Nativa, Sinop, v. 12, n. 2, p. 359-369, 2024.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nativa.v12i2.17526

ISSN: 2318-7670

Enhanced bioremediation of diesel oil contaminants in soil

Rafal Izzaldeen ABBAS *[1], Hussein Majeed FLAYEH [1]

[1] Department of Environment. Engineering College of Engineering, University of Baghdad, Baghdad, Iraq.
*E-mail: rafal.azz2111m@coeng.uobaghdad.edu.iq

Submitted on: 03/27/2024; Accepted on: 05/27/2024; Published on: 06/10/2024.

ABSTRACT: Compost-bioremediation was adopted to explore the sustainability of using a biological system of microorganisms in a mature, cured compost to break down diesel oil contaminants in soil. The process involved mixing agricultural wastes, including fruitwaste, sawdust, and sheep manure. Periodic sampling from vessels was carried out at the 15-day interval for TPH analysis and the isolation and enumeration of bacteria. Organic waste and soil contaminated with diesel oil were left outdoors for 90 days for degradation. The effectiveness of composting-bioremediation processes relies on diverse microbes. Therefore, parameters such as the C/N ratio (25–30), moisture content, and aerobic conditions are crucial. Manual rotating is necessary for optimal bioremediation. The results show that the biodegradation of diesel oil in soil was 86%–89%, higher than that of control (40%). This increase in biodegradation was due to nutrients in organic waste matter soil, which enhanced the microbes' degradation capabilities. The results from chemical and toxicity assays showed the efficacy of composting treatment for hydrocarbon removal with a germination index of 88.17%., 80.67%, and 92.04%, respectively. This research is the transformation of diesel oil contaminants into less detrimental chemicals by microorganisms in organic waste mixed into the soil.
Keywords: aerobic composting; organic waste; bioremediation; environmental recovery; degraded areas; environmental contamination.

Biorremediação aprimorada de contaminantes de óleo diesel no solo

RESUMO: A biorremediação foi adotada para explorar a sustentabilidade do uso de um sistema biológico de microrganismos em um composto maduro e curado para decompor os contaminantes do óleo diesel no solo. O processo envolveu a mistura de resíduos agrícolas, incluindo resíduos de frutas, serragem e esterco de ovelha. Amostras periódicas dos vasos foram realizadas com intervalo de 15 dias para análise de TPH e isolamento e enumeração de bactérias. Os resíduos orgânicos e o solo contaminado com óleo diesel ficaram ao ar livre por 90 dias para degradação. A eficácia dos processos de compostagem-biorremediação depende de diversos microrganismos. Parâmetros como a relação C/N (25–30), teor de umidade e condições aeróbicas são cruciais, sendo necessária a rotação manual para uma biorremediação ideal. Os resultados mostram que a biodegradação do óleo diesel no solo foi de 86% a 89%, superior à do controle (40%). Este aumento na biodegradação deveu-se aos nutrientes dos resíduos orgânicos do solo, que aumentaram a capacidade de degradação dos micróbios. Os resultados dos ensaios químicos e de toxicidade mostraram a eficácia do tratamento de compostagem para remoção de hidrocarbonetos com índice de germinação de 88,17%, 80,67% e 92,04%, respectivamente. Esta pesquisa consiste na transformação de contaminantes do óleo diesel em compostos químicos menos prejudiciais, por microrganismos presentes em resíduos orgânicos misturados ao solo.
Palavras-chave: compostagem aeróbica; resíduos orgânicos; recuperação ambiental; áreas degradadas; contaminação ambiental.

1. INTRODUCTION

Petroleum and petroleum products cause extensive environmental contamination. This is primarily due to human activities such as petroleum processing, extraction, transportation, storage, and usage. Bioremediation effectively removes pollutants like diesel, PAH, and TPH, but effectiveness depends on initial concentration and operation size. Composting enhances TPH remediation size without compromising efficiency.

Microorganisms can break down and convert petroleum and petroleum-related substances into minerals (ABENA et al., 2019). These species have been extracted from petroleum-contaminated areas and used in bioremediation research due to their secretion of enzymes like alkane hydroxylases and methane mono-oxygenases, which convert alkane C-H bonds into compounds. Compost bioremediation involves using mature, cured compost containing microorganisms to isolate or decompose pollutants in the soil.

Scientific literature shows that mesophilic microorganisms like *Acinetobacter calcoaceticus*, *Bacillus simplex*, *Paenibacillus pabuli*, *Bacillus pumilus* and *Pseudomonas aeruginosa* can break down petroleum pollutants in soils. Additionally, there are thermophilic microorganisms like *Bacillus megaterium*, *Aspergillus* sp, Pseudo *Xanthomonas* sp., *Mucor* sp, *Rhizopus* sp., and *Shigella flexneri* (ATAGANA, 2004; LOICK et al., 2009; GRAÇA et al., 2021; TRAn et al., 2021).

Biodegradation in composting and bioremediation methods are crucial in agricultural waste management, removing organic chemicals from contaminated wastes or substrates and replacing pesticides as primary pollutants. The bioremediation approach, known as ex-situ" is mostly used for the breakdown of polycyclic aromatic hydrocarbons (PAHs) (SOLANAS et al., 2009). The "in situ" bioremediation approach uses plant species to recover soil from hydrocarbon contamination, allowing them to thrive in organic chemicals and heavy metal-contaminated environments (MALDONADO-CHÁVEZ et al., 2010).

The phytoextraction process requires proper treatment of polluting plants, including composting, to ensure effective bioremediation, which requires a balanced carbon-to-nitrogen ratio, proper porosity, and moisture management. Composting for bioremediation requires optimal conditions, biological activity, and a balanced C/N ratio in agricultural wastes, adjusting moisture content and particle size. Bioremediation techniques are considered simple, environmentally friendly, and cost-effective methods for addressing persistent organic pollutants resistant to degradation (SINGH et al., 2017; KAEWLAOYOONG et al., 2020).

The rate of TPH reduction is influenced by various operational conditions such as the initial concentration of contaminants, soil-to-compost ratio, carbon-to-nitrogen ratio, presence of nutrients, moisture content, aeration rate, pH level, and temperature(LIN, 2008; WEI et al., 2017). The biodegradability of TPHs is influenced by factors such as carbon atom number, chemical structure, aromaticity, and affinity for soil particles (OSSAI et al., 2020). For example, compared to branched alkanes, linear alkanes are more easily broken down by various microbes (FUENTES et al., 2014).

Biodegradability of petroleum hydrocarbons in the following order: aromatics, branched alkanes, linear alkanes, and asphaltene compounds (OSSAI et al., 2020). The majority of TPHs consist of alkanes, with diesel oil, for instance, containing around (75) % alkane hydrocarbons, which include n-, iso-, and cyclo-alkanes, with approximately (25) % aromatic hydrocarbons, such as naphthalene and alkyl benzenes (MOHANTY et al., 2008). Gas chromatography-mass spectrometry GC-MS is used to comprehensively identify all the constituents present in petroleum hydrocarbons. The procedures used are quite specific, and the compounds may be verified by analyzing their mass spectra and retention time (AL NUAIMI et al., 2020).

Bioremediation is a cost-effective and efficient method for removing contaminants from petroleum-contaminated environments due to its wide applicability and ability to remove contaminants (AHMED et al., 2023) completely. Bacteria are key environmental degraders of spilled oil, primarily feeding on hydrocarbons, making them the primary agents responsible for environmental degradation (HIMANSHI et al., 2021).

