



Synergistic Application of Biochar and Biofertilizer to Restore Soil Structure and Mitigate Petroleum Hydrocarbons in Iranian Coastal Ecosystems

ARTICLE INFO

Article Type
Original Research

Authors

Navazollah Moradi, Ph.D.^{1*}
Ommolbanin Bazrafshan, Ph.D.²

How to cite this article

Moradi N., Bazrafshan O. Synergistic Application of Biochar and Biofertilizer to Restore Soil Structure and Mitigate Petroleum Hydrocarbons in Iranian Coastal Ecosystems. ECOPERSIA 2026;14(2): 161-178.

DOI:

10.48311/ecopersia.2026.119415.82879

¹ Assistant Professor, Department of Natural Resources Engineering, Faculty of Agriculture and Natural Resources, University of Hormozgan, Bandar Abbas, Iran.

² Professor, Department of Natural Resources Engineering, Faculty of Natural Resources and Agriculture, University of Hormozgan, Bandar Abbas, Iran.

* Correspondence

Address: Department of Natural Resources Engineering, Faculty of Agriculture and Natural Resources, University of Hormozgan, P.O. BOX: 3995, Bandar Abbas, Iran.

Tel: +989158263797

E-mail: nvz.moradi@hormozgan.ac.ir

Article History

Received: March 13, 2026

Revised: June 10, 2026

Accepted: June 11, 2026

Published: June 15, 2026

ABSTRACT

Aims: This study evaluated the combined effects of *Conocarpus* biochar and a *Bacillus*-based biofertilizer on TPH removal, aggregate stability index (ASI), and mean weight diameter of aggregates in diesel-contaminated mangrove sediments.

Materials & Methods: This study examined the synergistic effects of biochar (4% w/w) and a *Bacillus subtilis*-based biofertilizer (applied at 0%, 2%, and 5% v/w) on physicochemical recovery and total petroleum hydrocarbon (TPH) removal in diesel-contaminated (8% w/w) mangrove sediments collected from the Persian Gulf. A 70-day incubation experiment was conducted under controlled conditions (18–25°C, 50% field capacity).

Findings: The application of biochar significantly improved soil aggregate stability, as evidenced by increases in dry mean weight diameter (MWD_{dry}) and the Aggregate Stability Index (ASI). In contrast, the biofertilizer alone reduced clay dispersion and turbidity, indicating enhanced colloidal stability. Biochar alone achieved the highest TPH removal efficiency (95.5%), outperforming both the 2% biofertilizer treatment (90.5%) and the untreated control (93.5%), which demonstrated substantial indigenous microbial degradation. Notably, the combined application of biochar with 2% biofertilizer resulted in reduced remediation efficiency (86%), suggesting antagonistic interactions at lower microbial doses. However, when biochar was combined with the higher biofertilizer dose (5%), both structural integrity and TPH removal (95%) were effectively restored, revealing a dose-dependent synergy.

Conclusion: The results indicated that while biochar primarily functions as a physical stabilizer, especially under wet conditions, microbial amendments must be carefully dosed to avoid disrupting native microbial communities. Effective remediation of petroleum-polluted saline soils thus depends on a balanced integration of physical amendments and microbial ecology, rather than additive application alone.

Keywords: *Bacillus subtilis*, Bioremediation; Mangrove Sediments; Petroleum Contamination; Soil Aggregate Stability; TPH Removal.

CITATION LINKS

- [1] Goveas L.C., Krishna A., Salian A., Menezes J., Alva M., Basav... [2] Sarfo M.K., Gyasi S.F., Kabo-Bah A.T., Adu B., Mohhtar Q., App... [3] Rhykerd R., Crews B., McInnes K., Weaver R.W. Impact of bulkin... [4] Li Y., Li F.M., Li F.X., Yuan F.Q., Wei P.F. Effect of the ult... [5] Liu S., Zhu L.M.L., Jiang W.C., Qin J.Z., Lee H.S. Research on... [6] Novakovskiy A.B., Kanev V.A., ... [7] Li H.S., Sun H... [8] Kuppusamy S., Thavamani P., ... [9] Lominchar M.A., Santos A., de Miguel E., ... [10] Ren H.Y., Wei Z.J., Wang Y., Deng Y.P., Li M.Y., Wang B. Effec... [11] Chen Z.B., Reiche N., Vymazal J., Kusch P. Treatment of water... [12] Fahid M., Arslan M., Shabir G., ... [13] Bhatt P., Gangola S., Bhandari G., Zhang W.P., Maithani D., Mi... [14] Agarry S.E., Owabor C.N., Yusuf R.O. Studies on biodegradation... [15] Nwankwegu A.S., Obwosi C.O. ... [16] Varjani S.J. ... [17] Xu X.J., Liu W.M., Tian S.H., Wang W., Qi Q.G., Jiang P., Gao ... [18] Zhu X.M., Chen B.L., Zhu L.Z., Xing B.S. Effects and mechanism... [19] Dike C.C., Hakeem I.G., Rani A., ... [20] Tomczyk A., Sokołowska Z., ... [21] Khademalrasoul A., Mohammadi N. ... [22] Fang X., Zhang M., Zheng P., ... [23] Fakhrzadegan I., Hassanshahian M., Askari M. Isolation and ide... [24] Joo, M.H., Kim, J.Y. Characteristics of crude oil biodegradati... [25] Middleton H.E. Properties of soil that influence soil erosion... [26] Kemper W.D., Rosena R.C. ... [27] Moradi N., Emami H., ... [28] Wang F, Tang Y.A.,... [29] Amiri Tavassoli S., Moradi N., Bazrafshan O. ... [30] Saadati N., Davatgar N., Roodpeyma M., Mosaddeghi M.R., Bostan... [31] Song Y.F., Jing X., ... [32] Gul S., Whalen J.K., Thomas B.W., Sachdeva V., Deng H. Physico... [33] Samaei F., Asghari S., Aliasgharzad N. The effects of two arbu... [34] Moshtagh R., Moradi N., Gholami H. Investigation of the role o... [35] Kermanpour M., ... [36] Khademalrasoul A., Mohammadi N. Assessment of Zeoplant and bio... [37] Daryaei R., Moosavi S.A.A., ... [38] Beesley L., Moreno-Jiménez E., Gomez-Eyles J.L., Harris E., Ro... [39] Lehmann J., Rillig M.C., Thies J., Masiello C.A., Hockaday W.C... [40] Cheng J.M., Sun Z.H., ... [41] Sani J.E., Moses G., Musa S. Physicochemical evaluation of cocon... [42] Department of Petroleum... [43] Simpson S.L, Batley G.E, Chariton A.A. Revision of the ANZECC/... [44] Soleimani M., Jaberi N. Optimization of removal process of pet... [45] Van Hamme J.D., Singh A., Ward O.P. Recent advances in petrole...

Introduction

Petroleum contamination is a significant form of organic soil pollution, primarily resulting from anthropogenic activities such as extraction, refining, and transportation, as well as crude oil spills [1,2]. The constituents of crude oil, including alkanes, cycloalkanes, and aromatic hydrocarbons, are known to be carcinogenic, mutagenic, and teratogenic, posing significant threats to the integrity of soil ecosystems and the health of living organisms [3]. The introduction of these contaminants alters key soil physicochemical properties, disrupting nutrient availability, pH, aeration, and water-holding capacity [4]. These adverse effects are often exacerbated under adverse environmental conditions, such as saline soils [5,6]. Moreover, the bioaccumulation and biomagnification of petroleum-derived pollutants through the food chain pose serious risks to human health [7]. Among petroleum derivatives, diesel fuel presents a particularly critical remediation challenge due to its high toxicity and environmental persistence. Comprising 60–80% saturated hydrocarbons and 20–40% aromatic hydrocarbons, diesel has substantial potential to harm human health and soil ecosystems [8,9]. Diesel contamination suppresses soil microbial viability [10] and impairs seed germination and plant growth, thereby adversely affecting agricultural productivity and food chain safety. Therefore, developing effective and sustainable strategies for diesel remediation is of great importance.

