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Effect of Different Iron Fertilizers and *P.indica* Fungus Inoculation on Diesel Oil Bio-Degradation in Drought-Stressed Soil

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ABSTRACT

Background: This research investigated the effects of various iron fertilizers and the introduction of *Piriformospora indica* (*P. indica*) fungus on the biodegradation of diesel oil in soil experiencing water deficiency.
Methods: The treatments included applying pure Fe at rates of 0, 60, and 90 kg/ha, derived from Fe sulfate ($FeSO_4$) and Fe slag (containing 58.2 % Fe_2O_3), in a diesel oil-contaminated soil with concentrations of 0 % and 8 % (W/W). These treatments were implemented in a corn plant cultivation system, where the plants were inoculated with *P.indica* fungus under conditions of drought stress. After 90 days, the plant and soil Fe concentration was determined by atomic absorption spectroscopy (AAS). Furthermore, the extent of diesel oil bio-degradation in the soil was assessed.
Results: Applying 90 kg/ha pure Fe from Fe sulfate and Fe slag significantly enhanced the bio-degradation of diesel oil in the soil by 13.2 and 16.1 %, respectively. However, the efficiency of this process was lower under drought-stress conditions. In addition, Plant inoculation with *P.indica* significantly enhanced the diesel oil bio-degradation of the soil contaminated with 8 % (W/W) diesel oil under drought stress by 15.7 %. Moreover, the soil microbial activity was significantly increased by 14.1 %.
Conclusion: The combined use of *P.indica*, along with iron slag and iron sulfate, markedly enhances the biodegradation process of diesel oil within the soil.

1. Introduction

In order to develop societies and progress in industry and agriculture, humans produce and use chemical substances, including petroleum hydrocarbon compounds. However, the extraction, transmission, and utilization of these compounds as fuel have caused inevitable environmental risks (Muthukumar et al., 2023; Ossai, Ahmed, Hassan, & Hamid, 2020). The presence of petroleum hydrocarbons in soil not only limits plant growth but also poses dangerous consequences for human life (Ambaye, Vaccari, et al., 2023; Hosseini, Taghavi, Ghasemi, & Dehghani Ghanatghestani, 2023). In the past few decades, the release of petroleum hydrocarbons into the environment has heightened awareness of the dangers caused by these substances. As time passes, their adverse effects become increasingly apparent. (Adesina, Ewim, Lala, Ogunyemi, & Adeniyi, 2023; Ambaye, Formicola, Sbaffoni, Franzetti, & Vaccari, 2023). Therefore, we need to have information about the fate of these compounds, their effects on humans and the environment, as well as ways to remediate them (Kwon et al., 2023; Mfarrej et al., 2023). Petroleum hydrocarbons can enter the environment in different ways. Most of these pollutants enter the soil through extraction and leak from transmission pipes, storage tanks, and refineries. The physical and chemical characteristics of these compounds as well as their concentration are influenced by factors such as chemical reactions, microbial decomposition, and environmental conditions (e.g., temperature, light, oxygen, and solubility) (Ansari, Wrights, & Jaikishun, 2023; Saharan