This research utilized gas chromatography and GC-MS to analyze hydrocarbon degradation efficiency in diesel-polluted soils, aiming to enhance bioremediation efficiency through aerobic composting, a microcosm study. The amount of oil removed divided by the starting total petroleum hydrocarbon (TPH) concentration was used to determine the removal efficiency of diesel oil. Additionally, this study looks into how different biological activities, physicochemical processes, and biological process parameters affect the composting process. Through a thorough analysis and comprehension of these variables, we may gain significant knowledge regarding improving and refining the bioremediation procedure for soils contaminated with diesel.

2. MATERIAL AND METHODS

2.1. Soil characterization

The Environment and Water Research and Technology Laboratory of the Ministry of Science and Technology characterized soil samples' physical and chemical properties; the results are presented in Table 1.

Table 1. The soil's chemical and physical properties.
Tabela 1. Propriedades químicas e físicas do solo.

Soil parameters	Value
Soil texture	
Sand (%)	69.28
Clay (%)	17.40
Silt (%)	13.32
Moisture content (%)	2.13
Organic matter content (%)	1.90
Ash (%)	1.70
pH	7.53
Real density (g/cm^3)	2.37
Porosity	0.41
Surface Area (m^2/g)	21.2
SiO_2 (ppm)	46.31
Fe_2O_3 (ppm)	4.23
Al_2O_3 (ppm)	8.12
Cao (ppm)	16.94
SO_3 (ppm)	<0.06
L.O. I3 (ppm)	14.89
Na_2O (ppm)	1.2
K_2O (ppm)	1.4
CL (ppm)	0.07

2.2. Diesel oil

The Al-Dura oil refinery in Baghdad provided the characteristic data of the diesel oil used in this investigation. These criteria, shown in Table 2, were derived from the "Guide of Marketing Specifications for Iraqi Petroleum Products".

Table 2. Diesel oil properties.
Tabela 2. Proprieades do óleo Diesel.

Diesel property	Test results	Limit
Density (gm/cm^3) @15 °C	0.9060	Report[1]
Flashpoint (COC)°C	230	230[2]
Viscosity (Cst)@100 °C	12.92	12.5-16.3[3]
Pour point °C	-15	-9 max[4]

[1]ASTM-D4052; [2]ASTM-D92; [3]ASTM-D445-21; [4]ASTM-D97

2.3. Organic waste

Diesel oil-contaminated soil was studied using compost biodegradation of petroleum hydrocarbon-contaminated soil. A plastic container containing one kilogram of soil was filled with 0.5, 1, and 1.5 L of diesel oil in R, M, and B containers. Diesel oil was mixed with wastes and then allowed to stand undisturbed for two days to allow the hazardous components to volatilize. The raw waste materials were mixed to the proportions in the composition of the composting mixture according to Cornell Waste Management Institute© 1996. Composting involves mixing materials, with moisture being a critical factor. Literature suggests a 50%-60% moisture content for optimal conditions. It calculates the ratio (C/N) at the beginning of the composting process, aiming for a 25:1 ratio in all runs, ensuring the recommended range of 1/20-30/1 is followed.

In Table 3, the total weight of each vessel was 20.75 kg. Each diesel oil-polluted soil was treated separately with organic waste, added according to the mix ratio, Table 4 and mixed well. Diesel oil alone was added to the soil to determine the control weight; every three days, water was added to each vessel to modify the water-holding capacity and the contents were shaken twice a week to promote aeration and maintain a consistent moisture level of 50–60% throughout the experiment. Three identical iterations were conducted, and each container was sampled weekly for 90 days. Mature compost provided bacteria that accelerated the composting process. The composting method bioremediation trials denoted as R, M, and B, respectively, lasted about thirteen weeks.

Table 3. Cornell Waste Management Institute, 2014.
Tabela 3. Instituto de gestão de resíduos de Cornell, 2014.

Ing.	%Moisture	%Carbon	%Nitrogen	Mass(lbs)
SHM	60.0	43.2	2.7	22.0
LM	38.0	48.6	0.9	1.10
FW	62.0	56.0	1.4	22.00
SD	65.0	106.1	0.2	0.55
Calculated mixture moisture content:				60.5
Calculated mixture C/N ratio:				25.0

Ing. =Ingredient; LM =Leaves, Compacted, moist; FW = Fruit Wastes; SHM =Sheep manure; SD =Sawdust.

Table 4. Ratio and weight of composting material.
Tabela 4. Proporção e peso do material de compostagem

Nº	Exp. no.	Ingredients	Weight	Ratio %	SPc
1	R	SHM: LM: FW: SD	10:0.5:10:0.25	48:2.4:48:1.2	1 kg S +MCp + 0.5 L Do
2	M	SHM: LM: FW: SD	10:0.5:10:0.25	48:2.4:48:1.2	1 kg soil + MCp + 1L diesel oil
3	B	SHM: LM: FW: SD	10:0.5:10:0.25	48:2.4:48:1.2	1kg S + MCp + 1.5 L Do
4	Co.				1 kg S + 1L Do

Exp+Experiment; *MCp=Mixture compost, specifiaction = Spc; Soil=S; Diesel oil= Do; Control= Co.

2.4. Reactor design

Figure 1 illustrates the laboratory-scale composting system used in the experiment, which consists of rectangular plastic reactors. The experiment was run for 90 days, from March 26 to Jun 28, 2023. Composting is carried out in rectangular-shaped reactors made of plastic Polyethylene with dimensions 53.5 cm × 44 cm × 36.5 cm in height, length, and breadth, respectively, which were used as bioreactors and fitted with holes to guarantee sufficient ventilation and effective control of gaseous emissions. Every reactor has a removable lid cover. The containers are positioned at fifteen-degree tilts, with the center container's axis facing backward to collect and reuse any leachate. The openings in the containers allow air to pass through, oxygen to supply, gaseous emissions to escape, and the heat generated by decomposition to evacuate. Every three days, the compost material is transferred from the container to the container to guarantee the essential mixing for the speedy composting process that produces aerobic conditions and is more effective in reducing emissions and energy consumption.

Figure 1. Container shape in the composting process.
Figura 1. Container adotado no processo de compostagem.

2.5. Hydrocarbon analysis

Compost samples weighing about 20 g were collected from various container points, primarily the top, middle, and end terminals. To produce a homogenized sample, Triplicate homogenized samples were taken daily to measure the TPH in the compost. Each sample was combined to create a homogenized sample. Next, in addition to the first day, homogenized samples were obtained in triplicate on days 15, 30, 60 and 90. The sample was allowed to air dry in a sterile, well-ventilated laboratory. A 2.0 mm pore-size sieve was used to filter the materials. The soil sample was carefully mixed to ensure complete mixing of pollutants and achieve uniformity. Next, as stated in the Extraction Method, samples were taken from each pile to extract and measure the total petroleum hydrocarbons.

