processes

MDPI

Article

Sustainable Remediation of Polluted Soils from the Oil Industry Using Sludge from Municipal Wastewater Treatment Plants

Cristian Mugurel Iorga [1], Lucian Puiu Georgescu [1,2,*], Constantin Ungureanu [3] and Mihaela Marilena Stancu [4,*]

[1] Faculty of Sciences and Environment, "Dunarea de Jos" University of Galati, 111 Domneasca Street, 800201 Galați, Romania; cristian.iorga@ugal.ro
[2] REXDAN Research Infrastructure, "Dunarea de Jos" University of Galati, 98 George Cosbuc Street, 800385 Galati, Romania
[3] Faculty of Geology and Geophysics, University of Bucharest, 6 Traian Vuia Street, 020956 Bucharest, Romania; ungureanuconstantin@yahoo.com
[4] Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independenței, 060031 Bucharest, Romania
* Correspondence: lucian.georgescu@ugal.ro (L.P.G.); mihaela.stancu@ibiol.ro (M.M.S.)

Abstract: Soil pollution with hydrocarbons is a consequence of activities associated with the petroleum industry and related sectors. The effects of petroleum pollution are devastating, making the remediation of contaminated sites imperative. Consequently, soil decontamination represents a significant and costly challenge for the petroleum industry. The article proposes a dual-recovery bioremediation solution that is both efficient and cost-effective, exploring the potential use of dehydrated sewage sludge from municipal wastewater treatment plants to treat petroleum-contaminated soils. Over the three-month bioremediation experiment, changes in the density of indigenous bacteria in petroleum-contaminated soil samples, treated or untreated with sludge, were monitored along with the reduction in petroleum hydrocarbon concentrations. In parallel, the evolution of other contaminants, such as heavy metals, was monitored during the bioremediation experiment. Geotechnical tests were also conducted to evaluate the feasibility of returning the treated soil to its original location after the bioremediation experiment. Our results demonstrate that the proposed method effectively addresses both the remediation of petroleum-contaminated soils (hazardous waste) and the reuse of sewage sludge from municipal wastewater treatment plants.

Keywords: petroleum; pollution; sewage sludge; bioremediation; biopile

check for updates

Academic Editors: María Victoria Gallegos and Miguel Andrés Peluso

Received: 30 November 2024
Revised: 7 January 2025
Accepted: 10 January 2025
Published: 16 January 2025

Citation: Iorga, C.M.; Georgescu, L.P.; Ungureanu, C.; Stancu, M.M. Sustainable Remediation of Polluted Soils from the Oil Industry Using Sludge from Municipal Wastewater Treatment Plants. *Processes* **2025**, *13*, 245. https://doi.org/10.3390/pr13010245

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The growing global demand for petroleum has driven the expansion of the petroleum industry, now covering vast areas of soil in many countries. It is well known that the petroleum industry's activities are complex, starting with new crude oil deposit identification, exploitation, transportation, refining, storage, and the distribution of petroleum products. Accidents can often occur at any stage of this technological chain, causing soil pollution with harmful effects on human health and other biotic components [1]. Pollution caused by petroleum hydrocarbons occurs not only in the petroleum industry but also in other sectors that utilize petroleum products as an energy source or raw material [2]. Currently, soil pollution with petroleum hydrocarbons is among the most widespread and significant environmental issues worldwide. Petroleum hydrocarbons are a complex mixture of thousands of aliphatic and aromatic compounds with distinct physical and chemical properties [3–5]. Once petroleum

hydrocarbons pollute the soil, they undergo various transformations over time: physical (e.g., evaporation, adsorption), chemical (e.g., reactions with environmental chemical elements), and biological (e.g., interaction with aerobic and anaerobic microbiota). These transformation processes contribute to the degradation of petroleum hydrocarbons and further environmental pollution [2]. Consequently, large areas of soil polluted with petroleum hydrocarbons have become unusable [6]. Lack of timely intervention on spills allows petroleum hydrocarbons to migrate through the soil, contaminating the groundwater. Due to the complexity of these contaminants, their effects on soil are complex. Pollution with petroleum hydrocarbons negatively impacts the soil's physical (e.g., texture, compaction, hydraulic conductivity), chemical (e.g., mineral content), and microbiological properties [2]. In contaminated soil, hydrocarbons significantly impact indigenous microorganisms, affecting their diversity, abundance, and functional roles. Many hydrocarbons suppress sensitive microbial populations and promote the growth of hydrocarbon-degrading microorganisms. As a result, hydrocarbon-degrading bacteria often dominate contaminated soils, whereas microorganisms involved in nutrient cycling (e.g., nitrogen-fixing bacteria) are adversely affected, disrupting the ecosystem processes [7–11].

The remediation of petroleum-contaminated sites has become a global concern, aiming to limit negative impacts and restore affected soils. Several decontamination technologies have been developed, including physical, chemical, mechanical, and biological methods. Among these, biotechnologies are the most efficient, environmentally friendly, and cost-effective options used in many countries to treat soil contaminated with petroleum and petroleum products [12]. Biotechnologies generally depend on microorganisms that efficiently degrade hydrocarbons, ultimately breaking them down into carbon dioxide and water [7–9,13]. Bioremediation of soils using microorganisms can be achieved through either in situ or ex situ treatment techniques, each with advantages and disadvantages. The choice between in situ and ex situ bioremediation depends on factors such as soil conditions, contaminant type, and site-specific constraints. In situ treatment occurs directly at the contaminated site, without the need to excavate the soil. This method reduces costs and limits the release of pollutants into the atmosphere, though the processes are difficult to control and often slower. Advancements in biotechnological methods, such as engineered microorganisms and improved nutrient delivery, can enhance in situ treatments, particularly in variable soil conditions (e.g., humidity, pH, etc.). In situ treatment is generally suitable for sites with permeable soil, while ex situ approaches are more effective for heavily contaminated or low-permeability soils. Ex situ treatment involves removing contaminated soil for treatment at a bioremediation platform. While this method incurs higher costs and logistical challenges, it offers faster and more easily controlled processes and can treat a wide range of pollutants. Several methods, such as biostimulation, bioaugmentation, and composting, can be applied in this case. Additionally, the application of microorganisms and nutrients, as well as oxygen supply through soil aeration, is easier in ex situ treatment compared to in situ treatment [2,7–9,13].

Sewage sludge is often contaminated with petroleum hydrocarbons and heavy metals, posing significant environmental and public health hazards. Without proper treatment, storage, or disposal, these contaminants have the potential to leach into surrounding soil and water systems, resulting in prolonged ecological degradation. Petroleum hydrocarbons contribute to soil and water pollution, amplifying their environmental impact. Heavy metals such as lead, mercury, and cadmium also exhibit high toxicity even at trace concentrations. These metals can bioaccumulate in the food chain, posing severe health risks to humans and animals. Thus, implementing effective management and treatment strategies is essential to mitigate these risks [14]. Sewage sludge contains various microorganisms, including bacteria, fungi, protozoa, and viruses. While many of these organisms are essential in the biological treatment of wastewater by breaking down organic matter, some,

such as certain bacteria, can be pathogenic and pose health risks if not properly treated [15]. In recent decades, sewage sludge, a byproduct of municipal wastewater treatment, has emerged as a valuable resource in ecological restoration due to its high organic matter content, nutrients, and potential to improve soil structure. Traditionally considered a waste material, sewage sludge is now being repurposed for its capacity to rehabilitate degraded soils. The organic matter and nutrients present in sewage sludge can enhance soil fertility, stimulate microbial activity, and support plant growth, all of which are essential for recovering petroleum-contaminated ecosystems [16,17].

