ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2521-2931 (Print)
ISSN: 2521-293X (Online)
CODEN: MJSAEJ

Malaysian Journal of Sustainable Agriculture (MJSA)

DOI: http://doi.org/10.26480/mjsa.01.2024.20.27

RESEARCH ARTICLE

BIOREMEDIATION OF OIL CONTAMINATED DRILL-CUTTINGS USING DIFFERENT STRAINS OF NATIVE SOIL BACTERIA AND FUNGI FROM THE KURDISTAN REGION OF IRAQ

Tablo Abdulrahim Ahmed , Dilshad Ganjo.Ahmed

[a]Environmental Science & health Dept, College of Science, Univ. of Salahaddin,Erbil , KRI, Iraq.
[b]Biology Dept., College of Science, Univ. of Salahaddin,Erbil , KRI, Iraq.
**Corresponding Author Email: tablo.ahmed@su.edu.krd*

ARTICLE DETAILS

Article History:

Received 20 February 2024
Revised 15 March 2024
Accepted 04 April 2024
Available online 08 April 2024

ABSTRACT

Consortia of 4 bacterial and 4 fungal hydrocarbon-utilizing isolates was analyzed in remediation of water-based oil fields. Bio-augmentation experiments (in situ) were tested in 16 triplicates (excluding the control). The results (after two months of bioremediation) showed that; A consortium of isolates of Pseudomonas fluorescens-LR134300.1 and Aspergillus fumigatus-KU321562 was able to change the pH from strongly alkaline to almost neutral. A consortium of Kocuria rosea-MK648258 and Aspergillus niger-MK452260 isolates showed high biodegradation and high chloride tolerance. A consortium of Pseudomonas fluorescens-LR134300.1 and Penicillium chrysogenum-MK696383.1 isolates showed the highest percentage of total petroleum hydrocarbon (TPH) degradation and could degrade/utilize a range of carbon fractions from C6 to C36. A consortium of isolates of Kocuria rosea-MK648258 and Aspergillus flavus-MH270609.1 showed the highest lead-reducing capacity, while Bacillus subtilies-MK000710 and Penicillium chrysogenum-MK696383-KU321. The consortium extracts mineralized petroleum hydrocarbons as the sole source of energy and carbon, with mineralization rates statistically significant (Pandlt;0.05). The results of the Toxicity Characterization Leaching Procedure (TCLP) test showed that treating the drill cuttings with different consortia of bacterial and fungal strains proved to be a desirable disposal method.

KEYWORDS

Drill-cuttings, bioremediation, microbial consortia, pH, Cl, TPH, heavy metals, TCLP

1. INTRODUCTION

Iraq's oil reserves, including those in the Kurdistan Region, are among the largest in the world, holding more than 140 billion barrels of crude oil. Drilling operations may cause a significant amount of trash to be released into the surrounding environment at concentrations that are not typical of the natural world. Drilling wastes, which include drill-cuttings, are extremely harmful to the environment because they can cause mutation and/or cancer. A review of several international scientific studies found that lead, nikle, vanadium, zink, cadmium, mercury, and As are the primary trase elements found in oil-rich drill cutting. Lead is the most widely used. Lead is the most frequently detected heavy metal in oil spill incidents, with other metals following closely behind. The heavy metals most frequently connected to human toxicity are As, Hg, and Pb. Heavy metal poisoning can be caused by exposure to industries related to petroleum as well as air or water pollution. In the modern era, bioremediation of hydrocarbon-contaminated soils (using specific species of bacteria and fungi) is a well-established method that can be used in "in-situ" and "ex-situ" situations (Das and Chandran, 2011, 2011). Removing pollutants from the environment at a reasonable cost is possible when bioremediation is carried out in-situ. With little to no ecological impact, it is an enhanced technique for eliminating pollutants from the environment. Concerns about the potential environmental impact of drilling operations and crude oil have drawn more and more public attention. Landfills, burial pits, and thermal treatments like burning and thermal desorption have all been used to dispose of drill cuttings in addition to other non-biological methods. As a dependable alternative method for managing drilling waste and treating drill cuttings, bioremediation has been studied more recently. Still, there's been a lot of debate and research about how well consortia—mixed cultures—of different bacterial and fungal strains collaborate for oil-laden soil bioremediation work (Flemming and Wingender, 2010; Al-Mailem et al., 2013). It is typically necessary for multiple species to work together for the biodegradation of complex hydrocarbons. As it is intended for pollutants such as crude oil and petroleum, which are made up of different compounds, to fully mineralize into CO2 and H2O, this is significantly true. Single microorganisms are only capable of breaking down a small range of hydrocarbon substrates; to boost the rate and extent of petroleum biodegradation, mixtures of populations with diverse enzymatic capacities are required (Al-Oud and Ghoneim, 2018; Ite and Ibok, 2019). Because they are not considered hazardous, water-based mud (WBM) cuttings are usually disposed of overboard. Research has shown that this strategy has a number of serious adverse effects on the environment, such as changes to the natural soil biota, crop productivity disruptions, and soil degradation. Features of Hazardous Materials To determine whether a waste (after treatment) contains any hazardous elements, a chemical analysis process known as the Leaching Procedure (TCLP) is employed. The test can produce a rating that indicates whether or not the waste poses an environmental risk, simulating leaching through a landfill. After the waste has been rated, this rating may influence the waste management plan that is employed to dispose of it. Although not without some hardship, petroleum exploration and production in the KRI has contributed to a boom in the country's economy over the past 20 years. The principal objective of this study is to assess which natural soil bacterial and fungal strains, isolated from environments contaminated

Quick Response Code	Access this article online	
	Website: www.mjsa.com.my	**DOI:** 10.26480/mjsa.01.2024.20.27

Cite The Article: Tablo Abdulrahim Ahmed, Dilshad Ganjo.Ahmed (2024). Bioremediation of Oil Contaminated Drill-Cuttings Using Different Strains of Native Soil Bacteria and Fungi from The Kurdistan Region of Iraq. *Journal of Sustainable Agricultures, 8(1): 20-27.*

with oil, have the potential to be used in the bioremediation of water-based drill cuttings from oil fields. Additionally, the results will demonstrate the bioremediation capacity of these strains with respect to pH, chlorides, major ions (Na, K, Ca, and Mg), major ions (NO3, P-total, and SO4), heavy metals (Pb, Hg, As, Cu, Zn, Cd, Cr, Ba, and Ni), polyaromatic hydrocarbons (PAHs), moisture content percentage, and microbial population density. And lastly, To enhance the findings, TCLP was employed as an end-point. This work demonstrates how a collection of different native bacterial and fungal strains can be used for bioremediation. In reference to pH, TPH, chlorides, and specific dangerous heavy metals such as Pb, Hg, and As, as verified by the TCLP test (complete information is available from the corresponding author).