In recent years, bioremediation, particularly microbial-enhanced biodegradation, has gained significant attention as a cost-effective, eco-friendly, and sustainable solution for mitigating petroleum pollution [11,12]. Microorganisms are key agents in degrading

organic pollutants [13] and can be applied as bioaugmentation inoculants or biostimulants to enhance indigenous microbial populations and metabolic activity [14,15]. However, the efficacy of these approaches is highly dependent on environmental parameters such as temperature, moisture, and nutrient availability [16,17]. One way to address these physical and environmental limitations is through soil amendments that improve habitat conditions for degrading microorganisms.

In this context, biochar has emerged as a promising multifunctional soil amendment. Its porous structure and high specific surface area enhance cation exchange capacity, aeration, water retention, and soil pH, thereby creating a favorable microhabitat for microbial proliferation [10,18]. These attributes facilitate interactions between microbes and contaminants and provide stable carbon substrates that support metabolic processes. Studies show that combining biochar with petroleum-degrading microorganisms enhances the removal efficiency of total petroleum hydrocarbons (TPHs) and promotes overall soil functional recovery [19, 20]. Specifically, biochar application reduces soil bulk density, increases porosity, improves water-holding capacity, and mitigates hydrophobicity [21]. Optimal effects are often observed at an application rate of 6% and moisture levels equivalent to 25% of field capacity.

In a comprehensive study, Fang et al. (2024) reported a 76.60% reduction in TPHs using a combined biochar–*Bacillus*-based microbial agent [22]. Their findings indicated that biochar enriched the *Bacillus* population, which correlated positively with TPH degradation and improvements in soil chemical properties. Microbiome analyses

further revealed enhanced bacterial community richness and diversity, along with activation of carbohydrate and amino acid metabolic pathways, indicating heightened microbial stress-response mechanisms under petroleum-induced stress.

In Iran, a few studies have also explored this area. Fakhrzadegan et al. (2015) isolated hydrocarbon-degrading bacteria (*Vibrio azureus* and *Pseudomonas salomonii*) from mangrove sediments in Minab and Bandar Khamir, the Persian Gulf. Using these native strains alone, they achieved 66% crude oil removal within 15 days [23]. This confirms that mangrove sediments in the Persian Gulf harbor indigenous bacteria with significant potential for degradation.

In Iran, a few studies have been conducted. However, most Iranian studies have focused either on isolating degrading bacteria or on using conventional biochars (e.g., rice husk, date palm) in agricultural or industrial soils. What distinguishes the present study from previous work is the following: using *Conocarpus* wood waste biochar (a locally available feedstock in southern Iran), applying it to actual mangrove sediments (not farmland or industrial soils), combining it with a *Bacillus*-based biofertilizer, and specifically measuring physical properties such as the aggregate stability index (ASI) and the mean weight diameter of wet aggregates (MWD_{wet}), rather than focusing solely on TPH removal.

In this study, turbidity was used as a simple indicator of sediment stability. In mangrove ecosystems, healthy, stable sediments naturally have low turbidity because organic matter and roots bind particles together. When oil contaminates the sediment, it creates a water-repellent layer on the particles. This weakens the bonds between

particles and reduces their cohesiveness. As a result, the sediment becomes loose and unstable. Even small disturbances, such as tidal movements, can suspend particles, thereby increasing turbidity. Therefore, measuring turbidity provides a quick and low-cost way to evaluate how oil contamination affects mangrove sediments, complementing other physical indicators such as ASI and MWD_{wet} .

Despite significant advances in bioremediation technologies for petroleum-contaminated soils, practical efficacy remains limited by challenges such as slow degradation kinetics and process instability. Degrading microorganisms alone are often insufficient to break down hydrocarbons effectively because they require a suitable physicochemical environment for optimal activity.

Biochar can help address this limitation. It improves soil structure and serves as a microbial carrier due to its high surface area and porous architecture. As shown by Ren et al. (2020), biochar with a high specific surface area (up to $312 \text{ m}^2 \cdot \text{g}^{-1}$) and a microporous structure achieved a microbial immobilization rate of %71.2 and an oil removal rate of 70.7%. The same study also reported that immobilized microorganisms recovered 83.38% of their activity compared to free bacteria. Together, these findings suggest that biochar creates favorable microenvironments that enhance microbial activity and the accessibility of contaminants [10].

The present study focuses only on observable physical and chemical outcomes and does not investigate underlying microbial mechanisms. Therefore, this study was designed to evaluate the combined effects of *Conocarpus* wood waste biochar and

a *Bacillus*-based biofertilizer on (i) total petroleum hydrocarbon (TPH) removal efficiency, and (ii) selected soil physical properties, specifically the aggregate stability index (ASI) and the mean weight diameter of wet aggregates (MWD_{wet}), in diesel-contaminated mangrove sediments.

Materials & Methods

This study was conducted in several stages, as illustrated in the flowchart (Figure 1). Surface sediment samples were collected from an *Avicennia marina* mangrove forest in Kolghan, Bandar Abbas (Figure 2). The sampling depth was 10–0 cm. This depth was selected because, under natural conditions, most oil pollutants from human activities (such as boat spills and tidal currents) accumulate in the surface layer. In addition, this layer is biologically and chemically active, and it is the primary zone where mangrove roots interact with the surrounding sediment. Therefore, sampling at this depth allows the experimental setup to reflect field conditions more realistically. After collection, the sediment samples were air-dried and then spiked with diesel to a concentration of 8% (w/w). This concentration was not chosen randomly. Lower concentrations were also tested in preliminary pilot experiments. The 8% concentration was selected to represent a realistic scenario along the southern coasts of Iran. In this region, events such as tanker spills, pipeline ruptures, and extensive diesel smuggling, especially in coastal creeks, can create localized oil patches with diesel concentrations exceeding 8% in mangrove sediments. Therefore, this concentration was used as a critical (worst-case) condition to evaluate the maximum tolerance of the mangrove sediment

ecosystem under real-world conditions. The spiked samples were left to equilibrate for one week in closed containers.

The selection of the bacterial strain was also not random. Based on our preliminary studies and available data, *Bacillus subtilis* performs better than some common reference strains under combined salinity and oil stress [24]. This makes it a suitable choice for bioremediation in saline, oil-contaminated environments. In addition, the commercial availability of this biofertilizer also influenced our choice.

Following this, a factorial experiment was conducted using a completely randomized design. The treatments consisted of three application rates of the *Bacillus*-based biofertilizer (0%, 2%, and 5% v/w) combined with two levels of biochar (0% and 4% w/w). Each treatment was replicated three times, and 3 kg of the mixture was placed in individual plastic containers. To maintain stable moisture conditions, each container was covered with a perforated plastic sheet that was not airtight (small holes allowed partial gas exchange).

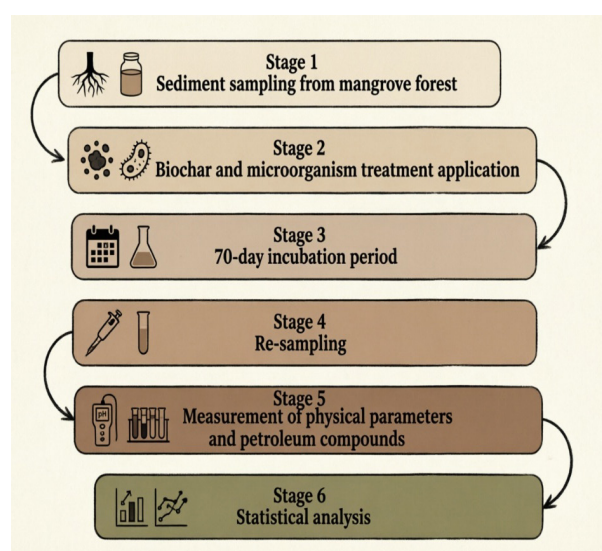


Figure 1) Schematic representation of the experimental design.



Figure 2) View of the sediment sampling site in the mangrove forest ecosystem.

The samples were incubated for 70 days. This duration was chosen based on principles of microbial ecology. Under the saline conditions of mangrove sediments, native bacteria were assumed to require a relatively long adaptation phase to overcome osmotic stress and hydrocarbon toxicity. A shorter period might capture only the adaptation phase without reflecting the actual degradation phase. This timeframe ensures that both the lag phase and the logarithmic decline in contamination are reflected in the results. Throughout the incubation period, the moisture content was maintained at approximately 50% of field capacity and adjusted weekly. To ensure adequate aeration and physical homogeneity, the contents of each container were manually mixed every two weeks. The experiment was conducted in the Soil and Water Laboratory at the University of Hormozgan under controlled conditions, with the temperature maintained at 18–25 °C.