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et al., 2023). Different compounds of petroleum hydrocarbons can influence the neurological system of humans. Skin diseases, lung diseases, digestive tract poisoning, blood diseases, headaches, and effects on vision are also other risks and toxicities of these dangerous polluting compounds in the environment (Adesina et al., 2023; Sarma, Goswami, Rabha, Patowary, & Devi, 2023). Therefore, it seems necessary to degrade such compounds in the soil and remove them from the food chain. Microbial populations in soil, water, and sediments contaminated with aromatic hydrocarbon compounds are capable of decomposing and destroying these pollutants. The decomposability of petroleum hydrocarbon compounds depends on their chemical characteristics and bio-availability (Yang et al., 2023). As the molecular weight of petroleum compounds increases, their degradability decreases. There is a wide range of different physical, chemical, and biological technologies to remediate contaminated soils, all of which aim to eliminate, reduce, or stabilize pollution and prevent their transfer to surface and underground water sources (Hoang et al., 2021; Ossai et al., 2020; Parus et al., 2023). Numerous industrial regions within Iran are experiencing the impacts of arid conditions and a scarcity of water resources (Jamali, Sohrabi, Mardeh, & Hoseinpanahi, 2020), which can increase the negative effects of petroleum hydrocarbons. Additionally, the availability of nutrients in these soils is very low (Kooch & Noghre, 2020), necessitating the use of organic and inorganic fertilizers such as Fe sources (Milashi, Javid, Alahdadi, & Darbandi, 2020). Iron slag, an industrial waste material generated (a byproduct of the Mobarakeh Steel Complex) during the production of iron in smelting furnaces, can be used as iron fertilizer after cooling (Karchegani, Hoodaji, & Kalbasi, 2014). Additionally, the presence of entophyte fungus, such as *Piriformospora indica (P.indica)* can increase a plant's resistance against abiotic stresses (Aslam, Karanja, & Bello, 2019; Gill et al., 2016), thereby enhancing plant growth which may increase the microbial population. As a result, this could increase the bio-degradation of petroleum hydrocarbons in the soil. However, it should be noted that the physical and chemical characteristics of the soil, environmental conditions, and plant genotype may change this process (Li et al., 2022). Thus, this research was conducted to evaluate the effects of different iron fertilizers and inoculation of plants with *P.indica* on diesel oil bio-degradation in soil under drought stress.

2. Materials and Methods

To investigate the effect of different iron fertilizers and inoculation of plants with *P.indica* on the bio-degradation of diesel oil in the soil under drought stress, a factorial experiment with a completely randomized block design with three replications was conducted as a greenhouse experiment. A soil sample with no salinity (EC = 1.1 dS/m) and low organic carbon (OC < 2 %) was selected as the experimental medium. The treatments included the application of 0, 60, and 90 kg/ha Fe from two sources: iron slag (containing 58.2 % Fe_2O_3) and iron sulfate ($FeSO_4$) (Masoumeh Abedi, Amir Hossein Baghaie, & Hamid Toranjzar, 2023). Additionally, the plants were inoculated with *P.indica*, which was cultivated in normal or drought-stress conditions. The control group received full irrigation, while the drought stress group experienced water depletion of 70 % of the field capacity. The contaminated soil was treated with diesel oil at concentrations of 0, 4, and 8 % (W/W). The maize plant seeds (Zea mays L. Single cross 704) were firstly soaked in water for a short time. After that, the seeds were submerged in 96 % alcohol and were sterilized by using sodium hypochlorite solution (1: 10 (v/v)) for 1min. Furthermore, the original *P.indica* inoculum used in this investigation was acquired from Malayer University, Hamadan Province, Iran. A portion of the fungus was selected from the growth media surface, stained with fuchsin acid, and observed under an optical microscope to confirm the presence of the fungus's spherical body and mycelium. Subsequently, *P. indica* was introduced into half of the seeds through immersion in inoculums (which were adjusted almost to 2×10^6) while being gently shaken for 3h. Furthermore, the non-inoculated seedlings were treated with sterile distilled water that had been treated with Tween 0.02 % (Mahdieh Abedi, Amir Hossein Baghaie, & Hamid Toranjzar, 2023). Following that, two seedlings of either non-inoculated or inoculated were planted in the 5 kg pot which was filled with the treated soil. After 90 days, the plants were harvested.

2.1 Plant and soil Fe concentration

The soil Fe concentration was measured using atomic absorption spectrometry after extraction with diethylenetriamine pentaacetate (DTPA) (Lindsay & Norvell, 1978). In addition, the plant samples were milled and 100 mg aliquots were incinerated at 550 °C for 6 h, and the ashes were dissolved in 1 mL of 13 M HNO_3 at 220 °C for 1 min (Takkar & Kaur, 1984). The concentrations of Fe in these samples were determined by atomic absorption spectrometry (PerkinElmer 3030; PerkinElmer, Wellesley, MA, USA). The accuracy of the Fe analyses was checked by analyzing certified standard materials and including blanks in all batches of separately processed samples.