2. Materials and Methods

2.1 Sampling site/sample collection

Drill-cutting samples were taken once from forty one drilling waste pits. Five major oil and gas fields—the provinces of Sulaymani, Halabja, Duhok, and Erbil—as well as the Garmiyan administration at KRI were all included in the pits. With the aid of a sterile hand auger, the drill cuttings were collected aseptically. Between 0 and 50 cm was the indicative sampling depth. Each sample was created by combining three to four samples that were collected over several square meters. For analysis, samples were gathered in triplicate (a backup copy was retained). The drill-cutting samples were placed in nylon bags in order to preserve petrolium hydrocarbon. They were then promptly returned for laboratory analyses, where a sterile spatula was used to homogenize the samples once more (Fontana et al., 2011).

2.2 Isolation and Enrichment of Hydrocarbon-Degrading Micro-Organims

By diluting 10 g of each soil sample in 90 ml of Basal Saline Medium (BSM) containing (grams per litre): (NH4)2HPO4, 2.7; Na2HPO4, 4.3; K2HPO4, 4.2; MgSO4.7H2O, 5.5; CaCl22H2O, 0.06; with 0.5% crude oil as the sole carbon source, oil-contaminated environments were used to isolate soil hydrocarbon-degrading bacterial populations. For forty days, the cultures were incubated at 30°C with 200 rpm of continuous agitation. BSM plates were inoculated using 100 microliters of the culture supernatant. After four more days of incubation at 30°C, individual colonies were removed from the plates and re-planted on fresh BSM plates to aid in molecular characterization and identification. Potato dextrose agar, or PDA, is used in soil enrichment techniques to isolate fungi (He et al., 2010). Utilizing a gas chromatography mass spectrophotometer (GC-MS) to examine soil-contaminated drill cuttings free of microorganisms, the hydrocarbon degrading activity in contaminated soils was verified.

2.3 Identification and Characterization of Microorganisms

Using a scanning electron microscope, molecular and morphological techniques, along with taxonomic keys, the microorganisms—native species of bacteria and fungi that cause oil in soil—were identified. To assess the dynamics of bacterial association, the polymerase chain reaction (PCR) technique was employed. Then, right after denaturing gradient gel electrophoresis (DGGE), the 16S rRNA gene fragment was reiterated. For the phylogenetic analysis, the MEGA 6 software package was utilized. However, for PCR amplification, Fungal isolates were contained in 0.5 g of hyphae (following a particular treatment). The PCR product sequencing of fungal isolates was submitted to GenBank. The phylogenetic tree and evolutionary analyses were then built by MEGA 4. The National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST) program was utilized to perform a homology search on the sequences (NCBI).

2.4 Consortia preparation and experimental set-up

Only four of the most effective native oil hydrocarbon-degrading bacterial isolates—Bacillus subtilies, MK000710 (labeled B1), Kocuria rosea, MK648258 (B2), Pseudomonas fluorescens, LR134300.1 (B3), and Pseudomonas putida, GQ303714.1 (B4)—were found to be among the 55 isolates. In contrast, it was discovered that four isolates out of the The most productive 68 native hydrocarbon-degrading fungi were F1, F2, F3, and F4. As demonstrated in Table 1, sixteen consortia comprising equal parts of the aforementioned pure bacterial and fungal cultures that were isolated from soil contaminated with hydrocarbons (not including the control) were created.

Table 1: Isolates of bacteria and fungi, accompanied by the consortium symbol

Pure Cultures of Bacterial (B) and Fungal (F) Isolates			Symbol
Bacillus subtilies-MK000710 (B1)	+	*Aspergillus niger*-MK452260 (F1)	B1+F1
B. subtilies-MK000710 (B1)	+	*A. fumigatus*-KU321562 (F2)	B1+F2
B. subtilies-MK000710 (B1)	+	*Penicillium chrysogenum*-MK696383 (F3)	B1+F3
B. subtilies-MK000710 (B1)	+	*A. flavus*-MH270609 (F4)	B1+F4
Kocuria rosea-MK648258 (B2)	+	*Aspergillus niger*-MK452260 (F1)	B2+F1
K. rosea-MK648258 (B2)	+	*A. fumigatus*-KU321562 (F2)	B2+F2
K. rosea-MK648258 (B2)	+	*Penicillium chrysogenum*-MK696383 (F3)	B2+F3
K. rosea-MK648258 (B2)	+	*A. flavus*-MH270609 (F4)	B2+F4
Pseudomonas fluorescens-LR134300.1 (B3)	+	*Aspergillus niger*-MK452260 (F1)	B3+F1
P. fluorescens-LR134300.1 (B3)	+	*A. fumigatus*-KU321562 (F2)	B3+F2
P. fluorescens-LR134300.1 (B3)	+	*Penicillium chrysogenum*-MK696383 (F3)	B3+F3
P. fluorescens-LR134300.1 (B3)	+	*A. flavus*-MH270609 (F4)	B3+F4
Pseudomonas putida-GQ303714.1 (B4)	+	*Aspergillus niger*-MK452260 (F1)	B4+F1
P. putida-GQ303714.1 (B4)	+	*A. fumigatus*-KU321562 (F2)	B4+F2
P. putida-GQ303714.1 (B4)	+	*Penicillium chrysogenum*-MK696383 (F3)	B4+F3
P. putida-GQ303714.1 (B4)	+	*A. flavus*-MH270609 (F4)	B4+F4

All bacterial and fungal isolates were combined for the 16 triplicate microcosms (35 by 11 cm plastic bowls) used in the bio-augmentation trials; the control group consisted solely of drill bits and soil. Before adding the working solution, each microcosm was filled with 4 kg of drill cuttings and 0.67 kg of top soil (about a 6:1 ratio) to start the microbial process. The microcosms were then allowed to settle for 7 days. Twenty milliliters of pure culture (cell density 7.6 x 1011 cfu/l) of bacterial (B1, B2, B3, and B4) and fungal (F1, F2, F3, and F4) species were placed in each microcosm. This working solution was used to fill each microcosm. Notable is the reality that each 14. After that the composite samples were checked biweekly (fortnightly) for pH, Cl, TPH, and heavy metals.

2.5 Methods and laboratory analyses

2.5.1 Extraction of TPH from drill-cutting samples

Dissolved in acetone, the nC16, nC32, and nC34 compounds were sprayed at a concentration of 600 mg/kg (200 mg/kg for each compound) onto 150 g of air-dried drill-cutting samples. Each sample was thoroughly mixed and allowed to stand for a day, being stirred occasionally, to ensure homogeneity and the removal of acetone. After being stored at 4°C, the samples were extracted using the subsequent techniques: 1) Mechanical shaking extraction: After weighing one gram of soil into each glass vial,

thirty milliliters of acetone:hexane (1:1, vol/vol.) were added. Using a reciprocating shaker set to 120 cycles per minute, the vials were sealed with a Teflon-lined cap and shaken for four hours. We allowed the particles to settle before extracting the sample. 2) Soxhlet extraction: 25 grams of drill cuttings were extracted in Soxhlet for 23 hours, four cycles per hour, using 160 ml of a 1:1 vol/vol mixture of acetone and hexane. Upon transferring the extract to a volumetric flask, its capacity was increased to 250 ml, with 30 ml reserved for analytical purposes.