The biochar was produced from pruning residues (leaves and thin branches) of *Conocarpus erectus* collected on the university campus (Table 1). Feedstock was oven-dried at 80 °C for 24 h, wrapped in aluminum foil to restrict oxygen exposure, and pyrolyzed in a muffle furnace at 450 °C for 3 h. After cooling, the material was ground and sieved to <0.25 mm. Total carbon, hydrogen, and nitrogen

contents were determined using a CHN elemental analyzer (Vario EL III, Elementar, Germany) (Table 2).

Table 1) Description of experimental treatments and their assigned codes.

Treatment	Code
Control	Control
Biofertilizer (2%)	BF 2
Biofertilizer (5%)	BF 5
Biochar	BC
Biochar+ Biofertilizer (2%)	BC+BF2
Biochar+ Biofertilizer (5%)	BC+BF5

Table2) The properties of the Biochar pruning waste were analyzed.

EC(dSm ⁻¹)	pH 1:10	O/C	H/C	N[%]	O[%]	H[%]	C[%]
2.25	8.85	0.49	0.73	0.18	37.5	3.52	57.2

Note: C: Carbon, H: Hydrogen, O: Oxygen, N: Nitrogen, pH: Acidity, EC: Electrical Conductivity.

Laboratory Analysis

Following a 70-day incubation period, soil samples from each treatment were collected with minimal disturbance to evaluate the effects of biochar and biofertilizer on soil physical properties. The samples were air-dried under controlled conditions before analysis. Key physical properties of the sediments, along with petroleum hydrocarbon removal efficiency, were then determined.

Aggregate stability was assessed by measuring the dispersion index of clay and silt (DC) in aqueous suspension using the Middleton dispersion index [25]. Additionally, the mean weight diameter (MWD) of aggregates was determined under both dry and wet sieving conditions. For this analysis, air-dried soil samples were first passed through a 4-mm sieve, and the fraction

retained between the 2-mm and 4-mm sieves was used. This fraction was then subjected to a nested series of sieves with apertures of 2, 1, 0.5, 0.25, and 0.1 mm, following the method of Kemper and Rosenau (1986).^[26] In the wet-sieving procedure, aggregates retained on each sieve were oven-dried at 105°C, weighed, and corrected for sand content. The MWD under wet conditions (MWD_{wet}) was calculated using Eq. (1).

$$MWD = \sum_{i=1}^k W_i \bar{x}_i \quad \text{Eq. (1)}$$

where X_i is the mean diameter between two consecutive sieves, and W_i is the proportion of aggregate mass retained on each sieve relative to the total corrected soil mass.

Based on the dry and wet MWD values (Kemper & Rosenau, 1986), the Aggregate Stability Index in water (ASI) was computed as the percentage ratio of MWD_{wet} to MWD_{dry} . ASI values approaching 100% indicate highly stable aggregates, while values below 50% denote very unstable aggregates that are highly susceptible to erosion.

Another key indicator, the Percent Aggregate Destruction (PAD), was evaluated as well. PAD was derived from dry and wet MWD measurements by comparing the mass of aggregates larger than 0.25 mm obtained under dry sieving (md) and wet sieving (mw). PAD was calculated using Eq. (2)^[27,28]. Lower PAD values reflect greater aggregate stability:

$$PAD = \frac{md - mw}{md} \times 100 \quad \text{Eq. (2)}$$

Turbidity measures light scattering in a solution and indicates the concentration of suspended solids. When soil is dispersed in water, the turbidity of the resulting suspension reflects the amount of suspended soil particles. To quantify this, a

Turbidimeter height in water (H.T.W) index was used. Briefly, 50 g of soil was placed in a 250 mL Erlenmeyer flask, mixed with approximately 200 mL of distilled water, and allowed to equilibrate for 24 h. The suspension was then transferred to a 1-L graduated cylinder and diluted to the final volume. After thorough homogenization and a 2-hour settling period, the depth of the clear suspension column was measured from the surface down to the point where a white plate at the bottom of the cylinder was no longer visible. This depth was recorded as the turbidity index value. Thus, higher values of this index (greater depth) indicate a lower sedimentation rate and a greater dispersion of suspended particles per unit of measurement time^[29].

Total petroleum hydrocarbons (TPHs) in the soil samples were quantified using a mechanical shaking extraction method, following established protocols^[30,31]. Specifically, 4 g of each sample was extracted with 10 mL of an acetone/n-hexane mixture (1:1, v/v) by mechanical shaking at 250 rpm for 2 h. After centrifugation at 2500 rpm for 10 min, the supernatant was collected. The extraction was repeated four times per sample. The supernatants were combined, homogenized, and transferred to a pre-weighed glass container. Solvents were evaporated to dryness in an oven for ≥ 24 h. TPH content was then determined gravimetrically from the residual mass.

Statistical Analysis

All statistical analyses were performed using SPSS (version 22; IBM Corp.). Treatment effects were evaluated by one-way analysis of variance (ANOVA). Post hoc comparisons between group means were conducted using Duncan's multiple range test, with statistical significance defined as $p < 0.05$.

Findings

Effects of Biochar and Biofertilizer on Soil Physical Properties and Removal of TPH

The application of biological amendments, including biochar and microbial inoculants (applied individually or in combination), markedly altered soil physical properties and total petroleum hydrocarbon (TPH) removal in diesel-contaminated soil. Analysis of variance (ANOVA) showed significant variations in soil physical properties in response to biochar, biofertilizer, and their interaction in diesel-contaminated soil, as shown in Table 3. Biochar significantly influenced TPH degradation, PAD, ASI, MWD_{dry} , and MWD_{wet} ($p < 0.05$ to $p < 0.01$), with no significant effect on DC or H.T.W. This pattern indicates that biochar primarily enhanced aggregate stability under both dry and wet conditions, while exerting minimal influence on fine-particle dispersion. The biofertilizer significantly affected TPH, PAD, DC, H.T.W., and MWD_{dry} ($p < 0.05$ to $p < 0.01$), whereas ASI and MWD_{wet} remained unchanged. These results show that the microbial amendment mainly reduced particle dispersion and improved dry-state aggregation, without altering overall stability or wet-condition performance. The biochar-biofertilizer interaction was significant for TPH, DC, H.T.W., and MWD_{dry} ($p < 0.05$ to

$p < 0.01$), but not for PAD, ASI, or MWD_{wet} . Overall, biochar served as the primary driver of structural stabilization. At the same time, the biofertilizer contributed selectively to dispersion control and dry aggregate formation, with limited synergistic effects across the full set of measured properties.

Influence of *Bacillus*-Based Biofertilizer on Measured Soil Parameters

Applying biofertilizer improved the physical structure and biological activity of diesel-contaminated soil. Higher values of dry and wet mean weight diameter (MWD_{dry} and MWD_{wet}) suggested better aggregate formation (Figures 3A, B). The increase in the aggregate stability index (ASI) and decrease in percentage of aggregate disruption (PAD) indicated that soil aggregates became larger and more resistant to breakdown (Figures 3C, D). This enhanced stability reduced clay and silt dispersion (DC) and lowered turbidity in soil suspensions (Figures 3E, F), meaning fewer fine particles were detached. Consequently, the treatment helped reduce physical degradation and limited the transport of clay-bound contaminants to nearby water bodies. Overall, the biofertilizer supported aggregate stability, preserved colloidal integrity, and maintained pore

Table 3) The ANOVA results for the effects of experimental treatments on measured soil physical properties.