2.2 APX and POX enzyme activity

After weighing 0.2 g of fresh leaf tissue, 4 cc of ice-cold extraction buffer was used, and the mixture was centrifuged for 15min at 16,000 rpm. Following that, the amount of enzyme activity was ascertained by application of the upper layer of centrifuged solution (Malar, Shivendra Vikram, JC Favas, & Perumal, 2016). Then the Peroxidases (POX) and ascorbate peroxidase (APX) were calculated.

2.3 Diesel oil bio-degradation

Extraction of PHC from soil was done based on the method used by Hatim *et al.* (2019) (Hatami, Abbaspour, & Dorostkar, 2019). To extract Diesel oil from the soil, 2 g of soil was mixed with 10 mL of dichloromethane and shaken for 10 min at 500 rpm. Then, to separate the supernatant from the soil the suspension was centrifuged at 3000 rpm for 10 min. The described step was repeated two times and the supernatant was added to the previous one. Thereafter, the supernatant was kept at laboratory temperature for 24h to evaporate dichloromethane and the remaining was weighed to estimate the amount of PHC using the gravimetric method. The diesel oil bio-degradation percentage in the soil was estimated according to Hatami *et al.'s* method (Hatami et al., 2019).

2.4 Soil microbial activity

The Zamani *et al.* (2016) approach was applied to measure the respiration of soil microbes (Zamani et al., 2016). For seven days at 27 °C, 15 g of soil treatments were incubated in 500 mL glass containers with rubber stoppers. To collect the produced CO_2, 10 mL of a 0.5 M NaOH solution was placed inside the containers as a blank sample. Titrating the excess alkali with HCl allowed for the identification of the trapped CO_2, and three glass containers free of soil were incubated as controls (Zamani et al., 2016).

2.5 Statistical analysis

Statistical analyses according to the ANOVA test were applied using SAS V.9.1. To evaluate the variance in means, the least significant difference (LSD) test was employed. The $P < 0.05$ value was considered to estimate the considerable differences.

3. Results and Discussion

The highest concentration of Fe in soil (Table 1) belonged to the soil that amended with the highest rate of iron slag, while the lowest was observed in soil that did not receive any iron fertilizer. Increasing the soil application of iron slag from 0 to 60 and 90 kg/ha pure Fe considerably elevated the Fe availability in the soil by 13.5 and 18.4 %, respectively. In addition, using organic and inorganic iron sources has significantly increased the bio-availability of soil Fe availability. In addition, the interaction effects of *P.indica* and iron sources had significant effects on enhancing the soil Fe concentration which can be attributed to the role of *P.indica* fungus in increasing the soil Fe availability (Baghaie & Aghilizefreei, 2020).

Table 1. Treatment effects on soil Fe concentration (mg/kg)

Plant inoculation	Diesel oil (W/W)	Normal condition						Drought stress					
		Iron sources (kg/ha pure Fe)											
		Iron slag			Iron sulfate			Iron slag			Iron sulfate		
		0	60	90	0	60	90	0	60	90	0	60	90
+ *P.indica*	0	41.3c'*	78.9d	83.4a	41.5c'	73.2g	80.6b	34.8f'	68.6k	74.2f	34.3f'	65.8n	70.2
	4	38.2d'	75.4e	78.1d	38.6d'	70.2i	72.1h	30.7i'	65.4n	68.2k	30.3i'	60.1s	67.4l
	8	34.1f'	72.1h	75.3e	34.5f'	66.7m	69.2j	31.2h'	60.3s	64.1o	31.8h'	56.8u	61.7r
- *P.indica*	0	37.2e'	73.6g	79.3	37.8e'	68.2k	72.6h	33.7g'	68.3k	63.1p	33.1g'	60.7s	63.7p
	4	33.1g'	62.4q	67.6l	33.8g'	60.2s	63.8p	29.8j'	58.7t	54.4w	29.3j'	53.6x	48.3z
	8	30.5i'	63.7p	66.7m	30.9i'	55.8v	58.5t	25.7l'	51.7y	53.5x	25.3l'	42.3b'	44.1a'

* The similar letters indicate no significant differences ($P < 0.05$).