2.5.2 Sample Clean-Up And Analyses

Analyses and sample cleanup were carried out using gas chromatography. The extracts underwent a column procedure for the purpose of eliminating polar organic compounds [6]. To summarize, every sample was subjected to a silica gel column consisting of approximately 63.5 mm of Grade 60 Å activated silica gel (70-230 mesh) (Fisher 5826-1) heated to 101°C for 12 hours and approximately 24.5 mm of ASC anhydrous sodium sulphate (Fisher 5415-1) dried at 400°C for 4 hours. The silica gel column was conditioned with hexane before the extract was run through it to ensure that all of the compounds of interest were collected in an evaporating vessel. A different solvent was then used to flush the column. Hexane was then added to the solvent extracts to bring the volume up to 2.0 ml after they had evaporated to a volume of roughly 1 to 1.5 ml.

2.5.3 Gas chromatography analysis for the TPH

The USEPA's method number 8015, which outlines the procedure, was duly implemented. The samples were examined using GC-MS. The Restek Corporation, Bellefonte, PA, CP 3800 model includes a flame ionization detector (FID) and a 30 m MXT-1 column with an internal diameter of 0.53 mm and a film thickness of 0.25 µm. The injector port (Model 1079 PTV) temperature rose to 350°C after being maintained at 120°C for two minutes, with a 200°C per minute increase following. Then, it cooled to the starting temperature after dropping to 250°C and being held there for five minutes. 330°C was reached at a rate of after the column was held at 35°C for four minutes.

2.5.4 Toxicity characteristic leaching procedure (TCLP)

Leaching samples, leaching samples, preparing leachate for analysis, and leachate analysis were the four crucial steps in the confirmatory toxicity characteristic leaching procedure (TCLP), and supplemented the current bioremediation process's end results. A particle size of roughly 1 mm in diameter was achieved by grinding drill cuttings that had undergone bioremediation. Using reagent water, the roughly 5 g weighted ground or crusted sample was homogenized. Following crushing, the 100 g sample was extracted for 18 hours at 30 rpm and 22°C using the chosen extraction fluid. The filtrate is the TCLP extract that was obtained by filtering the mixture after the agitation period.

2.5.6 pH and chloride

Drill-cutting sample pH was measured using the glass electrode pH meter (Adwa pH-Adwa Microprocessor pH meter-Hungary) with a soil to water ratio of 1:1. protocol for the silver nitrate titration method, also referred to as the Argentometric method. wherein drill-cutting samples were treated with K_2CrO_4 to cause it to react with chloride and function as an indicator. Then, using the following formula, the samples were titrated against $AgNO_3$ to extract chloride ions:

Chloride = *(A-B) × N × 1000/ Wt*

Where N is the normalcy of $AgNO_3$ (0.014), Wt is the weight of air-dried drill-cutting (g), and A, B, and N are the volumes of titrant used for the sample, blank, and normalcy, respectively.

2.5.7 Electrical conductivity (mmhos/cm)

To measure electrical conductivity, a portable EC-meter model (HANNA instruments, HI98129, CE; Made in Mauritius) was utilized. The same instrument manufacturer's prepared standard solutions were used to calibrate the device before each sampling. The findings were expressed in mmhos/cm, according to the American Public Health Association.

2.5.8 Sodium adsorption ratio (SAR)

The sodium-to-calcium and magnesium-to-magnesium ratio (mill equivalents/l) of the liquid extracted from a paste of saturated soil is known as the sodium adsorption ratio, or SAR. The following equation was used to calculate $SAR_{,}$:

$$SAR = \frac{Na^{+}}{\sqrt{\left(Ca^{2+} + Mg^{2+}\right)/2}}$$

2.5.9 Exchangeable sodium percentage (ESP)

With the use of the following empirical relationship, the exchangeable sodium percentage (ESP) was calculated from the saturated paste extracts' SAR.

ESP *= 100 (- 0.0126 + 0.01475. SAR) / (1 + [- 0.0126 + 0.01475. SAR])*

2.5.10 Heavy metals

The heavy metals were measured using an ICP-OES–Varian model 735-Es, 8300 (Perkin Elmer Company, USA) following the digestion of drill-cut samples. The assay was conducted in-situ using the direct air-acetylene flame method, and As, Hg, and Pb were measured using a spectrophotometer. In order to decrease the quantity of metals that adhere to the container walls, a one-liter suction-filtered sample was mixed with four milliliters of concentrated HNO3.

2.5.11 Hydrocarbon degradation percentage (%)

2.5.11.1 Degradation (%) is equal to (a - b) / an x 100.

The weights of (a) and (b) represent the initial, undervalued weight of crude oil and the residual weight of crude oil following degradation.

2.5.12 Quality assurance and quality control (QA/QC)

Strict adherence to accepted practices and protocols for quality assurance and control has been upheld during the course of this investigation. Data collection, labeling, analysis, and confirmation were all done using QA/QC protocols.

2.5.13 Statistical analysis

To assess significant differences between the different treatment options, the standard deviation ±SD and two-way analysis of variance (ANOVA) multiple comparison test were used at the 95% confidence level ($P<0.05$). Three repetitions per treatment (n=3) are indicated by triplicates.

3. RESULTS

As previously indicated, morphological, molecular, and taxonomic keys were used to identify the native species of the bacterial and fungal isolates assessed in this investigation (data not provided). Ahmad and Ganjo provide comprehensive information on molecular techniques for partial pairwise alignment of the isolated genes and partial sequencing results of bacterial and fungal species of concern. The primary objective of this part of the study was to assess the synergistic efficiency of the four bacterial and fungal isolates that were identified as hydrocarbon-degrading/utilizing consortiums in order to determine which one would be most effective for bioremediation of oil-contaminated drill cuttings. Furthermore, little to no changes were seen between biweekly intervals, and after two months, little to no changes were seen, so only the outcomes of the first two weeks and two months of bioremediation are presented here. Table 2 displays. The mean concentration values of pH, Cl and TH (milligrams per kilogram) with ± standard deviation that were noted during the bioremediation phase in relation to the reference.

