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The potential of organic wastes in eliminating old-aged petroleum pollution in saline soils: A case study in Khuzestan province

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ABSTRACT

Petroleum contains carcinogens and toxic substances that can have an unfavorable impact on environmental quality and human health when the soil becomes contaminated with crude oil. BBiostimulation and bioaugmentation are the main strategies in the bioremediation of oil-contaminated soils. To decompose old-aged petroleum pollution in saline soil, a full factorial experiment was utilized. The experiment was designed using a completely randomized design with four factors: bacterial inoculum, sugarcane bagasse, chemical fertilizer, and molasses. The application of these factors was conducted in four separate experiments: pretreatment of agricultural soil and spent mushroom compost, pretreatment of spent mushroom compost, pretreatment of agricultural soil, and no pretreatment. After a 60-day incubation period at 28 ˚C, the results showed that the organic wastes of molasses and spent mushroom compost in combination with bacterial inoculum reduced total petroleum hydrocarbons 38 and 33.3%, respectively. Molasses had a considerable impact on increasing the efficiency of bacterial inoculum 1 and bacterial inoculum 3. Similarly, spent mushroom compost was found to significantly affect bacterial inoculum 1. In addition, bagasse was observed to accelerate the bioremediation process by improving the physical conditions of the soil. In the pretreatment of agricultural soil, bagasse in combination with bacterial inoculum 1 and chemical fertilizer reduced the total petroleum hydrocarbons significantly (38%) compared to the control treatment. These results highlight the effectiveness of organic wastes as biostimulation agents in promoting the growth and reproduction of the soil microbial community, as well as establishing the bacterial inoculum.

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INTRODUCTION

The oil industry plays an important role in the global energy supply and economy. However, the extraction, transportation, and other activities related to petroleum products can lead to their release into the soil environment, causing damage to agricultural, residential, or recreational land, and water resources. Iran, with over 100 years of oil drilling history and more than 20,000 km of pipelines, faces critical issues with soil pollution (Gitipour et al., 2015; Khudur et al., 2018). Petroleum compounds accumulate in the soil and are released into the atmosphere and water resources, disrupting the biological, hydrological, and ecological functions of the soil and the food chain (Hewelke et al., 2018; Chen et al., 2020). Such pollution poses a serious threat to the environment and human health, making it imperative to take appropriate measures to mitigate the risks and address the issue of soil contamination in Iran (Gitipour et al., 2015).

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The decomposition of petroleum compounds in the soil presents significant challenges due to their hydrophobicity, structural complexity, low bioavailability, and multiphase nature (Speight et al., 2012; Biswas et al., 2017). Particularly in the bioremediation of old-pollution soils, the reduced bioavailability of pollutants and the inability of the microbial community to break them down are significant hurdles. It is important to note that while pollutants in most artificially contaminated soils or those that have not been polluted for a long time can significantly decompose during bioremediation, this is not always observed in soils with long-term pollution (Lladó et al., 2013). This is because the bioavailability of pollutants decreases with time in soil contamination, making it increasingly difficult to eliminate pollution in soils that have been contaminated for extended periods (Garousin et al., 2021). Therefore, bioremediation of old-age contaminated soils poses significant challenges, and innovative approaches and technologies may be required to address this problem.

Some soil characteristics, such as salinity, can also restrict the potential of soil remediation. Because the high concentration of salt in the soil can be fatal for many microorganisms (Ravanipour et al., 2015). Soil salinity can negatively impact the supply of oxygen and hydrocarbon to the soil (Kalami et al., 2021), reducing the amount of biomass, activity, and diversity of the soil microbial community due to osmotic and ionic stresses. Consequently, the biological decomposition of pollutants is significantly disrupted (Azadi et al., 2021). However, some salt-tolerant bacteria have developed different strategies to survive under environmental stress, such as assembling compatible osmolytes to maintain intracellular osmotic balance (Etesami et al., 2018). Moreover, some microorganisms are halophiles and must thrive in high osmotic pressure. For instance, the aerobic and gram-positive *Bacillus zhangzhouensis* bacterium can survive in salinity ranging from zero to 12% NaCl with optimal conditions between 1-3% (Liu et al., 2016).

Since the 1970s, numerous methods for soil remediation have been proposed, which can be broadly categorized into three groups: physical, chemical, and biological (Visentin et al., 2019; Lu et al., 2019) (Fig. 1).

While physical and chemical methods are fast and effective, especially in highly polluted environments, they can be expensive and environmentally hazardous (Abdollahinejad et al., 2020). Bioremediation is generally preferred over the other two methods due to its cost-effectiveness and environmental friendliness (Feng et al., 2021). In bioremediation, bioaugmentation and biostimulation strategies are commonly employed (Ossai et al., 2020). Depending on the soil conditions and equipment, they can be used alone, in combination, or conjunction with non-biological methods.

Bioaugmentation involves adding microbial culture to enhance the existing populations and promote the biodegradation of pollutants. The microbial culture can be prepared from exogenous bacteria, the autochthonous soil microbial community, or genetically engineered microbes (Nwankwegu et al., 2017; Poi et al., 2017). Recent studies have identified more than 79 bacterial genera including *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Arthrobacter*, *Pseudomonas*, *Rhodococcus*, and *Mycobacterium* that can degrade petroleum hydrocarbons (Tremblay et al., 2017; Xu et al., 2018). However, the vast potential of native bacterial strains for pollutant degradation is still unknown.

Biostimulation increases the growth and reproduction of the microbial community of contaminated soil by adding chemical fertilizers, bio-surfactants, bulking agents, oxygen, or electron donors to degrade pollutants (Lim et al., 2016; Wu et al., 2017). Biostimulation agents enhance the compatibility of bacteria in overcoming bioremediation limitations and promote pollutant decomposition in bioremediation projects (Bodor et al., 2020).

Biostimulation and bioaugmentation may not always be efficient due to the low activity of microorganisms and the low availability of pollutants (Kalami et al., 2021). Therefore, it is necessary to enhance pollutant bioavailability and microbial bioactivities in oil-contaminated soils. There are different ways to achieve this, such as strengthening the microbial community