2.6. Extraction method for total petroleum hydrocarbons

10 g of the Composted samples were sieved and mixed with 10 g of solid anhydrous sodium sulfate at a ratio of 1:1. Next, the mixture was transferred to a cellulose thimble, which was lowered into a Soxhlet extractor vessel (SHIN, 2012). A volume of 200 mL of hexane, which serves as the extraction solvent, was introduced into a round-bottomed flask with a capacity of 250 mL. The extraction process lasted 24 hours, with a frequency of 5 cycles per hour (UDOINYANG et al., 2019). The solvent/extract was then condensed in the concentrator using rotational evaporation until it reached a final volume of roughly 2 mL. Replicas of the extract were generated from each composite extract sample for examination using GC/MS.

2.7. Gas chromatography-mass spectrometry

After the last step of the extraction process, the extracts were analyzed using a gas chromatography-mass spectrometry (GC-MS) device (model QP2020 NX, Shimadzu Corporation, Japan), as described by (ALMALKI et al., 2022). Helium was used as the carrier gas in a 5 MS capillary column 30 meters long, 0.25 millimeters in diameter, and 0.25 micrometers thick in film. This allowed for the successful separation of the samples. A 1.0 µL sample volume was injected using the AOC-20i auto-injector. The injector and detector temperatures were set to 280 and 300 °C, respectively. The following programming was used to determine the GC oven's temperature: After being held at 60 °C for three minutes, the temperature was ramped up to 180 °C at 10 °C/per minute and then down to 300 °C at 10 °C/per minute. In MS, the interface was adjusted to 280°C and the ion source to 230°C.

The relative amount of each component is determined by comparing each component's peak area to the chromatogram's total peak area. Material components are separated using gas chromatography, and the findings are shown as a chromatogram. Band intensity indicates the presence of different compounds and their respective quantities under analysis. Where the band intensity informs about the amount of the analytes and the number of bands representing different molecules in the mixture, using discrete numbers in the form of peaks, mass spectrometry can determine the mass of individual components in a mix as well as the mass of a molecule or its fragments., which is characteristic of particular elements in the mix (OSINOWO et al., 2020).

3. RESULTS

Tables 1, 2, and 3 detail the chemical and physical characteristics of the soil, diesel oil, and organic material used in bioremediation research. It is important to remember that the soil chosen for the bioremediation procedure had a 25:1 C: N ratio. This ratio is optimal for achieving efficient diesel oil biodegradation in the soil. Therefore, organic wastes must be included as an extra source of nutrients, particularly nitrogen N and phosphorus P, to promote the best possible biodegradation conditions. Among the four organic wastes employed, it is noteworthy that sheep manure exhibited the highest nitrogen content.

This revelation is particularly significant as nitrogen is recognized as one of the most influential limiting nutrients that significantly contributes to the successful occurrence of biodegradation processes, as suggested by (OKOH, 2006; KIM et al., 2005). In addition, it is essential to note that 60% of sheep dung is moisture. 60% of sheep dung is moisture, potentially containing vital microbes that could enhance oil biodegradation efficiency. Temperature is a crucial environmental component, affecting the physicochemical characteristics of pollutants and controlling organic matter and bacterial numbers, thus influencing the transformation of organic matter.

3.1. Temperature

The temperature in the three containers R, M, and B increases from room temperature to a point where a mesophilic phase transitions to a thermophilic phase. Over five days, the temperature increased by 69, 66, and 68 in containers R, M, and B, suggesting the initiation of the composting process. Oil includes easily decomposable substances.

Elevated temperatures increase volatility while reducing TPH viscosity, allowing for quicker bioavailability of diesel oil pollutants due to increased volatility. If the composting mixture temperature exceeds 50°C, the survival and existence of mesophilic and pathogenic bacteria may be threatened and potentially compromised. Ambient temperature significantly impacts microbial activity levels and degradation rates. Low temperatures cause a decline in the bioavailability of TPHs, complicating their biodegradation process, and their presence can be detrimental to the environment. As a consequence, the temperature of the containers carefully decreased until it was close to the surrounding temperature; on the 8th-16th, the temperature entered a second mesophilic phase, resulting in the maturation process, as depicted in Figure 2.

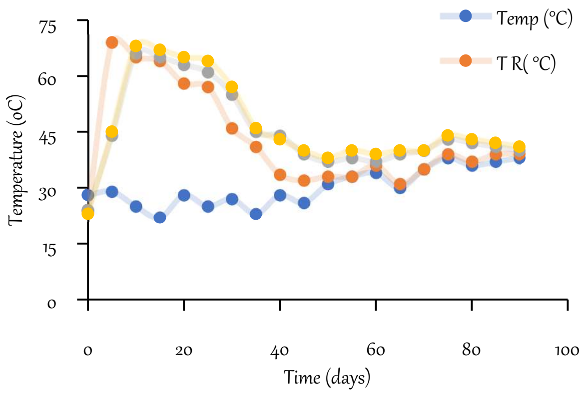


Figure 2. Temperature variations vs time in a composting system.
Figura 2. Variações de temperatura versus tempo em sistema de compostagem.

3.2. pH value

The pH value of a compost mixture, particularly diesel oil-contaminated soil, is significantly influenced by composting materials and soil. The pH value in composting varies depending on the stage, with lower values observed in the mesophilic phase due to the production of organic acids from decomposing microbes. The pH drops to 4.3 in the initial days of composting, causing rapid temperature rise, organic mass reaching room temperature, and local mesophilic organisms multiplying. The pH of mature compost decreases due to the production of simple organic acids, which are converted into carbon dioxide by microbial activity. Figure 3 suggests it was high-quality and within the recommended range of 6–8.5.

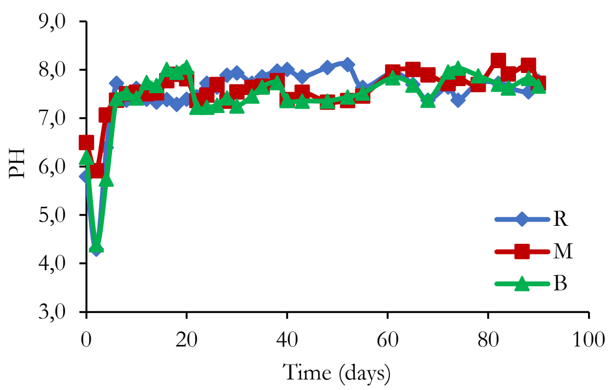


Figure 3. Change in pH value in a composting system.
Figura 3. Mudança no valor do pH em um sistema de compostagem.

3.3. Moisture content

Moisture is crucial for microorganisms to thrive in composting. Initial substrates had high moisture content (66%, 68%, and 65%) for R, M, and B. Still, the ideal moisture content depends on the feedstock's physical features, such as particle size and water-holding ability. The moisture content in composting is constantly altered by temperature and aeration levels, which can be adjusted through waste mixture blending or water addition, with a gradual decrease in moisture content, especially in the thermophilic phase (Figure 4).

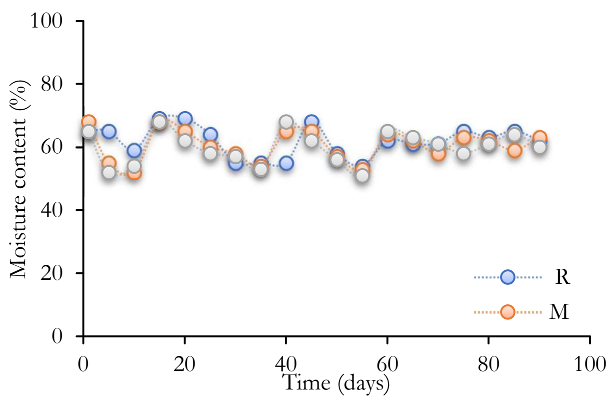


Figure 4. Moisture contents vs time.
Figura 4. Conteúdo de umidade versus tempo.