3.1 Hydronium ion concentration (pH)

In line with Table 2, at A. fumigatus-KU321562 and Pseudomonas fluorescens-LR134300.1 mixed pure cultures formed the consortium of B3+F2, which altered the pH during the first two weeks of bioremediation from strongly alkaline 12.86±0.02 to acidic 4.66±0.5 by a significant ($P < 0.05$) margin. Two months of bioremediation (mixed pure cultures of Pseudomonas putida-GQ303714.1 + Aspergillus niger-MK452260) resulted in a mean pH value adjustment to approximately neutral ($P < 0.05$) by the consortium of B4+F1 isolates.

Table 2: Following two weeks and two months of bioremediation, (n=3) pH, chloride, and TPH concentration values (mg/kg) were noted while treating drill-cuttings using an alternative consortium of bacteria (HUB) and fungi (HUF). The values were recorded with ± standard deviation. in contrast to the control (bold values indicate lowest reductions)

HUB + HUF + Soil + Drill-cuttings	two weeks			two months		
	pH	CL	T.H	pH	CL	T.H
B1+F1	4.92±0.4	12073±379	11044±1137	5.78±0.7*	1794±895*	5990±571
B1+F2	6.00±0.6	10371±255	12744±775	5.68±0.9*	21102±1559	6629±783
B1+F3	6.18±0.5	21986±2206	10740±1404	6.16±0.7	21125±1573	10031±609
B1+F4	5.94±0.6	21136±2240	16258±2377	6.86±0.4	1210±107*	4614±657
B2+F1	4.72±0.5	21415±2023	14554±1967	6.98±0.1	**1027±38***	7671±543
B2+F2	5.72±0.6	7873±751	12578±1461	6.86±0.4	20359±2619	1948±781*
B2+F3	6.00±0.2	9153±655	11722±1610	5.96±0.9*	1945±603*	4868±928
B2+F4	5.18±0.1	21773±2256	17274±2141	6.96±0.3	4481±845	2572±515
B3+F1	5.38±0.2	**4862±1011***	13404±1810	7.34±0.3	21967±2431	4805±873
B3+F2	**4.66±0.5***	22011±2111	15688±2397	7.54±0.7	1992±859*	2751±915
B3+F3	5.02±0.3	22416±3582	12168±655	6.46±0.6	1480±294*	**1133±410***
B3+F4	5.62±0.3	10055±422	17733±1327	7.30±0.2	1267±182*	3641±869
B4+F1	4.88±0.7	21081±1548	17118±1019	**7.72±0.6***	1275±234*	3829±507
B4+F2	5.20±0.4	21672±1904	**10235±1725***	7.36±0.6	22164±2928	14545±512*
B4+F3	5.90±0.1	14351±1471	17862±1171	6.44±0.3	21082±1548	2130±781
B4+F4	5.70±0.6	22109±2681	17940±1121	6.16±0.7	21235±2611	8118±838
Control	12.86±0.02	24850±154.1	16393±1158	12.86±0.02	24850±154.1	16393±1158

** Treatment with control was significant (P <0.05).*

3.2 Chloride content

Following a fortnight of bioremediation, there was a significant (P < 0.05) reduction in the chloride content of the B3+F1 consortium isolates (mixed pure cultures of Pseudomonas fluorescens-LR134300.1 and Aspergillus niger-MK452260) as compared to the control sample, which had a mean value of 24850±154 mg/kg. However, the B2+F1 consortium (mixed pure cultures of Kocuria rosea-MK648258 and Aspergillus niger-MK452260) isolates demonstrated the highest biodegradation activity with a high tolerance for chloride and further decreased the chloride content significantly (P < 0.05) from the control sample mean of 24850±154 to the mean value of 1027±38 mg/kg. In contrast, Table 2 showed minimal change after two months of bioremediation.

3.3 Total petroleum hydrocarbons (TPH)

The group known as B3+F3, which consisted of mixed pure cultures of (LR134300.1) and (MK696383.1), showed the most promise in lowering TPH. after two months of bioremediation. After two months, the mean TPH in the control sample dropped to 1133±410 mg/kg, a significant reduction (P < 0.05). Table II. The overall mean TPH concentration (mg/kg) measured in 16 triplicates after treatment with different HUB and HUF consortiums is shown in Figure 1. The figure indicates that the only sources of energy and carbon that the consortium isolates are the hazardous mineralized petroleum hydrocarbons, and that the rates of mineralization differ statistically significantly (P<0.05 In order to bioremediate drill cuttings, an analysis of hydrocarbon chromatographic data interpretation was carried out. Fig. 2 employs a Gas chromatography/FID technique to quantify and interpret TPH for the consortium B3+F3 (only). Further consortia can obtain the full TPH results of the GC/FID method from the corresponding author.

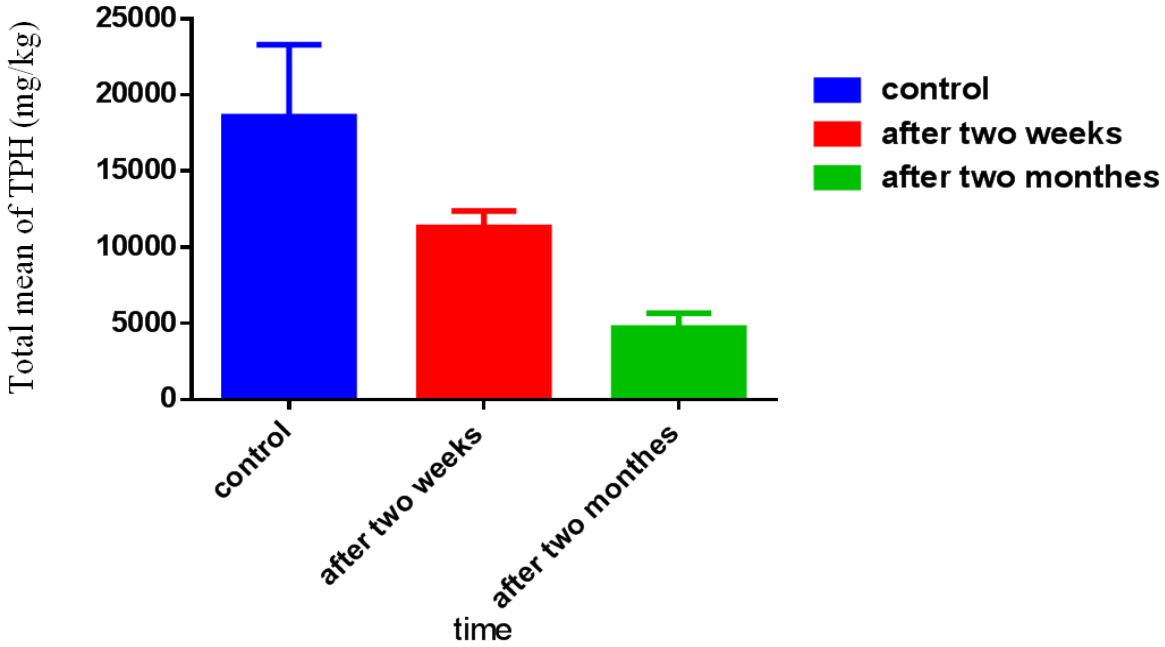
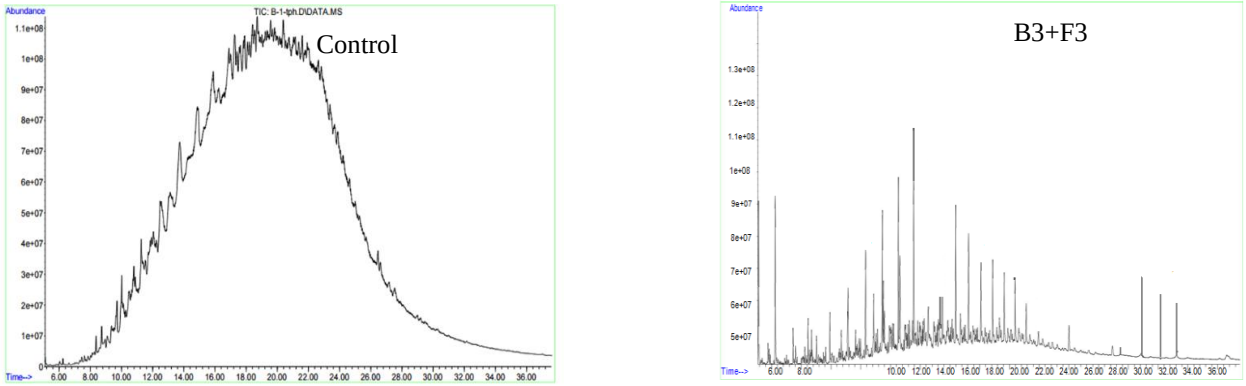


