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Bioremediation of Soil Contaminated with Diesel using Biopile 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. The capability of mixed bacterial culture was examined to remediate the diesel-contaminated soil in bio piling system. For fast ***ex-situ*** treatment of diesel-contaminated soils, the bio pile system was selected. Two pilot scale bio piles (25 kg soil each) were constructed containing soils contaminated with approximately 2140 mg/kg total petroleum hydrocarbons (TPHs). The amended soil: (contaminated soil with the addition of nutrients and bacterial inoculum), where the soil was mixed with 1.5% of sawdust, then supplied with the necessary nutrients and watered daily to provide conditions promoting microorganism growth. Unamended soil was prepared as a control (contaminated soil without addition). Both systems were equipped with oxygen to provide aerobic conditions, incubated at atmospheric temperature and weekly sampling within 35 days. Overall 75% of the total petroleum hydrocarbons were removed from the amended soil and 38 % of the control soil at the end of study period.

The study concluded that ***ex-situ*** experiment (Bio pile) is a preferable, economical, and environmentally friendly procedure, thus representing a good option for the treatment of soil contaminated with diesel.

Keywords: *Bioremediation, bio piles, Diesel, Soil pollution.*

1. Introduction

Oil pollution accidents had become a universal phenomenon and have caused serious environmental problems, such as the introduction of toxic compounds in 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 common product for distillation of crude oil with a very complex composition. It consists mainly of low molecular weight alkanes and polycyclic aromatic hydrocarbons (PAHs) [2] . With the increasing interest in the conservation of the environment, biological 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]. 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]. 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 [5].

Remediation technologies can be divided into ***ex-situ*** and ***in-situ*** methods [6]. Amongst ***ex-situ*** methods available to bioremediate soils, bio piles have been described as an effective way to remove hydrocarbons which exist in diesel [7]. In bio piling, 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 [8]. Aeration is carried out by the air compressor which drives the oxygen through the perforated tubes that are placed throughout the pile. 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.

2. Materials and Methods

2.1 Measuring the Physio-Chemical Properties of Soil

Physio-chemical analyses 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) [10]. The pH was determined by taken 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. All the analyses were conducted in the laboratory at the Environment and Water Research and Technology directorate/ Ministry of Science and Technology.

2.2 Preparation of Contaminated Soil

Twenty five kilogram of the uncontaminated soil sample was collected at a depth of (0- 30) cm. Soil was air dried, manually homogenized by removing any material such as stones. The soil passed through a 2-mm sieve and autoclaving at 121°C for 35min for two times [11].

The prepared soil was mixed by a hand trowel and contaminated with 1% of diesel/ soil (v/w) then thoroughly mixed to distribute diesel throughout the soil particles to achieve complete semi-artificial contamination. The contaminated soil was mixed with 1.5% sawdust to maintain moisture [16]. For the nutrient addition, the soil was fertilized with nutritious salts solution [$(NH_4)_2SO_4$ and KH_2PO_4] in order to provide the C: N: P ratio of approximately (100:5:1) [12].

Fresh bacterial suspension enriched with biodegradable isolates were sprayed on the soil surface and homogenized thoroughly with soil mixture [13].The treated soil was manually mixed with a clean trowel to enhance aeration and daily irrigated to maintain its moisture content within (50-60%) during the 35 days of experiment period. Unamended contaminated soil (without additions) was used as the control.

2.3 Bacterial Inoculum

Specialized bacterial cultures had been developed in the laboratory to form a bacterial mixture capable of decomposing diesel contaminating the soil. The mixed culture was consisted of four selected bacterial isolates of Sphingomonas paucimobilis, Pentoae species, Staphylococcus aureus, and Enterobacter cloacae isolated from diesel-contaminated soil. These degradable bacteria after isolated and identified were cultivated under controlled conditions. For the **ex-situ** soil remediation , a dense suspension was prepared as shown below [14] :

Sphingomonas paucimobilis was inoculated into sterile tube containing 5 ml of normal saline solution until a cell concentration of 2.1 x 10^8 CFU.ml^{-1} (7 McFarland Standard) was reached. The 5 ml bacterial suspension was transferred into 100 ml of Bushnell-Haas Medium (BHM). This was repeated using Pentoae 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. These were used for field work [15].