This study aimed to assess the potential of using dehydrated sewage sludge from a wastewater treatment plant to enhance the bioremediation efficiency in petroleum-contaminated soil. The evolution of bioremediation parameters, along with the dynamics of indigenous hydrocarbon-degrading bacteria, was monitored using physicochemical and microbiological methods. Furthermore, geotechnical analyses were conducted to examine the influence of dehydrated sewage sludge on soil quality, assessing its potential for reuse as a filling material in the ecological restoration of petroleum-contaminated sites.

2. Materials and Methods

2.1. Bioremediation Experiment of Petroleum-Contaminated Soil Treated with Sewage Sludge

The bioremediation experiment was conducted in an area located in Constanta County, Romania, that is highly contaminated with petroleum products. The site was previously used as a storage facility for petroleum products, including oil, gasoline, and light liquid fuels, as well as engine and transmission oils. The contamination at the site has been present for an estimated 40 to 50 years. The petroleum-contaminated soil sample was collected from a depth of 0.8 to 2 m. Untreated petroleum-contaminated soil was used as the control. The bioremediation experiment was conducted for three months and was initiated by mixing the petroleum-contaminated soil (denoted S) with dehydrated sewage sludge (denoted N), in different proportions: S1:N1 (1:1, v/v), S2:N1 (2:1, v/v), and S1:N2 (1:2, v/v) for each mixture forming a pile (biopile). A biopile formed only from the contaminated soil was used as a control. The dehydrated sewage sludge was collected from a wastewater treatment plant (Galați County, Romania). The experiment was conducted on a bioremediation platform, and during the three months, at one-week intervals, the petroleum-contaminated soil was treated with sewage sludge, and the control soil contaminated was aerated by using an excavator. For microbiological and physico-chemical analyses, samples were collected in sterile containers and stored at 4 °C for further analyses.

2.2. Physicochemical Analysis of Petroleum-Products-Contaminated Soil Treated with Sewage Sludge

The **moisture** content of samples (triplicate) was determined by the oven-dry (at 105 °C) method [11]. The **soil organic matter** (SOM) content of samples was determined by using combustion (440 °C, 6 h) and oxidation (20% hydrogen peroxide) methods [18]. The **particle size** distribution of the samples was determined by the combined method (sieving and sedimentation) [19]. The percentage distribution by grain fractions is graphically represented on a semi-logarithmic graph by the granulometric distribution curve and on a ternary plot.

Heavy metal concentrations in samples were determined using the inductively coupled plasma mass spectrometry (ICP-MS) method [20] by using a NexION 2000 ICP Mass Spectrometer (Waltham, MA, USA). Oven-dried samples (at 80 °C) were grounded and then sieved using a 2 mm to 0.25 mm sieve. The extracts for ICP-MS analysis were obtained by mineralization with HNO_3 (65%) and H_2O_2 (30%), using a Milestone ETHOS EASY—Advanced Microwave Digestion System (Milestone, Sorisole, Italy).

Total petroleum hydrocarbon (TPH) concentration in the samples was determined through infrared spectroscopy (IR). Sample extracts were obtained by using S-316 solvent. After the removal of polar compounds (with activated aluminum oxide), non-polar compounds were determined by measuring the absorbance at a wavelength of 2930 cm^{-1}, using an IR spectrometer InfraCal 2 (Wilks Enterprise, Norwalk, CT, USA), by the baseline method [21].

2.3. Enumeration and Isolation of Bacteria from Petroleum-Contaminated Soil Treated with Sewage Sludge

Samples were mixed (1:1 g/v) with phosphate-buffer saline (PBS, [22]) and incubated at room temperature on a rotary shaker (200 rpm) for one hour. Then, serial dilutions (10^{-1}–10^{-12}) were performed in PBS. The pH of the samples was determined by using a Hanna pH 213 (Woonsocket, RI, USA).

The enumeration of the **hydrocarbon-tolerant** and **hydrocarbon-degrading bacteria** in the samples (initially and after two and three months of treatment) was performed through the most probable number (MPN) method [23]. Serial dilutions of each sample (10^{-1}–10^{-12} in PBS) were inoculated into 96-multiwall plates containing LB [22] supplemented with 5% (*v*/*v*) diesel for hydrocarbon-tolerant bacteria enumeration and minimal medium [4,5] supplemented with 5% (*v*/*v*) diesel for hydrocarbon-degrading bacteria enumeration. Multiwall plates were incubated for 1–14 days at 30 °C. The viability of the bacteria (cell g^{-1} soil) was determined using 0.3% (*w*/*v*) triphenyl tetrazolium chloride (TTC) dye as a redox indicator of cellular respiration, as previously described by Stancu and Grifoll [23].

The enumeration of the **enterobacteria** was performed through the plate count agar (PCA) method. Serial dilutions of the samples (10^{-1}–10^{-6} in PBS) were inoculated onto EMB agar. Petri dishes were incubated for 1–5 days at 30 °C. Then, the number of enterobacteria present per g of sample (cfu g^{-1} soil) was determined.

The **hydrocarbon-degrading** bacteria were isolated from the samples (initially, after two and three months of treatment) by the enrichment culture method. The samples (5% *v*/*v*) were used to initiate enrichment cultures in a liquid minimal medium [4,5] supplemented with 5% (*v*/*v*) diesel as the sole carbon source. The tubes were incubated for 14 days on a rotary shaker (200 rpm) at 30 °C. The obtained enrichment cultures (5% *v*/*v*) were then transferred into fresh minimal medium with 5% (*v*/*v*) diesel. The tubes were incubated under the same conditions for another 14 days. The isolated hydrocarbon-degrading bacteria were stored at −80 °C in 25% (*v*/*v*) glycerol. The growth of the hydrocarbon-degrading bacteria was determined by measuring the optical density at 660 nm (OD_{660}) and cell viability [4,5] on LB agar and EMB agar. Biosurfactant production by isolated bacteria was studied by using the emulsification index, diesel overlay agar, and CTAB blue agar method, as described earlier [4,5]. Diesel biodegradation by the isolated bacteria was established by diesel film fragmentation and by the determination of the free carbon dioxide (CO_2 mg L^{-1}) [4].

2.4. Geotechnical Tests for Petroleum-Contaminated Soil Treated with Sewage Sludge

Normal Proctor tests were performed on the samples at the end of the bioremediation experiment using a normal Proctor hammer (manual) and a normal Proctor die to determine the degree of compaction (https://www.studocu.com/bo/document/escuela-militar-de-ingenieria/mecanica-de-suelos-1/astm-d698-12-compactacion-estandar-compress/33534831 accessed on 9 January 2025). To determine the deformability characteristics, samples (duplicate) with the highest dry density, obtained in the normal Proctor test, were loaded into the odometer by increasing (from 12.5 kPa to 500 kPa) the vertical stress (starting at a contact pressure of 12.5 kPa, following the loading steps of 50 kPa, 100 kPa, 200 kPa,

300 kPa, and 500 kPa). The deformation was measured (0.01 mm precision) after 24 h for each loading step.