Figure 1: During the two months of bioremediation, the total mean concentration (mg/kg) of TPH (derived from 16 triplicates) was monitored while drill cuttings were being treated with consortia of (HUB + HUF) isolates, in comparison to the control.



Before bioremediation (control group) After bioremediation

Figure 2: Using isolates from the B3+F3 consortium (mixed pure cultures of Pseudomonas fluorescens-LR134300.1 and Penicillium chrysogenum (MK696383.1) prior to and following bioremediation, the TPH content (mg/kg) was determined by gas chromatography (GC-FID).

3.4 Heavy metals (Pb, Hg and As)

Table 3 Using a consortium of various (HUB+HUF) isolates, the average (n=3) lead (Pb), mercury (Hg), and arsenic (As) concentrations (mg/kg) were measured prior to, during, and following the drill-cuttings bioremediation treatment.

Table 3: demonstrates the average (n = 3) concentration values (mg/kg) of lead, mercury, and arsenic with ± SD of the isolates used to treat drill-cuttings using a consortium of various (HUB + HUF) isolates, along with the comparison that

HUB + HUF + Soil + Drill-cuttings	two weeks			two months		
	Pb	Hg	As	Pb	Hg	As
B1+F1	15.50±3.0	N.D	6.08±0.4	5.42±0.2	N.D	2.6±0.5
B1+F2	14.19±3.2	N.D	4.78±0.3	7.40±0.2	N.D	0.9±0.01*
B1+F3	14.85±2.5	N.D	5.91±0.3	4.00±0.2	N.D	**0.6±0.05***
B1+F4	10.33±1.2	N.D	4.65±0.2	5.60±0.1	N.D	1.4±0.8
B2+F1	12.16±3.5	N.D	4.87±0.1	3.40±0.1*	N.D	2.2±0.3
B2+F2	13.08±2.7	N.D	**3.67±0.3***	6.40±0.2	N.D	0.8±0.06*
B2+F3	12.96±4.0	N.D	4.49±0.1	4.00±0.8	N.D	1.1±0.2
B2+F4	13.89±4.0	N.D	4.18±0.4	**2.80±0.3***	N.D	1.5±0.4
B3+F1	12.71±3.0	N.D	4.88±0.1	5.17±0.2	N.D	1.1±0.1
B3+F2	13.90±3.0	N.D	5.26±0.1	9.08±0.9	N.D	2.3±0.5
B3+F3	12.09±4.1	N.D	5.46±0.1	8.26±0.8	N.D	1.6±0.5
B3+F4	14.32±3.0	N.D	4.49±0.1	9.09±0.3	N.D	1.4±0.05
B4+F1	15.50±2.2	N.D	4.18±0.3	4.97±0.6	N.D	0.7±0.03*
B4+F2	**8.60±0.8***	N.D	5.71±0.2	3.80±0.1*	N.D	2.1±0.3
B4+F3	9.20±0.8	N.D	4.85±0.2	3.70±0.2*	N.D	0.5±0.04*
B4+F4	10.40±0.7	N.D	6.05±0.2	4.70±0.2	N.D	1.7±0.05
Control	26.70+4.8	00.12 ±0.1	11.67+1.7	26.70+4.8	00.12 ±0.1	11.67+1.7

** Significantly with control (P <0.05).*

The level that cannot be detected is 0.001 mg/kg (N.D.). Detection limit apparatus

Afterwards The isolates from consortium B4+F2, consisting of mixed pure cultures, revealed that following two weeks of bioremediation, lead had the highest potential reduction. At 8.6±0.8 mg/kg, the mean Pb concentration was significantly (P < 0.05) lower than that of the control group. In contrast, Table 3 revealed only minor changes after a two-month bioremediation period. The B2+F4 consortium, on the other hand, demonstrated the greatest ability to significantly (P < 0.05) reduce lead from the 26.70±4.8 to 2.8±0.3 mg/kg. This consortium was made up of mixed pure cultures of Kocuria rosea-MK648258 and A. flavus-MH270609.1. After two weeks of bioremediation, the mean value of arsenic (As) in the B2+F2 consortium isolates (pure cultures of Kocuria rosea-MK648258 and A. fumigatus-KU321562) was 3.67±0.3 mg/kg, a significant (P < 0.05) decrease from the control sample's mean value of 11.67+1.7 mg/kg. Like in the previous example, after two months of bioremediation, there was little change observed in the arsenic (As) content of the consortium B1+F3 isolates (mixed pure cultures of Bacillus subtilies-MK000710 and Penicillium chrysogenum-MK696383-KU321562). This reduction was significant (P < 0.05) and reached a mean of 0.6±0.05 mg/kg (compared to the control sample). Following the remediation procedure, mercury (Hg) was not detectable (below the instrument's detection limit of 0.001 mg/kg) Table 3.

3.5 Toxicity characteristic leaching procedure (TCLP) experiments

The TCLP test was performed in order to investigate further confirmatory leaching experiments to the bio-remediated drill-cuttings. A number of chemical and physical tests Table 4 were performed on different mixtures. Since the Ministry of Natural Resources (MNR) and local government (KRG) in Iraq have not yet established any regulations or guidelines regarding TCLP limits, the USEPA's 2005 "Contaminated Sediment Remediation Guidance for Hazardous Waste Sites" standards were used as a guide during the assessment.