2.4 Bio pile Tanks

Several studies have been conducted on biopiles system of various sizes, ranged from pilot scale systems 100 kg [16] to field studies 500 kg of polluted soil [17]. The diverse nature of the soil components is difficult to capture in the laboratory scale as well the processes related to the distribution of oxygen and hydrocarbons through soil texture and soil moisture are varied with bio pile sizes. So for this study, 25kg was

chosen as the same amount was used by (Sharma et al.,2014) [2]. At this mass, the soil can still represent field soil and its temperature will remain almost equal and not create parts of soil that have a high temperature which will negatively affect the bacteria**.**

2.5 Experimental Design

Two rectangular stainless steel tanks pilot-scale bio pile were used, as shown in Figure1. The dimensions of the tank are 52 cm in length, 33 cm wide and 26 cm deep. The soil was filled up to 15 cm from the bottom of the tank shown in Figure 2 [7]. In order to provide aerobic conditions, an air compressor was connected to the tank. The air flow was controlled by valves and air flow rate was adjusted to 2L/h. The air 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 0.5 out-diameter with perforations 3 mm diameter, spaced 50 mm apart. To prevent soil from entering the ventilation pipes, they designed so that the holes were directed downward [18].

Fig. 1. The pilot-scale stainless steel tanks of Biopile system.

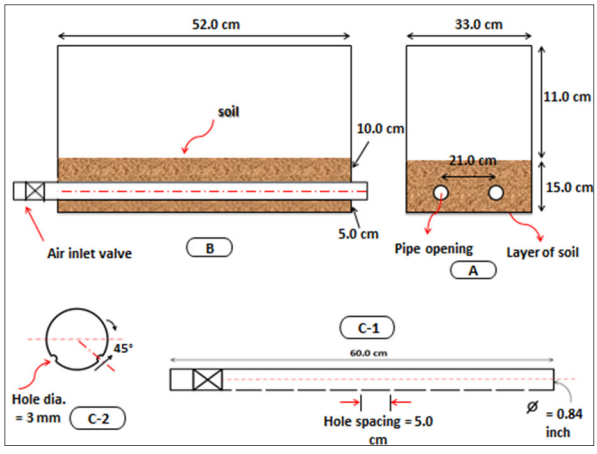

Fig. 2. the dimensions of the stainless steel Biopile system. A: front view B: side view C-1: air supply tube C-2: cross section of air supply tube

2.6 Monitoring the Bioremediation Experiment

2.6.1 Determination of Physiochemical Parameters

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 [19]. 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.

2.6.2 The Bacterial Count

The number of diesel-utilizing bacteria in soil was obtained using the method of pour plate as follows: each soil sample was prepared by adding 1gram of soil to 9 mL sterile distilled water and shaking for 2 minutes. After settling down for 10 min, serial dilutions of samples up to 10^{-4} dilution were performed. Aliquot (1.0 ml) of dilution was inoculated into a petri dish containing modified Bushnell-Haas medium (BHM with agar). Plates were incubated at 30°C for 48 hour; the formation colonies were then counted using Digital Colony Counter device [20, 21] .

2.6.3 Total Hydrocarbons Analysis by Extraction Method

Twenty-five gram 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[12]. The amount of residual diesel was determined by weight to quantify the amount of degraded diesel over time as follows:

$$\text{Weight of Residual diesel} = \text{weight of baker containingextracted diesel} - \text{weight of empty beaker} \quad \ldots (1)$$

$$\text{Weight of diesel degraded} = \frac{\text{Weight of residual diesel} \times 1000}{\text{weight of soil sample} / 1000} \quad \ldots (2)$$

3. Results and Discussion

3.1 Physical and Chemical Properties of the Soil

The results of the soil characterization are presented in Table 1. 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 1,
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 %

3.2 Ex-situ Experiment Bioremediation (Bio Piles)

Biopile 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. Among the main advantages associated with the use of the biopile 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 [22]. Soil contaminated with diesel initially was contained 2140 mg /kg at zero days for both amended soil 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/kg when compared with that of the control soil 2000 mg /kg. At the end of treatment period, the reduction of TPHs reached to 520 mg /kg for amended soil while reached to 1430 mg /kg for the control as shown in Table 2.

Table 2,
Bioremediation of a soil contaminated with diesel during 35 days.