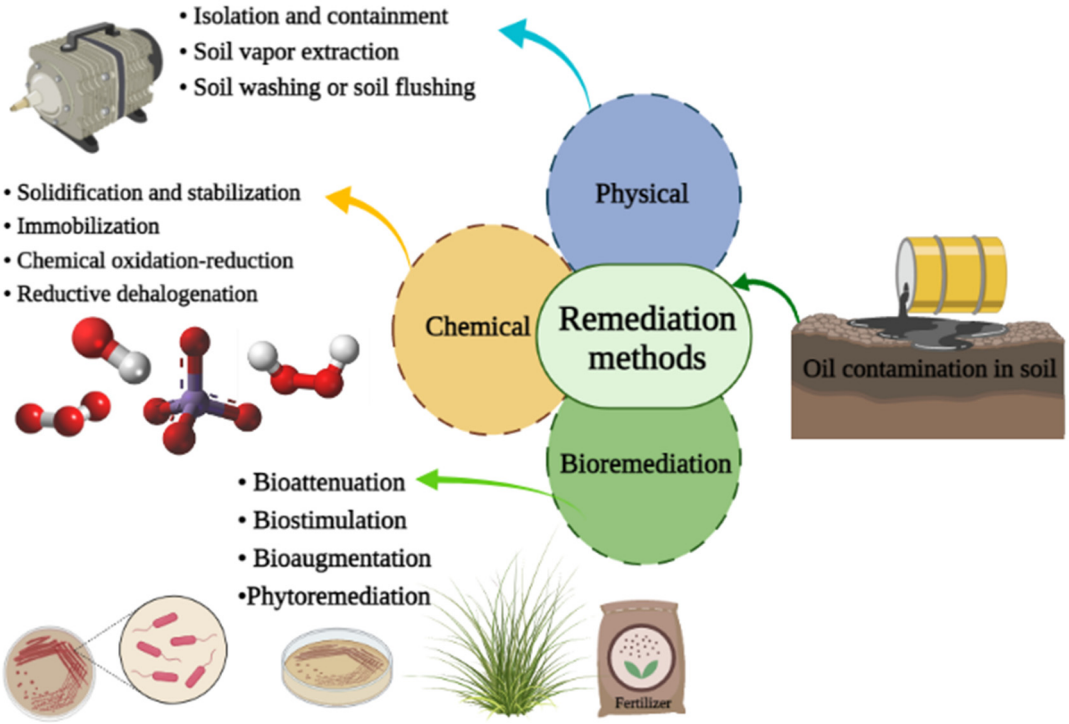

Fig. 1. Remediation methods of oil-contaminated soil(Ossai et al., 2020)

with lignocellulosic materials in old-contaminated soil containing creosote, which can significantly increase the biodegradation of polycyclic aromatic hydrocarbons with high molecular weight (Lladó et al., 2013). Generally, organic wastes are nutrient-rich and have a high potential for bioremediation of oil-polluted soils by promoting bacterial growth (Hoang et al., 2021). Furthermore, adding organic carbon sources to saline soils can improve enzyme activity and soil biological function (Azadi et al., 2021).

Various types of organic wastes such as plant residues, green manures, animal manures, biosolids, composted organic materials, and biochar have been successfully used to restore oil-contaminated soil (Hoang et al., 2021). The organic waste type affects the decomposition rate of diesel fuel. The selection of efficient organic waste can be a beneficial alternative to chemical fertilizers (Horel et al., 2015). Researchers have investigated the potential of various organic wastes for the bioremediation of oil-contaminated soils. Alvim and Pontes (2018) showed that Sawdust and Simultaneous aeration had a significant effect on improved ventilation and diesel oil degradation. Additionally, adding digested sewage sludge had more potential for aliphatic hydrocarbon degradation in comparison with chemical fertilizer (Gielnik et al., 2021). Rice husk, as a bulking agent, with the suitable microbial consortium and aeration under a biopile system can remediate oil-polluted soil (Zhang et al., 2021). Goat manure compost contains a valuable microbial population that can help degrade crude oil in the soil. It is also a nutrient resource and a biostimulation agent with high potential in the bioremediation of total petroleum hydrocarbons (Ani et al., 2021).

In Khuzestan province, Iran, substantial quantities of oil-polluted soils have piled up and are exposed to the environment and the local community. These soils have a long history of pollution, spanning over sixty years, and have very high salinity levels, which pose significant challenges to their restoration. In this oil-rich province, the Haft Tappeh sugarcane factory produces sugar products from sugarcane. As a byproduct of sugar production, the factory generates more than two million tons of molasses and bagasse annually. About 75% of molasses

is simple carbohydrates. Other nutrients, such as potassium, magnesium, and calcium, are abundant in molasses (USDA, 2019). Additionally, about 700 to 800 thousand tons of mushroom compost is generated annually when producing edible mushrooms in Iran (https://zaya.io/sy1ka). The mushroom composts are rich in protein, cellulose, amino acids, mycelium, and bacteria, making them valuable sources of nutrition (Aona et al., 2017). Despite their nutritional value, the enormous quantities of these organic wastes in Iran are mainly disposed of, causing severe environmental concerns (Mohammadi et al., 2020). However, given their potential, these organic wastes can be utilized in oil-contaminated soil bioremediation to develop a circular bioeconomy in the study area.

Based on the issues raised, there are limitations in the remediation of oil-contaminated soil, especially in saline and old-aged polluted soil. Native bacterial strains have the potential to overcome soil limitations in bioremediation projects. Additionally, there is an opportunity to implement a circular bioeconomy in the study area. Therefore, various bioremediation methods were investigated within the framework of biostimulation and bioaugmentation strategies using industrial and agricultural organic waste and native bacterial strains.

MATERIALS AND METHODS

Contaminated soil characteristics

The study site is located at 31° 22′ 27″ N and 48° 42′ 01″ E around the Ahvaz oilfield in Khuzestan province, Iran, and has a long history of crude-oil pollution spanning over sixty years. Soil samples were collected from five points at specific distances within a contaminated area of 100 m^2 and a depth of 0 ~ 20 cm. Then, these contaminated soil samples were mixed and transported to the laboratory under sterile conditions. At first, some physical and chemical soil properties were measured (Table 1).

To determine the texture of oil-contaminated soil, the Gee and Bauder hydrometer method, which involves the analysis of soil particles in a sedimentation cylinder, was used (Gee et al., 1986). The soil pH using a pH meter (Thomas, 1996) and the electrical conductivity (EC) using a conductivity meter (Rhoads, 1996) were measured. The sodium adsorption ratio (SAR) was calculated using sodium, calcium, and magnesium ions concentrations in the soil extract (Sparks, 1996). The Total Petroleum Hydrocarbon (TPH) content of oil-contaminated soil was measured by the spectrophotometry method at 420 nm (Song et al., 2002; Adesodun et al., 2008). Also, the organic carbon was determined using the oxidation of the organic carbon in the soil with a mixture of sulfuric acid and potassium dichromate, followed by titration with ferrous ammonium sulfate (Olsen et al., 1982). The total nitrogen through the soil digestion with sulfuric acid (Bremner, 1996) and the available phosphorus through the soil extraction with a solution of ammonium fluoride and hydrochloric acid, followed by the measurement of

Table 1. Basic properties of the studied soil

Variable	Unit	soil
Sand	%	66
Silt	%	24
Clay	%	10
Texture	-	Sandy loam
EC	$dS.m^{-1}$	80.6
pH	-	7.52
SAR	$(mmol.l^{-1})^{0.5}$	183.3
Total nitrogen	%	0.261
Organic carbon	%	8.77
Available phosphorus	%	0.0025
C:N:P	-	336:10:1
TPH	$mg.kg^{-1}$	82895

Table 2. The accession number of strains in GenBank and the closest phylogenetic similarity

Strain	Similarity (%)	Closest phylogenetic similarity	Accession number
BK111	99.71	*Bacillus zhangzhouensis*	MF540569
SNP5	95.49	*Rhodococcus fascians* LMG3623	OL759129
Q-SH1	99.80	*Halobacillus dabanensis* D-8(T)	JQ305105.1
Q-SH3	99.16	*Acidovorax delafieldii* AF078764	KC178692
Q-SH14	98.67	*Bacillus rhizosphaerae* FJ233848	KC178693

phosphorus content by colorimetric analysis (Nelson et al., 1996), was determined.