3. Results and Discussion

3.1. Bioremediation Experiment of Petroleum-Contaminated Soil Treated with Sewage Sludge

The bioremediation experiment was conducted in an area highly contaminated with petroleum products due to the existence in the past of an old deposit of petroleum products. As we mentioned in the material and methods section, during the three months of the bioremediation experiment, at one-week intervals, the petroleum-contaminated soil treated or untreated with dehydrated sewage sludge was aerated by using an excavator (Figure 1a) to promote aerobic degradation. Generally, the bioremediation efficiency depends on various factors, like the geological and geographical characteristics of the petroleum-contaminated site, environmental conditions (e.g., pH, temperature, availability of nutrients and oxygen, and contaminants bioavailability), and the native microbial community structure. Oxygen is the key electron acceptor in aerobic bioremediation, and, if it is not present in adequate concentrations, it can significantly limit the biodegradation potential of aerobic microorganisms, including bacteria. In the absence of oxygen, the anaerobic degradation of petroleum hydrocarbons ensues at a slower rate than that of aerobic microbial degradation. Hence, providing adequate concentrations of oxygen in the contaminated soil is essential for higher biodegradation rates [24].



Figure 1. Bioremediation experiment of petroleum-contaminated soil treated with sludge. (**a**) Biopile (BP), soil aeration (SA), soil sampling (SS). (**b**) Enumeration and isolation of bacteria, initial samples (PI), after two (PII) and three (PIII) months of treatment; microbiological analysis of samples by MPN or PCA methods; hydrocarbon-degrading bacteria isolation by enrichment culture (EC) method from initial samples (CI), after two (CII) and three (CIII) months of treatment, bacteria growth (BG) on agar media (LB, EMB).

3.2. Physicochemical Analysis of Petroleum-Products-Contaminated Soil Treated with Sewage Sludge

Before starting the bioremediation experiment, some physicochemical parameters (i.e., pH, humidity, organic matter, heavy metals, and total petroleum hydrocarbon) of the soil contaminated with petroleum products and sludge were determined (Table 1). The physicochemical parameters determined in this study are very important for the bioremediation process of soils contaminated with petroleum products and for the use of sludge in ecological remediation. As we mentioned in the introduction, sewage sludge represents an important source of both micronutrients and macronutrients, as well as of water, which could have a positive effect on the activity of microorganisms that exist in the petroleum-contaminated soil [25]. Previously, it was reported that several parameters, such as pH (below 6.5), humidity (below 40%), high concentrations of hydrocarbons and heavy metals, and low nutrient content (nitrogen, phosphorus, and potassium) can limit the biodegradation process by inhibiting the development of bacteria capable of degrading hydrocarbons [9–11,25–27].

Table 1. Physico-chemical characterization of petroleum-contaminated soil and sludge.

Parameters		Samples	
		Soil	**Sludge**
pH		7.3	6.8
Moisture (W, %)		19.06	305
Density of the mineral skeleton (ρ_{smediu}, g cm^{-3})		2.662	2.674
Organic matter (%)	Loss-on-ignition	5.2	38.2
	Oxidation	5.5	39.3
Heavy metals (mg/kg ds)	Cadmium	<0.8	<0.8
	Cromium	19.9	30.6
	Copper	21.6	107.0
	Nickel	26.1	28.6
	Lead	12.2	27.0
	Zinc	50.0	348.0
Petroleum hydrocarbons (mg kg^{-1} ds)		4630	3810

At the initiation of the bioremediation experiment, both soil (pH 7.3) and sludge (pH 6.8) exhibited near-neutral **pH** values (Table 1). Soil contamination with petroleum products can significantly influence pH levels. Acidic compounds form in petroleum and their derivatives through chemical and/or biochemical oxidation, reducing pH in the contaminated sites. In contrast, the presence of soil minerals and salts of large organic acid molecules can undergo basic hydrolysis, raising pH levels in contaminated sites [28].

The initial **moisture** content of the soil was 19.06%, while the sludge exhibited a moisture content of 305% (Table 1). Soil moisture is inherently variable, influenced by climatic conditions and petroleum contamination [29]. In contrast, sludge moisture content is determined by wastewater treatment processes and the organic matter content [30]. The high moisture content of sewage sludge provides a significant advantage by reducing the requirement for additional water during the bioremediation process [17].

The **soil organic matter** (SOM) content was analyzed using combustion and oxidation methods. The results, detailed in Table 1, show slight differences between the two methods, with values of 5.2% and 5.5% for soil and 38.2% and 39.3% for sludge, respectively. The

combustion method yielded marginally lower values (0.3% for soil and 0.9% for sludge), likely due to water loss from clay mineral structures during heating and the water retention properties of organic matter [31]. Despite these minor discrepancies, the combustion method is faster and provides reliable results.

The **particle size** distribution of the petroleum-contaminated soil significantly affects the bioremediation process. For example, sandy soil, due to its higher porosity, allows for better oxygenation, enhancing bioremediation efficiency. In contrast, clay soils retain water and other substances, obstructing oxygen diffusion and slowing the bioremediation process [24]. Periodic soil aeration was used to maintain adequate oxygen levels in this study. The contaminated soil consisted of approximately 64% silt, 7% sand, and 29% clay, classifying it as silty clay or clayey silt. Similarly, the sludge contained 68% silt, 8% sand, and 24% clay, categorizing it as clayey silt or silty clay. Both materials showed overlapping particle size distribution curves, with slight variations in the clay fraction (Figure 2a). The ternary diagram (Figure 2b) further corroborates the similarity in granulometric composition.

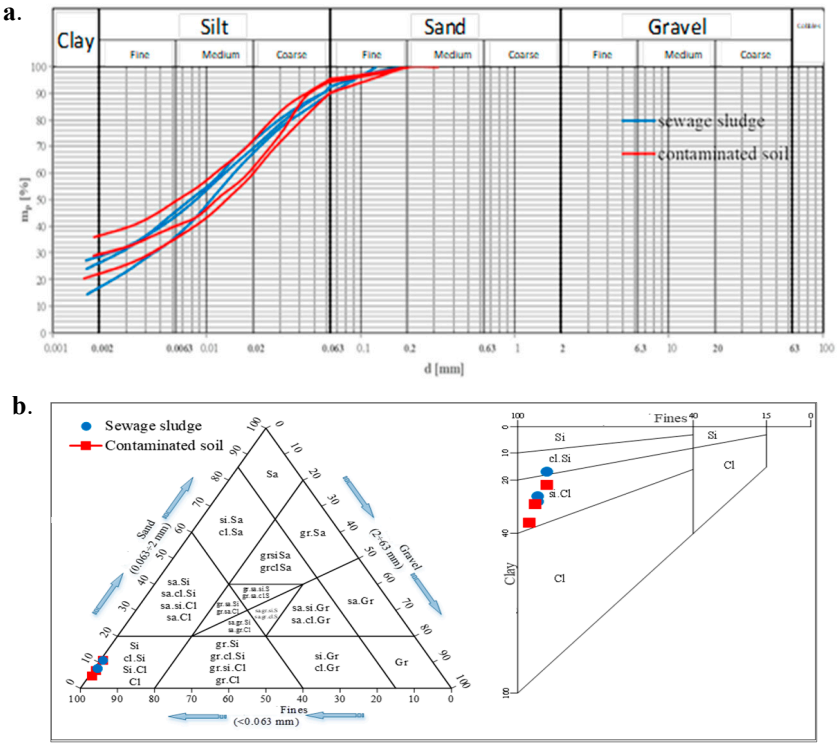


Figure 2. Granulometric distribution of the petroleum-contaminate soil and sludge and ternary diagram. (**a**) Granulometric distribution; (**b**) ternary diagram for the soil and sludge classification.

