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Effect of Intercropping System and *Piriformospora Indica* Fungus on Biodegradation of Petroleum Hydrocarbons under Drought and Salinity Stress in a Pb and Cd Contaminated Soil

Amir Hossein Baghaie [a*]

a. Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran.

***Corresponding author:** Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran. Postal code: 38361-1-9131.
E-mail address: a-baghaie@iau-arak.ac.ir

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ABSTRACT

Background: This research was conducted to evaluate the effect of *Piriformospora indica* (*P.indica*), drought, and soil salinity stress on diesel oil biodegradation in a soil that was polluted with Pb and Cd.
Methods: Treatments consisted of corn inoculated with *P.indica* and white clover intercropping system under the salinity (3.1 and 6 dS/m) and drought (normal and intensive) stress which cultivated in the Pb and Cd polluted (naturally) soil which simultaneously contaminated with diesel oil at the rates of 0 and 4 % (W/W). After 10 weeks, plants were harvested and the diesel oil biodegradation in the soil was determined. In addition, the soil microbial activity, the APX and POX enzyme activates were measured.
Results: Diesel oil biodegradation significantly increased in the soil under cultivation of plants inoculated with *P.indica*, while it significantly decreased under salinity and drought stress by 12.9 and 9.8%. However, the intercropping system had positive effects on increasing the soil microbial activity and biodegradation of diesel oil in the soil.
Conclusion: Corn inoculation with *P.indica* significantly affected the biodegradation of diesel oil in the heavy metals and petroleum hydrocarbon polluted soil that is under salinity and drought stress.

1. Introduction

Today, environmental pollution is one of the most serious problems on the earth. Since the beginning of the industrial revolution, daily production and commercial activities have caused serious problems in the field of water and soil pollution [1-3]. Everyday human activities can enter the large amounts of pollutants in the environment and various components of the ecosystem [4-6], including air, water, and soil. Soil pollutants are chemically divided into two groups of organic and inorganic pollutants. Pollution of the environment by crude oil and its derivatives is a serious worldwide problem. In general, accumulation and contaminants in the soil can have devastating effects on the environment and human health [7, 8]. Environmental pollution can enter the food chain in the soil and cause a serious threat to animal and human health. Petroleum hydrocarbons are one of the most common organic pollutants in the environment due to their high solubility and biodegradability which are known to be toxic to many organisms and unfortunately, these pollutants have entered the soil and groundwater through runoff and fuel tanks,

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endangering human health [9, 10].

Various physical, chemical, and biological methods have been used to clean up petroleum hydrocarbons pollution. Among these, phytoremediation is a friendly, low-cost, and innovative method to use the plant to reduce many organic and inorganic pollutants and has been used to monitor soil pollution [11, 12]. However, soil contamination can negatively affect phytoremediation efficiency by reducing plant biomass. On the other hand, in the central regions of the country, salinity and drought stress [13, 14] as two plant stressors have a negative impact on plant growth. Thus, it is necessary to look for appropriate solutions to deal with these stressors. Accordingly, Mousavi Kouhi *et al.* (2020) investigated the phytoremediation potential of native plant species naturally growing in a heavy metal-polluted saline-sodic soil and concluded that saline soils can increase the availability of heavy metals and can decrease the plant biomass that is an important factor in phytoremediation efficiency. However, they did not consider the simultaneous effect of heavy metal contamination with petroleum compounds and their role on phytoremediation efficiency [15]. Therefore, it is necessary to look for a suitable solution to reduce the negative effects of plant stress such as salinity and drought which can help increase the efficiency of phytoremediation. In this regard, Zamani *et al.* (2016) reported that plant rhizosphere microorganisms can help to increase the plant resistance to abiotic stresses. However, they did not consider the plant abiotic stresses such as drought or salinity [16]. On the other hand, the results of several researchers showed that plant inoculation with *piriformospora indica* (*P.indica*) can help to increase plant growth which may enhance the soil microbial activity that a positive role on biodegradation of petroleum hydrocarbons in the soil [16, 17]. *P. indica* has been a well-known and most studied entophytic fungus for vegetative growth and resistance to plants since past decades. Zamani *et al.* (2018) investigated the effect of *P.indica* on the degradation of petroleum hydrocarbons in the soil and concluded that inoculation of corn with *P.indica* had a significant effect on increasing the plant growth and enhancing the biodegradation of petroleum hydrocarbons in the soil [16]. On the other hand, the physiology and plant cultivation system can have a positive effect on soil microbial activity and thus the decomposition of petroleum compounds in the soil, which needs to be studied in separate studies for each plant and in different soil physic-chemical conditions [18].