Sources of Variations	Mean Squares							
	df	MWD_{wet}	MWD_{dry}	ASI	HTW	DC	PAD	TPH
BC	1	1.377*	0.041**	1525.472*	0.642 ^{ns}	2.254 ^{ns}	942.77**	20.056*
BF	2	0.395 ^{ns}	0.009**	330.820 ^{ns}	4.132*	41.118**	690.714**	63.167**
BF + BC	2	0.176 ^{ns}	0.008*	113.393 ^{ns}	10.140**	28.045**	244.983 ^{ns}	19.056*
Error	12	0.186	0.0010	182.660	0.636	0.966	73.886	3

Note: MWD: Mean Weight Diameter under dry and wet conditions, PAD: Percentage Aggregate Degradation, ASI: Aggregate Stability Index in water, DC: Dispersible Clay and Silt, H.T.W: Turbidimeter height, TPH: Total Petroleum Hydrocarbons Removal Rate.

connectivity, contributing to functional soil recovery.

Although the *Bacillus*-based biofertilizer improved soil physical properties, total petroleum hydrocarbon (TPH) removal did not follow a simple linear trend, reflecting

interactions between soil structure and microbial communities. The highest TPH removal occurred in the unamended control, suggesting that native microbes were already well-adapted to diesel degradation (Figure 3G). Without added organic Carbon, these

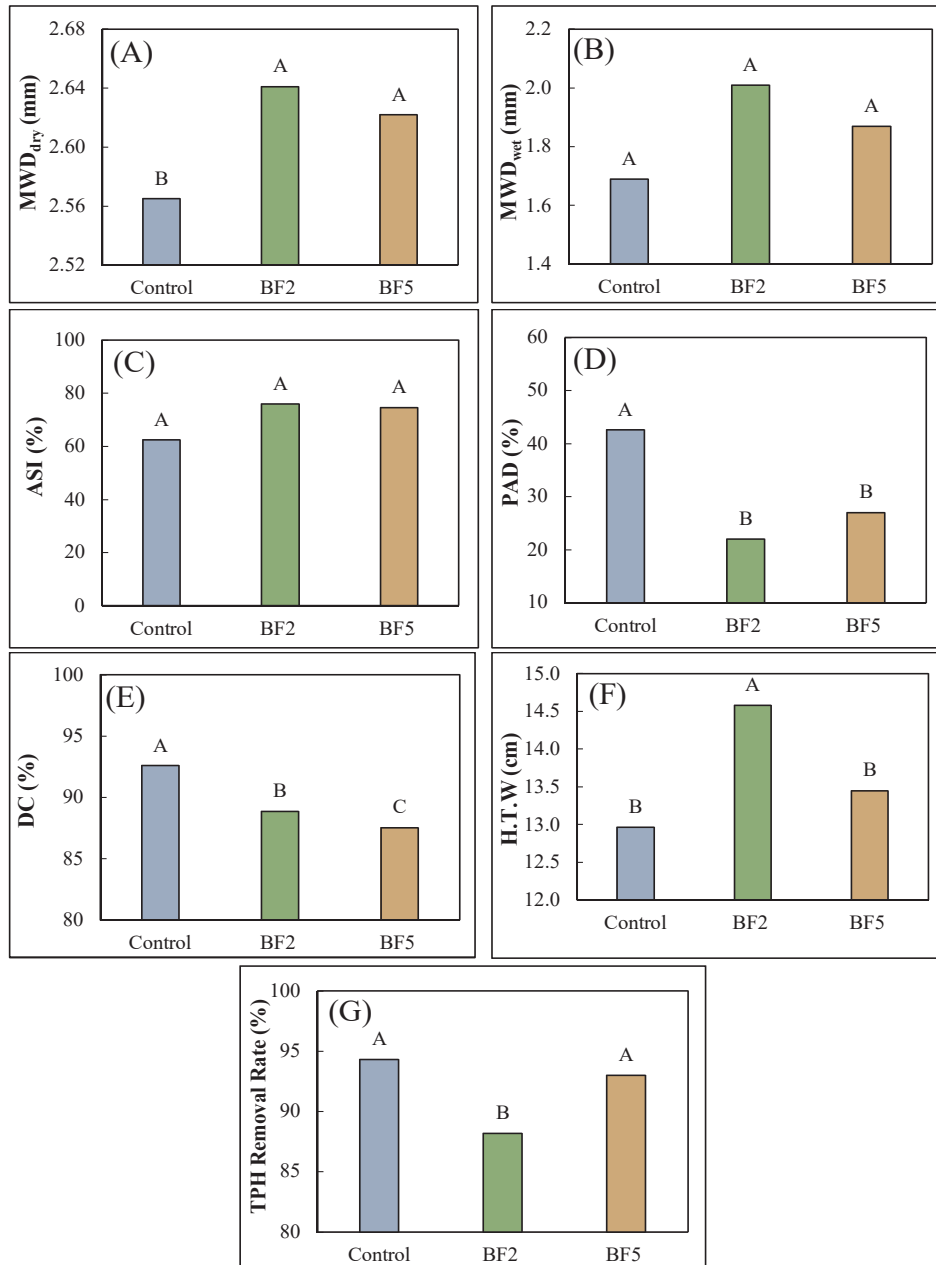


Figure 3) Effects of biofertilizer application at different concentrations (0%, 2%, and 5% v/w) on physical properties and remediation efficiency in diesel-contaminated mangrove sediment.

(A, B) Mean Weight Diameter (MWD) of aggregates under dry and wet conditions. (C) Aggregate Stability Index in water (ASI). (D) Percentage Aggregate Degradation (PAD). (E) Clay and Silt Dispersion (DC). (F) Turbidimeter height in water (H.T.W). (G) Total petroleum hydrocarbons. Values labeled with different letters differ significantly at $p < 0.05$ (Duncan's test).

indigenous microbes efficiently used diesel as their energy source. At the 2% application rate, organic compounds from the biofertilizer may have shifted microbial activity toward more readily available carbon sources or temporarily altered the soil environment, affecting the native community and resulting in lower hydrocarbon removal. At 5%, however, the biofertilizer appeared to be effective. *Bacillus* strains produced sufficient extracellular polymeric substances (EPS) to stabilize aggregates and reduce particle dispersion, while also fostering cooperation with native microbes. Under these conditions, *Bacillus* contributed to hydrocarbon degradation and supported soil recovery by adding organic matter. These findings suggest that successful bioremediation depends on balancing soil physical stability, contaminant availability, and compatibility between introduced and native microbial populations. In this study, this balance was achieved at the 5% biofertilizer rate.

Concurrent Effects of Biochar and Biofertilizer on Measured Soil Properties Impact of Amendments on Wet and Dry Mean Weight Diameter (MWD)

Statistical analysis indicated that while the addition of *Bacillus*-based biofertilizer increased wet mean weight diameter (MWD_{wet}), this increase was not statistically significant at the 0.05 level (Figure 4A). The combination of biochar with either 2% or 5% biofertilizer yielded the highest MWD_{wet} values (2.21 mm); however, these values did not differ significantly from the control or other treatments. Thus, although a positive trend was observed, the data did not support a statistically significant synergistic effect. Claims of synergy would therefore be premature based on the current dataset. Detecting significant effects in future studies may require increased replication, extended incubation periods, or enhanced

statistical power.

Biofertilizer application alone did not significantly affect dry mean weight diameter (MWD_{dry}). In contrast, combined biochar and biofertilizer treatments significantly increased MWD_{dry} ($P < 0.05$; Figure 4B). This improvement indicates that biochar complements microbial amendments to enhance soil structural stability. The highest MWD_{dry} was recorded at the 2% biofertilizer + biochar rate, suggesting an optimal application level. Higher doses did not confer additional benefits, likely due to microbial carrying-capacity limits or competitive interactions at elevated inoculum densities.