The density of the **mineral skeleton** (ρ_S) is critical in determining soil porosity, void ratio, and pedotransfer functions. The mineral skeleton density for soil and sludge samples was determined as 2.662 g cm^{-3} and 2.674 g cm^{-3}, respectively, consistent with the typical range for mineral soils (2.4–2.9 g cm^{-3}) [32,33].

Hence, **heavy metal** concentrations in the soil and sludge were analyzed before initiating the bioremediation process (Table 1). The results showed similar cadmium concentrations (<0.8 mg kg^{-1} ds) in both samples and comparable nickel concentrations (26.1 mg kg^{-1} ds in soil, 28.6 mg kg^{-1} ds in sludge). However, concentrations of chromium (30.6 mg kg^{-1} ds), copper (107 mg kg^{-1} ds), lead (27.0 mg kg^{-1} ds), and zinc (348 mg kg^{-1} ds) were significantly higher in sludge than in soil, with the latter showing values of 19.9 mg kg^{-1} ds, 21.6 mg kg^{-1} ds, 12.2 mg kg^{-1} ds, and 50 mg kg^{-1} ds, respectively. The most pronounced difference was observed for zinc, with sludge concentrations

exceeding soil levels by approximately seven-fold. Analyzing the values of the concentrations of heavy metals in the soil sample, it was found that, only in the case of copper and nickel, exceedances slightly above the normal values were recorded, which means that the pollutants specific to the activity were not heavy metals. For the sludge sample, all the heavy metal concentration values exceeded the normal values, and in the case of zinc and copper, some values exceeded the normal values by 3.5 times and 5 times, respectively. The obtained results were evaluated and interpreted based on the provisions of the national regulation on soil pollution assessment. This regulation establishes the reference values and standards used to determine the level of soil contamination, ensuring compliance with locally applicable guidelines and requirements.

Total petroleum hydrocarbon (TPH) content is a key indicator of environmental pollution. The soil sample exhibited a TPH concentration of 4630 mg kg^{-1} ds (Table 1), approximately 50 times higher than the normal value, attributed to accidental petroleum and their derivative spills during warehouse operations. The sludge sample also contained significant petroleum hydrocarbons (3810 mg kg^{-1} ds, Table 1), likely originating from wastewater contamination from the anthropogenic release of petroleum product pollutants. Petroleum hydrocarbons may reach wastewater treatment plants through various pathways, including industrial effluents, accidental spills, urban stormwater runoff, and improper disposal of petroleum-based products by households or businesses [14].

3.3. Enumeration and Isolation of Bacteria from Petroleum-Contaminated Soil Treated with Sewage Sludge

Microorganisms play a key role in sustaining soil ecological functions. The bioremediation of petroleum-contaminated soil by indigenous microorganisms is considered an efficient, environmentally friendly, and cost-effective technology, as compared with other physicochemical treatment methods. Bioremediation of contaminated soils depends on the composition and concentration of petroleum, the presence of suitable microorganisms, and environmental factors (e.g., pH and temperature) [8–10,13]. At the initiation of the bioremediation experiment, all analyzed samples had a neutral pH (6.8–7.3). The soil sample untreated with sludge (control) had a higher pH value (7.2–7.3) compared with those obtained for the soil samples treated for two and three months with sludge (pH 6.5–6.9). The pH can be highly variable in soils and should be taken into consideration when we try to improve the biological treatment methods in sites contaminated with petroleum hydrocarbons [8]. The microorganisms that are frequently involved in the decontamination of petroleum-polluted soils are bacteria, fungi, and yeasts. Bacteria play the most important role in the bioremediation of petroleum-contaminated soils [9–11,27]. In the soil samples treated or untreated with sludge (initially and after two and three months, Figure 1b), by using the most probable number (MPN) method, we revealed the existence of hydrocarbon-tolerant and hydrocarbon-degrading bacteria, and by the plate culture method, we identified the existence of enterobacteria. The number of hydrocarbon-tolerant and hydrocarbon-degrading bacteria varied from one sample to another (10^4–10^{12} cell g^{-1} soil) during the bioremediation experiment (Table 2). In the samples collected at the beginning of the experiment, the number of hydrocarbon-tolerant bacteria was higher (10^{11} cell g^{-1}) compared to the number of hydrocarbon-degrading bacteria (10^4–10^{10} cell g^{-1}). Thus, not all bacteria existing in the samples could degrade petroleum hydrocarbons. In the samples collected after two months, the number of hydrocarbon-tolerant bacteria (10^{11}–10^{12} cell g^{-1}) and the number of hydrocarbon-degrading bacteria (10^{11} cell g^{-1}) had very close values. Most of the hydrocarbon-tolerant bacteria present in the analyzed samples were also capable of growing on a minimal medium in the presence of 5% diesel as a sole carbon source. In the samples collected after three months, the number of hydrocarbon-tolerant bacteria was higher (10^{10}–10^{11} cell g^{-1}) compared to the number of hydrocarbon-degrading

bacteria (10^5 cell g^{-1}). The variations observed in the number of hydrocarbon-degrading bacteria in the analyzed samples are explainable because the number of these bacteria is higher when the concentration of petroleum hydrocarbons is high and subsequently decreases with the reduction in hydrocarbon contamination. Like the other two tested bacteria, the number of enterobacteria varied from one sample to another (0–10^6 ufc g^{-1} soil) (Table 2). In the samples collected at the initiation of the experiment, the number of enterobacteria was higher (10^4–10^5 ufc g^{-1}), compared with their numbers in samples collected after two and three months (0 cell g^{-1}). In the samples collected after two and three months, we observed the presence of filamentous fungi that are more resistant to stress conditions than other microorganisms. The presence of filamentous fungi in soils contaminated with petroleum hydrocarbons has a beneficial effect on bioremediation efficiency [8,34]. Sometimes they were described as more efficient than bacteria in the degradation of high molecular weight hydrocarbons in contaminated soils [13]. Fungi such as *Aspergillus*, *Penicillium*, and *Graphium* are microorganisms that can degrade persistent petroleum pollutants [8].

Table 2. Hydrocarbon-tolerant, hydrocarbon-degrading, and enterobacteria in the petroleum-contaminated soil treated with sludge.