Today, the problem of simultaneous soil contamination with heavy metals and petroleum compounds is one of the main issues of the country's industrial areas and remediation of these areas depends on the physical and chemical characteristics of the soils of each region and its climatic characteristics. Among these, salinity and drought stresses in arid and semi-arid regions are two plant abiotic stresses that can restrict the plant biomass [19] and thereby decrease the plant phytoremediation efficiency [20] or biodegradation of petroleum hydrocarbons in the soils [21]. One of the effective solutions is changing the cultivation pattern such as intercropping system or plant inoculation with symbiotic bacteria such as *P. indica* which can reduce the negative effects of salinity, drought, or heavy metals stresses. However, the plant type, climate condition, and soil physico-chemical properties can have different effects on phytoremediation efficiency that should be considered in different researches. Thus, this research was carried out to evaluate the effect of plant inoculation with *P. indica* fungus, drought, and salinity stress on biodegradation of diesel oil in a Pb and Cd contaminated soil under cultivation of corn and white clover intercropping system.

2. Materials and Methods

To investigate the effect of *P. indica* fungus, drought, and salinity stress on biodegradation of diesel oil in a Pb and Cd contaminated soil under cultivation of corn and white clover intercropping system, a soil with low organic carbon that was naturally polluted with Pb and Cd was selected. Selected physicochemical properties of studied soil were shown in Table 1.

Treatments consisted of corn and white clover intercropping system which were as a main crop and intercrop, respectively, with three plant densities of white clover (0, 20, and 30 seeds per pots). In addition, corn was inoculated with *P.indica* under soil salinity (initial salinity and 6 dS/m) and water stress (Normal (D_0)) and intensive (D_1) and was cultivated in the soil that was artificially polluted with diesel oil at the ratio of 0 and 4 % (W/W) and naturally contaminated with Pb (600 mg/ kg soil) and Cd (mg/kg soil). This research was done as factorial experiments in the layout of a randomized completely block design with three replications. To perform this experiment, the Pb and Cd-polluted soil was contaminated with diesel oil at the rates of 0 and 4 % (W/W) and incubated for one month to equilibrium. After that, soil salinity was applied and the initial salinity of soil was considered as a treatment.

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Table 1: Selected soil physico-chemical properties

Characteristic	Unit	Amount
Soil texture	-	Silt loam
pH	-	7.0
EC	dS/m	3.1
Pb available	mg/kg soil	600
Cd available	mg/kg soil	15
As available	mg/kg soil	ND*
Organic carbon	%	0.1
$CaCo_3$	%	8

*ND: Not detectable by atomic absorption spectroscopy (AAS)

After that, soil salinity was applied and the initial salinity of soil was considered as a treatment. For increasing the soil salinity from 3.1 (initial salinity of soil) to 6 dS/m, the studied soil was irrigated several times with saline water to reach the equilibrium of soil EC equal to 6 dS/m. these rates of salinity were selected to find out the role of experimental treatments on plant growth in saline soils (EC>4 dS/m). The climate condition in the greenhouse was set up in 14h photoperiod, relative air humidity of 45-45% and temperature of 22/17 ºC (day/night). Soil moisture in normal conditions was controlled at 70% of the total water holding capacity (FC) during the experiment by daily watering. The intensive drought stress (D_1) was designed at the permanent wilting point (PWP) when the plant has a problem with water supply.

Three days after seed germination on filter paper, the corn seedlings (as the main crop) and white clover (as an intercrop) were transferred into 5 kg plastic pots (two seedlings in each pot) as a monoculture and intercropping system and filled with the treated soil. Two fungal plugs of 10 mm in diameter were placed at a distance of 1 cm below the corn and white clover seedlings in the soil at sowing time. *P. indica* was prepared from the soil and water research institute, Tehran, Iran. Plants were irrigated under normal (full irrigation (D_0)) and intensive (70% water depletion of field capacity) drought stress (D_1). After 10 weeks of plant growth, the percentage of diesel oil degradation of diesel oil in the soil was measured using the GC with a Delsi DI 200 chromatograph equipped with a direct injection port and an FID detector at 340 C. The carrier gas was helium under 0.08 MPa and the column was a CP Sil 5 CB (Chrompack) capillary column (50 m by 0.32 mm, film thickness 0.25 µm). The soil microbial respiration as an index of soil activity was determined as evolved CO2 according to the method that was described by Besalatpour *et al.* (2011) [22]. Peroxidase (POX) [23] and ascorbate peroxidase (APX) enzyme activity were also determined [24].