In this regard, Abedi *et al.* (2023) showed that using iron sources from iron slag has a significant effect on increasing the Fe concentration in the soil and plant. However, they mentioned that soil physic-chemical properties have a significant effect on the amount of nutrient availability in the soil (Masoumeh Abedi et al., 2023). On the other hand, soil application of iron sulfate at the rate of 90 kg/ha pure Fe has considerably enhanced ($P < 0.05$) the soil iron concentration by 11.1 %. The highest efficiency of iron slag relative to iron sulfate in increasing the iron concentration in the soil can be attributed to the gradual release of iron in the soil from the iron slag source thus preventing the precipitation of iron in the soil. In this line, Bagheri *et al.* (2017) indicated that using iron slag has a significant effect on increasing the soil Fe concentration (Bagheri, Baghaei, & Niei, 2017). It is noteworthy that in the central parts of Iran, due to the low availability of iron in the soil, the use of iron compounds can greatly help to increase the availability of iron in the soil (Gao et al., 2023). The Fe concentration of the plant (Table 2) showed similar findings.

Table 2. Treatment effects on Fe concentration of the plant (mg/kg)

Plant inoculation	Diesel oil (W/W)	Normal condition						Drought stress					
		Iron sources (kg/ha pure Fe)											
		Iron slag			Iron sulfate			Iron slag			Iron sulfate		
		0	60	90	0	60	90	0	60	90	0	60	90
+ *P.indica*	0	41.4w*	82.7d	91.3a	41.8w	78.1g	86.7b	35.9y	78.1g	85.2c	35.4y	75.1i	80.3f
	4	37.8x	78.2g	86.3b	37.9x	75.2i	82.2d	32.4a'	72.1j	78.7g	32.6a'	65.3o	71.5k
	8	33.1z	72.6j	81.2e	33.7z	65.7o	71.7k	30.6c'	66.4n	69.5m	30.9c'	60.4	63.7q
- *P.indica*	0	35.6y	77.1h	85.4c	35.7y	72.1j	80.4f	31.4b'	70.3l	81.2e	31.7b'	66.5n	70.2l
	4	32.1a'	72.1j	81.7e	32.7a'	66.7n	70.2l	28.1e'	66.2n	72.8j	28.6e'	60.3s	62.5e
	8	29.8d'	66.1n	70.2l	29.4d'	60.2s	64.6p	25.9f'	55.3u	59.4t	25.4f'	50.3v	55.1u

* The similar letters indicate no significant differences ($P < 0.05$).

Drought stress has a negative effect on Fe concentration of the soil, as, the results of our study have indicated that the soil Fe concentration in the drought stress as compared to normal conditions has significantly decreased by 15.7 % which could be related to the effect of drought stress on the microbial activity in the soil (Table 3). Decreasing the soil microbial activity by 15.8 % in the soil affected by drought stress as compared to the normal conditions confirms our results. In this regard, Abdi Ardestani *et al.* (2021) mentioned that drought stress and species variation have a considerable effect on the microbial activity of the soil (Abdi Ardestani, Khalili, & Majidi, 2021).

Table 3. Treatment effects on microbial respiration of the soil (mg C-CO_2/kg soil)