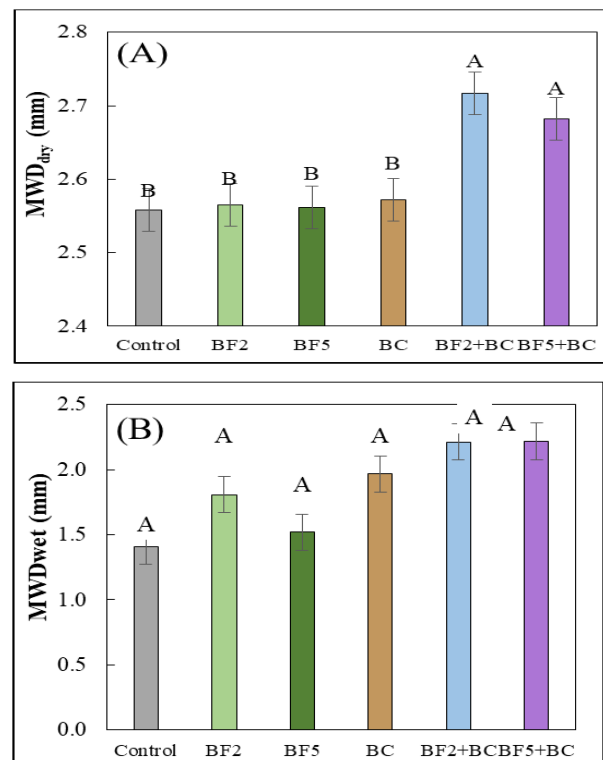


Figure 4) Effects of biochar and biofertilizer on sediment properties in a petroleum-contaminated mangrove forest. (A) Mean Weight Diameter of aggregates under dry conditions (MWD_{dry}). (B) Mean Weight Diameter of aggregates under wet conditions (MWD_{wet}). Different letters indicate significant differences at $p < 0.05$ (Duncan's test). Control (unamended contaminated sediment); BF2 and BF5 (biofertilizer at 2% and 5% v/w); BC (biochar); BF2+BC and BF5+BC (combined biochar and biofertilizer applications).

Impact of Amendments on Aggregate Stability Index (ASI)

Post hoc mean comparison showed that biofertilizer application, regardless of the presence of biochar, increased the aggregate stability index (ASI) (Figure 5D). However, this increase was statistically significant ($p < 0.05$) only in treatments that received biochar, compared with the control. In other words, without biochar, the biofertilizer alone did not significantly improve aggregate stability. The highest ASI value was observed in the treatment combining 5% biochar with biofertilizer. However, this value was not statistically different from treatments that received only biochar or only biofertilizer. The lack of significance could be due to high within-treatment variability, limited sample size, or a nonlinear response of soil to amendment rates. Despite these limitations, several key findings remain robust and can be stated with certainty. First, the positive effect of biochar on ASI is clearly statistically significant. Second, adding biofertilizer on top of biochar did not lead to a statistically significant additional improvement, although the numerical trend was positive. Therefore, we should interpret the combined treatment as promising but not conclusively synergistic. Further studies with larger sample sizes or longer incubation periods may help detect smaller effects if they exist.

Impact of Amendments on Clay and Silt Dispersion in Water

A comparison of the dispersion data demonstrated that the application of biofertilizer, both alone and combined with biochar, significantly ($p < 0.05$) reduced the dispersion of clay and silt particles in soil suspension (Figure 5A). This indicates

enhanced aggregate stability and lower susceptibility to water erosion following the biological treatments. Notably, biofertilizer alone exhibited a stronger inhibitory effect on dispersion than did the combination with biochar. The lowest dispersion value was observed with the 5% biofertilizer treatment, which differed significantly ($p < 0.05$) from all other treatments. This suggests that a high-concentration biofertilizer alone can effectively stabilize fine soil particles and restrict their dispersion. While biochar generally improves soil physical properties, its combination with biofertilizer may slightly reduce this stabilizing potential, or the amendment ratios in the combined treatments may not have been optimized.

Impact of Amendments on Turbidity

Statistical analysis showed that biofertilizer application, with or without biochar, increased turbidimeter readings of the soil suspension (Figure 5B). This increase was statistically significant ($p < 0.05$) for all treatments, except for the combination of biochar with 5% biofertilizer, compared to the control. In other words, higher turbidimeter readings indicate a lower concentration of colloidal particles in suspension and a clearer supernatant.

Impact of Amendments on Percent Aggregate Destruction (PAD)

Mean comparison analysis indicated that microbial biofertilizer application, either alone or combined with biochar, tended to reduce percent aggregate destruction (PAD) in diesel-contaminated soil (Figure 5C). However, this reduction was not statistically significant relative to the control. The lowest PAD values were observed in the treatment combining biochar with 5% biofertilizer (Figure 5C). Notably, no significant differences were detected between treatments with

and without biochar. While the data suggest a favorable trend, they do not support a statistically verifiable synergistic effect; claims of synergy would be speculative at this stage. The absence of statistical significance likely reflects similar treatment means, overlapping variability, or limited replication. Although these findings are inconclusive, the observed trends warrant further investigation under varied experimental conditions or with increased sample sizes.

Impact of Amendments on Diesel Removal Efficiency

The effectiveness of biochar and biofertilizer in diesel bioremediation was evaluated by measuring total petroleum hydrocarbon (TPH) removal. The unamended control achieved 93.5% removal, indicating the

presence of active indigenous microbial communities and/or natural attenuation. Adding 2% *Bacillus subtilis* biofertilizer (BF2) slightly reduced removal to 90.5%, possibly due to competition with native microbes. Increasing the biofertilizer dose to 5% (BF5) raised TPH removal to 95%, showing that higher application rates can enhance biodegradation. Biochar alone achieved 95.5% removal, comparable to BF5. However, combining BF2 with biochar reduced removal to 86%, suggesting an antagonistic interaction at the lower biofertilizer dose. Statistical analysis confirmed these differences. Overall, the results indicate that effective bioremediation depends on optimizing amendment concentration and compatibility (Figure 6).

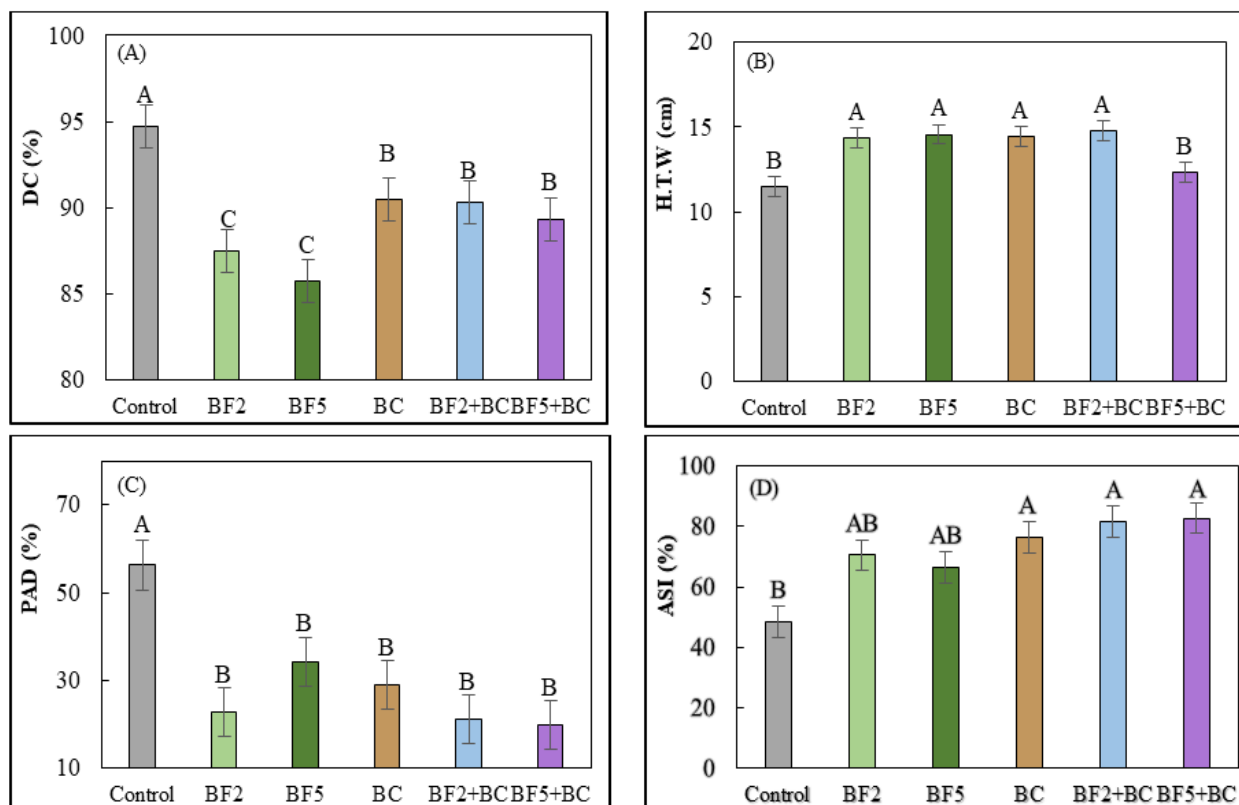


Figure 5) Effects of biochar and biofertilizer on sediment properties in petroleum-contaminated mangrove forest. (A) Dispersible clay and silt (DC). (B) Turbidimeter height in water (H.T.W). (C) Percentage Aggregate Degradation (PAD). (D) Aggregate Stability Index in water (ASI). Different letters indicate significant differences at $p < 0.05$ (Duncan's test). Control (unamended contaminated sediment); BF2 and BF5 (biofertilizer at 2% and 5% v/w); BC (biochar); BF2+BC and BF5+BC (combined biochar and biofertilizer applications).

