amended soil was 28.78%, while from the 14 day to the 28 day the degradation rate falls in the range 41.3% to 43.93%. This is because the low molecular weight compounds were degraded extensively during the first week of treatment, while higher molecular weight compounds were partially degraded (24) . Usually, all types of bacteria attack compounds with low molecular weight more readily. Among the hydrocarbons, aliphatic compounds are expected to be more efficiently degraded. Also, between branched and straight chain alkanes, bacteria favors degrading straight chain hydrocarbons (25) . At the end of remediation period (after 35 days), diesel-contaminated soil which amended with (mixed bacterial culture and nutrient) showed the highest degradation rate which reached to 75.71 % while for unamended soil (control) the degradation rate reached to 33.18 % . The percentage of removing diesel through remediation period is shown in Fig.9 and 10.

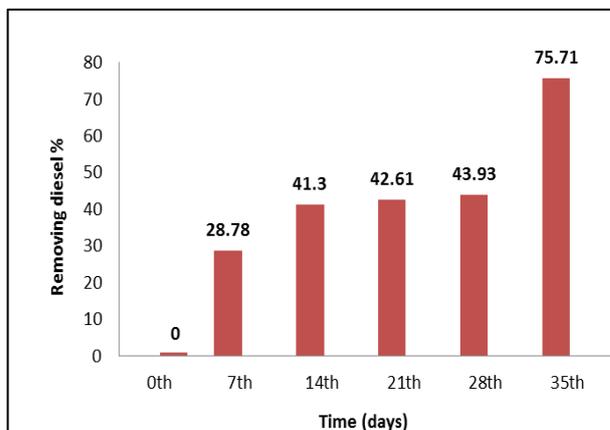


Figure.9 Percentage of removing diesel from amended soil through remediation time - 35 days

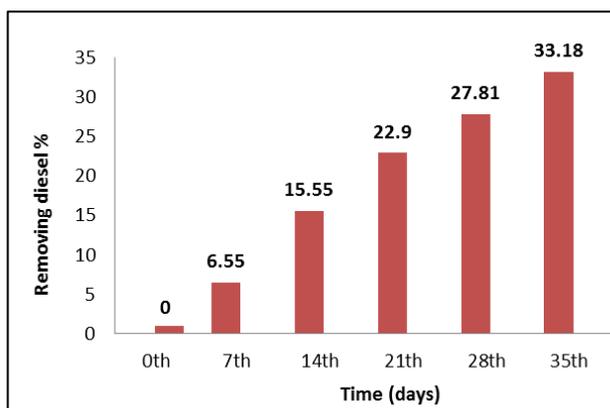


Figure.10 Percentage of removing diesel from unamended soil (Control) through remediation time - 35 days

Evaluation Changes in Soil Properties

The variation in pH of soil contaminated with diesel can be attributed to metabolic processes. However, the pH range observed in this study still falls within the pH range suitable for microbial growth indicating that these isolates showed optimal growth in the pH range from 7.2 to 6.8. Riskuwa and Ijah reported that the growth of most microorganisms is usually greatest within a pH range of 6 to 8 (22). The moisture content is an important factor and was measured to ensure that the air which was injected through the perforated pipes of bio pile system did not dry the soil and thus determining the bacterial growth. There was little change in soil moisture content of the bio piles over time.

Biological processing helps to maintain moisture within the allowable level because microorganisms during the metabolism of hydrocarbons produce water (16). The pH and moisture content data of the polluted soil agree with earlier work by Umar et al. studied biodegradation of waste lubricating oil polluted soil (10). Moreover, temperature does not appear to have a greater effect on biodegradation, as biodegradation is found to occur in soil with moderate temperatures. The results of pH, moisture content and temperature are listed in Table 4 and Table 5.

Table.4 pH, moisture content, and temperature of amended soil during 35 days

Times (days)	pH value	Moisture content %	Temperature °C
0 th	7.2	61.6	29.4
7 th	7.3	68.3	37.6
14 th	7.3	55	36.4
21 th	7.3	56.6	33.3
28 th	7.3	53.3	32.9
35 th	7.1	65	33.1

Table.5 pH, moisture content, and temperature of unamended soil during 35 days

Times (days)	pH value	Moisture content %	Temperature °C
0 th	7.12	50	31.3
7 th	6.9	50	29.9
14 th	6.8	65	30.8
21 th	6.8	60	32.3
28 th	6.8	73.3	31.8
35 th	6.8	61.6	29.8

The Bacterial Counting

A bacterial count was conducted for soil samples that were contaminated with diesel. It seems that the bacteria utilizing diesel were adapted to this oil level because they have shown high numbers. For amended soil during the bioremediation period, the bacteria utilizing diesel

increased progressively from 3.1×10^5 CFU/gm soil in the first week to 1.20×10^6 CFU/gm soil in the fourth week and then decreased to 1.04×10^6 CFU/gm soil in the fifth week. The highest populations of bacteria utilizing diesel were 1.20×10^6 CFU/gm soil at the fourth week. Bacteria utilizing diesel, in the beginning, were stimulated by simple hydrocarbon sources (straight chain hydrocarbons) which caused a good percentage of degradation. As those components reduced, the mixed bacterial cultures had to utilize the more resistant hydrocarbons (aromatic hydrocarbons) (26). It is probable that biodegradation of higher molecular weight hydrocarbons may create toxic intermediates that can prohibit the bacteria utilizing diesel.