Number of Bacteria		Samples			
		Soil	Soi and Sludge Mixtures (*v*/*v*)		
			S1:N1	S2:N1	S1:N2
Hydrocarbon-tolerant bacteria (cell g^{-1})	PI	2.5×10^{11}	2.5×10^{11}	2.5×10^{11}	2.5×10^{11}
	PII	1.6×10^{12}	2.5×10^{11}	2.5×10^{11}	2.5×10^{11}
	PIII	3.0×10^{10}	2.5×10^{11}	2.5×10^{11}	2.5×10^{11}
Hydrocarbon-degrading bacteria (cell g^{-1})	PI	1.7×10^{10}	1.7×10^{9}	1.7×10^{7}	9.5×10^{4}
	PII	2.0×10^{11}	2.5×10^{11}	2.5×10^{11}	2.5×10^{11}
	PIII	3.0×10^{5}	8.0×10^{5}	9.5×10^{5}	1.7×10^{5}
Enterobacteria (cfu g^{-1})	PI	2.0×10^{5}	3.6×10^{5}	3.5×10^{4}	2.5×10^{4}
	PII	0, Ff	0, Ff	0, Ff	0, Ff
	PIII	0, Ff	0, Ff	0, Ff	0, Ff

Initial samples (PI), after two (PII) and three (PIII) months of treatment; filamentous fungi (Ff) on EMB agar.

Using the enriched culture method, we isolated twelve hydrocarbon-degrading bacterial consortia from the petroleum-contaminated soil samples treated or untreated with sludge (Figure 1b, Table 3). Consortia C1.1, C1.3, C1.4, and C1.5 were isolated from samples collected at the initiation of the bioremediation experiment (PI), and consortia C2.1, C2.3, C2.4, C2.5 and C3.1, C3.3, C3.4, and C3.5 were isolated from the samples collected after two (PII) and three (PIII) months, respectively. The growth of hydrocarbon-degrading bacterial consortia in the presence of 5% diesel as the sole carbon source varied from one sample to another (OD_{660} 0.58–0.97). In the samples collected at the beginning of the experiment, the growth of hydrocarbon-degrading bacteria was higher (DO_{660} 0.89–0.97) compared to the growth of these bacteria in the samples collected after two and three months (DO_{660} 0.58–0.82). No significant differences in hydrocarbon-degrading bacteria viability were observed from one sample to another. All the isolated hydrocarbon-degrading consortia showed very good viability (100%) on the LB agar supplemented or not with diesel. Furthermore, all these consortia showed very good viability on EMB agar, a medium which is a selective culture medium used for the identification of Gram-negative bacteria, specifically

enterobacteria. Our results prove that some of the hydrocarbon-degrading bacteria from the isolated consortia could belong to the Enterobacteriaceae.

Table 3. Hydrocarbon-degrading bacteria isolated from the petroleum-contaminated soil treated with sludge.

Hydrocarbon-Degrading Bacteria		CI				CII				CIII			
		C1.1	C1.3	C1.4	C1.5	C2.1	C2.3	C2.4	C2.5	C3.1	C3.3	C3.4	C3.5
Growth on diesel	Absorbance ($OD_{660\ nm}$)	0.97	0.90	0.95	0.89	0.82	0.80	0.75	0.69	0.76	0.70	0.68	0.58
	Viability (LB, %)	100	100	100	100	100	100	100	100	100	100	100	100
	Viability (LB-diesel, %)	100	100	100	100	100	100	100	100	100	100	100	100
	Viability (EMB, %)	100	100	100	100	100	100	100	100	100	100	100	100
Biosurfactants	Emulsification index (E_{24}, %)	100	50	10	10	50	50	10	10	10	10	10	10
	Diesel overlay	+	+	–	–	+	+	–	–	–	–	–	–
	CTAB blue	–	+	–	–	+	+	+	–	–	–	–	+
Diesel biodegradation	Diesel fragmentation	+	+	+	+	+	+	+	+	+	+	+	+
	CO_2 (mg L^{-1})	1848	1748	1660	1630	1748	1748	1560	1460	1460	1460	1416	1416

Bacteria isolated from initial samples (CI), after two (CII) and three (CIII) months of treatment; consortia (C1.1, 1.3, 1.4, 1.5; C2.1, 2.3, 2.4, 2.5; C3.1, 3.3, 3.4, 3.5); biosurfactants, diesel overlay, CTAB blue method, positive reaction (+), negative reaction (–); diesel fragmentation, positive reaction (+); CO_2 production (CO_2 mg L^{-1}).

We further investigate if the isolated bacterial consortia produced biosurfactants (Table 3). Some of the bacterial consortia isolated from the samples collected at the initiation of the experiment (i.e., C1.1 and C1.3) and from the samples collected after two months (C2.1 and C2.3) produced a higher amount of biosurfactants (E_{24} 50–100%) compared to the rest of consortia (E_{24} 10%). These four bacterial consortia (C1.1, C1.3, C2.1, and C2.3) gave a positive reaction when the diesel overlay agar assay was used as a screening method, confirming biosurfactant production. Moreover, five of the bacterial consortia (C1.3, C2.1, C2.3, C2.4, and C3.5) gave a positive reaction when they were grown on CTAB blue agar, confirming biosurfactant production.

The biodegradation of diesel oil by hydrocarbon-degrading bacterial consortia was confirmed by breaking up the diesel film from the surface of the minimal liquid medium and by monitoring the free CO_2 (Table 3). All isolated consortia were able to break the diesel film when grown in a medium with a minimum of 5% diesel. The amount of free CO_2 released in the growth medium varied from one sample to another, from 1416 to 1848 mg L^{-1}. Similar results were earlier reported by Stancu [4] when different strains of the genera *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, and *Bacillus* were grown in a medium with a minimum of 3% diesel. *Pseudomonas* along with other bacterial genera, such as *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Corynebacterium*, *Enterobacter*, *Flavobacterium*, *Lysinibacillus*, *Micrococcus*, and *Rhodococcus* were reported to have a good ability to degrade petroleum hydrocarbons [9].

Ecological restoration of petroleum-contaminated sites has emerged as a pivotal aspect of environmental remediation efforts, focusing on restoring the health, biodiversity, and functionality of affected soils. A comprehensive understanding of the interactions between petroleum-based pollutants and indigenous microbial communities is essential for the development and optimization of effective bioremediation strategies. At the start of the

bioremediation experiment, TPH concentrations varied from one sample to another (Table 4, Figure 3). The highest TPH concentration (6190 mg kg^{-1} ds) was observed in the S1:N1 mixture, followed by S (4630 mg kg^{-1} ds), S1:N2 (4350 mg kg^{-1} ds), and the lowest in the S2:N1 mixture (3200 mg kg^{-1} ds). A significant reduction in TPH concentrations occurred during the first two months. The largest decrease was recorded in the S1:N1 mixture (3290 mg kg^{-1} ds), followed by S (2100 mg kg^{-1} ds), S1:N2 (1970 mg kg^{-1} ds), and S2:N1 (1200 mg kg^{-1} ds). In the later stages of the experiment, TPH reduction slowed, with decreases observed in S1:N1 (900 mg kg^{-1} ds), S1:N2 (540 mg kg^{-1} ds), S (400 mg kg^{-1} ds), and S2:N1 (290 mg kg^{-1} ds). These findings indicate that mixtures with higher initial TPH concentrations exhibited greater reductions. Degradation rates were particularly high during the first two months, with approximately 80% reductions observed across mixtures, while the control soil exhibited a slightly higher rate of 85%. In the last month, degradation rates decreased to approximately 20% for the mixtures and 15% for the control soil. The degradation rates over the entire period of the experiment were as follows: in the mixture, S1:N1 (67.7%), followed in order by S1:N2 (58%), S (54.6%), and S2:N1 (53.4%). Our findings are consistent with the existing literature, which indicates that the addition of various organic amendments, including composted materials, can enhance hydrocarbon degradation. Specifically, our results align with studies reporting a significant reduction in TPH concentrations, particularly during the initial stages of bioremediation. These observations support the conclusion that bioremediation can effectively decrease TPH levels, although the degradation rate tends to decline as the process advances. As the experiment progressed, the rate of TPH reduction diminished, which follows the results of other bioremediation studies, where degradation rates typically slow as the more readily degradable components are removed and the remaining hydrocarbons become more resistant to degradation [35,36].