Time (days)	Total petroleum hydrocarbons (mg/kg soil)	
	Amended soil	**Unamended soil (control)**
0^{th} day	2140	2140
$7^{th.}$ day	1524	2000
14^{th} day	1256	1800
21^{th} day	1228	1650
28^{th} day	1200	1545
35^{th} day	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 [21]. 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 [23].The result agrees with those of (Shabir et al., 2008) [21] , who found that the biodegradation rate of kerosene-contaminated soil was significantly enhanced by the addition of inorganic amendments along with the addition of mixed bacterial culture through 6weeks and reached to 65 %.

The biodegradation rate in the biopile systems increased with time. After the first 7 days of remediation, the degradation rate of amended soil was 28%, while from the 14 day to the 28 day the degradation rate falls in the range 41.3% to 43.9 %. 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 oxidize 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% while for unamended soil (control) the degradation rate reached to 38 %. The percentage of remaining diesel through remediation period showed in Figures 3 and 4.

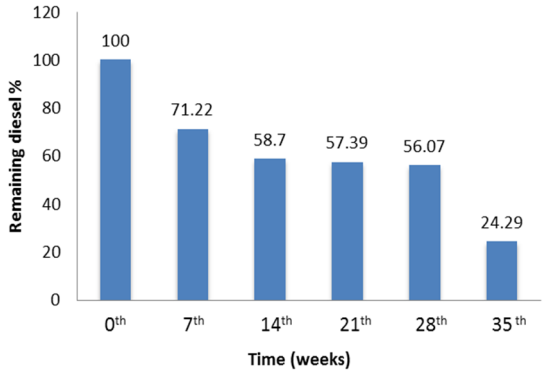

Fig. 3. Percentage of remaining diesel through remediation time in amended soil.

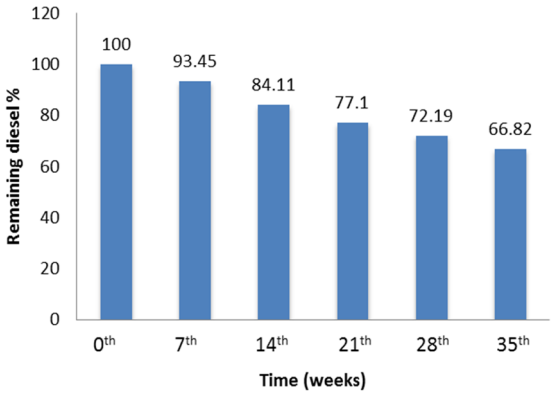

Fig. 4. Percentage of remaining diesel through remediation time in unamended soil (control).

3.3 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 [26]. 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 biopiles over time.

Biological processing helps to maintain moisture within the allowable level because microorganisms during the metabolism of hydrocarbons produce water [19]. The pH and moisture content data of the polluted soil agree with earlier work by Umar et al. who studied biodegradation of waste lubricating oil polluted soil [20]. 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 were listed in Table 3 and Table 4.

Table 3,
PH, moisture content, and Temperature of amended soil

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

Table 4,
PH, moisture content, and Temperature of unamended soil

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

3.4 The Bacterial Counting

A bacterial count was conducted for soil samples that were contaminated with diesel. It seems that soil bacteria were adapted to this oil level because they have shown high numbers of bacteria. For amended soil during the bioremediation period, the diesel-utilizing bacteria increased progressively from 3.1x10^5 CFU/g in the first week to1.20 x10^6 CFU/g in the fourth week and then decreased to 1.04 x10^6 CFU/g in the fifth week. The highest populations of diesel-utilizing bacteria were 1.20 x10^6 CFU/g 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) [27]. It is probable that biodegradation of higher molecular weight hydrocarbons may create toxic intermediates that can prohibit the diesel-utilizing bacteria.

The numbers of diesel-utilizing bacteria in soil samples amended with nutrients were higher compared to counts for unamended soil. This could be attributed to the presence of considerable quantities of nitrogen and phosphorous, two necessary nutrients for bacterial biodegradation activities [28]. The use of microorganisms that already have been adapted to hydrocarbons and increase their abundance helped to reach to the best bioaugmentation approach. Diesel degradation was most related to the number of microorganisms degrading diesel. With the increased number of bacteria and the addition of nutrients, this way reduces the cleaning time considerably [4] . 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 diesel-utilizing bacteria was range from $1.6x10^4$ CFU/g to $3.5x10^4$ CFU/g. Changes in the counts of diesel-utilizing bacteria during the 35-day of bioremediation study are represented in Figures 5 and 6. These results matched with Obiakalaije and Makinde who studied the biostimulation effect on the degradation of crude oil-contaminated soil. The number of hydrocarbon degrading bacteria was increased from $2.51x10^5$ to $1.74x10^6$ CFU/ g [21] .