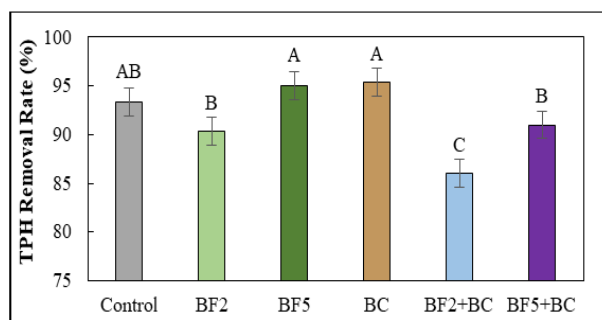


Figure 6) Effects of biochar and biofertilizer amendments on total petroleum hydrocarbon (TPH) removal efficiency in contaminated mangrove sediments.

Different letters indicate significant differences at $p < 0.05$ (Duncan's test). Control (unamended contaminated sediment); BF2 and BF5 (biofertilizer at 2% and 5% v/w); BC (biochar); BF2+BC and BF5+BC (combined biochar and biofertilizer applications).

Discussion

This study evaluated the combined effects of biochar and a *Bacillus subtilis* biofertilizer on soil physical properties and TPH degradation in diesel-contaminated *Avicennia marina* mangrove sediments. The combined amendments improved soil physical structure and enhanced TPH removal. However, because this study did not include direct measurements of microbial community composition, biomass, enzymatic activity, or extracellular polymeric substances (EPS), the mechanisms driving these improvements remain uncertain. The observed relationships should therefore be interpreted as correlational rather than mechanistic. Future studies should incorporate targeted microbial and biochemical assays to establish causal links. Additionally, the ecological thresholds of these amendments and the contribution of indigenous microbial communities warrant further investigation.

In this study, the mean weight diameter (MWD) of soil aggregates increased following biochar application. This improvement may be attributed to the porous structure and

surface chemistry of biochar, which can provide nucleation sites for the association of soil particles. The increase in MWD was more pronounced when biochar was applied together with a microbial inoculant, suggesting a synergistic effect. One plausible mechanism is that biochar retains microorganisms in the soil matrix, where their metabolic activity produces extracellular polymeric substances, such as polysaccharides, that bind particles into stable aggregates. This interpretation aligns with previous studies reporting increases in MWD after biofertilizer application [32, 33]. However, because microbial biomass, EPS production, and enzymatic activities were not quantified in the present work, the proposed biological mechanisms remain indirect and should be verified through targeted measurements. The observed enhancement in aggregate size is consistent with published evidence that biochar can promote the formation and stabilization of larger soil aggregates [34]. In this study, the combined application of biochar and microorganisms was associated with greater aggregate water stability compared to controls without amendments. One possible explanation is that biochar's porous structure, surface area, and functional groups may influence soil physicochemical properties such as pH, water retention, and cation exchange capacity. These changes could, in turn, affect microbial activity and stimulate the production of extracellular polymeric substances (EPS), which bind soil particles and enhance aggregate stability [32]. However, direct measurements of EPS, microbial biomass, enzyme activities, or microbial community composition were not conducted in this study. Therefore, the above interpretation remains speculative

and requires verification in future research. The combined application of biochar and biofertilizer was associated with notably lower clay dispersion than either amendment applied alone. Based on previous studies [32], this may be explained by biochar adsorbing exchangeable cations and microbial activity promoting the production of binding agents, such as exopolysaccharides, that coat clay surfaces. However, because cation exchange, exopolysaccharide synthesis, and microbial activity were not directly measured in this study, these mechanisms remain hypothetical.

Consistent with earlier reports that petroleum hydrocarbons can naturally suppress clay dispersion across diverse soil types [29,35,37], our results show that the biochar–biofertilizer combination further reduced dispersion relative to untreated contaminated sediments. This suggests that the combined amendments improved structural stability even under hydrocarbon stress.

From a physicochemical perspective, higher turbidimeter readings indicate lower colloidal concentration and improved solution clarity, as reduced colloidal load allows greater light penetration [28]. Our results showed that soil amendments improved the physical quality of the soil solution by reducing colloidal load. The highest light penetration depth was observed in the combined treatment of biochar with 2% biofertilizer. Although this treatment did not differ significantly from biofertilizer-only applications, the overall trend suggests that these amendments reduce the dispersion of suspended particles and increase soil solution transparency.

In addition, the addition of biochar and biofertilizer was associated with increased aggregate formation, larger aggregate

diameter, and greater aggregate stability, which corresponded to a lower percentage of aggregate breakdown. Previous studies have similarly reported that soil amendments such as biochar [32], nanomaterials, and conventional mineral amendments [26] can reduce aggregate disruption and improve soil structure.

The influence of biochar on soil structural stability in petroleum-contaminated environments varies across studies. While petroleum hydrocarbons can increase aggregate stability through hydrophobic interactions [28,34], Khademalrasoul and Mohammadi (2021b) observed a decrease in mean weight diameter (MWD) after biochar amendment in contaminated soils [36]. They proposed that biochar may alter soil physicochemical properties or interfacial interactions with petroleum residues, potentially disrupting soil aggregation. In the present study, conducted using the same A. In Marina sediment, as in our previous work [29], the combined application of biochar and a *B. subtilis*-based biofertilizer yielded higher MWD values than contamination alone. This result suggests that co-amendment was associated with improved structural stability compared to the contaminated control. However, the current data do not identify the specific mechanisms driving this improvement. Potential factors include changes in soil physical properties, microbial activity, or interfacial interactions, but direct measurements (e.g., EPS production, enzymatic activity, microbial biomass) would be required to test these hypotheses. These findings indicate that the efficacy of biochar in polluted systems should be evaluated alongside complementary amendments, such as microbial inoculants, rather than inferred from single-factor studies.

Natural attenuation in the control treatment achieved 93.5% TPH removal, demonstrating the robust degradation capacity of indigenous microorganisms under aerobic conditions. This finding aligns with earlier studies on mangrove sediments in the same region. For instance, Fakhrzadegan et al. (2015) isolated nine hydrocarbon-degrading bacterial strains from sediments near Minab and Bandar Khamir in the northern Persian Gulf. Three of these strains, identified as *Vibrio azureus* and *Pseudomonas salomonii*, removed more than 50% of crude oil within 15 days, with strain NP16 achieving the highest removal rate (66%). Together, these findings^[23] indicate that Persian Gulf mangrove sediments host native bacterial communities adapted to hydrocarbon exposure, likely due to chronic, low-level inputs from maritime activities and urban runoff.

The high removal efficiency observed in the control likely reflects three factors: (i) prior adaptation of indigenous microbes to hydrocarbons, (ii) the presence of established degrading genera such as *Vibrio* and *Pseudomonas*, and (iii) incubation conditions (temperature, moisture, and aeration) that favored aerobic metabolism. Although the 93.5% removal rate in this study exceeds the 66% reported by Fakhrzadegan et al. (2015), the difference can be attributed to variations in incubation duration, initial contaminant concentration, or site-specific microbial community composition. Overall, both studies confirm that mangrove sediments in this region possess a strong intrinsic capacity for petroleum hydrocarbon degradation. The robust natural attenuation observed here has practical implications for evaluating amendment strategies. Adding

2% biofertilizer resulted in slightly lower TPH removal than the control, suggesting competition rather than synergy between the introduced strains and the native microbial community. When indigenous degraders are already highly active, external inoculants may not enhance biodegradation rates and could temporarily suppress them. These findings indicate that natural attenuation may be a sufficient and cost-effective approach for mangrove sediments with robust native microbial communities. Future studies should identify the conditions under which biochar or biofertilizer amendments provide measurable benefits, such as in sediments with lower intrinsic degradation capacity or under field conditions with additional stressors (e.g., salinity fluctuations and tidal dynamics).