2.1. Statistical analysis

A completely block randomized design in three replications was used. The statistical analyses of data were performed using the ANOVA procedure. Differences between means were evaluated using the least significant difference (LSD test). The 0.05 probability value was used to determine the significant difference.

3. Results and Discussion

The greatest degradation percentage of diesel oil in the soil (Table 2) belonged to the soil under cultivation of plants (intercropping system) inoculated with *P.indica,* while the lowest was measured in the absence of *P.indica* under salinity and drought stress. Using corn and white clover intercropping system significantly increased the biodegradation of diesel oil in the soil, as, the results of the current study showed that using corn in intercropping relative to mono-culture system significantly increased the degradation of diesel oil in the soil by 13.1% that may be related to the role of plant resistance to abiotic stresses [25] and consequently increase the plant root exudate that can help to increase the diesel oil degradation in the soil [26]. The results of a study by Bian *et al.* (2021) indicated that [18] using the intercropping system can enhance the phytoremediation of heavy metal efficiency through changes in the soil rhizosphere properties that are similar to our results [18].

In addition, increasing the soil microbial respiration (Table 3) with increasing the diesel oil percentage in the soil can indicate that petroleum hydrocarbons in the soil can be used as a carbon source for soil micro-organisms [27]. However, the highest level of petroleum hydrocarbon pollution may soil microbial respiration adversely. In this regard, Mitter *et al.* (2021) investigated the impact of diesel and biodiesel contamination on soil microbial community activity and structure and concluded that increasing the soil pollution with petroleum hydrocarbons had adverse effects on soil microbial respiration [28].

Table 2: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on biodegradation of diesel oil (%) in the soil in the presence and absence of *P.indica*

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	NC**	NC	NC	NC
		4	68.1d*	62.7i	64.3g	61.7j
	1:20	0	NC	NC	NC	NC
		4	70.1c	65.4f	67.7e	64.1g
	1:30	0	NC	NC	NC	NC
		4	72.4a	68.3d	71.1b	65.2
D_1	1:0	0	NC	NC	NC	NC
		4	65.1f	60.5k	62.1i	59.7l
	1:20	0	NC	NC	NC	NC
		4	68.3d	62.5i	64.2g	61.3j
	1:30	0	NC	NC	NC	NC
		4	70.3c	65.4f	67.3e	63.8h

*Data with similar letters are not significant ($P < 0.05$), **NC: Not measured, D0 and D1 are normal and intensive drought stress, respectively.

Table 3: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on soil microbial respiration (mg C-CO_2/kg soil)

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	14.0z*	13.7	13.6b´	13.2e´
		4	16.5h	16.3j	16.0	15.4o
	1:20	0	14.9s	14.6u	14.5v	14.0z
		4	17.2d	16.9g	17.0f	16.5h
	1:30	0	15.2p	15.0r	14.7t	14.3w
		4	18.4a	18.1b	18.1b	17.8c
D_1	1:0	0	13.5c´	13.1f˝	13.1f˝	12.5g´
		4	16.1l	16.0m	15.5n	15.1q
	1:20	0	14.1y	13.7a´	13.6b´	13.3d´
		4	17.0f	16.4i	16.3j	16.0m
	1:30	0	14.6u	14.2x	14.1y	13.7a´
		4	17.1e	16.2k	16.5h	16.0m

*Data with similar letters are not significant ($P < 0.05$), D0 and D1 are normal and intensive drought stress, respectively.