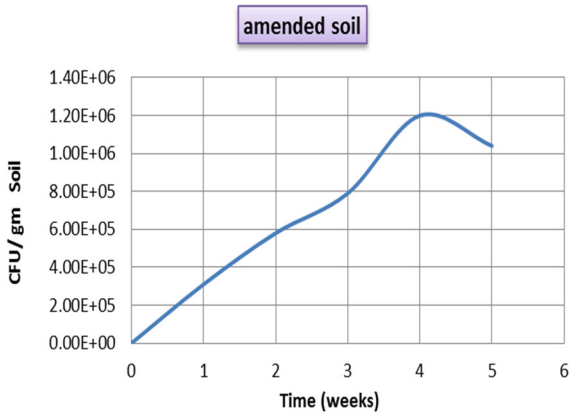

Fig. 5. Changes in counting of diesel-utilizing bacteria for amended soil.

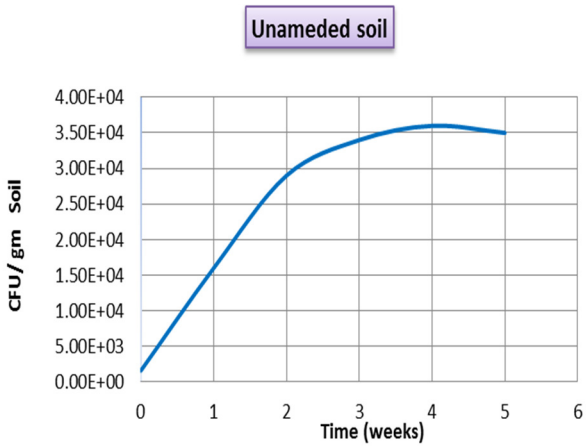

Fig. 6. Changes in counting of diesel-utilizing bacteria for unamended soil (control).

4. Conclusion

The present study showed that the ***ex-situ*** bioremediation (bio piling) of diesel-polluted soil performed under aerobic conditions has shown to be an effective remediation method for hydrocarbons contaminated soils. 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 % of the total petroleum hydrocarbons were removed from the amended soil and 38 % of the control soil at the end of study period. The bioremediation procedure for contaminated soil with petroleum hydrocarbons especially with diesel is applicable in a field scale because of its low cost and environment-friendly.

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المعالجة البايولوجية للتربة الملوثة بالديزل باستخدام منظومة Biopile

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الخلاصة

ركزت هذه الدراسة على معالجة التربة الملوثة بالمركبات النفطية (الديزل) و التي تسببت في مشاكلات 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 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 (٢٥ كيلوغراماً من التربة لكل منهما) و يحتويان على التربة الملوثة بما يقرب من ٢١٤٠ ملغم / كغم من الهيدروكربونات النفطية. التربة المعدلة: (التربة الملوثة مع إضافة المغذيات واللقاح البكتيري) حيث تخلط التربة مع ١,٥٪ من نشارة الخشب، ثم زودت بالمغذيات اللازمة وتُسقى يومياً لتوفير الظروف التي تعزز نمو الكائنات الحية الدقيقة. تم تحضير التربة غير المعدلة بوصفها عنصر سيطرة (التربة الملوثة بدون إضافة). جُهز كلا النظامين بالأوكسجين لتوفير الظروف الهوائية،و تم الحضن في درجة حرارة الجو وسُحبت العينات اسبوعياً خلال مدة ٣٥ يوما. تم إزالة ٧٥٪ من إجمالي الهيدروكربونات البترولية من التربة المعدلة و ٣٨٪ من تربة السيطرة في نهاية مدة الدراسة. خلصت الدراسة الى أن تجربة المعالجة البيولوجية خارج الموقع هي الإجراء المفضل،وهي اقتصادية وصديقة للبيئة، وبالتالي تمثل خيارا جيدا لمعالجة التربة الملوثة بالديزل.