Table 4. Physico-chemical characterization of petroleum-contaminated soil treated with sludge.

Parameters			**Samples**			
			Soil	**Soil and Sludge Mixtures (*v*/*v*)**		
				S1:N1	**S2:N1**	**S1:N2**
Organic matter (%)	Combustion	PI	5.2	ND	ND	ND
		PIII	4.5	11.3	8.1	13.9
Heavy metals (mg kg^{-1} ds)	Cadmium (Cd)	PI	<0.8	<0.8	<0.8	<0.8
		PIII	<0.8	<0.8	<0.8	<0.8
	Chromium (Cr)	PI	19.9	22.6	20.5	24.0
		PIII	23.5	24.3	25.6	24.2
	Copper (Cu)	PI	21.6	37.9	28.5	48.1
		PIII	22.7	37.8	33.8	42.4
	Nickel (Ni)	PI	26.1	26.7	25.2	27.2
		PIII	26.8	25.8	27.4	26.4
	Lead (Pb)	PI	12.2	16.3	17.4	17.1
		PIII	14.2	16.8	16.3	18.0
	Zinc (Zn)	PI	50.0	110.0	74.2	141.0
		PIII	54.8	110.0	91.4	121.0
Petroleum hydrocarbons (mg kg^{-1} ds)		PI	4630	6190	3200	4350
		PII	2500	2900	2000	2380
		PIII	2100	2000	1710	1840

Not determined (ND).

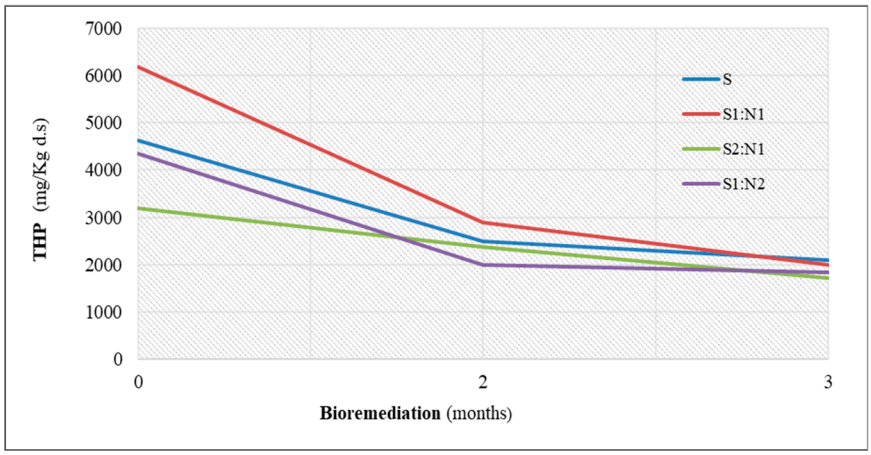


Figure 3. Bioremediation of petroleum-contaminated soil treated with sludge.

Heavy metal concentration varied from one sample to another in the bioremediation experiment (Table 4). The sludge exhibited higher levels of heavy metals than the prepared mixtures (S1:N1, S2:N1, S1:N2), reflecting dilution effects in the mixtures. Throughout the bioremediation experiment, heavy metal concentrations in the mixtures remained relatively stable, with minor variations attributed to sampling and measurement uncertainties. The concentrations of chromium and lead in all mixtures remained below the normal allowable limits. Zinc concentrations in the S2:N1 mixture also fell within normal limits. However, for the other samples, concentrations of copper, nickel, and zinc exceeded normal levels, but remained below regulated thresholds. Cadmium concentrations were consistently below the detection limit across all samples, indicating negligible presence. Heavy metal concentrations can negatively impact soil health, plant growth, and human health [37,38]. In addition, excessive heavy metal concentrations inhibit the activity of hydrocarbon-degrading microorganisms, reducing biodegradation efficiency [25].

3.4. Geotechnical Tests for Petroleum-Contaminated Soil Treated with Sewage Sludge

After the completion of the bioremediation process, it is necessary to know the geotechnical characteristics for the reintroduction of the bioremedied soils in the voids from which they were extracted by excavation. A key parameter in this context is the degree of compaction. The results of the compaction tests are presented graphically, with a curve plotted for each mixture to determine the maximum dry density and the optimal compaction moisture content under an energy of 0.6 J cm^{-3} (Figure 4). The Proctor test results indicate a strong correlation between soil organic matter content, optimal compaction moisture, and maximum dry density. For soil with 4.5% organic matter, the maximum dry density and optimal compaction moisture were 1.55 g cm^{-3} and 22.5%, respectively. Similarly, for the S2:N1 mixture containing 8.1% organic matter, the values were 1.48 g cm^{-3} and 25%, and for S1:N2 with 13.9% organic matter, the corresponding values were 1.42 g cm^{-3} and 25.8%. As the organic matter content increased, the maximum dry density decreased, while the optimal compaction moisture content increased. This trend aligns with the known properties of organic matter, which retains significant moisture and exhibits a lower density compared to mineral particles [30].

Analysis of compressibility curves (Figure 5) also demonstrated a clear relationship between density, oedometric modulus values, and organic matter content. The increased organic matter resulted in decreased density and correspondingly lower oedometric modulus values. Consequently, the material transitioned from medium compressibility (E_{oed} = 10,000–20,000 kPa) to high compressibility (E_{oed} = 5000–10,000 kPa), as defined by international standards [39]. Previous studies have shown that soil organic matter

significantly influences the physical and mechanical properties of clay [30]. At the end of the experiment, the organic matter content in the soil treated with sludge was evaluated. The findings revealed that higher sludge proportions in the mixtures corresponded to higher organic matter contents: S (4.5%), S2:N1 (8.1%), S1:N1 (11.3%), and S1:N2 (13.9%). Although the initial organic matter content in the contaminated soil was 5.2%, its final value was lower after the bioremediation process (4.5%). A similar reduction in organic matter may have occurred in the mixtures, though the final values, which influence their suitability for excavation backfill, were the focus of this assessment. For the evaluated moisture content of 305%, with an organic matter contribution of 39% from the sludge and 5% from the contaminated soil, the observed organic matter percentages in the mixtures closely aligned with theoretical predictions. This consistency supports the feasibility of utilizing these treated materials for geotechnical applications in excavation backfill. The observed reduction in density and oedometric modulus with increased organic matter content aligns with the findings in the literature, indicating a shift towards medium-to-high compressibility. This is primarily due to the lighter nature of organic material and its capacity to retain water, which influences soil structure and mechanics. However, the suitability of the treated soil for its intended purpose is assessed based on its ability to provide adequate support for ecological restoration.