Plant inoculation	Diesel oil (W/W)	Normal condition						Drought stress					
		Iron sources (kg/ha pure Fe)											
		Iron slag			Iron sulfate			Iron slag			Iron sulfate		
		0	60	90	0	60	90	0	60	90	0	60	90
+ P.indica	0	10.1d'*	14.7	15.8	10.1d'	14.1	15.2	9.7e'	14.1	15.0	9.7e'	13.8k	14.2
	4	9.6f'	14.3	15.2	9.6f'	13.4o	14.2	9.2g'	13.7l	14.0i	9.2g'	13.3p	13.8k
	8	9.2g'	14.1	14.7	9.2g'	13.1r	13.8k	8.5h'	13.1r	14.0i	8.5h'	12.5w	13.1r
- P.indica	0	7.2i'	13.5n	13.9j	7.2i'	13.1r	13.3p	6.8j'	13.1r	13.6m	6.8j'	12.7u	13.0s
	4	6.6k'	13.1r	13.3p	6.6k'	12.6v	13.1r	6.3l'	12.6v	13.1r	6.3l'	12.1y	12.5w
	8	6.2m'	12.7u	13.1r	6.2m'	12.2x	12.8y	5.7n'	11.5a'	11.9z	5.7n'	11.1b'	10.7c'

* The similar letters indicate no significant differences ($P < 0.05$).

The results indicate that drought stress can lead to a decrease in plant Fe concentration, likely due to its effect on reducing soil Fe concentration (Table 1). However, the application of iron compounds, specifically 60 and 90 kg/ha pure Fe Kg/ha from iron slag, significantly (P < 0.05) enhanced the Fe concentration in plants under drought stress by 16.2 and 19.1 %, respectively. This finding is consistent with a study by Kazemi *et al.* (2014) which showed, that using Zn and Fe foliar fertilizer had a significant influence on plant growth and nutrient uptake by plants (Kazemi, Baradaran, Seghat Eslami, & Ghasemi, 2014). Increasing the application rate of diesel oil had a negative effect on soil and plant Fe concentration. Based on the results of our study, by increasing the soil pollution to diesel oil from 0 to 4 and 8 % (W/W), the soil Fe concentration was significantly (P < 0.05) reduced by 14.2 and 17.1 %, respectively. Plants Fe concentration also decreased by 12.2 and 15.1 %, respectively. In the meantime, the inoculation of plants with *P.indica* was able to moderate the negative effects of diesel oil pollution. Inoculated plants with *P.indica* showed a 12.5 and a 15.5 % increase in soil Fe concentration. Based on the results of Baghaie *et al.* (2020) plant inoculation with *P.indica* can enhance the nutrient uptake of the plants which they related to the role of *P.indica* in promoting the soil Fe availability and thus its uptake by the plant (Baghaie & Aghilizefreei, 2020). However, our results have shown that this effect was less in the plant under stress than in the plant exposed to normal conditions. Regardless of soil contamination with diesel compounds, the type of iron source also had a significant effect on the amount of iron absorbed by plants. The use of iron slag compared to iron sulfate was more effective in increasing iron availability in soil and plants, which can be attributed to the relatively high percentage of iron in the slag composition (Das et al., 2020). Additionally, inoculation of plants with *P.indica* had a significant effect on enhancing the plant's resistance to abiotic stress, such as drought stress. Inoculated plants with *P.indica* under stress showed a 14.2 % increase in Fe concentration compared to plants under normal conditions. This highlights the role of beneficial soil organisms, like *P.indica* in establishing symbiotic relationships with plants and increasing their resistance to environmental stresses. Entophytic fungus such as *P.indica*, which are considered to be the most important soil microorganisms, increase plant growth with physiological genetic changes in their host plants and increase the possibility of developing their cultivation in dry saline soils or climates with abiotic and biotic stresses (Jogawat et al., 2013; Xu, Wang, Wang, Wei, & Zhang, 2017). Regarding diesel oil bio-degradation in the soil, the treatments significantly influenced the process (Table 4). The highest diesel oil bio-degradation in the soil was observed in soils that received the highest rate of Fe from Fe slag. Using 60 and 90 Kg/ha pure Fe from iron slag sources significantly (P < 0.05) increased the bio-degradation of diesel oil in soil contaminated with 8 % (W/W) diesel oil by 13.4 and 15.6 %, respectively.