Experimental description

To determine the most efficient bioremediation methods, four factors were applied to the contaminated soils, including bacterial inoculum (In), chemical fertilizer (CF), sugarcane bagasse (B), and molasses (Mo).

Bacterial inoculum (In) factor comprised four levels: no bacterial inoculum (In_0), BK111 (In_1), SNP5 (In_2), and a blend of Q-SH1, Q-SH3, and Q-SH14 strains (In_3) (Table 2). The BK111 strain was isolated from slaughterhouse sewage by FMA (Feather meal agar) medium (Korouzhdehi et al., 2018). SNP5 strain was isolated from rhizosphere of *Sparganium erectum* L. grown in textile effluent contaminated sites, using MSM (Mineral salt medium) containing C.B. Red EB and T. Red 3BL-01 (Nikkhah et al., 2023). Additionally, Q-SH1, Q-SH3, and Q-SH14 strains were isolated from petroleum-contaminated saline and sodic soils in the oil and gas exploitation area of Sarajeh, Qom, central Iran, using modified 6SW-Vit-agar medium (Pourbabaee et al., 2019).

To perform molecular identification, the 16S rRNA gene was amplified using the universal bacterial primers 27F and 1492R. After amplification, the purified PCR products were sequenced by Macrogen Company in South Korea, using the ABI system 3730XL. This system is a capillary electrophoresis platform that allows for accurate and reliable sequencing of DNA fragments. The identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarity was achieved using the EzTaxon-e server. This server is a bioinformatics tool that can identify bacterial species based on 16S rRNA gene sequences (Korouzhdehi et al., 2018; Pourbabaee et al., 2019).

The capability of these bacteria to decompose complex and resistant petroleum compounds has been demonstrated (Yam et al., 2010; Korouzhdehi et al., 2018; Levy-Booth et al., 2019; Pourbabaee et al., 2019), and therefore, they were chosen for this study. The selected strains were inoculated into the nutrient broth medium (Bridson et al., 1970) and incubated in the fermenter at 30 ˚C for 16 hours. Then, based on the experimental design, the strains were inoculated into the treatments at a rate of 10% (Wu et al., 2012).

Chemical fertilizer (CF) was defined at two levels, consisting of no chemical fertilizer (CF_0) and the addition of nitrogen and phosphorus (CF_1) that were required. Nitrogen and phosphorus were supplied from urea and triple superphosphate, respectively, for the chemical fertilizer (CF) factor. The C:N:P ratio was modified and adjusted to a ratio of 100:10:1 (Martínez Álvarez et al., 2015), and 1.83% urea and 0.42% triple superphosphate were applied to the soil.

The study used organic wastes from the Haft Tappeh sugarcane factory near the Iranian city of Shush in oil-rich Khuzestan Province. Two levels of sugarcane bagasse (B) factor were considered: without (B_0) and with 5% sugarcane bagasse (B_1). The sugarcane bagasse was in the oven at 100 °C for 24 hours and then crushed to a size of 2 mm for use (Hamzah et al., 2014). The molasses (Mo) factor levels included without (Mo_0) and with 0.5% v/w molasses (Mo_1). Additionally, a 5% v/v solution of the molasses was prepared for application to treatments (Sutigoolabud et al., 2005).

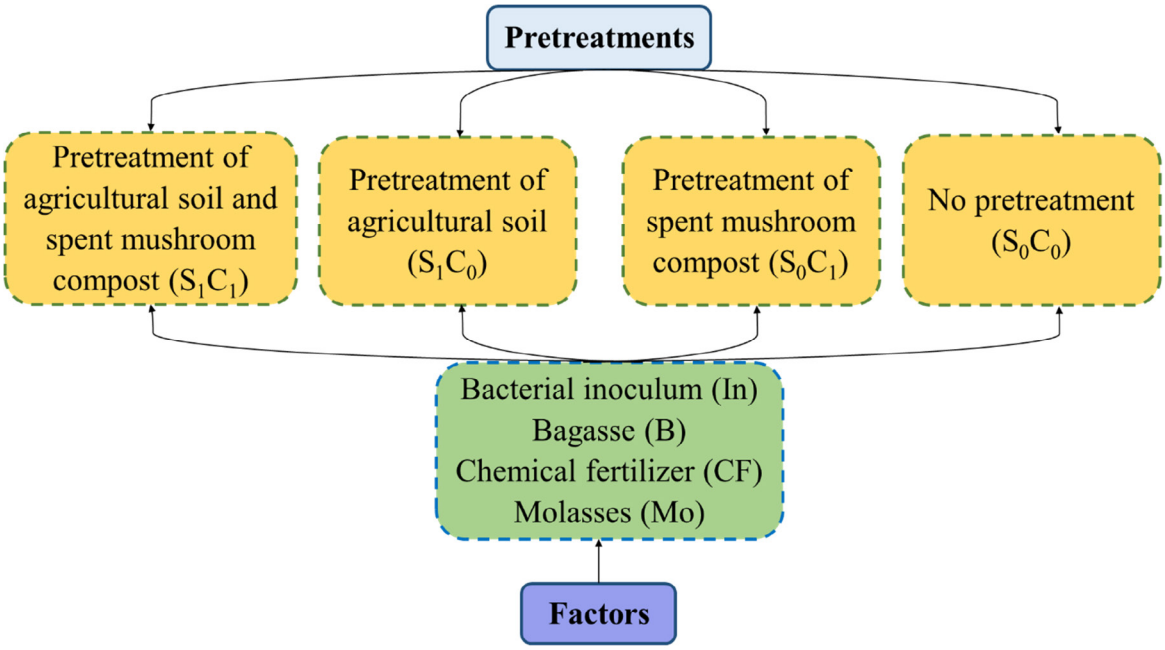

Fig. 2. Experimental Scheme to investigate the effect of bacterial inoculum (In), bagasse (B), chemical fertilizer (CF), and molasses (Mo) on the elimination of total petroleum hydrocarbons (TPH) in an old-aged oil-polluted saline soil in different pretreatments.

Table 3. The treatment combinations for eliminating TPH in each pretreatment (n = 3).