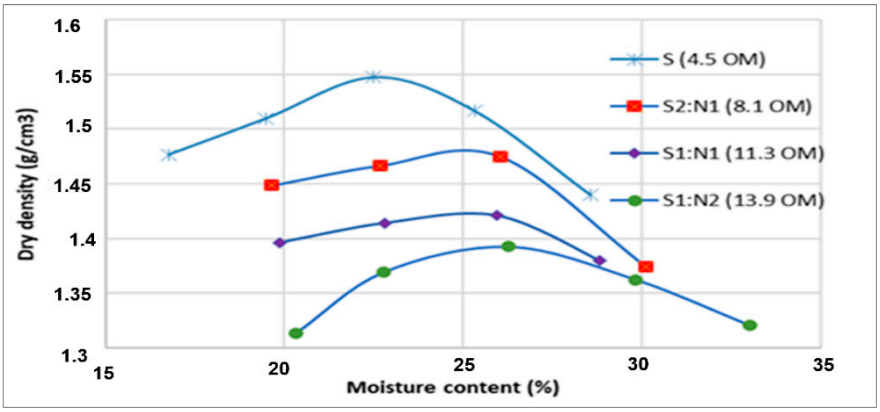
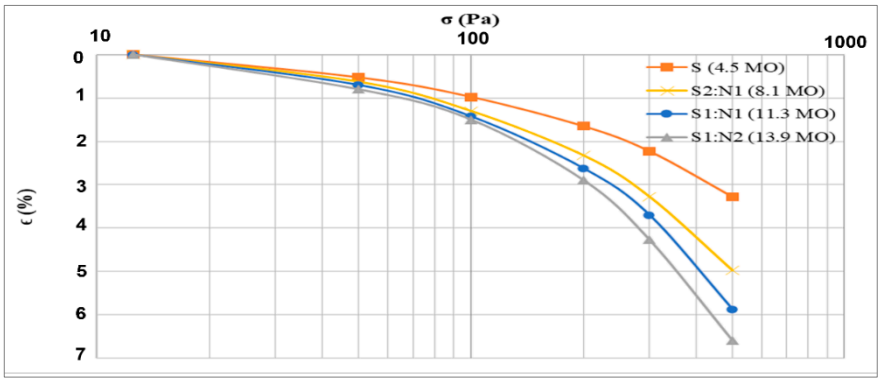


Figure 4. Compaction tests for the petroleum-contaminated soil treated with sludge.



Vertical Stress σ (kPa)	Oedometer Modules	Samples			
		Soil	Soil and Sludge Mixtures (*v*/*v*)		
			S1:N1	S2:N1	S1:N2
100	E_{oed} 100–200	14,815	8333	9756	7143
200	E_{oed} 200–300	17,391	9302	10,526	7273
300	E_{oed} 300–500	19,048	9195	11,765	8602

Figure 5. Compressibility modules for petroleum-contaminated soil treated with sludge.

4. Conclusions

This comprehensive analysis underscores the physicochemical and biological conditions that influence the bioremediation process, highlighting the interplay of soil and sewage sludge properties in petroleum hydrocarbon degradation. The bioremediation experiment demonstrated that TPH reduction was strongly influenced by initial concentrations, with the highest reduction observed in the S1:N1 mixture (67.7%). Degradation was most rapid during the first two months (~80%), followed by a slower phase (~20%) in the later stages. Mixtures with higher initial TPH concentrations exhibited greater overall degradation efficiency, highlighting their potential for accelerated bioremediation processes. Bacteria, such as hydrocarbon-tolerant, hydrocarbon-degrading, and enterobacteria are found in a wide range of natural environments, including in petroleum-contaminated soils and dehydrated sewage sludges. During the bioremediation experiment, the number of hydrocarbon-tolerant bacteria, hydrocarbon-degrading bacteria, and enterobacteria varied from one sample to another. Incorporating sewage sludge into petroleum-contaminated sites as a soil amendment not only helps restore soil health but also contributes to bioremediation by stimulating the degradation of hydrocarbons through enhanced indigenous microbial activity. Using treated soil as fill material for excavated pits is crucial, as it reduces the need for natural resources and prevents ecological imbalances. This paper provides a detailed discussion of the test results, highlighting the optimal soil-to-sludge ratio of 1:1 by volume for the cases studied. Further studies will be conducted at another petroleum-contaminated site to validate the proposed method, and the findings will be incorporated into future work.

Author Contributions: Conceptualization, C.M.I. and L.P.G.; methodology, M.M.S., C.M.I. and C.U.; validation, M.M.S., C.M.I., L.P.G. and C.U.; investigation, C.M.I., M.M.S. and C.U.; data curation, C.M.I., M.M.S. and C.U.; writing—original draft preparation, C.M.I. and M.M.S.; writing—review and editing, M.M.S.; supervision, L.P.G. All authors have read and agreed to the published version of the manuscript.

Funding: The study was funded by project no. 197/2023 from the "Dunărea de Jos" University of Galați and project no. RO1567-IBB05/2023 from the Institute of Biology Bucharest of Romanian Academy.

Data Availability Statement: The data presented in this study are available on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Mustafa, A.D.; Juahir, H.; Yunus, K.; Amran, M.A.; Hasnam, C.N.C.; Azaman, F.; Abidin, I.Z.; Azmee, S.H.; Sulaiman, N.H. Oil spill related heavy metal: A review. *Malays. J. Anal. Sci.* **2015**, *19*, 1348–1360.
2. Ossai, I.C.; Ahmed, A.; Hassan, A.; Hamid, F.S. Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environ. Technol. Innov.* **2020**, *17*, 100526. [CrossRef]
3. Stancu, M.M. Kerosene tolerance in *Achromobacter* and *Pseudomonas* species. *Ann. Microbiol.* **2020**, *70*, 8. [CrossRef]
4. Stancu, M.M. Characterization of new diesel-degrading bacteria isolated from freshwater sediments. *Int. Microbiol.* **2023**, *26*, 109–122. [CrossRef]
5. Stancu, M.M. Characterization of a new *Pseudomonas aeruginosa* strain isolated from petroleum-polluted soil. *Waste Biomass Valorization* **2024**, 1–15. [CrossRef]
6. Polyak, Y.M.; Bakina, L.G.; Chugunova, M.V.; Mayachkina, N.V.; Gerasimov, A.O.; Bure, V.M. Effect of remediation strategies on biological activity of oil-contaminated soil–A field study. *Int. Biodeterior. Biodegrad.* **2018**, *126*, 57–68. [CrossRef]
7. Das, N.; Chandran, P. Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnol. Res. Int.* **2011**, *2011*, 941810. [CrossRef]
8. Al-Hawash, A.B.; Dragh, M.A.; Li, S.; Alhujaily, A.; Abbood, H.A.; Zhang, X.; Ma, F. Principles of microbial degradation of petroleum hydrocarbons in the environment. *Egypt. J. Aquat. Res.* **2018**, *44*, 71–76. [CrossRef]