Following their chemical characterization, the main contaminants found in the bio-remediated drill-cuttings were identified. Among these pollutants were pH, EC, SAR, ESP, TPH, and Cl in addition to heavy metals (As, Br, Cd, Cr, Cu, Ni, Pb, Hg, and Zn). According to the initial mean values of the chemical characterization of the drill-cuttings, most of the parameters, particularly the four essential criteria (EC, SAR, ESP, and Cl) for assessing water quality for irrigation, far surpass the USEPA limits. The untreated drill cuttings are therefore dangerous to use on agricultural land.

Table 4: USEPA limits were compared with mean values (n=3) with ± SD of the toxicity characteristic leaching procedure (TCLP) test result, comparing the initial (before bioremediation) and bio-remediated drill-cuttings using different consortia of HUB/HUF isolates.

S/N	Parameter	Initial Mean Value (Control)	Mean Value (Bio-remediated Drill-cuttings)	USEPA 2005 limit
	Moisture %	63.64±9%	21.2±3%	<50%
	pH	12.86±0.02	7.33±0.02	6.5 - 9.0
	EC	54.37±9.34	4.08±2.18	8 mmhos/cm
	SAR	108.52±51.22	7.14±2.73	12 meq/l
	ESP	46%	6%	15%
	Leachate testing for:			
	TPH	16393±1158	24±2.91	100 mg/l
	Cl	24850±154.1	3923±116	5000 mg/l
	As	11.67±1.7	3.88±1.3	5 mg/l
	Br	1944±78	84±4.5	100 mg/l
	Cd	1.1±0.2	0.06±0.1	1 mg
	Cr	227.5±36	4.1±0.8	5 mg/l
	Cu	43.0±3.1	4.2±0.3	5 mg/l
	Ni	27940.±4504	688.±25.11	5000 mg/l
	Pb	26.70±4.8	3.83±0.9	5 mg/l
	Hg	00.12	ND	12 mg/l
	Zn	63.64±9.9	8.72±1.8	50 mg/l

However, the mean values of the chemical characterization of the bio-remediated drill-cuttings showed that most of the parameters were generally far below limits compared to the USEPA. During the bioremediation of the drill cuttings in this investigation, it appears that all toxicity was completely eliminated, no TCLP leachates were seen, and stable safe limits were maintained.

4 Discussion

4.1 Hydronium ion concentration (pH)

The chemical and physical characteristics of soil, as well as the mineral components that are available to soil microorganisms (like bacteria and fungi), are all determined by the concentration of hydrogen ions, or pH (He et al., 2010). It's possible that higher metabolic activity during the first two weeks of bioremediation led to the creation of intermediate metabolites that are acidic and a drop pH in the process and/or Organic acids are produced when aliphatic and aromatic hydrocarbons are biodegraded both aerobically and anaerobically (Kjeilen and Aabel, 1996; Walworth and Reynolds, 1995; Reineke, 2001). whereas the gradual increase afterword (i.e., after two months) can be attributed to the subsequent release of intermediate final products, which most likely had increasing effects on the pH of the treatment sets (Zhang and Bennett, 2005; Varjani, 2017, 223).

4.2 Chloride content

The water-based mud (WBM) amended with KCl are the more readily utilizable substrate for the growth of bacterial and fungal isolates (Arce-Ortega et al., 2004). The observation of high counts in consortia-containing tests suggested that, as opposed to single isolates, a high degree of chloride content biodegradation can be accomplished (Ahmad and Ganjo, 2020; Ahmad and Ganjo, 2020). This may because WBMs does not contain oil in their liquid phase and such they are non-toxic and also readily degradable. On the other handhigh chloride concentrations (CaCl2, MgCl2, KCl, etc.) have been shown to negatively impact some microorganisms' growth; nonetheless, some species are more resilient to increased chloride concentrations (Haddadi and Shavandi, 2013. Numerous studies showed that the ability of some microorganism species to withstand chloride changes within species (Al-Mailem et al., 2013).

4.3 Total petroleum hydrocarbons (TPH)

Petroleum products have a complicated chemical makeup that can alter over time after being released into the environment (Katoh and Standley, 2013; Srivastava et al., 2014). Additionally, the susceptibilities of petroleum hydrocarbons to microbial attacks vary according to their molecular structures and overall differences in susceptibilities (Huerta-Cepas et al., 2016; Huerta-Cepas et al., 2016). Actually, a number of factors strictly limit the amount of petroleum hydrocarbon-chemical wastes that can be broken down by microbes. These include concentration of combinations and bioavailability among the pollutants, The exchanges between organic contaminants and soil, organic matter, temperature, pH, availability of nutrients (especially nitrogen and phosphorus), soil moisture level, availability of oxygen, and redox potential (Spormann and Widdel, 2000; Widdel and Rabus, 2001). In addition, biodegradation is the process by which complex physiological reactions, largely catalyzed by microorganisms, lead to the partial or total mineralization of environmental organic pollutants (Koshlaf et al., 2016; Abdel-Shafy and Mansour, 2018).

Compounds below C6 are generally not quantitatively detectable using GC-based techniques due to their extreme volatility and solvent peak interference potential. The B3+F3 consortium proved that it could decompose and utilise a range of carbon fractions, from C6 to C36. Gasoline from C6 to C12, kerosene from C9, engine oils from C12 to C25–30, lubricant heavy goods and oils up to C17, diesel from C10 to C28, and C20 through >C36 were the principal reduced carbon fractions. Studies conducted in natural environments have shown that the biodegradation of petroleum hydrocarbons is mainly caused by a range of bacteria and fungi. This suggests that natural Microbial communities in drill cuttings affected by hydrocarbons are naturally able to break down hydrocarbons since they can use the elements of crude oil as a source of carbon and energy (Chandra et al., 2013). More research is needed to determine how much the HUBs and HUFs can use various hydrocarbon fractions, though.

4.4 Heavy metals (Pb, Hg and As)

The concentrations of almost all heavy metals under investigation typically reached their lowest safe limits in respect to national and international standards following bioremediation treatment. Table 3 displays the various ways in which the selected consortia in this study demonstrated that the bioremediation process decreased the levels of heavy metals of concern. API. in 2009 asserted that bioremediation techniques are recognized as a more cost-effective and environmentally favorable alternative to traditional physicochemical methods for the restoration and reclamation of heavy metal-contaminated sites (API, 2009). Similar discoveries regarding the bioremediation of heavy metals using different strains of microorganisms have been made previously (Ite, 2012; Ite et al., 2016) .