In this regard, Mitter *et al.* (2021) investigated the impact of diesel and biodiesel contamination on soil microbial community activity and structure and concluded that increasing the soil pollution with petroleum hydrocarbons had adverse effects on soil microbial respiration [28]. It seems that the effect of petroleum hydrocarbons on soil microbial activity in different areas depends on the soil physic-chemical properties that should be studied in different conditions. In this regard, Baghaie *et al.* (2020) investigated the effect of corn and white clover intercropping on biodegradation of diesel oil in arsenic-contaminated soil in the presence of *P.indica* and concluded that cultivation of corn and white clover in an intercropping system can increase the biodegradation of diesel oil in the contaminated soil. However, they did not consider the role of plant abiotic stresses such as drought or salinity toxicity and their role in degradation of petroleum hydrocarbons in the soil [29]. In addition, increasing the corn to white clover can improve the biodegradation of diesel oil in the soil. The results of the present study showed that increasing the corn to white clover ratio from 1:0 to 1:20 and 1:30 significantly increased the biodegradation of diesel oil in the soil by 14.5 and 13.5% under normal conditions (whit out any abiotic stress), respectively. A significant increase by 20 % in biodegradation of diesel oil in the soil was observed, when the plant biomass was improved by 17.3% in the intercropping system which indicates that increasing the plant biomass may increase the plant root exudate which is an important carbon source for enhancing the soil microbial activity [30, 31] and thereby improve the biodegradation of diesel oil in the soil. In this regard, Jin *et al.* (2019) investigated the effect of plants and their root exudate on bacterial activities during rhizobacterium–plant remediation of petroleum hydrocarbons and concluded that plant root exudate has a significant effect on increasing the biodegradation of petroleum hydrocarbons that is in line with our results [32]. The presence of *P.indica* had a significant effect on increasing the biodegradation of diesel oil in the soil (Table 2). However, salinity and drought stress had an adverse effect on biodegradation of diesel oil in the soil. Based on the results of this study, the biodegradation of diesel oil significantly increased by 15.1% in the soil under cultivation of the plants inoculated with *P.indica* in the intercropping system under normal conditions (without any stress). For salinity and drought stress, they were increased by 10.1 and 12.2%, respectively. Generally, *P.indica* has been a well-known and most studied entophytic fungus for vegetative growth and resistance to plants in past decades. Accordingly, Su *et al.* (2017) reported that *P.indica* can enhance the quality, seed yield, and growth of oilseed plants. However, they did not mention the interaction effects of *P.indica* with plants' abiotic stresses that is the main objective of our study [33].

Salinity and drought stress had a significant effect on decreasing the biodegradation of diesel oil in the soil. Based on the results of our study, increasing soil salinity from 2 to 6 dS/m significantly decreased the biodegradation of diesel oil in the soil by 14.3% that can be related to the role of soil salinity on increasing the heavy metal availability and thereby decrease the soil microbial activity that has an important role on biodegradation of diesel oil in the soil [34]. Khoshgoftarmanesh *et al.* (2004) investigated the effects of soil salinity on soil and plant Cd concentration and concluded that saline irrigation water can increase the soil cd availability and thereby increase the plant Cd uptake [35]. In addition, Zhang *et al.* (2020) evaluated the effect of salinity on Cd distribution and accumulation in two contrasting halophytes, *Suaeda glauca* and *Limonium aureum* and concluded that soil salinity had a significant effect on Cd uptake by plants [36]. Generally, salinity can influence the Cd solubility by the formation of Cd-chloride complexes or instigating sodium competition at the adsorption sites of soil particles, which have been shown to increase the activity and bioavailability of Cd, causing more Cd to be absorbed by plants [37].

The interaction effects of drought and salinity stress on diesel oil biodegradation in soil were also significant. However, the cultivation system had also a significant effect on bio-degradation of diesel oil in the soil. Accordingly, a significant decrease was observed in biodegradation of diesel oil in the soil by 17.3%, when the corn (mono-culture system) was exposed to salinity and drought stress. Although this reduction was less in the intercropping system which can be attributed to the role of the intercropping system on increasing the plant resistance to environmental stresses such as salinity or drought stresses and possibly increases the soil microbial activity via consumption of carbon compounds in root secretions. Increasing soil microbial activity (Table 3) in corn and white clover intercropping system relative to corn mono-culture confirms our results. Minai *et al.* (2006) investigated the role of salinity on bioremediation of petroleum hydrocarbon in soil and concluded that soil salinity had a negative effect on biodegradation of crude oil in the soil. However, they did not mention the role of salinity on petroleum hydrocarbon degradation in heavy metal polluted soil [38].

The highest APX (Table 4) and POX (Table 5) enzyme activity has belonged to the corn cultivated in a mono-culture system under salinity and drought stress in the absence of *P.indica*, while the lowest was measured in the corn cultivated in the intercropping system under normal condition. Regardless of the cultivation system, plant cultivation under drought or salinity stress significantly increased the APX or POX enzyme activity. However, the APX or POX enzyme activity was lower under intercropping relative to monoculture system. Accordingly, a significant decrease in APX enzyme activity was observed under drought and salinity stress by 11.3 and 14.2%, respectively, when the corn was cultivated in intercropping relative to monoculture system which can be related to the role of white clover as a intercrop plant on increasing plant resistance to abiotic stress.

At this time, increasing the corn to white clover ratio also helped to increase the plant resistance to abiotic stresses. Increasing the corn to white clover ratio from 1:10 to 1:30 significantly decreased the APX enzyme activity by 13.1 and 15.2 %, respectively.