Beyond its adsorption capacity, biochar's influence in contaminated soils depends on its physicochemical properties, such as porosity, specific surface area, and surface functional groups. These characteristics facilitate pollutant retention and create protective microhabitats for microorganisms^[38,40]. Additionally, biochar can supply labile carbon, potentially sustaining microbial metabolism during hydrocarbon degradation^[40]. Consequently, beyond direct adsorption, biochar may enhance biodegradation by improving contaminant bioavailability and supporting microbial activity. However, validating these mechanisms requires direct measurements, including microbial community profiling, enzyme assays, or quantification of extracellular polymeric substances (EPS).

For context, Sani et al. (2023) studied crude oil-contaminated soil from the Kaduna Refinery in Nigeria, which initially contained approximately 2045 mg.kg⁻¹ TPH.

They amended the soil with coconut shell activated carbon (BET surface area: $567 \text{ m}^2 \cdot \text{g}^{-1}$) at rates of 1–3.5%, incubated the mixtures for 36 days at ambient temperature, and homogenized them three times daily. At the highest amendment rate (3.5%), TPH removal reached 78.14%, reducing concentrations to $447 \text{ mg} \cdot \text{kg}^{-1}$, which falls below Nigeria's regulatory threshold of $1000 \text{ mg} \cdot \text{kg}^{-1}$ [41]. In the present study, application of *Conocarpus* wood waste biochar to diesel-contaminated mangrove sediments resulted in approximately 95% TPH removal. However, direct comparison between these outcomes is not methodologically appropriate, as the studies differed in soil matrix, amendment type, initial contamination levels, incubation duration, and experimental conditions. The higher removal percentage observed here, therefore, reflects treatment and site-specific efficacy rather than inherent superiority. Nevertheless, the performance of *Conocarpus*-derived biochar in mangrove sediments indicates its potential as a locally available amendment for remediating oil-impacted coastal ecosystems. Definitive comparisons among amendment types would require standardized, side-by-side testing under identical conditions.

Despite the observed removal efficiencies, residual TPH concentrations under the most effective treatment remained at approximately $4,000 \text{ mg} \cdot \text{kg}^{-1}$ (from an initial $80,000 \text{ mg} \cdot \text{kg}^{-1}$), exceeding the Sediment Quality Guidelines upper limit of $550 \text{ mg} \cdot \text{kg}^{-1}$ [43,44]. This indicates that, under the tested conditions, the treatment did not meet regulatory standards. Although the combined application of biochar and biofertilizer accelerated TPH degradation, it was insufficient to achieve compliance in heavily contaminated mangrove sediments.

Compared with energy-intensive physical methods such as microwave-assisted desorption [45], the biological approach evaluated here offers practical advantages, including a lower risk of secondary contamination and reliance on naturally occurring microbial processes. It should be noted, however, that this study did not directly compare the two approaches, and treatment efficacy will likely vary with sediment characteristics. Within the measured parameters, the biochar-biofertilizer combination improved both TPH removal and key physical properties. These results suggest that such amendments may be useful components of integrated remediation strategies for petroleum-impacted sites. However, further work is needed to evaluate their performance across different sediment types and contamination levels.

Conclusion

This study evaluated the combined effects of *Conocarpus*-derived biochar and a *Bacillus*-based biofertilizer on total petroleum hydrocarbon (TPH) removal and the physical properties of diesel-contaminated mangrove sediments. Among all treatments, the standalone applications of 4% biochar and 5% biofertilizer each achieved 95% TPH removal, representing the highest efficiency observed. Notably, the unamended control achieved 93.5% TPH removal via natural attenuation, a result comparable to that of the amended treatments. This suggests that the indigenous microbial community in mangrove sediments is inherently capable of degrading hydrocarbons. Thus, autochthonous microorganisms appear to drive baseline pollutant degradation, a capacity that should be considered in

remediation planning. However, the highest-performing treatment still resulted in a residual TPH concentration of 4000 mg.kg⁻¹, exceeding the recommended ecological threshold of 550 mg.kg⁻¹ [43].

Regarding sediment physical properties, all biofertilizer treatments applied alone or with biochar improved structural indicators relative to the control, indicating potential utility under site-specific conditions. These results suggest that integrating biochar and biofertilizer can concurrently enhance soil structure and biological activity in contaminated coastal sediments. A limitation of this work is that exopolysaccharide (EPS) production and microbial community composition were not assessed. Future studies should incorporate microbial sequencing and EPS quantification to clarify the mechanisms driving these improvements. For coastal ecosystems such as the Persian Gulf mangroves, long-term field trials are required before field-scale implementation.

Acknowledgments

The authors gratefully acknowledge Shaghayegh Amiri Tavassoli for her valuable assistance and technical expertise in conducting the experimental work.

Authors' Contributions

Both authors contributed equally to this work. **N Moradi** and **O Bazafshan**: jointly conceived and designed the study, performed the experiments, analyzed the data, and wrote the manuscript. Both authors reviewed and approved the final version of the manuscript.

Ethical Permission

No Ethical approval was required for this study.

Funding/ Support

This research did not receive any specific grant from funding agencies in the public,

commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

Declaration Use AI

The authors have used AI (Qwen 3.6) for language editing to improve the readability of the work and Figure 1. The authors have reviewed and edited the output and take full responsibility for the publication's content.

References

1. Goveas L.C., Krishna A., Salian A., Menezes J., Alva M., Basavapattan B., Sajankila S.P. Isolation and characterization of bacteria from refinery effluent for degradation of petroleum crude oil in seawater. *J. Pure. Appl. Microbiol.* 2020; 14(1):473-84.
2. Sarfo M.K., Gyasi S.F., Kabo-Bah A.T., Adu B., Mohktar Q., Appiah A.S., Serfor-Armah Y. Isolation and characterization of crude-oil-dependent bacteria from the coast of Ghana using Oxford Nanopore sequencing. *Heliyon.* 2023; 9(2): e13075.
3. Rhykerd R., Crews B., McInnes K., Weaver R.W. Impact of bulking agents, forced aeration, and tillage on remediation of oil-contaminated soil. *Bioresour. Technol.* 1999; 67(3):279-85.
4. Li Y., Li F.M., Li F.X., Yuan F.Q., Wei P.F. Effect of the ultrasound-Fenton oxidation process with the addition of a chelating agent on the removal of petroleum-based contaminants from soil. *Environ. Sci. Pollut. Res.* 2015; 22(23):18446-18455.
5. Liu S., Zhu L.M.L., Jiang W.C., Qin J.Z., Lee H.S. Research on the effects of soil petroleum pollution concentration on the diversity of natural plant communities along the coastline of Jiaozhou Bay. *Environ. Res.* 2021; 197:111127.
6. Novakovskiy A.B., Kanev V.A., Markarova M.Y. Long-term dynamics of plant communities after biological remediation of oil-contaminated soils in the far north. *Sci. Rep.* 2021; 11(1):4888.
7. Li H.S., Sun H., Wang X.P., Li F.J., Cao L.X., Li Y., Dong R., Sun Y., Sun P., Bao M. Temporal and spatial variation of petroleum hydrocarbons and microbial communities during static release of oil pollution sediments. *Front. Mar. Sci.* 2022; 9(1):1025612.
8. Kuppusamy S., Thavamani P., Venkateswarlu K., Lee Y.B., Naidu R., Megharaj M. Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future