When compared to other consortia, the B2+F4 consortium (Kocuria rosea-MK648258 and A. flavus-MH270609) The isolates with the highest potential for biodegradation and/or accumulation of heavy metals were not disclosed. Microorganisms, including bacteria and fungi, may bio-accumulate and partially immobilize heavy metals in their cells prior to biodegradation. When heavy metals start to break down in cells, it could happen as their concentrations threaten the survival of the microorganisms, especially in enclosed areas or conditions with low oxygen (Ite et al., 2016; Boulding and Ginn, 2016; Joutey et al., 2013; Alloway, 2013). The extent to which HUBs and HUFs heavy metals and the processes that cause them to bioaccumulate.

However, Alloway, 2013, asserted that there is a chance that the ways in which bacteria and fungi may react to heavy metals in oil-contaminated soils vary, possess unique mechanisms for adaptation, and employ distinct approaches to bioremediation are all connected to the degradation of the heavy metals (Alloway, 2013; Kapahi and Sachdeva, 2019; Wang et al., 2017; Jin et l., 2018; Wang et al., 2017). However, several investigations carried out worldwide found that WBM drill-cuttings had no mercury (Hg). Li S,2017, given that the main sources of mercury in drilling waste samples are grease and lubricant (Li et al., 2017).

4.5 Toxicity characteristic leaching procedure (TCLP) experiments

It is demonstrated that the toxicity characteristic leaching procedure (TCLP) can be used to determine the mobility of a given list of organic and inorganic contaminants (metals and solvents) in liquid, solid, and multiphasic wastes (Öz et al., 2019; Rzger, 2017). The slow leaching of these contaminants in a landfill environment is modeled by TCLP. Prior to being dumped in landfills that have been approved, waste must undergo testing by TCLP. The purpose of testing is to determine if a waste has characteristics that make it uniquely hazardous (Kogbara et al., 2014). This leads to the main conclusion that treating drill cuttings with bacterial and fungal strains using consortia of related native soil hydrocarbon has proven to be a desirable way to get rid of drill cuttings. However, as contaminant leaching concentrations of concern decreased over time, drill-cuttings were classified as non-reactive, non-hazardous waste, and safe for irrigation. Comparing the EC, Cl, SAR, and ESP values of the remediated drill-cuttings to the USEPA safe limits of 8 mmhos/cm, 5000 mg/l, 12 meq/l, and 15%, respectively, showed that the values were 4.08±2.18 mmhos/cm, 3923±116 mg/l, 7.14±2.73 meq/l, and 6%, respectively. Although high salinity (EC) and chlorides in irrigation water are the most common causes of crop toxicity, high sodium ions in water can change soil permeability and cause problems with infiltration. This dispersion leads to the breakdown of soil aggregates. When soil dries out, it becomes compacted and harder, and the rate at which water and air penetrate it decreases, affecting the structure of the soil.

5. Conclusion

The B4+F1 consortium (mixed pure cultures of Pseudomonas putida-GQ303714.1 + Aspergillus niger-MK452260) isolates brought the pH down to almost neutral. The B2+F1 consortium demonstrated the highest chloride tolerance and biodegradation activity among mixed pure cultures of Aspergillus niger (MK452260) and Kocuria rosea (MK648258).

The mixed pure cultures of Kocuria rosea-MK648258 and A. flavus-MH270609 isolates from the B2+F4 consortium showed the highest capacity for the biodegradation and/or bioaccumulation of heavy metals when compared to isolates from other consortiums. To precisely ascertain the extent to which these microbes able to accumulate trase metals and the process that trigger their decomposition, more investigation is necessary.

The consortium B3+F3 (mixed pure cultures of Pseudomonas fluorescens-LR134300.1 and Penicillium chrysogenum-MK696383.1) isolates were able to degrade/use a wide range of carbon fractions from C6 to C36.

More research should be done on these organisms' synergistic properties and their catabolic genes or plasmids, which give them the ability to degrade, in order to enhance strains and possibly employ them for biotechnological applications.

Acknowledgements

We express our gratitude to all of the MaCrogen, Korea/Beotkkot-to, Geumcheon-gu, Seoul (Gasan-dong, World Meridian I) laboratory staff for their gracious assistance in verifying the results of the GC-MS and GC-FID analyses, as well as the genomic DNA extraction from isolates of bacteria and fungi.

References

Abdel-Shafy, H.I., Mansour, M.S., 2018. Microbial Degradation of Hydrocarbons in the Environment: An Overview. Microbial Action on Hydrocarbons: Springer, Pp. 353-86.

Ahmad, T.A., Ganjo, D.G., 2020. bacterial isolates and their bioremediation for pH, chloride, tph and some toxic heavy metals. biochemical and cellular archives. 20(1).

Ahmad, T.A., Ganjo, D.G., 2020. Fungal isolates and their bioremediation for pH, chloride, tph and some toxic heavy metals. EurAsian Journal of BioSciences. 14: Pp. 149-60.

Alloway, B.J., 2013. Heavy metals and metalloids as micronutrients for plants and animals. Heavy metals in soils: Springer. Pp. 195-209.

Alloway, B.J., 2013. Sources of heavy metals and metalloids in soils. Heavy metals in soils: Springer. Pp. 11-50.

Al-Mailem, D., Eliyas, M., Radwan, S., 2013. Bioremediation of oily hypersaline soil and water via potassium and magnesium amendment. Canadian journal of microbiology. 2013;59(12): Pp. 837-44. https://doi.org/10.1139/cjm-2013-0698

Al-Mailem, D., Eliyas, M., Radwan, S.,2013.Oil-bioremediation potential of two hydrocarbonoclastic, diazotrophic Marinobacter strains from hypersaline areas along the Arabian Gulf coasts. Extremophiles. 17(3): Pp. 463-70. https://doi:10.1007/s10482-012-9830-z

Al-Oud, S., Ghoneim, A., 2018. Lead Transport in Soils Amended with Municipal Solid Waste Ash. Polish Journal of Environmental Studies. 27(6). https://DOI: 10.15244/pjoes/76030

API. 2009. 580 Recommended Practice for Risk-Based Inspection. American Petroleum Institute, Washington, DC. 2009.

Arce-Ortega, J., Rojas-Avelizapa, N.G., Rodríguez-Vázquez, R., 2004 Identification of recalcitrant hydrocarbons present in a drilling waste-polluted soil. Journal of Environmental Science and Health, Part A. 39(6): Pp. 1535-45. https://doi.org/10.1081/ESE-120037852

Boulding, J.R., Ginn, J.S., 2016.Practical handbook of soil, vadose zone, and ground-water contamination: assessment, prevention, and remediation: CRC Press. https://doi.org/10.1201/9781420032147

Chandra, S., Sharma, R., Singh, K., Sharma, A., 2013. Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. Annals of microbiology. 63(2):Pp. 417-31.

Das, N., Chandran, P., 2011;2011. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnology research international.. https://doi:10. 4061 /2011 /941810

Flemming, H-C., Wingender, J., 2010. The biofilm matrix. Nature reviews microbiology. 8(9):623-33.