Table 4. Treatment effects on the diesel oil bio-degradation in the soil (%)

Plant inoculation	Diesel oil (W/W)	Normal condition						Drought stress					
		Iron sources (kg/ha pure Fe)											
		Iron slag			Iron sulfate			Iron slag			Iron sulfate		
		0	60	90	0	60	90	0	60	90	0	60	90
+ P.indica	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	4	35.8o**	59.2b	60.8a	35.8o	55.1d	57.1c	27.2u	40.6l	52.7g	27.2u	36.2n	40.8l
	8	30.1s	53.2f	54.1e	30.1s	50.3h	52.4g	25.2v	36.4n	40.8l	25.2v	35.8o	38.4m
- P.indica	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	4	30.1s	54.4e	57.1c	30.1s	50.9h	54.3e	24.7w	35.3o	46.7j	24.7w	31.4r	34.8p
	8	27.2u	48.4i	53.5f	27.2u	45.7k	48.2i	21.7y	30.5s	33.5q	21.7y	22.5x	28.4t

* Not detectible, ** The similar letters indicate no significant differences ($P < 0.05$).

In the study, it was observed that the soil microbial activity increased by 12.7 and 15.4 %, respectively, which confirms our results. Regardless of Fe sources, using Fe fertilizer had a significant influence on increasing the diesel oil bio-degradation in the soil. Specifically, using 90 Kg/ha pure Fe from iron slag and Fe sulfate sources significantly increased the diesel oil bio-degradation in the soil by 16.3 and 13.5 %, respectively. This improvement can be related to the role of iron sources in stimulating soil microbial activity, thereby enhancing the biodegradation of diesel oil in the soil. The present research also demonstrated a significant increase of 14.7and 17.2 %, respectively, in soil microbial activity due to using 90 kg/ha pure Fe from Fe sulfate and iron slag sources. However, under conditions of drought stress, the bio-degradation of diesel oil in the soil was lower in corn cultivation. Furthermore, the inoculation of plants with *P.indica* considerably (P < 0.05) promoted the bio-degradation of diesel oil in the soil. Our results revealed that the presence of *P.indica* considerably (P< 0.05) increased the diesel oil bio-degradation in the soil polluted with 8 % (W/W) diesel oil by 13.2 %. Consequently, the application of iron fertilizers such as Fe slag and Fe sulfate to the soil effectively enhanced Fe availability, leading to increased microbial community activity and, consequently, the bio-degradation of diesel oil. This aligns with the findings of Zamani *et al.* (2016), who reported that the inoculation of plants with *P.indica* enhanced microbial respiration, thereby promoting the bio-degradation of diesel oil in the soil (Zamani et al., 2016). In addition, Sarvi Moghanlo et al. (2012) investigated the effect of mycorrhiza and degrading bacteria on promoting the phytoremediation of petroleum hydrocarbons in contaminated soil and concluded that inoculation of plants with mycorrhiza fungus had a significant effect on increasing the bio-degradation of petroleum hydrocarbons in the soil via increasing the microbial activity (Sarvi Moghanlo, Chorom, Falah, & Motamedy, 2012) that is similar to our results. Conversely, the activity of plant enzymes showed an opposite trend to the diesel oil bio-degradation in the soil. A significant increase in the bio-degradation of diesel oil in the soil by 14.5 % with a decrease the 11.4 % in APX (Table 5) enzyme activity was observed when the soil was amended with 90 kg/ha pure Fe from Fe slag source. For POX (Table 6) enzyme activity, it was decreased by 16.7 %. Soil application of Fe fertilizer has reduced the plant enzyme activity which suggested the reduction of stress conditions for the plants. However, inoculation of the plant with *P.indica* also decreased the enzyme activity of the plant such as POX and APX. In this regard, Ghorbani *et al.* (2018) suggested that plant inoculation with *P.indica* can alleviate the negative effects of biotic and abiotic stress (Ghorbani, Razavi, Omran, & Pirdashti, 2018) confirms our results. Similarly, Yaghobian *et al.* (2014) conducted research that yielded comparable results (Yaghoubian et al., 2014). Furthermore, our study demonstrated that the inoculation of plants with *P.indica* significantly reduced the plant enzyme activities. Specifically, under drought stress conditions, the APX and POX enzyme activities of *P.indica* inoculated plants were significantly decreased. These findings align with the results of Mojdehi *et al.* (2020), which further support our observations (Mojdehi, Taghizadeh, Baghaie, Changizi, & Khaghani, 2020).