3.4. Biodegradation Rate of TPH

The study demonstrates that after 90 days of soil amendment with organic wastes, used diesel oil showed a high biodegradation rate compared to the control soil treatment (Figure 5). Each container's soil was sampled at 15-day intervals for total petroleum hydrocarbon analysis. After 90 days of biodegradation, soil contaminated with diesel and treated with a nutrient blend showed the highest rate of disintegration, reaching 89.49, 88.8, and 86.07%, respectively. The study found that diesel oil-contaminated soil amended with organic waste showed higher oil biodegradability than untreated control soil, with a degradation rate of 40.2%.

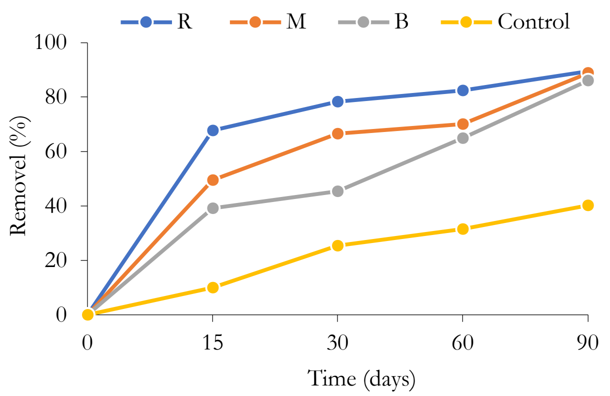


Figure 5. Percentage removal of TPH in diesel-contaminated soil through bioremediation.
Figura 5. Porcentagem de remoção de TPH em solo contaminado com diesel por meio de biorremediação.

The main difference in oil biodegradation between treated and untreated soil occurred between 15 and 30 days, with biostimulation leading to a significant increase in oil biodegradation. Adding nutrients stimulates the indigenous microorganisms' degradative capabilities, allowing them to break down organic pollutants faster (AUSMA et al., 2002).

The findings showed that, compared to C (control bioreactor), the overall elimination percentage of TPHs after 15 days was 67.67%, 49.53%, and 39.09% for the bioreactors R, M, and B, respectively. There was a 10.03% drop in total particulate hydrocarbons (TPHs).

The diesel removal% through biodegradation of TPHs in the polluted soil was much higher than in the control soil, Table 5. Bacterial activity in composting aids is low, and diesel concentration drop may be due to degradation, volatilization, or adsorption to organic compounds. Soil bacteria utilize diesel oil as a nutrient source, with the highest rate of mineralization observed in soil supplemented with organic wastes. The high concentration of diesel oil in contaminated soil negatively impacts microbial biodegradation activities, potentially causing a low percentage of degradation in the control C bioreactor due to the toxicity of the soil's microbial flora (IJAH; ANTAI, 2003; RAHMAN et al., 2002).

Table 5. The Percentage is removing diesel oil through bioremediation.
Tabela 5. Percentual de remoção de óleo diesel por biorremediação.

Time	R	M	B	Control
0	0	0	0	0
15	67.6777287	49.537	39.09307989	10.03
30	78.24685515	66.475	45.32059128	25.42
60	82.42698215	70.06	64.85756805	31.45
90	89.49186322	88.816	86.078	40.2

The GC-MS mass spectrum data analysis was compared with the NIST library version 14 database, revealing that each sample had significantly fewer hydrocarbons than naturally occurring diesel oil, as shown in Figure 6. Chromatograms from days 1 to 90 in Figures (7A, 7B and 7C) show that specific components, like n-alkanes and lower molecular weight constituents, were degraded preferentially. Bacteria are more inclined to attack items with low molecular weight, with aliphatic compounds having a higher degradation rate and preferring straight-chain hydrocarbons over branched-chain alkanes.

3.5. Microbial counts analysis

The results from the diesel oil-polluted soil treated with composting showed that applying composting bioremediation affected soil bacteria growth and that the average bacteria growth in the treatment samples was different from the bacteria growth in the control sample. After four weeks, the highest bacteria growth was observed due to fast standard paraffin decomposition, organic nutrition materials, and bacteria activity, resulting in maximum petroleum degradation. At the same time, soil (control) had the lowest count. For compost during the bioremediation of diesel oil, the results obtained from the analysis indicated that the initial population of bacterial cells in the contaminated soil was ($1.93*10^3$, $6*10^7$, and $2.2*10^7$) CFU/mL, respectively. After the bioremediation period, the counts increased significantly, reaching ($2.45*10^9$, $2.05*10^8$, and $2.2*10^9$) CFU/ml, respectively. Notably, these biodegradation rates were considerably higher than those observed in the control soil, which exhibited a bacterial population of (5.6×10^6 -3.53×10^6) CFU/mL.

The presence of essential nutrients in contaminated soil is believed to have significantly increased the proliferation of microbial life compared to the control soil. This observation indicates a substantial growth in biomass, underscoring the remarkable adaptability of bacteria to the prevailing substrate and environmental conditions. The efficient degradation of organics and hydrocarbons in oil-contaminated areas can be attributed to the combination of powerful degrading bacteria, Pseudomonas aeruginosa and Staphylococcus. The rapid increase in hydrocarbon-degrading bacterial populations due to high hydrocarbon availability has allowed microorganisms to adapt to soil contaminated with diesel, resulting in significant degradation.