Factors			In_0	In_1	In_2	In_3
B_0	CF_0	Mo_0	$In_0B_0CF_0Mo_0$	$In_1B_0CF_0Mo_0$	$In_2B_0CF_0Mo_0$	$In_3B_0CF_0Mo_0$
		Mo_1	$In_0B_0CF_0Mo_1$	$In_1B_0CF_0Mo_1$	$In_2B_0CF_0Mo_1$	$In_3B_0CF_0Mo_1$
	CF_1	Mo_0	$In_0B_0CF_1Mo_0$	$In_1B_0CF_1Mo_0$	$In_2B_0CF_1Mo_0$	$In_3B_0CF_1Mo_0$
		Mo_1	$In_0B_0CF_1Mo_1$	$In_1B_0CF_1Mo_1$	$In_2B_0CF_1Mo_1$	$In_3B_0CF_1Mo_1$
B_1	CF_0	Mo_0	$In_0B_1CF_0Mo_0$	$In_1B_1CF_0Mo_0$	$In_2B_1CF_0Mo_0$	$In_3B_1CF_0Mo_0$
		Mo_1	$In_0B_1CF_0Mo_1$	$In_1B_1CF_0Mo_1$	$In_2B_1CF_0Mo_1$	$In_3B_1CF_0Mo_1$
	CF_1	Mo_0	$In_0B_1CF_1Mo_0$	$In_1B_1CF_1Mo_0$	$In_2B_1CF_1Mo_0$	$In_3B_1CF_1Mo_0$
		Mo_1	$In_0B_1CF_1Mo_1$	$In_1B_1CF_1Mo_1$	$In_2B_1CF_1Mo_1$	$In_3B_1CF_1Mo_1$

In_0 no bacterial inoculum; In_1 *Bacillus zhangzhouensis*; In_2 *Rhodococcus fascians* LMG3623; In_3 blend of *Halobacillus dabanensis* D-8(T), *Acidovorax delafieldii* AF078764, and *Bacillus rhizosphaerae* FJ233848; B_0 without sugarcane bagasse; B_1 with 5% sugarcane bagasse; CF_0 no chemical fertilizer; CF_1 adding nitrogen and phosphorus fertilizers; Mo_0 without molasses; Mo_1 with 0.5% v/w molasses.

All the factors mentioned above were applied under four different pretreatment conditions: (1) no pretreatment (S_0C_0), (2) pretreatment of spent mushroom compost (S_0C_1), pretreatment of agricultural soil (S_1C_0), and pretreatment of agricultural soil and spent mushroom compost (S_1C_1). In the S_1C_1, S_1C_0 and S_0C_1 pretreatment conditions, polluted soil was amended with 10% an equal combination of agricultural soil and spent mushroom compost, 5% agricultural soil, and 5% spent mushroom compost, respectively. The S_0C_0 pretreatment condition was left un-amended.

The agricultural soil used in the study contained 0.157% total nitrogen (Bremner, 1996) and 0.0015% available phosphorus (Olsen et al., 1982). This soil was sampled from the agricultural soils of the College of Agriculture and Natural Resources Campus at University of Tehran. After air-drying, it was passed through a 2 mm sieve before being applied to treatments. The spent mushroom compost was collected from edible mushroom cultivation centers and contained 63.8% organic matter (Tandon, 2005) and 62.3% water content (Estefan, 2013). Additionally, it had a pH of 6.5 (Sundberg et al., 2004).

Experimental implementation

The experimental setup is illustrated in Figure 2. Experiments were conducted as a 4×2×2×2 full factorial experiment using a completely randomized design with three replications for each treatment combination. Thus, 32 treatment combinations were present for each pretreatment condition (Table 3). The old-polluted soil and associated factors were thoroughly mixed to ensure even distribution within the contaminated soil matrix for the experiment. The experimental units were incubated at 25 ˚C for 60 days, and tap water was added to adjust the soil moisture to approximately 70% of water holding capacity during the incubation period.

The total petroleum hydrocarbons (TPH) content of oil-polluted soil was determined after the incubation. For this purpose, a solvent extraction method was used. Specifically, 20 ml of a mixture of dichloromethane and acetone with a 1:1 ratio was added to 10 grams of soil. The mixture was shaken for 30 minutes to ensure thorough extraction of the TPHs from the soil. After shaking, the mixture was allowed to settle, and the solvent layer was separated from the soil using filter paper. Finally, the TPH content in the soil extract was measured using spectrophotometry at 420 nm (Song et al., 2002; Adesodun et al., 2008).

Statistical analysis

The data were analyzed statistically to determine the effects of independent factors (In, B, Mi, and Mo) and their interactions using the GLM procedure through a four-way analysis of variance (ANOVA), after the testing for normality of distribution and homogeneity of variance in Minitab 16 software. Additionally, the means (n=3) were compared to determine significant differences through Tukey's test. Finally, the partial effect size index (Eta^2_p) was calculated to determine the variance contribution of each factor and their interaction. It ranges from 0 to 1, with higher values indicating a stronger effect of the factor on the dependent variable (Tabachnick et al., 2012):

$$\mathrm{Eta}_{\mathrm{p}}^{2} = \frac{\mathrm{SS}_{\mathrm{effect}}}{\mathrm{SS}_{\mathrm{effect}}+\mathrm{SS}_{\mathrm{error}}}$$

where Eta^2_p is the partial effect size; SS_{effect} is the sum of squares for the effect of interest; and SS_{error} is the sum of squares for error term associated with the effect (Tabachnick et al., 2012).

RESULTS AND DISCUSSION

Four experiments, namely S_0C_0, S_0C_1, S_1C_0, and S_1C_1, were designed as pretreatments to determine the most effective TPH bioremediation methods. S_0C_0 represented no pretreatment, S_0C_1 for pretreatment of spent mushroom compost, S_1C_0 for pretreatment of agricultural soil, and S_1C_1 for pretreatment of agricultural soil and spent mushroom compost. Based on the experimental scheme, four factors, bacterial inoculum (In), chemical fertilizer (CF), bagasse (B), and molasses (Mo), were added to each experimental unit. Finally, after the measurement of TPH, the data were analyzed statistically.

The results of the ANOVA for the oil-contaminated soil bioremediation treatments are presented in Table 3. In the S_0C_0 pretreatment, bacterial inoculation ($p < 0.001$), bagasse ($p < 0.01$), chemical fertilizer ($p < 0.05$), and molasses ($p < 0.001$) had significant effects on the TPH levels in the soil (Table 3). Among all the factors, bacterial inoculation had the most significant effect in reducing TPH with the highest partial effect size parameter (Eta^2_p). After that, molasses, bagasse, and chemical fertilizer were also found to be efficient in reducing the TPH levels in the soil, respectively (Table 3).

In the S_0C_0, the mean values of treatments were compared and are shown in Table 4. In_1 alone did not have a significant effect on TPH reduction, but the combination of In_1 and

Table 3. Mean square values of ANOVA in four experiments for the effect of independent factors (bacterial inoculum (In), bagasse (B), chemical fertilizer (CF), molasses (Mo), and their interaction) on TPH in an old-polluted soil by crude oil.