The numbers of bacteria utilizing diesel in soil samples amended with nutrients were higher compared to counts for unamended soil. This attributed to the presence of considerable quantities of nitrogen and phosphorous, two necessary nutrients for bacterial biodegradation activities (27). Low bacterial counts and low contaminants levels can indicate that biodegradation were successful and that the bacteria are declining off because the contamination (food source) is decreasing. For unamended soil, the population of bacteria utilizing diesel ranged from 1.6×10^4 CFU/gm soil to 3.5×10^4 CFU/gm soil. Changes in the counts of bacteria utilizing diesel during the 35-day of bioremediation study are represented in Fig.11. These results matched with Obiakalaje *et al.* (2015) who studied the biostimulation effect on the degradation of crude oil-contaminated soil. The number of hydrocarbon degrading bacteria was increased from 2.51×10^5 CFU/gm soil to 1.74×10^6 CFU/gm soil (10).

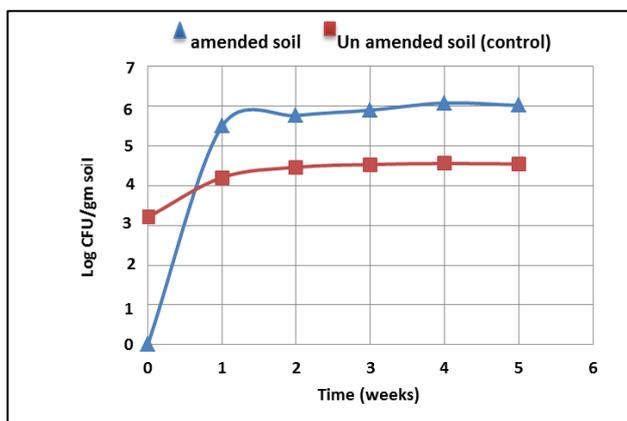


Figure.11 Changes in counting of bacteria utilizing diesel for both amended soil and unamended soil (Control)

Conclusions:

The results of this study showed that:

Three strains of gram-negative bacteria were isolated from diesel-contaminated soil which were *Sphingomonas paucimobilis*, *Pentaoe species*, *Enterobacter cloacae*, and one strain of Gram-positive bacteria as *Staphylococcus aureus*. The metabolic activity of isolated bacteria showed a good level of biodegradability when used individually. Also they demonstrated the best biodegradability when used together in a mixed culture which reached 88.4%. The addition of nutrients to the soil contaminated with diesel stimulated the microbial population and showed an increase in degradation rates, especially during the early stages of degradation. An overall **75.71 %** of the total petroleum hydrocarbons (TPH_s) were removed from the amended soil and **33.18 %** of the control soil at the end of study period. An *ex-situ* bioremediation (bio piles) of diesel polluted soil performed under aerobic conditions has shown to be an effective remediation method for hydrocarbons contaminated soils.

Conflicts of Interest: None.

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المعالجة البيولوجية للتربة الملوثة بالمركبات الهيدروكربونية باستخدام منظومة Bio piles

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الخلاصة:

ركزت هذه الدراسة على معالجة التربة الملوثة بالمركبات النفطية (الديزل) و التي تسببت في مشاكل بيئية خطيرة. ومن الطرق الواعدة و الأكثر فعالية لمعالجة التربة الملوثة بالنفط هي المعالجة البيولوجية بل هي الخيار الذي يوفر إمكانية تدمير الملوثات الضارة باستخدام النشاط البيولوجي. تم عزل أربعة سلالات بكتيرية من عينات التربة الملوثة بالديزل. العزلات التي تم تشخيصها بواسطة نظام Vitek 2، هي كل من *Enterobacter cloacae*، *Staphylococcus aureus*، *Sphingomonas paucimobilis*، و *Pentocae species*. تم اجراء اختبار قابلية إنتاج المستحلبات الحيوية باستخدام Sigma 703D stand-alone tensiometer و أظهر الاختبار أن هذه العزلات هي من منتجي المستحلبات الحيوية. أفضل النتائج لاختبار انخفاض الشد السطحي تم تحصيلها عند استخدام الخليط البكتيري حيث اظهرت النتائج ان المستحلبات الحيوية المنتجة من قبل الخليط البكتيري قامت بخفض الشد السطحي للوسط من 66 (ملي نيوتن/متر) الى 33.89 (ملي نيوتن/متر). وللحصول على مزيد من الأدلة على تأثير التحلل البيولوجي لهذه العزلات بشكل فردي وبشكل خليط بكتيري، تم استخدام تقنية كروماتوغرافيا الغاز التي أكدت حدوث التحلل البيولوجي. تم فحص قدرة الخليط البكتيري لمعالجة التربة الملوثة بالديزل في منظومة bio piling. ومن أجل معالجة سريعة خارج الموقع للتربة الملوثة بالديزل تم اختيار منظومة bio piling. وقد تم إنشاء منظومتين biopiles على نطاق تجريبي (25 كيلو غرام من التربة لكل منهما) و يحتويان على التربة الملوثة بما يقرب من 2140 ملغم / كغم من الهيدروكربونات النفطية. جُهِز كلا النظامين بالأوكسجين لتوفير الظروف الهوائية، و تم الحضان في درجة حرارة الجو و سُحبت العينات اسبوعياً خلال فترة 35 يوماً. تم إزالة 75٪ من إجمالي الهيدروكربونات البترولية من التربة المعدلة و 33٪ من تربة السيطرة في نهاية فترة الدراسة. خلصت الدراسة الى أن تجربة المعالجة البيولوجية خارج الموقع هي الإجراء المفضل، وهي اقتصادية وصديقة للبيئة، وبالتالي تمثل خياراً جيداً لمعالجة التربة الملوثة بالديزل.

الكلمات المفتاحية: التحلل الحيوي، Bio piles، المعالجة البيولوجية، الديزل، تلوث التربة.