Fontana, A.R., Camargo, A., Martinez, L.D., Altamirano, J.C., 2011. Dispersive solid-phase extraction as a simplified clean-up technique for biological sample extracts. Determination of polybrominated diphenyl ethers by gas chromatography–tandem mass spectrometry. Journal of Chromatography A. 1218(18): Pp. 2490-6. https://doi:10.1016/ j.chroma. 2011.02.058

Haddadi, A., Shavandi, M., 2013. Biodegradation of phenol in hypersaline conditions by Halomonas sp. strain PH2-2 isolated from saline soil. International biodeterioration & biodegradation. 85: Pp. 29-34. https://doi.org/10.1016/j.ibiod.2013.06.005

He, Z., Deng, Y., Van Nostrand, J.D., Tu, Q., Xu, M., Hemme, C.L., 2010. GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. The ISME journal. 4(9): Pp. 1167-79.

Huerta-Cepas, J., Serra, F., Bork, P., 2016. ETE 3: reconstruction, analysis, and visualization of phylogenomic data. Molecular biology and evolution.33(6): Pp. 1635-8. https://doi.org/10.1093/molbev/msw046

Huerta-Cepas, J., Szklarczyk, D., Forslund, K., Cook, H., Heller, D., Walter, M.C., 2016.eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic acids research. 44(D1): Pp. D286-D93. https://doi.org/10.1093/nar/gkv1248

Ite, A.E., 2012. Biodegradation and ecotoxicity of petroleum-derived chemical wastes in the environment: Lancaster University.https://DOI: 10.12691/jephh-6-2-2

Ite, A.E., Adebisi, O.O., Hanney, N.F., Semple, K.T., 2016. The Effect of Rhizosphere Soil and Root Tissues Amendment on Microbial Mineralisation of Target 14 C–Hydrocarbons in Contaminated Soil. International Journal. 4(2): Pp. 21-34. https://DOI:10.12691/ijebb-4-2-1

Ite, A.E., Ibok, U.J., 2019. Role of plants and microbes in bioremediation of petroleum hydrocarbons contaminated soils. Int J. 7(1): Pp. 1-19. https://DOI:10.12691/ijebb-7-1-1

Ite, A.E., Ubong UU, Etesin UM, Nsi EW, Ukpong EJ, Ekanem AN, et al. Heavy metals in epiphytic lichens and mosses of Oil–Producing communities of eket and ibeno, akwa ibom State–Nigeria. American Journal of Environmental Protection. 2016;4(2):Pp.38-47. https://DOI:10.12691/env-4-2-1

Jin, Y., Luan, Y., Ning, Y., Wang, L., 2018.Effects and mechanisms of microbial remediation of heavy metals in soil: A critical review. Applied Sciences. 8(8):1336. https://doi.org/10.3390/app8081336

Joutey, N.T., Bahafid, W., Sayel, H., El Ghachtouli, N., 2013. Biodegradation: involved microorganisms and genetically engineered microorganisms. Biodegradation-life of science. Pp. 289-320. https://dx.doi.org/105772/52777

Kapahi, M., Sachdeva, S., 2019. Bioremediation Options for Heavy Metal Pollution. Journal of Health and Pollution. 9(24):Pp.191203.

Katoh, K., Standley, D.M.,2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution.30(4): Pp. 772-80. https://doi.org/10.1093/molbev/mst010

Kjeilen, G., Aabel, J., 1996. Cripps S. Disposal of Oil-based Drilling Mud and Cuttings: A Pre-study: RF-Rogaland Research.

Kogbara, R.B., Al-Tabbaa, A., Stegemann, J.A., 2014 Comparisons of operating envelopes for contaminated soil stabilised/solidified with different cementitious binders. Environmental Science and Pollution Research. 21(5): Pp. 3395-414.

Koshlaf, E., Shahsavari, E., Aburto-Medina, A., Taha, M., Haleyur, N., Makadia, T.H., et al., 2016. Bioremediation potential of diesel-contaminated Libyan soil. Ecotoxicology and environmental safety. 133:Pp. 297-305. https:/ /doi.org /10.1016 /j.ecoenv. 2016.07.027

Li, S., Wang, W., Liang, F., Zhang, W-x., 2017. Heavy metal removal using nanoscale zero-valent iron (nZVI): theory and application. Journal of hazardous materials. 322:Pp.163-71. https://doi.org/10.1016/j.jhazmat.2016.01.032

Öz, N., Kadizade, G., Yurtsever, M., 2019. Investigation Of Heavy Metal Adsorption On Microplastics. https://doi.org/10.15666/aeer/1704_73017310

Reineke, W., 2001. Aerobic and anaerobic biodegradation potentials of microorganisms. Biodegradation and Persistence: Springer Pp. 1-161.

Rzger, A.A., 2017. HeavyOilinIraq:Review.JournalofScientificandEngineeringResearch. 4(8):Pp. 134–41.

Spormann, A.M., Widdel, F., 2000. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. Biodegradation. 11(2-3): Pp. 85-105.

Srivastava, J., Naraian, R., Kalra, S., Chandra, H., 2014. Advances in microbial bioremediation and the factors influencing the process. International Journal of environmental science and technology. 11(6): Pp. 1787-800.

Varjani, S.J.,2017.Microbial degradation of petroleum hydrocarbons. Bioresource Technology. 223: Pp. 277-86. https://doi.org/10.1016/j.biortech.2016.10.037

Walworth, J.L., Reynolds, C., 1995 Bioremediation of a petroleum-contaminated cryic soil: Effects of phosphorus, nitrogen, and temperature. Soil and Sediment Contamination. 4(3):Pp. 299-310. https://doi.org/10.1080/15320389709383580

Wang, C-q., Lin, X-y., Zhang, C., Mei, X-d., 2017. Environmental security control of resource utilization of shale gas' drilling cuttings containing heavy metals. Environmental Science and Pollution Research. 24(27): Pp. 21973-83.

Wang, X., Zhang, D., Pan, X., Lee, D-J., Al-Misned, F.A., Mortuza, M.G., et al., 2017 Aerobic and anaerobic biosynthesis of nano-selenium for remediation of mercury contaminated soil. Chemosphere. 170: Pp. 266-73.https://doi.org/ 10.1016/ j.chemosphere. 2016.12.020

Widdel, F., Rabus, R., 2001. Anaerobic biodegradation of saturated and aromatic hydrocarbons. Current opinion in biotechnology. 12(3): Pp. 259-76. https://doi.org/ 10.1016/S0958-1669(00)00209-3

Zhang, C., Bennett, G.N., 2005. Biodegradation of xenobiotics by anaerobic bacteria. Applied Microbiology and Biotechnology. 67(5): Pp. 600-18.