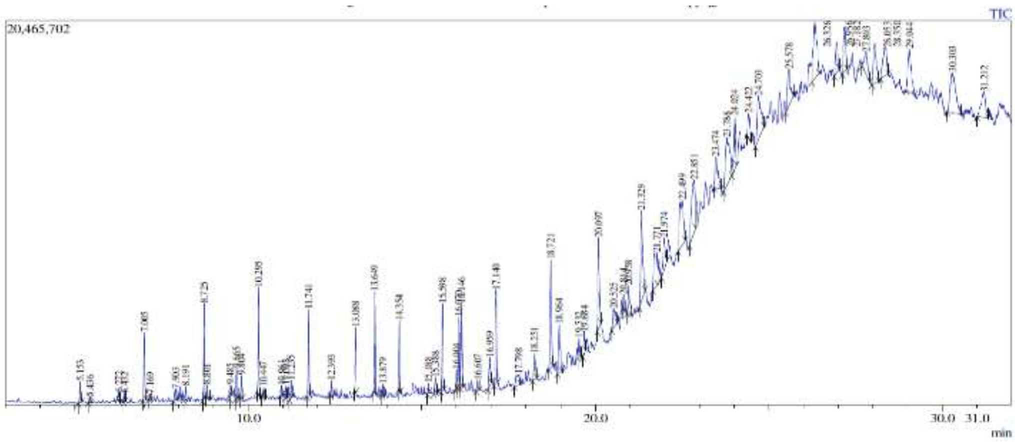
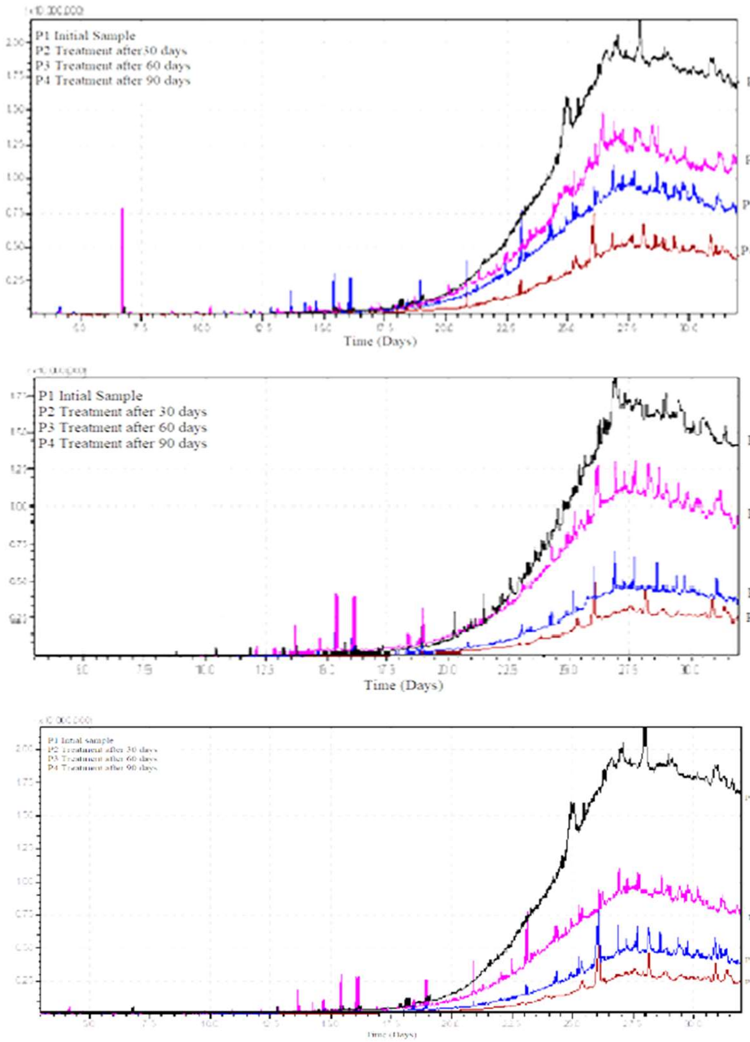


Figure 6. Chromatogram chart of undegraded diesel oil.
Figura 6. Gráfico do cromatograma do óleo diesel não degradado.



(A)

(B)

(C)

Figure 7. Chromatogram chart of samples R (A), M (B) and B (C).
Figura 7. Gráfico cromatograma das amostras R (A), M (B) e B (C).

The study found that nitrogen and phosphorous in nutrient-amended soil samples led to higher bacteria counts due to their essential role in bacterial biodegradation activities. Low bacterial counts and contaminant levels indicate successful biodegradation, reducing bacterial activity due to less contamination. Organic wastes improve soil aeration, providing oxygen for microbial communities and promoting bacteria growth in the soil. Bacteria significantly contributed to the biodegradation of TPH, leading to a reduction in TPH concentrations. Bacteria with a specific number of cells can be used to degrade TPH in various fields. Microbial count in the experiment was significantly correlated to TPH removal, and Table (6) shows information on bacteria that appeared in petroleum hydrocarbon bioremediation.

Table 6. Bacteria are used for petroleum hydrocarbon bioremediation at different diesel-contaminated waste composting temperatures.
Tabela 6. Bactérias utilizadas para biorremediação de hidrocarbonetos de petróleo em diferentes temperaturas de compostagem de resíduos contaminados com diesel.

contaminants TPHs Phases	(PH)	Bacteria types of bacteria	Counts CFU/ml
Mesophilic	3.5-4.5		
R1	4.3	*Klebsiella pneumonia* ssp *pneumonia*	$1.93*10^3$
		Pseudomonas aeruginosa	$1.5*10^6$
		Bacillus	$1.75*10^6$
		Acitinomysics	$1.25*10^6$
M1	5.92	*Klebsiella pneumonia* ssp *pneumonia*	$6*10^7$
		Pseudomonas aeruginosa	$6*10^7$
		Bacillus	$4.5*10^7$
		Acitinomysics	$6*10^7$
		Staphylococcus Lentus+	$6*10^7$
		Enterobacter aerogenes -	$2.1*10^5$
B1	4.39	*Klebsiella pneumonia* ssp *pneumonia*	$2.2*10^7$
		Pseudomonas aeruginosa	$5*10^7$
		Bacillus	$5*10^7$
		Acitinomysics	$2.2*10^7$
		Enterobacter aerogenes	$2.6*10^3$
C		*Pseudomonas aeruginosa*	$3.53*10^6$
		Bacillus	$4.5*10^4$
		Acitinomysics	$2.7*10^3$
		Klebsiella pneumonia ssp *pneumonia*	$4.2*10^3$
Thermophilic	7.2-7.7		
R3		*Klebsiella pneumonia* ssp *pneumonia* -	$8.5*10^6$
		Pseudomonas aeruginosa	$8.5*10^6$
		Bacillus	$1.15*10^7$
		Acitinomysics	$2.7*10^6$
		Enterobacter cloacae complex -	$1.87*10^3$
M3	7.38	*Klebsiella pneumonia* ssp *pneumonia* -	$2.3*10^5$
		Pseudomonas aeruginosa	$6*10^7$
		Bacillus	$4.2*10^7$
		Acitinomysics	$8*10^6$
		Staphylococcus Lentus +	$2.3*10^5$
B3,	7.41	*Klebsiella pneumonia* ssp *pneumonia* -	$1.95*10^4$
		Pseudomonas aeruginosa	$2.2*10^7$
		Bacillus	$6*10^7$
		Acitinomysics	$6*10^6$
		Staphylococcus Lentus +	$6*10^7$
Maturation		*Klebsiella pneumonia* ssp *pneumonia*	$2.45*10^9$
R		*Bacillus*	$6*10^6$
		Pseudomonas aeruginosa	$1.5*10^6$
		Acitinomysics	$8*10^8$
		Pseudomonas oleovorans	$2.45*10^9$
		Staphylococcus lentus	$2.45*10^9$
M		*Klebsiella pneumonia* ssp *pneumonia*	$2.05*10^8$
		Pseudomonas aeruginosa	$2*10^6$
		Bacillus	$1.5*10^7$
		Acitinomysics	$2.5*10^6$
		Pseudomonas oleovorans	$2.05*10^8$
		Staphylococcus lentus	$2.05*10^8$
B		*Klebsiella pneumonia* ssp *pneumonia*	$2.2*10^9$
		Pseudomonas aeruginosa	$9*10^7$
		Bacillus	$8.4*10^7$
		Acitinomysics	$6*10^7$
C		*Pseudomonas aeruginosa*	$5.6*10^6$
		Bacillus	$2.3*10^5$
		Acitinomysics	$3.8*10^4$
		Klebsiella pneumonia ssp *pneumonia*	$2.2*10^4$

3.6. Germination Index GI

All experiments were run in triplicate. The germination test was repeated with deionized water as a control, as shown in Figure 8. After 40 days at 65% moisture and a C/N ratio of 25, the compost obtained from R, M, and B trials was chosen to carry out the germination test to check the phytotoxic effect on plant growth.