Source	df	Mean square			
		S_0C_0	S_0C_1	S_1C_0	S_1C_1
In	3	131373372 (0.29)***	206928481 (0.43)***	228151980 (0.47)***	59339088 (0.19)**
B	1	156657873 (0.14)**	235672853 (0.22)***	218157009 (0.23)***	131850768 (0.15)**
CF	1	75684029 (0.07)*	48520090 (0.05)ns	119225121 (0.14)**	124439609 (0.14)**
Mo	1	375889198 (0.28)***	18629563 (0.02)ns	212144060 (0.22)***	4368788 (0.006)ns
In×B	3	181858421 (0.36)***	67563966 (0.20)**	36622724 (0.12)*	24275394 (0.09)ns
In×CF	3	114872879 (0.26)***	51468370 (0.16)*	115042021 (0.31)***	212505746 (0.46)***
In×Mo	3	345629973 (0.51)***	43470489 (0.13)*	185402596 (0.42)***	83527652 (0.25)***
B×CF	1	404335856 (0.29)***	282617 (0.0003)ns	223488640 (0.23)***	17581265 (0.02)ns
B×Mo	1	218896083 (0.18)***	79350720 (0.09)*	92464355 (0.11)**	815509 (0.001)ns
CF×Mo	1	648280887 (0.40)***	11813 (0.00001)ns	62814738 (0.08)*	22619594 (0.03)ns
In×B×CF	3	9086039 (0.03)ns	93653796 (0.25)***	29277462 (0.10)ns	155759743 (0.39)***
In×B×Mo	3	131245503 (0.28)***	86497300 (0.24)**	72879399 (0.22)**	137557582 (0.36)***
In×CF×Mo	3	70615380 (0.18)**	69032010 (0.20)**	112588810 (0.31)***	51038009 (0.17)**
B×CF×Mo	1	241320792 (0.20)***	138648051 (0.14)**	987235 (0.001)ns	95890933 (0.11)**
In×B×CF×Mo	3	66280651 (0.17)**	256886750 (0.48)***	108540871 (0.30)***	78106082 (0.24)**
Error	64	15367233	12989842	11829730	11577537
C.V. (%)	-	12.5	10.6	11.1	10.4
R^2	-	76.7	69.0	74.2	70.0

S_0C_0 no pretreatment; S_0C_1 pretreatment of spent mushroom compost; S_1C_0 pretreatment of agricultural soil; S_1C_1 pretreatment of agricultural soil and spent mushroom compost. In: bacterial inoculum; B: sugarcane bagasse; CF: chemical fertilizer; Mo: molasses.
Values in parentheses represent effect size (the partial Eta^2) for each source of variability.
ns: not significant.
df: degree of freedom.
C.V.: coefficient of variation.
* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

molasses resulted in 38% TPH elimination compared to the control treatment. Meanwhile, the factor of molasses alone reduced only 19% of soil TPH. In_1 and chemical fertilizer did not have a significant effect, but In_1 and bagasse diminished 25% of TPH compared to the control treatment. However, bagasse alone did not have a significant impact (Table 4). In_2 reduced 20% of the soil TPH compared to the control treatment, but the TPH elimination rate for the combination of In_2 and Mo was 29%, for In_2 and B was 25.4%, and for treatment of In_2, B, and CF was 26.2% (Table 4). In_3 did not significantly remove TPH, but the treatment of In_3 and Mo eliminated 31.2%, and the combination of In_3, B, and Mo reduced 26% of the soil TPH compared to the control treatment.

In_1 and In_3, when used alone, had no significant effect on TPH degradation, while In_2 was able to remove about one-fifth of soil TPH within 60 days. However, the soil's salinity, severe pollution, and long history of contamination posed significant challenges to the bioremediation process. Severe oil pollution can be toxic and inhibitory to many microorganisms, reducing soil microbial biodiversity and the survival (Huang et al., 2021). As time passes, pollutants interact with the organic and mineral particles of soil, which are absorbed by the surfaces of organic and mineral soil particles and diffused into the micro and nano pores. Therefore, long history of contamination reduces the bioavailability of pollutants for microorganisms and affects biodegradation (Garousin et al., 2021). Additionally, salinity harms the soil microbial community and wastes the microorganisms' energy for establishing osmotic balance and survival. On the other hand, salinity decrease the hydrocarbon and oxygen bioavailability (Kalami et al., 2021). Nevertheless, In_2 was able to eliminate soil TPH despite inhibiting factors of bioremediation.

The native strain *Rhodococcus fascians* was present in In_2. This bacterium can grow in

Table 4. Effect of bacterial inoculum (In), bagasse (B), chemical fertilizer (CF), and molasses (Mo) on TPH (mg kg^{-1} soil) in an oil-contaminated soil. Mean values (n=3) are shown together with standard deviations.

Treatment	B_0				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	78858±3064^{a}	71148±3781^{a-f}	63277±3543^{c-i}	72809±3406^{a-d}	71088±6005^{a}
CF_0Mo_1	63954±3876^{c-i}	49008±4002^{j}	55825±3721^{hij}	54180±3462^{ij}	55742±6463^{c}
CF_1Mo_0	77406±2548^{ab}	67293±4495^{a-h}	61970±3847^{c-i}	67729±4695^{a-h}	68600±6735^{a}
CF_1Mo_1	73535±4364^{a-d}	77116±3711^{ab}	61728±2938^{d-i}	65841±4091^{b-i}	69990±7666^{a}
mean	73003±6434^{A}	66577±11952^{B}	60700±4252^{C}	65140±7880^{BC}	
	B_1				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	73100±4011^{a-d}	59309±4610^{e-j}	58825±4659^{f-j}	74551±3287^{abc}	66446±8510^{ab}
CF_0Mo_1	58535±5151^{f-j}	65116±4688^{b-i}	71793±2507^{a-e}	58486±3463^{f-j}	63482±6722^{b}
CF_1Mo_0	61922±3994^{c-i}	61099±2954^{d-j}	58148±3093^{g-j}	67196±4566^{a-h}	62091±4652^{b}
CF_1Mo_1	58970±4299^{f-j}	70584±4460^{a-g}	67680±4318^{a-h}	55486±4072^{hij}	63180±7405^{b}
mean	63132±7211^{BC}	64027±5790^{BC}	64111±6863^{BC}	63930±8494^{BC}	

In_0 no bacterial inoculum; In_1 *Bacillus zhangzhouensis*; In_2 *Rhodococcus fascians* LMG3623; In_3 blend of *Halobacillus dabanensis* D-8(T), *Acidovorax delafieldii* AF078764, and *Bacillus rhizosphaerae* FJ233848; B_0 without sugarcane bagasse; B_1 with 5% sugarcane bagasse; CF_0 no chemical fertilizer; CF_1 adding nitrogen and phosphorus fertilizers; Mo_0 without molasses; Mo_1 with 0.5% v/w molasses.