Table 5. Treatment effects on APX enzyme activity (Unit/mg protein)

Plant inoculation	Diesel oil (W/W)	Normal condition						Drought stress					
		Iron sources (kg/ha pure Fe)											
		Iron slag			Iron sulfate			Iron slag			Iron sulfate		
		0	60	90	0	60	90	0	60	90	0	60	90
+ *P.indica*	0	8.4h'*	8.1k'	7.9l'	8.4h'	8.3i'	8.2j'	9.3z	8.9d'	8.2j'	9.3z	9.2a'	8.7e'
	4	9.0c'	8.6f'	8.3i'	9.0c'	8.9d'	9.1b'	10.1t	9.1b'	8.7e'	10.1t	9.6x	9.4y
	8	11.3i	10.7n	10.2s	11.3i	11.1k	10.6o	11.9g	11.1k	10.8m	11.9g	11.7h	11.3i
- *P.indica*	0	8.9d'	8.5g'	8.1k'	8.9d'	8.8e'	9.2a'	9.8v	9.2a'	9.6x	9.8v	9.8v	9.6x
	4	9.9u	9.3z	9.7w	9.9u	9.7w	10.4q	10.7n	10.4q	10.1t	10.7n	10.5p	10.3r
	8	12.2e	11.3i	10.9l	12.2e	11.7h	11.2j	13.1a	12.4c	12.1f	13.1a	12.8b	12.3d

* The similar letters indicate no significant differences (P< 0.05).

Table 6. Treatment effects on POX enzyme activity (Unit/mg protein)

Plant inoculation	Diesel oil (W/W)	Normal condition						Drought stress					
		Iron sources (kg/ha pure Fe)											
		Iron slag			Iron sulfate			Iron slag			Iron sulfate		
		0	60	90	0	60	90	0	60	90	0	60	90
+ *P.indica*	0	14.1g'	13.8i'	13.4k'	14.1g'	13.9h'	13.6j'	14.7d'	14.1g'	13.9h'	14.7d'	14.5e'	14.2f'
	4	15.9u	15.4z	15.2a'	15.9u	15.7w	15.4z	16.2r	15.8v	15.5y	16.2r	16.1s	15.9u
	8	17.8g	16.4p	16.1s	17.8g	16.9m	16.5o	18.3c	17.8g	17.2k	18.5b	18.1e	17.8g
- *P.indica*	0	15.6x	15.1b'	14.8c'	15.6x	15.4z	15.1b'	16.1s	15.8v	15.2a'	16.1s	16.0t	15.5y
	4	16.8n	16.3q	15.9u	16.8n	16.5o	16.1s	17.4j	16.8n	16.5o	17.4j	17.1l	16.8n
	8	18.1e	17.5i	17.1l	18.1e	17.9f	17.4j	18.6a	18.2d	17.8g	18.6a	18.5b	18.1e

* The similar letters indicate no significant differences (P< 0.05).

4. Conclusion

The diesel oil bio-degradation of the soil is affected by the Fe sources. Using 90 kg / ha pure Fe from an iron slag source considerably enhanced the diesel oil bio-degradation in the soil. However, this increase was higher in soil under the normal relative to drought stress. Inoculation of plants with *P.indica* had also increased the diesel oil bio-degradation in the soil which was more effective in normal conditions. Increasing soil pollution to diesel oil significantly reduced the soil microbial activity and thereby decreased the diesel oil bio-degradation in the soil. While, the type of soil pollutant, plant genotype, and physic-chemical properties of the soils have different impacts on the bio-degradation of petroleum hydrocarbons in the soil that could be considered in the field study.

Authors' Contributions

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Conflicts of Interest

There is no conflict of interest between the authors.

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Ethical considerations

The Islamic Azad University, Arak Branch Ethics Committee gave its approval to this article (IR.IAU.ARAK.REC.1402.016).

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