The results of the germination test are given in Table 7, which shows Growth Index G (%) is 92%, 88%, and 94%, respectively; the calculated value of germination index (GI) is 88.17%., 80.67%, and 92.04%, respectively. As shown in Figure 9, diesel oil has a volatile component that contains light hydrocarbons capable of entering quickly through the plant cell walls.

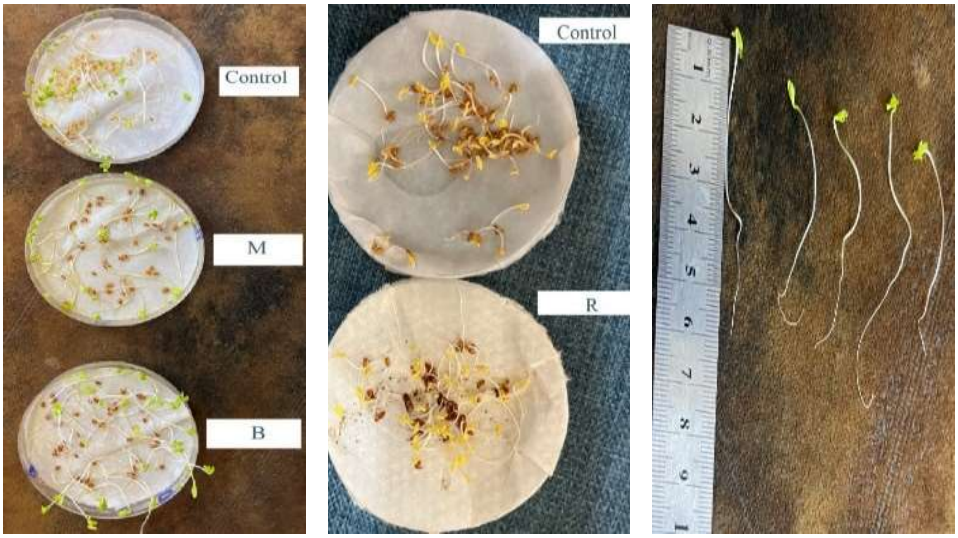


Figure 8. Germination index.
Figura 8. Taxa de germinação.

These small hydrocarbon molecules that penetrate the plants can be phytotoxic, explaining the delay in germination and decrease in seed germination (OGBO, 2009). In the present study, the higher germination in treatments R and B was probably due to the reduction of volatile hydrocarbons

Table 7. Germination Index.
Tabela 7. Taxa de germinação.

Mix	Mean, no of germinating seeds	Growth Index G (%)	Mean root length (c)	Root length Index L (%)	Germination Index GI (%)
R	46	92	1.95	95.83	88.17
M	44	88	2.69	91.67	80.67
B	47	94	2.44	97.92	92.04
Co	48/50	96	1.09		

*Mix= miture; Co= Control; GrI= Growth Index; GI= Germination Index; Root length Index= L; (c)= Mean root length; Mn= Mean, no of germinating seeds.

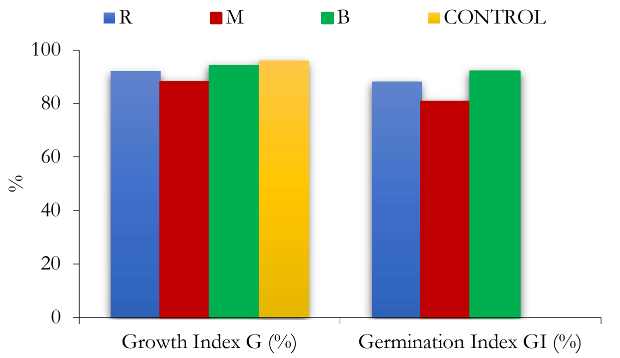


Figure 9. Growth index and Germination index.
Figura 9. Taxa de crescimento e de germinação.

4. DISCUSSION

Composting increases temperatures between 45 and 70°C, enhancing the dissolution of pollutants and stimulating more metabolic activity in the compost pile (GIBB et al., 2001). Bacteria use hydrocarbons as an energy source, enhancing their metabolic processes and significantly increasing temperatures (MARÍN et al., 2006).

Low temperatures negatively impact microbial growth, propagation, and degradation rates (GIBB et al., 2001). As organic compounds decayed, the piles became richer in stable chemicals and less accessible to microorganisms (ELANGO et al., 2009).

The temperature entered a second mesophilic phase from the 8th-16th, resulting in the maturation process during the final composting stage - Figure 2 (AL-SAEDI; IBRAHIM, 2019).

The pH value increases in the thermophilic phase due to the formation of NH4 and the degradation of organic acids into VOCs, CO_2, and H_2O (LIN, 2008). A study found that pH values significantly impact the biodegradation efficiency of crude oil-contaminated soil. Moisture is crucial in composting, as microorganisms need sufficient moisture to thrive. Water is essential for nutrient movement and microorganism availability, while moisture impacts temperature, nutrition, oxygen intake, and air penetration.

The higher moisture content (often >70%) during the composting process leads to anaerobic conditions and the creation of waterlogs. Composting's biological process may be hindered by its lower moisture content, often less than 40%, which can cause early dehydration. A range of 55–65% has been used by most composting studies handling different kinds of organic materials (TRAN et al., 2021; LIN et al., 2021).

Gas chromatography-mass spectrometry (GC-MS) is a popular analytical technique used to identify significant TPHs like organic and alkane, iso-, and cyclo-alkanes, and aromatic hydrocarbons like naphthalene and alkyl benzene compounds. Analyzing samples of diverse extracts using this method might assist in determining their composition and enhance their biological characteristics (YASEEN et al., 2024).

Contaminated soil containing essential nutrients encourages microbial life proliferation, demonstrating their adaptability to substrate and environmental conditions, with Staphylococcus being known for its petroleum degradation capabilities (SHEKHAR et al., 2015; WU et al., 2018). Hinchee et al. (1995) showed that bioremediation would not proceed at a substantial if the population of native microorganisms that can degrade the target contamination is less than 105 colony-forming units (CFU g^{-1} of soil).

The counts of hydrocarbon degraders in oil-polluted soil, reported by (IJAH; ANTAI, 2003b), were 9*106 CFU/g, comparable to our findings. However, the counts obtained by (Antai; Mgbomo, 1989) in hydrocarbon-contaminated soil were higher at 9*108 CFU g^{-1}.

The variation in outcomes might be attributed to disparities in the microbial ecology of the soil, as well as the distinct features or types used (TIQUIA et al., 1996). A higher than 80% GI indicates a phytotoxic-free and mature compost. On the other hand, poor relative seed germination (60%) and root growth are indications that the compost was immature (MAREK et al., 2003). Similar results were found by Bossert; Bartha (1985), who registered low germinability of corn seeds in soil contaminated by crude oil.

5. CONCLUSIONS

Research shows that aerobic bioremediation of diesel-polluted soil can effectively remediate hydrocarbon-contaminated soils, with an 89.5% elimination of total petroleum hydrocarbons. Composting can accelerate bioremediation and remove organic waste simultaneously, making it a practical solution. Composting techniques can effectively manage waste and improve soil conditions, with economic and scientific support for waste removal and soil remediation composting from both financial and scientific perspectives. It aims to provide valuable insights for local governments regarding waste treatment and management. Key characteristics include maintaining a balanced C/N ratio, appropriate moisture, and particle size for successful compost-based bioremediation.