Means with similar uppercase letters, lowercase and italic have no significant difference (Tukey's test at a = 0.05).

nutrient-poor and high C/N environments. Additionally, the catabolic pathways of pollutant decomposition do not stop with a sufficient supply of nutrients. *Rhodococcus fascians* can also adapt to hydrophobic substrates, such as oil, by producing surfactants (Kuyukina et al., 2019). In_1 contained *Bacillus zhangzhouensis*, a gram-positive and aerobic bacterium that can survive in salinity ranging from zero to 12% sodium chloride. Optimal conditions for its growth are 1 to 3% salinity (Liu et al., 2016). On the other hand, In_3 was a consortium of three strains of relatively halophilic bacteria that can tolerate a sodium chloride concentration of 0.5 to 28%. These strains are also capable of decompose polycyclic aromatic hydrocarbons (Pourbabaee et al., 2019). Despite their high potential, In_1 and In_3 alone were not effective in breaking down soil TPH, unlike In_2. It appears that In_1 and In_3 had difficulties establishing themselves in the contaminated soil and utilizing their potential. However, the combination of In_1 and In_3 with organic wastes was able to significantly eliminate soil TPH.

The study results indicate that biostimulation can enhance the bioremediation ability of the bacterial inoculums under investigation. Furthermore, combining biostimulation and bioaugmentation strategies can lead to successful bioremediation of oil-contaminated soils, as demonstrated in previous research (Wei et al., 2021). The dried *Eichhornia crassipes* straw powder enhanced the efficacy of bacterial consortium in oil-polluted soil remediation. The removal percentage was 51.7%, which is superior to using either *Eichhornia crassipes* dried powder (37.0%) or a bacteria solution (36.0%) alone (Tao et al., 2019). Incorporating biochar and compost improved the establishment of the microbial consortium in oil-contaminated soil, leading to the highest decomposition rate of total hydrocarbons (85%) in the Italian ryegrass plant rhizosphere when the microbial consortium inoculation, biochar, and compost were combined, as observed in the study by Hussain et al. (2018). Furthermore, Zeneli et al. (2019) found that the bioaugmentation – biostimulation treatment was the most effective method for decontaminating refinery solid waste, with a maximum TPH elimination rate.

In this study, biostimulation (by organic waste and chemical fertilizers) and bioaugmentation (by bacterial inoculum) left significant positive effects. For instance, in S_0C_0, the use of molasses as a biostimulation agent significantly impacted the efficiency of the bioaugmentation strategy. The best-performing treatments in S_0C_0 were In_1 and molasses (with 38% TPH decomposition)

and In_3 and molasses (with 31.2% TPH decomposition). However, In_1 and In_3 alone were not effective in soil bioremediation. The bioremediation capacity of In_2 was also enhanced in the presence of molasses, leading to 29.2% of TPH decomposition.

Molasses, a byproduct of sugar refining, is rich in significant resource nutrients. It contains simple sugars, such as sucrose, glucose, and fructose, in abundance. Molasses is also a rich source of nutrients like potassium, magnesium, calcium, and phosphorus, as well as microelements such as iron, manganese, copper, zinc, and selenium. 5% of the non-sugar portion of molasses comprises nitrogen compounds, half of which are absorbable (USDA, 2019). As an available carbon resource, molasses facilitates the establishment of the bacterial inoculum in the soil. In a study conducted by Yousefi et al. (2021), it was observed that molasses demonstrated the highest percentage removal (40%) of total petroleum hydrocarbons over 15 weeks when compared to other organic wastes. In another study, bacterial inoculum and glucose eliminated 80% of soil TPH (Pham et al., 2018). Additionally, the nitrogen, phosphorus, and potassium content in molasses regulate the nutrient ratio and pave the way for bacteria to break down hydrocarbon compounds.

In S_0C_1 pretreatment, bacterial inoculum and bagasse significantly affected ($p < 0.001$) the soil TPH, with a higher effect observed for bacterial inoculum ($Eta^2_p = 0.43$) compared to bagasse ($Eta^2_p = 0.22$). Additionally, the interaction effects of the In, B, CF, and Mo factors were significant ($p < 0.001$) (Table 3). Table 5 shows the comparison results of the mean values of treatments in S_0C_1. In this pretreatment, In_1 alone decomposed 33.3% of soil TPH compared to the control treatment. Mo, B, and CF also showed significant reductions in TPH, but no synergistic effect was observed with In_1. In S_0C_1, In_2 reduced 23.2% of soil TPH compared to the control treatment, while In_2 and B combination resulted in a 28.5% reduction in soil TPH (Table 5). In_3 treatment led to a 26.3% reduction in soil TPH, while the combination of In, B, Mo, and CF reduced it to 33% compared to the control treatment (Table 5).

In S_0C_1, the highest TPH decomposition was observed with In_1, suggesting a positive interaction between spent mushroom compost and In_1. Spent mushroom compost is abundant in amino acids, mycelium, and bacteria. During the mycelium growth process, various sugars,

Table 5. Effect of bacterial inoculum (In), bagasse (B), chemical fertilizer (CF), and molasses (Mo) on TPH (mg kg^{-1} soil) in an oil-contaminated soil under pretreatment of spent mushroom compost. Mean values (n = 3) are shown together with standard deviations.

Treatment	B_0				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	79201±2879^{a}	52077±2766^{fg}	60761±2875^{b-f}	58293±3611^{b-f}	63258±11531^{ab}
CF_0Mo_1	58632±2882^{b-f}	62019±3569^{b-f}	66552±4237^{bcd}	67100±3084^{bcd}	63576±4685^{ab}
CF_1Mo_0	59115±3970^{b-f}	60374±3121^{b-f}	53841±3836^{ef}	64922±3430^{b-e}	59563±5143^{bc}
CF_1Mo_1	69519±2920^{b}	62212±4420^{b-f}	59503±3786^{b-f}	67341±3682^{bc}	64644±5240^{a}
mean	67117±10010^{A}	59346±5028^{CDE}	60165±5683^{B-E}	64414±4827^{AB}	
	B_1				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	61438±3252^{b-f}	57180±2957^{c-f}	56551±3386^{c-f}	63422±3409^{b-f}	59648±4092^{bc}
CF_0Mo_1	68987±3495^{b}	55583±3763^{def}	63180±3834^{b-f}	56793±4296^{c-f}	61136±6506^{abc}
CF_1Mo_0	63712±3812^{b-f}	56454±3814^{c-f}	65019±4423^{b-e}	56986±4310^{c-f}	60543±5332^{abc}
CF_1Mo_1	58003±3449^{b-f}	56938±3445^{c-f}	60761±3518^{b-f}	53018±4089^{f}	57180±4246^{c}
mean	63035±5132^{ABC}	56539±3062^{E}	61378±4639^{BCD}	57555±5209DE	

In_0 no bacterial inoculum; In_1 *Bacillus zhangzhouensis*; In_2 *Rhodococcus fascians* LMG3623; In_3 blend of *Halobacillus dabanensis* D-8(T), *Acidovorax delafieldii* AF078764, and *Bacillus rhizosphaerae* FJ233848; B_0 without sugarcane bagasse; B_1 with 5% sugarcane bagasse; CF_0 no chemical fertilizer; CF_1 adding nitrogen and phosphorus fertilizers; Mo_0 without molasses; Mo_1 with 0.5% v/w molasses.