- directions. *Chemosphere*. 2017; 168(1):944-968.
9. Lominchar M.A., Santos A., de Miguel E., Romero A. Remediation of aged diesel-contaminated soil by alkaline-activated persulfate. *Sci. Total Environ*. 2018; 622(1): 41-48.
 10. Ren H.Y., Wei Z.J., Wang Y., Deng Y.P., Li M.Y., Wang B. Effects of biochar properties on the bioremediation of the petroleum-contaminated soil from a shale-gas field. *Environ. Sci. Pollut. Res*. 2020; 27(29): 36427-36438.
 11. Chen Z.B., Reiche N., Vymazal J., Kuschik P. Treatment of water contaminated by volatile organic compounds in hydroponic root mats. *Ecologic. Engineer*. 2017; 98(1):339-345.
 12. Fahid M., Arslan M., Shabir G., Younus S., Yasmeen T., Rizwan M., Siddiqe K., Ahmad S.R., Tahseen R., Iqbal S., Ali S., Afzal M. *Phragmites australis*, in combination with hydrocarbon-degrading bacteria, is a suitable option for the remediation of diesel-contaminated water in floating wetlands. *Chemosphere*. 2020; 240(1):124890.
 13. Bhatt P., Gangola S., Bhandari G., Zhang W.P., Maithani D., Mishra S., Chen S. New insights into the degradation of synthetic pollutants in contaminated environments. *Chemosphere*. 2020; 268(1):128827.
 14. Agarry S.E., Owabor C.N., Yusuf R.O. Studies on biodegradation of kerosene in soil under different bioremediation strategies. *Bioremedia. J*. 2010; 14(3): 135-141.
 15. Nwankwegu A.S., Onwosi C.O. Bioremediation of gasoline-contaminated agricultural soil by bioaugmentation. *Environ. Technol, Innov*. 2017; 7(1):1-11.
 16. Varjani S.J. Microbial degradation of petroleum hydrocarbons. *Bioresour. Technol*. 2017; 223(1): 277-286.
 17. Xu X.J., Liu W.M., Tian S.H., Wang W., Qi Q.G., 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(1):2885.
 18. Zhu X.M., Chen B.L., Zhu L.Z., Xing B.S. Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: A review. *Environment. Pollut*. 2017; 227(1): 98-115.
 19. Dike C.C., Hakeem I.G., Rani A., Surapaneni A., Khudur L., Shah K, Ball A.S. The co-application of biochar with bioremediation for the removal of petroleum hydrocarbons from contaminated soil. *Sci. Total. Environ*. 2022; 849(1):157753.
 20. Tomczyk A., Sokołowska Z., Boguta P. Biochar physicochemical properties: Pyrolysis temperature and feedstock kind effects. *Review. Environment. Sci. Bio/Technol*; 19(1):191-215.
 21. Khademalrasoul A., Mohammadi N. Effects of biochar and zeoplant on physical properties of soils contaminated with total petroleum hydrocarbons (TPHs). *Appl. Soil. Res*. 2021a; 9(1): 14-27. (In Persian).
 22. Fang X., Zhang M., Zheng P., Wang H., Wang K., Lv J., Shi F. Biochar-bacteria-plant combined potential for remediation of oil-contaminated soil. *Front. Microbiol*. 2024; 15(1):1343366.
 23. Fakhrzadegan I., Hassanshahian M., Askari M. Isolation and identification of crude oil-degrading bacteria from the mangrove forests ecosystem in Khamir and Minab ports, north of the Persian Gulf. *Aquat. Ecol*. 2015; 5(3): 70-79. (in Persian).
 24. Joo M.H., Kim J.Y. Characteristics of crude oil biodegradation by biosurfactant-producing bacterium *Bacillus subtilis* JK-1. *J. Korean Soc. Appl. Biol. Chem*. 2013; 56(2), 193-200.
 25. Middleton H.E. Properties of soil that influence soil erosion. *USDA. Tech. Bull*. 1930; 178(1): 1-6.
 26. Kemper W.D., Rosena R.C. Aggregate stability and size distribution. In: Klute A, editor. *Methods of soil analysis. Part 1. Physical analysis*. Madison: SSSA; 1986. p. 425-42.
 27. Moradi N., Emami H., Astarai A.R., Fotovat A., Ghahraman B. The effect of nanoparticles of aluminum oxide and silicon oxide on soil structural stability indicators. *J. Water. Soil. Conserv*. 2016; 23(5):253-265. (In Persian).
 28. Wang F., Tang Y.A., Zhang J.S., Gao P.C, Coffie J.N. Effects of various organic materials on soil aggregate stability and soil microbiological properties on the Loess Plateau of China. *Plant Soil. Environ*. 2013; 59(4):162-168.
 29. Amiri Tavassoli S., Moradi N., Bazrafshan O. Assessing the impact of petroleum pollutants on the physical properties of mangrove forest sediments. *Environ. Eros. Res*. 2025; 14(4): 80-95. (In Persian).
 30. Saadati N., Davatgar N., Roodpeyma M., Mosaddeghi M.R., Bostani A. Evaluation of petroleum contamination effects on soil water repellency intensity in Bakhtiardasht of Isfahan. *Iran. J. Soil. Res*. 2015; 29(3): 359-369.
 31. Song Y.F., Jing X., Fleischmann S., Wilke B.M. Comparative study of extraction methods for the determination of PAHs from contaminated soils and sediments. *Chemosphere*. 2002; 48(10): 993-1001.
 32. Gul S., Whalen J.K., Thomas B.W., Sachdeva V., Deng H. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. *Agric. Ecosyst. Environ*. 2015; 206(1): 46-59.
 33. Samaei F., Asghari S., Aliasgharzad N. The effects of two arbuscular mycorrhizal fungi on some physical properties of a sandy loam soil and nutrient uptake by spring barley. *Soil. Environ*.

- 2015; 34(1): 1-9.
34. Moshtagh R., Moradi N., Gholami H. Investigation of the role of biochar from eggplant plant residues and shrimp waste on some soil stability characteristics. *Environ. Eros. Res.* 2022; 12(1): 1-17. (In Persian). URL:
35. Kermanpour M., Mosaddeghi M.R., Afyuni M., Hajabassi M A. Effect of Petroleum Pollution on Soil Water Repellency and Structural Stability in Bakhtiardasht Plain, Isfahan. *J. Water. Soil. Sci.* 2015; 73(19): 139-149. (In Persian).
36. Khademalrasoul A., Mohammadi N. Assessment of Zeoplant and biochar of sugarcane residual on mean weight diameter and Atterberg limits of soil contaminated with total petroleum hydrocarbon. *Iran. J. Soil. Water. Res.* 2021b; 52(2): 395-407.
37. Daryaei R., Moosavi S.A.A., Ghasemi R., Riazzi M. Evaluation of aggregate stability as influenced by petroleum compounds in texturally different soils. *J. Water. Soil. Conserv.* 2022; 29(2): 25-45.
38. Beesley L., Moreno-Jiménez E., Gomez-Eyles J.L., Harris E., Robinson B., Sizmur T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* 2011; 159(12): 3269-82.
39. Lehmann J., Rillig M.C., Thies J., Masiello C.A., Hockaday W.C., Crowley D. Biochar effects on soil biota – A review. *Soil. Biol. Biochem.* 2011; 43(9): 1812-1836.
40. Cheng J.M., Sun Z.H., Yu Y.Q., Li X.R., Li T.T. Effects of modified carbon black nanoparticles on plant-microbe remediation of petroleum and heavy metal co-contaminated soils. *Int. J. Phytoremediat.* 2019; 21(7):634-42.
41. Sani J.E, Moses G, Musa S. Physicochemical evaluation of coconut shell biochar remediation effect on crude oil contaminated soil. *Cogent. Eng.* 2023; 10(2): 2269659.
42. Department of Petroleum Resources. Environmental guidelines and standards for the petroleum industry in Nigeria (EGASPIN). Vol 2. Lagos: Author; 2002: 320 p.
43. Simpson S.L, Batley G.E, Chariton A.A. Revision of the ANZECC/ARMCANZ sediment quality guidelines. CSIRO Land Water. 2013; Report No: 8/07
44. Soleimani M., Jaberi N. Optimization of removal process of petroleum hydrocarbons from soil using microwave radiation and its effect on some soil biological characteristics. *Iran. J. Soil. Water. Res.* 2017; 48(2): 397-404.
45. Van Hamme J.D., Singh A., Ward O.P. Recent advances in petroleum microbiology. *Microbiol. Mol. Biol. Rev.* 2003; 67(4):503-549.