Further studies are needed to enhance and develop new uses for composting organic waste, ultimately benefiting local governments. The microbial counts of different organic waste types vary based on the temperature during composting, influenced by chemical and biological reactions. A decrease in the C: N ratio can indicate compost maturity.

6. REFERENCES

ABENA, M. T. B.; LI, T.; SHAH, M. N.; ZHONG, W. Biodegradation of total petroleum hydrocarbons (TPH) in highly contaminated soils by natural attenuation and bioaugmentation. **Chemosphere**, v. 234, p. 864-874, 2019. https://doi.org/10.1016/j.chemosphere.2019.06.111

ABIOYE, O. P.; AGAMUTHU, P.; ABDUL-AZIZ, A. R.; Biodegradation of used motor oil in soil using organic Waste Amendments. **Biotechnology Research International**, v. 2012, e 587041, 2012. https://doi.org/10.1155/2012/587041

AHMED, W. A.; ALZUBAIDI, S. F.; HAMZA, J. S. Biodegradation of crude oil in contaminated water by local isolates of Enterobacter cloacae. **Iraqi Journal of Science**, v. 55, n. 3A, p. 1025-1033, 2023.

AL NUAIMI, M. T. H.; TAHER, T. A.; AL-JANABI, Z. Z.; ADEL, M. M. High-resolution GC/MS study of biodegradation of crude oil by *Bacillus megaterium*. **Research on Crops**, v. 21, n. 3, p. 650-657, 2020. https://doi.org/10.31830/2348-7542.2020.101

ALMALKI, G.; RABAH, S.; AL-FAIfi, Z.; ALHARBI, A. SHARMA, M. Phytochemistry screening, antioxidant and antimicrobial activities of *Euphorbia inarticulata* Schweinf plant extract. **Pharmacophore**, v. 13, n. 1, p. 91-99, 2022.

AL-SAEDI, Z. Z.; IBRAHIM, J. A. K. Aerobic municipal solid waste compost quality according to different layers of composting bioreactor. **Journal of Engineering Sciences**, v. 26, n. 3, p. 7-16, 2019. https://doi.org/10.33261/jaaru.2019.26.3.002

ANTAI, S. P.; MGBOMO, E. Distribution of hydrocarbon utilizing bacteria in oil-spill areas. **Microbios Letters**, v. 40, n. 159, p. 137-143, 1989.

ANTHONY, O. Biodegradation alternative in the Cleanup of petroleum hydrocarbon pollutants. **Biotechnology and Molecular Biology Review**, v. 1,n. 2,p. 38-50, 2006. https://doi.org/10.5897/BMBR.9000002

ATAGANA, H. I. Co-composting of PAH-contaminated soil with poultry manure. **Letters in Applied Microbiology**, v. 39, n. 2, p. 163-168, 2004. https://doi.org/10.1111/j.1472-765X.2004.01554.x

AUSMA, S.; EDWARDS, G. C.; FITZGERALD-HUBBLE, C. R.; HALFPENNY-MITCHELL, L.; GILLESPIE, T. J.; MORTIMER, W. P. Volatile hydrocarbon emissions from a diesel fuel contaminated soil bioremediation facility. **Journal of the Air & Waste Management Association**, v. 52, n. 7, p. 769-780, 2002. https://doi.org/10.1080/10473289.2002.10470819

BERTRAN, E.; SORT, X.; SOLIVA, M.; TRILLAS, I. Composting winery waste: sludges and grape stalks. **Bioresource Technology**, v. 95, p. 203-208, 2004. https://doi.org/10.1016/j.biortech.2003.07.012

BOSSERT, I.; BARTHA, R. Plant growth on soil with a history of oil sludge disposal. **Soil Science**, v. 140, p. 75-77, 1985.

CORNELL WASTE MANAGEMENT INSTITUTE_ Cornell University. **Calculate C/N Ratio For Three Materials**. Cornell University Ithaca, NY, 1996. https://compost.css.cornell.edu/calc/2.html.

ELANGO, D.; THINAKARAN, N.; PANNEERSELVAM, P.; SIVANESAN, S. Thermophilic composting of municipal solid waste. **Applied Energy**, v. 86, n. 5,p. 663 668, 2009. https://doi.org/10.1016/j.apenergy.2008.06.009

GIBB, A.; CHU, A.; WONG, R. C.; GOODMAN, R. Bioremediation kinetics of crude oil at 5° C. **Journal of Environmental Engineering**, v. 127, p. 818-824, 2001. https://doi.org/10.1061/(ASCE)0733-9372(2001)127:9(818)

GRAÇA, J.; MURPHY, B.; PENTLAVALLI, P.; ALLEN, C. C. R.; BIRD, E.; GAFFNEY, M.; DUGGAN, T. KELLEHER, B. Bacterium consortium drives compost stability and degradation of organic contaminants in in-vessel composting process of the mechanically separated organic fraction of municipal solid waste (MS-OFMSW). **Bioresource Technology Reports**, v. 13, e100621, 2021. https://doi.org/10.1016/j.biteb.2020.100621

HINCHEE, R. E.; FREDRICKSON, J.; ALLEMAN, M. B. C. (Eds). **Bioremediation of chlorinated solvents.** Columbus, OH: Battelle Press, 1995. 221p.

IJAH, U. J. J.; ANTAI, S. P. The potential use of chicken-drop microorganisms for oil spill remediation. **The Environmentalist**, v. 23, n. 1, p. 89-95, 2003a. https://doi.org/10.1023/A:1022947727324

IJAH, U. J. J.; ANTAI, S. P. Removal of Nigerian light crude oil in soil over 12 months. **International Biodeterioration & Biodegradation**, v. 51, n. 2, p. 93-99, 2003b. https://doi.org/10.1016/S0964-8305(01)00131-7

KIM, S.; CHOI, D. H.; SIM, D. S.; OH, Y. S. Evaluation of bioremediation effectiveness on crude oil-contaminated sand. **Chemosphere**, v. 59, n. 6, p. 845-52, 2005. https://doi.org/10.1016/j.chemosphere.2004.10.058

KAEWLAOYOONG, A.; CHENG, C. Y.; LIN, C.; CHEN, J. R.; HUANG, W. Y.; SRIPROM, P. White rot fungus *Pleurotus pulmonarius* enhanced bioremediation of highly PCDD/Fcontaminated field soil via solid state fermentation. **Science ot The Total Environment**, v. 738, e139670, 2020. https://doi.org/10.1016/j.scitotenv.2020.139670

LIN, C. A negative-pressure aeration system for composting food wastes. **Bioresource Technology**, v. 99, n. 16, p. 7651-7656, 2008. https://doi.org/10.1016/j.biortech.2008.01.078

LIN, C.; CHERUIYOT, N. K.; BUI, X-T.; NGO, H. H. Composting and its application in bioremediation of organic contaminants. **Bioengineered**, v. 13, n. 1, p. 1073-1089, 2022. https://doi.org/10.1080/21655979.2021.2017624

LIN, C. T.; CHERUIYOT, N. K.; HOANG, H. G.; HOANG, H-G.; LE, T. H.; TRAN,H. T. BUI, X. T. · Benzophenone biodegradation and characterization of malodorous gas emissions during co-composting of food waste with sawdust and mature compost. **Environmental Technology & Innovation**, v. 21, e101351, 2021. https://doi.org/10.1016/j.eti.2020.101351

LOICK, N.; HOBBS, P. J.; HALE, M. D.; JONES, D. L. Bioremediation of poly-aromatic hydrocarbon (PAH)-contaminated soil by composting. **Critical Reviews in Environmental Science and Technology**, v. 39, n. 4, p. 271-332, 2009. https://doi.org/10.1080/10643380701413682

MALDONADO-CHÁVEZ, E.; RIVERA-CRUZ, M. C.; IZQUIERDO-REYES, F. PALMA-LÓPEZ, D. J. Effects of rhizosphere, microorganisms, and fertilization in the bioremediation and phytoremediation of soils with new and weathered crude oils. **University and Science**, v. 26, n. 2, p. 121-136, 2010.