Means with similar uppercase letters, lowercase and italic have no significant difference (Tukey's test at a = 0.05).

organic acids, enzymes, and bioactive substances are added to it. This organic waste has a higher nutritional value compared to other agricultural wastes (Umor et al., 2021). Spent mushroom compost can increase nutrient availability and biostimulation in soil bioremediation (Asemoloye et al., 2020). Moreover, lignin-decomposing fungi found in spent mushroom compost play a crucial role in breaking down complex crude oil compounds (Okerentugba et al., 2015). Therefore, by adding organic acids, enzymes, and bioactive substances, the spent mushroom compost not only increases the nutrient availability and stimulates microorganisms' growth but also enhances the bioavailability of petroleum hydrocarbons and mitigates the limitation caused by the age of pollution.

A recent study found that the combination of spent mushroom and water hyacinth composts had the most significant stimulatory effect on TPH biodegradation. The study reported a decomposition rate of 89% for soil oil pollutants (Udume et al., 2023). Liu et al. (2019) demonstrated the successful bioremediation of agricultural soil contaminated with aged polycyclic aromatic hydrocarbons by combining *Paracoccus* sp. LXC with humic acid (HA) and spent mushroom compost. Moreover, Antón-Herrero et al. (2022) showed that the application of spent mushroom compost significantly improved the degradation of aliphatic and aromatic hydrocarbons ranging from C_{10} to C_{35}.

In S_1C_0 pretreatment, bacterial inoculum ($p < 0.001$), bagasse ($p < 0.001$), chemical fertilizer ($p < 0.01$), and molasses ($p < 0.001$) had significant effects on soil TPH, as shown in Table 3. Furthermore, the interaction of these four factors was also significant ($p < 0.001$). According to Eta^2_p values, the bacterial inoculum factor (0.47) was found to be the most effective factor in TPH decomposition, followed by the bagasse (0.23), molasses (0.22), and chemical fertilizer (0.14) (Table 3). In S_1C_0, In_1 alone reduced soil TPH by 29.2% compared to the control treatment, as shown in Table 6. Additionally, the combination of In_1 and bagasse eliminated 26% of soil TPH, whereas bagasse alone did not have a significant effect. Molasses and chemical fertilizers reduced soil TPH by 19.2% and 17%, respectively (Table 6).

In this pretreatment, In_1, bagasse and chemical fertilizer significantly reduced soil TPH by 38% compared to the control treatment (Table 6). In_2 alone decreased TPH by 16%, but in combination with the molasses, the TPH elimination increased to 35%. Additionally, In_2, bagasse, molasses, and chemical fertilizer together decomposed 31% of soil TPH. In_3 reduced

Table 6. Effect of bacterial inoculum (In), bagasse (B), chemical fertilizer (CF), and molasses (Mo) on TPH (mg kg^{-1} soil) in an oil-contaminated soil under pretreatment of agricultural soil. Mean values (n = 3) are shown together with standard deviations.

Treatment	B_0				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	78713±3241^{a}	55728±3424^{c-i}	66229±3605^{bcd}	55148±3690^{d-i}	63955±10455^{ab}
CF_0Mo_1	63567±3635^{b-g}	66325±3622^{bc}	51131±4342^{hi}	60712±3298^{b-h}	60434±6779^{bc}
CF_1Mo_0	65358±3316^{b-e}	66906±3527^{b}	67874±3337^{ab}	64632±3369^{b-f}	66192±3180^{a}
CF_1Mo_1	65116±2785^{b-e}	55341±3463^{c-i}	53986±2716^{f-i}	64922±3258^{b-f}	59841±6030^{bcd}
mean	68188±6965^{A}	61075±6520^{BC}	59805±8241^{BCD}	61353±5046^{BC}	
	B_1				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	68793±3357^{ab}	58390±3142^{b-i}	59890±3520^{b-h}	60228±3533^{b-h}	61826±5152^{abc}
CF_0Mo_1	66277±3409^{bc}	63470±3389^{b-g}	60035±3655^{b-h}	60761±3748^{b-h}	62636±3974^{abc}
CF_1Mo_0	58003±3728^{b-i}	48760±3112^{i}	66374±3450^{bc}	60325±3582^{b-h}	58366±7247^{cd}
CF_1Mo_1	59357±3747^{b-i}	55486±3279^{c-i}	54519±3202^{e-i}	52777±3184^{ghi}	55535±3815^{d}
mean	63108±5634^{B}	56527±6527^{D}	60205±5286^{BCD}	58523±4588^{CD}	

In_0 no bacterial inoculum; In_1 *Bacillus zhangzhouensis*; In_2 *Rhodococcus fascians* LMG3623; In_3 blend of *Halobacillus dabanensis* D-8(T), *Acidovorax delafieldii* AF078764, and *Bacillus rhizosphaerae* FJ233848; B_0 without sugarcane bagasse; B_1 with 5% sugarcane bagasse; CF_0 no chemical fertilizer; CF_1 adding nitrogen and phosphorus fertilizers; Mo_0 without molasses; Mo_1 with 0.5% v/w molasses.

soil TPH by 30% compared to the control treatment. However, combining In_3 with other factors did not significantly enhance the potential of TPH decomposing (Table 6).

Means with similar uppercase letters, lowercase and italic have no significant difference (Tukey's test at a = 0.05).

In S_1C_1, the soil TPH was significantly affected by bacterial inoculum ($p < 0.01$), bagasse ($p < 0.01$), and chemical fertilizer ($p < 0.01$), whereas the molasses factor was insignificant ($p < 0.05$) (Table 3). The interaction among bacterial inoculum, bagasse, chemical fertilizer, and molasses was also significant at the 0.1% level ($p < 0.01$). According to Eta^2_p, the bacterial inoculum factor (0.19) had the most substantial impact on the dependent variable, followed by bagasse (0.15) and chemical fertilizer (0.14) factors.

Table 7 shows the comparison results of the mean values of treatments in S_1C_1. Mo, In_1, and B alone had no significant effects on TPH degradation, but CF decreased soil TPH by 17%. The combination of molasses and chemical fertilizer was successful in promoting TPH decomposition by 36% compared to the control treatment in S_1C_1 (Table 7). In_2 reduced soil TPH by 16%, but in treatment of In_2, B, and CF, soil TPH decomposition increased to 28.1% (Table 7). In_3 brock down 29.2% of soil TPH compared to the control treatment. However, the combination of other factors with In_3 did not affect the potential of TPH decomposition (Table 7).