9. Xu, X.; Liu, W.; Tian, S.; Wang, W.; Qi, Q.; Jiang, P.; Gao, X.; Li, F.; Li, H.; Yu, H. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. *Front. Microbiol.* **2018**, *9*, 2885. [CrossRef]
10. Ravi, A.; Ravuri, M.; Krishnan, R.; Narenkumar, J.; Anu, K.; Alsalhi, M.S.; Devanesan, S.; Kamala-Kannan, S.; Rajasekar, A. Characterization of petroleum degrading bacteria and its optimization conditions on effective utilization of petroleum hydrocarbons. *Microbiol. Res.* **2022**, *265*, 127184. [CrossRef]
11. Jia, W.; Cheng, L.; Tan, Q.; Liu, Y.; Dou, J.; Yang, K.; Yang, Q.; Wang, S.; Li, J.; Niu, G.; et al. Response of the soil microbial community to petroleum hydrocarbon stress shows a threshold effect: Research on aged realistic contaminated fields. *Front. Microbiol.* **2023**, *14*, 1188229. [CrossRef] [PubMed]
12. 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* **2019**, *234*, 864–874. [CrossRef] [PubMed]
13. Chicca, I.; Becarelli, S.; Di Gregorio, S. Microbial involvement in the bioremediation of total petroleum hydrocarbon polluted soils: Challenges and perspectives. *Environments* **2022**, *9*, 52. [CrossRef]
14. Mohammadi, L.; Rahdar, A.; Bazrafshan, E.; Dahmardeh, H.; Susan, M.A.B.H.; Kyzas, G.Z. Petroleum hydrocarbon removal from wastewaters: A review. *Processes* **2020**, *8*, 447. [CrossRef]
15. Fijalkowski, K.; Rorat, A.; Grobelak, A.; Kacprzak, M.J. The presence of contaminations in sewage sludge–the current situation. *J. Environ. Manag.* **2017**, *203*, 1126–1136. [CrossRef]
16. Kicińska, A.; Kosa-Burda, B.; Kozub, P. Utilization of a sewage sludge for rehabilitating the soils degraded by the metallurgical industry and a possible environmental risk involved. *Hum. Ecol. Risk Assess.* **2018**, *24*, 1990–2010. [CrossRef]
17. Gielnik, A.; Pechaud, Y.; Huguenot, D.; Cebron, A.; Esposito, G.; van Hullebusch, E.D. Functional potential of sewage sludge digestate microbes to degrade aliphatic hydrocarbons during bioremediation of a petroleum hydrocarbons contaminated soil. *J. Environ. Manag.* **2021**, *280*, 111648. [CrossRef]
18. Satoh, Y.; Ishizuka, S.; Hiradate, S.; Nagano, M.A.A.H.; Koarashi, J. Sequential loss-on-ignition as a simple method for evaluating the stability of soil organic matter under actual environmental conditions. *Environ. Res.* **2023**, *239*, 117224. [CrossRef]
19. *EN ISO 14688-1*; Geotechnical Research and Testing—Soil Identification and Classification Part 1: Identification and De-Scription. NEN: Delft, The Netherlands, 2018.
20. Simionov, I.A.; Călmuc, M.; Iticescu, C.; Călmuc, V.; Georgescu, P.L.; Faggio, C.; Petrea, Ş.M. Human health risk assessment of potentially toxic elements and microplastics accumulation in products from the Danube River Basin fish market. *Environ. Toxicol. Pharmacol.* **2023**, *104*, 104307. [CrossRef]
21. Okparanma, R.N.; Mouazen, A.M. Determination of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) in soils: A review of spectroscopic and non-spectroscopic techniques. *Appl. Spectrosc. Rev.* **2013**, *48*, 458–486. [CrossRef]
22. Sambrook, J.; Russel, D. *Molecular Cloning: A Laboratory Manual*, 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 2001.
23. Stancu, M.M.; Grifoll, M. Multidrug resistance in hydrocarbon-tolerant Gram-positive and Gram-negative bacteria. *J. Gen. Appl. Microbiol.* **2011**, *57*, 1–18. [CrossRef] [PubMed]
24. Mekonnen, B.A.; Aragaw, T.A.; Genet, M.B. Bioremediation of petroleum hydrocarbon contaminated soil: A review on principles, degradation mechanisms, and advancements. *Front. Environ. Sci.* **2024**, *12*, 1354422. [CrossRef]
25. Chibuike, G.U.; Obiora, S.C. Heavy metal polluted soils: Effect on plants and bioremediation methods. *Appl. Environ. Soil Sci.* **2014**, *2014*, 752708. [CrossRef]
26. Silva-Castro, G.A.; Calvo, A.R.; Laguna, J.; González-López, J.; Calvo, C. Autochthonous microbial responses and hydrocarbons degradation in polluted soil during biostimulating treatments under different soil moisture. Assay in pilot plant. *Int. Biodeterior. Biodegrad.* **2016**, *108*, 91–98. [CrossRef]
27. Patowary, K.; Patowary, R.; Kalita, M.C.; Deka, S. Development of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. *Front. Microbiol.* **2016**, *7*, 1092. [CrossRef]
28. Onojake, M.C.; Omokheyeke, O.; Osakwe, J.O. Hydrocarbon contamination to environment. *Bull. Earth Sci. Thail.* **2014**, *6*, 67–79.
29. Devatha, C.P.; Vishnu, A.V.; Rao, J.P.C. Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation. *Appl. Water Sci.* **2019**, *9*, 89. [CrossRef]
30. Gui, Y.; Zhang, Q.; Qin, X.; Wang, J. Influence of organic matter content on engineering properties of clays. *Adv. Civ. Eng.* **2021**, *2021*, 6654121. [CrossRef]
31. Bot, A.; Benites, J. *The Importance of Soil Organic Matter Key to Drought-Resistant Soil and Sustained Food Production*; FAO Soils Bulletin 80; FAO: Rome, Italy, 2005.
32. Ruehlmann, J. Soil particle density as affected by soil texture and soil organic matter: 1. Partitioning of SOM in conceptional fractions and derivation of a variable SOC to SOM conversion factor. *Geoderma* **2020**, *375*, 114542. [CrossRef]
33. Ruehlmann, J.; Körschens, M. Soil particle density as affected by soil texture and soil organic matter: 2. Predicting the effect of the mineral composition of particle-size fractions. *Geoderma* **2020**, *375*, 114543. [CrossRef]

34. Abena, M.T.B.; Chen, G.; Chen, Z.; Zheng, X.; Li, S.; Li, T.; Zhong, W. Microbial diversity changes and enrichment of potential petroleum hydrocarbon degraders in crude oil-, diesel-, and gasoline-contaminated soil. *3 Biotech* **2020**, *10*, 42. [CrossRef]
35. Gandolfi, I.; Sicolo, M.; Franzetti, A.; Fontanarosa, E.; Santagostino, A.; Bestetti, G. Influence of compost amendment on microbial community and ecotoxicity of hydrocarbon-contaminated soils. *Bioresour. Technol.* **2010**, *101*, 568–575. [CrossRef] [PubMed]
36. Dados, A.; Omirou, M.; Demetriou, K.; Papastephanou, C.; Ioannides, I.M. Rapid remediation of soil heavily contaminated with hydrocarbons: A comparison of different approaches. *Ann. Microbiol.* **2015**, *65*, 241–251. [CrossRef]
37. Briffa, J.; Sinagra, E.; Blundell, R. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon* **2020**, *6*, e04691. [CrossRef]
38. Swain, C.K. Environmental pollution indices: A review on concentration of heavy metals in air, water, and soil near industrialization and urbanization. *Discov. Environ.* **2024**, *2*, 5. [CrossRef]
39. *EN ISO 17892-5*; Geotechnical Investigation and Testing—Laboratory Testing of Soil—Part 5: Incremental Loading Oedometer Test. NSAI: Dublin, Ireland, 2017.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.