MANGLA, H.; SHARMA, H.; DAVE, S. SUDAN, J.; PATHAK, H. Microbial mechanism of petroleum hydrocarbons degradation: An Environmental perspective. **Applied Environmental Biotechnology,** v. 6, n. 2, p. 32-42, 2021. https://doi.org/10.26789/AEB.2021.02.005

MAREK, S.; MAGDALENA, J. ROMAN, Z. In-Vessel composting for utilizing of municipal sewage sludge. **Applied Energy**, v. 75, n. 3-4, p. 249-256, 2003. https://doi.org/10.1016/S0306-2619(03)00038-2

MARÍN, J. A.; MORENO, J. L. HERNÁNDEZ, T. GARCÍA, C. Bioremediation by composting of heavy oil refinery sludge in semiarid conditions. **Biodegradation,** v. 17, p. 251-261, 2006. https://doi.org/10.1007/s10532-005-5020-2

MOHANTY, G.; MUKHERJI, S. Biodegradation rate of diesel range n-alkanes by bacterial cultures *Exiguobacterium aurantiacum* and *Burkholderia cepacia*. **International Biodeterioration & Biodegradation**, v. 61, n. 3, p. 240-250, 2008. https://doi.org/10.1016/j.ibiod.2007.06.011

OGBO, E. M. Effects of diesel fuel contamination on seed germination of four crop plants: *Arachis hypogala*, *Vigna unguiculata*, *Sorghum bicolor* and *Zea mays*. **African Journal of Biotechnology**, v. 8, n. 2, p. 250-253, 2009.

OSINOWO, I.; ABIOYE, O. P.; OYEWOLE, O. A. OYELEKE, S. B. The bioremediation of diesel-contaminated soil using bacterial cocktail and organic nutrients. **Journal of Microbiology, Biotechnology and Food Sciences**, v. 10, n. 2, p. 150-158, 2020. https://doi.org/10.15414/jmbfs.2020.10.2.150-158

OSSAI, I. C.; AHMED, A.; HASSAN, A. HAMID, F. S. Remediation of soil and water contaminated with petroleum hydrocarbon: a review. **Environmental Technology & Innovation**, v.17, e100526, 2020. https://doi.org/10.1016/j.eti.2019.100526

RAHMAN, K. S. M.; BANAT, I. M.; THAHIRA, J.; THAYUMANAVAN, T.; LAKSHMANAPERUMALSAMY, P. Bioremediation of Gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith, and rhamnolipid biosurfactant. **Bioresource Technology**, v. 81, n. 1, p. 25-32, 2002. https://doi.org/10.1016/S0960-8524(01)00105-5

SHIN, H. Determination of MTBE, TBA, and BTEX in Soil by Headspace Gas Chromatography-Mass Spectrometry. **Bulletin of The Korean Chemical Society**, v. 33, p. 1693-1698, 2012. https://doi.org/10.5012/bkcs.2012.33.5.1693

SINGH, P.; JAIN, R., SRIVASTAVA, N.; BORTHAKUR, A.; PAL, D.; SINGH, R.; MADHAV, S.; SRIVASTAVA, P.; TIWARY, D.; MISHRA, P.K. Current and emerging trends in bioremediation of petrochemical waste: a review. **Critical Reviews in Environmental Science and Technology**, v. 47, n. 3, p. 155-201, 2017. http://dx.doi.org/10.1080/10643389.2017.1318616

SOLANAS, A. M.; RIERA, M.; VIDAL, G. **Guide to bioremediation of soils contaminated by petroleum hydrocarbons**. University of Barcelona: Ed. Agènciad Residus de Catalunya, pp. 127, 2009.

SUDHIR, S. H.; JAI, G.; DINESH, M. Hydrocarbon bioremediation efficiency by five indigenous bacterial strains isolated from contaminated soils. **International Journal of Current Microbiology and Applied Sciences**, v. 4, n. 3, p. 892-905, 2014.

TIQUIA, S. M.; TAM, N. F. Y.; HODGKIS, I. J. Effect of composting on phytotoxicity of spent pig-manure sawdust litter. **Environmental Pollution**, v. 93, n. 3, p. 249-256, 1996. https://doi.org/10.1016/S0269-7491(96)00052-8

TRAN, H-T.; LIN, C.; BUI, X-T.; NGO, H. H.; CHERUIYOT, N. K.; HOANG, H-G.; VU, C-T.

Aerobic composting remediation of petroleum hydrocarbon-contaminated soil. Current and Future Perspectives. **Sci of The Total Environment**, v. 753, e142250, 2021. https://doi.org/10.1016/j.scitotenv.2020.142250

UDOINYANG, U.; EDEM, D. AKABIO, J. H. U. Petroleum hydrocarbon contents in tropical soils near crude oil exploration and processing site in Niger Delta, Nigeria. **Journal of Environmental Science: Current Research**, v. 2, e7, 2019. https://doi.org/10.24966/ESCR-5020/100007

WEI, Y.; LI, J.; SHI, D.; LIU, G.; ZHAO, Y.; SHIMAOKA, T. Environmental challenges impeding the composting of biodegradable municipal solid waste: A critical review. **Resources, Conservation and Recycling**, v. 122, p. 51-65, 2017. https://doi.org/10.1016/j.resconrec.2017.01.024

WU, T.; XU, J.; XIE, W.; YAO, Z.; YANG, H.; SUN, C.; LI, X. *Pseudomonas aeruginosa* L10: A Hydrocarbon-degrading, biosurfactant-producing, and plant-growth-promoting endophytic bacterium isolated from a reed (*Phragmites australis*). **Frontiers in Microbiology**, v. 9, e1087, 2018. https://doi.org/10.3389/fmicb.2018.01087

YASEEN, M. M.; MERAH, M. H. M.; GHAZI, A. M. GC-Mass characterization of tomato ethyl acetate extract and its antibacterial and antioxidant properties. **Nativa**, v. 11, n. 4, p. 592-597, 2024. http://dx.doi.org/10.31413/nat.v11i4.16634

Acknowledgments: Thank and appreciate all who supported and helped until we reached publishing from the Department of Environment. Engineering College of Engineering, University of Baghdad, Baghdad, Iraq.

Authors contribution: Both authors participated in all stages of the project/article execution. The authors read and agreed to the published version of the manuscript.

Data availability: Study data can be obtained by e-mail from the corresponding author or the second author upon request. It is not available on the website as the research project is still under development.

Conflicts of Interest: The authors declare no conflict of interest. Supporting entities had no role in the study's design; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.