In the S_1C_0 pretreatment, In1, bagasse, and chemical fertilizer decomposed more than one-third of soil TPH. However, in S_1C_1, combining molasses and chemical fertilizer proved to be successful for TPH decomposition. Bagasse, by improving physical conditions, especially soil aeration, increases oxygen availability, which is necessary for hydrocarbon degradation. It also helps establish a microbial community in polluted soil and promote the success of soil bioremediation programs (Hamzah et al., 2014; Liu et al., 2015). Moreover, bagasse contains various types of microorganisms that can produce enzymes such as protease, cellulase, hemicellulase, lignin peroxidase, manganese peroxidase, and lactase. These enzymes play an essential role in breaking down complex organic compounds, including petroleum hydrocarbons, into simpler compounds that can be utilized by microorganisms. So, they are effective in enhancing soil TPH degradation rate (Babaei et al., 2020).

In a microcosm unit, Fenton oxidation was utilized in combination with biostimulation using

Table 7. Effect of bacterial inoculum (In), bagasse (B), chemical fertilizer (CF), and molasses (Mo) on TPH (mg kg^{-1} soil) in an oil-contaminated soil under pretreatment of agricultural soil and spent mushroom compost. Mean values (n = 3) are shown together with standard deviations.

Treatment	B_0				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	71213±3351^{a}	62261±3849^{a-f}	59890±3817^{b-h}	50357±3434^{hi}	60930±8345^{a}
CF_0Mo_1	66180±3691^{ab}	53260±3045^{e-i}	64825±2566^{a-d}	62599±3374^{a-f}	61716±5934^{a}
CF_1Mo_0	59261±3127^{b-h}	62261±3670^{a-f}	53841±3699^{d-i}	59938±3886^{b-h}	58825±4454^{abc}
CF_1Mo_1	45663±2575^{i}	62503±3580^{a-f}	56793±3191^{b-h}	65261±3419^{abc}	57555±8313^{abc}
mean	60579±10395^{A}	60071±5103^{A}	58837±5120^{A}	59539±6603^{A}	
	B_1				
	In_0	In_1	In_2	In_3	mean
CF_0Mo_0	64196±3595^{a-e}	61583±2345^{a-g}	54373±4265^{e-i}	59503±3212^{b-h}	59914±4766^{ab}
CF_0Mo_1	55099±3702^{c-i}	56357±3384^{b-i}	58099±3370^{b-h}	55777±3467^{b-i}	56333±3190^{bc}
CF_1Mo_0	53454±3667^{e-i}	54760±3446^{c-i}	51180±2680^{ghi}	62696±3608^{a-f}	55523±5364^{c}
CF_1Mo_1	64583±3419^{a-d}	56309±3629^{b-i}	51906±2575^{f-i}	58728±3380^{b-h}	57881±5537^{abc}
mean	59333±6139^{A}	57252±3861^{AB}	53889±3982^{B}	59176±3888^{A}	

In_0 no bacterial inoculum; In_1 *Bacillus zhangzhouensis*; In_2 *Rhodococcus fascians* LMG3623; In_3 blend of *Halobacillus dabanensis* D-8(T), *Acidovorax delafieldii* AF078764, and *Bacillus rhizosphaerae* FJ233848; B_0 without sugarcane bagasse; B_1 with 5% sugarcane bagasse; CF_0 no chemical fertilizer; CF_1 adding nitrogen and phosphorus fertilizers; Mo_0 without molasses; Mo_1 with 0.5% v/w molasses.

Means with similar uppercase letters, lowercase and italic have no significant difference (Tukey's test at a = 0.05).

nutrients and oil palm bagasse to remove total petroleum hydrocarbons from soil artificially contaminated with crude oil. The treatment resulted in the degradation of 55% of TPH, as reported by Guzmán-López et al. (2021). Furthermore, a study conducted by Sadañoski et al. (2020) revealed that biostimulation by supplementing sugarcane bagasse and myco-augmentation demonstrated the highest PCBs-removal rate (90%) with a significant reduction in toxicity after 90 days. This treatment also improved the oxidable organic matter, phosphorous contents, and dehydrogenase activity of the soil.

Due to the nature of the oil pollutants, nitrogen and phosphorus are often deficient in oil-polluted soil, which can limit the bioremediation process. Adding nutrients can help increase the rate of bioremediation (Sarkar et al., 2020). Accordingly, by adjusting the C/N ratio, chemical fertilizers had a positive effect on the establishment and growth of the bacterial inoculum under favorable conditions (Obieze et al., 2020). According to Wu et al. (2019), there is a strong positive correlation between nitrogen and phosphorus content and TPH degradation, highlighting the significance of regulating the C/N ratio.

Organic wastes and chemical fertilizers provided a favorable environment for bacterial inoculums by addressing the limitations of polluted soil, importing valuable nutrient resources to the soil, and increasing the bioavailability of petroleum pollutants. A recent study found that under optimal conditions, the combination of *Bacillus subtilis* X-12, wheat bran, and swine wastewater led to a degradation rate of 68.27% of TPH (Zhang et al., 2020). In another study, Dadrasnia and Ismail (2015) reported that *Bacillus* 139SI and tea leaf had significant potential in the degradation of crude oil in the soil, achieving a degradation rate of 89%. Furthermore, a study conducted by Zhang et al. (2012) demonstrated that sewage sludge amendments at a low dose of 0.1% and cattle manure at 1% had prominent effects (42.4%) on soil high-molecular-weight PAHs degradation and stimulation of PAHs-degrading microbes after 60 days of incubation. The organic waste effect on soil PAHs degradation was attributed to the interaction of nutrients and dissolved organic matter in organic waste with soil microorganisms.

However, some reports suggest that the biostimulation strategy may not have a significant impact on the success of the bioaugmentation process. For instance, the *Rhodococcus erythropolis* alone was effective in the bioremediation of oil-contaminated soil, and no significant difference was observed when compared to the biostimulation treatment (Wei et al., 2021). The differences observed could be attributed to several factors as the initial conditions of the contaminated soil, the limitations and challenges during the bioremediation process, the type of pollutants, the type of bacteria used, and the biostimulation agent employed.

CONCLUSION

In S_0C_0, it was found that two treatments, In_1Mo_1 and In_3Mo_1, were more efficient than others. In S_0C_1, In_1 proved to be more successful than the other factors. In the S_1C_0 state, the superior treatment contained In_1, chemical fertilizer, and bagasse, while in the S_1C_1 state, the interaction of chemical fertilizer and molasses was beneficial. In general, it can be stated that organic waste and chemical fertilizers, used as biostimulation agents, can improve the conditions of old-aged, oil-polluted, and saline soil for the establishment and growth of bacteria. As a result, the activity of the microbial community is enhanced, leading to increased TPH decomposition. Additionally, the utilization of the aforementioned organic wastes has economic benefits. They are found abundantly in the study area, and often, there is no comprehensive management system for them. Therefore, using molasses, bagasse, and spent mushroom compost in the bioremediation of oil-contaminated soils can not only play a crucial role in soil restoration but also provide waste management and economic benefits for the soil bioremediation projects. However, it is important to note that this research was conducted on a laboratory scale, and further studies are necessary to evaluate the effectiveness of these methods on a larger scale.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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