For the POX enzyme, similar results were observed. A significant decrease by 15.3 and 17.1% in APX and POX enzyme activity was observed when the corn cultivated in intercropping system inoculated with *P.indica* indicating that inoculation of plant with *P.indica* can improve the plant resistance among the abiotic stresses such as salinity or heavy metals toxicity.

Increasing the plant biomass (Table 6) in corn intercropping relative to monoculture system has significantly decreased the APX and POX enzyme activity and increased the biodegradation of diesel oil in the soil.

The remarkable point of this research is that the white clover planting as an intercrop has been able to increase the microbial respiration activity of the rhizosphere plant, and this has been able to increase the biodegradation of petroleum compounds in the soil. Increasing the plant biomass (Table 6) and soil microbial respiration (Table 3) in intercropping system indicated that using intercropping relative to monoculture system can increase the plant resistance to abiotic stress via increasing plant biomass and consequently increase the biodegradation of petroleum compounds in heavy metal polluted soil which is an important factor in environmental studies. In the meantime, increasing the corn to white clover ratio through increasing the white clover density in the intercropping system can help to increase the biodegradation of diesel oil in the soil. However, the salinity and drought stress had a significant effect on degradation of diesel oil in the soil. Generally, white clover as an intercrop plant can elevate the nitrogen fixation which can help to improve the biomass and nutrient uptake of the main crop (corn) and consequently can increase the plant resistance to abiotic stress and enhance the biodegradation of diesel oil in the soil [39].

Li *et al.* (2009) compared the effect of intercropping and mono-culture system on the plant growth in Cd polluted soil and concluded that using intercropping relative to monoculture system had a significant effect on the plant growth which attributed to the role of intercrop plants on

Table 4: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on APX enzyme activity (Unit/mg protein)

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	10.8w	11.1u	11.3s	11.5q
		4	11.9n	12.1l	13.1c	13.4b
	1:20	0	10.5y	10.7x	10.8w	11.2t
		4	11.6p	11.9n	12.6g	13.0d
	1:30	0	10.0b´	10.2a´	10.4z	10.8w
		4	11.2t	11.6p	11.9n	12.4i
D_1	1:0	0	11.5q	11.8o	11.9n	12.1l
		4	12.5h	12.8e	13.4b	13.5a
	1:20	0	11.0v	11.3s	11.4r	11.8o
		4	12.0m	12.2k	12.7f	13.1c
	1:30	0	10.5y	10.8w	10.8w	11.4r
		4	11.5q	11.8o	11.9n	12.3j

*Data with similar letters are not significant (P < 0.05), D0 and D1 are normal and intensive drought stress, respectively.

Table 5: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on POX enzyme activity (Unit/mg protein)

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	12.2s*	12.5q	12.7o	13.0
		4	13.4j	13.9e	13.7g	14.2c
	1:20	0	12.0t	12.2s	12.6p	12.7o
		4	13.1n	13.6h	13.3k	13.8f
	1:30	0	11.5x	11.8v	11.8v	12.0t
		4	12.7o	13.1n	13.0	13.5i
D_1	1:0	0	12.0t	12.3r	12.3r	12.6p
		4	13.7g	14.2c	14.1d	14.4a
	1:20	0	11.6	11.9u	11.8v	12.2s
		4	13.4j	13.9e	13.7g	14.3b
	1:30	0	11.0a´	11.4y	11.3z	11.6
		4	13.1n	13.7g	13.2l	13.8f

*Data with similar letters are not significant ($P < 0.05$), D0 and D1 are normal and intensive drought stress, respectively.

nitrogen fixation and thereby increase the plant growth of the main crop (corn) and improve the plant resistance to abiotic stress such as heavy metals that is similar to our results. However, they did not consider the interaction effects of other abiotic stresses such as salinity and drought [39]. Increasing the plant growth in the intercropping system has been mentioned by different researchers [30, 40]. The results of Hamzei *et al.* (2015) showed that chickpea-barley intercropping improved all canopy growth indices in comparison to sole cropping that is in line of our results [40].

The greatest plant Cd (Table 7) and Pb (Table 8) concentration was measured in the corn cultivated in the intercropping system under normal conditions, while the lowest belonged to the corn in monoculture system under salinity and drought stress.

Regardless of salinity and drought stress, corn cultivation in the intercropping system significantly increased the Pb and Cd concentration. The results of our studies showed that corn cultivation in the intercropping system significantly increased the Pb concentration under normal, salinity, and drought stress by 15.3, 12.5, and 13.2%, respectively. For plant Cd concentration, it was increased by 13.1, 8.3, and 11.9%, respectively which can be attributed to the role of intercrop plant in increasing the growth of main plant and thereby enhancing the heavy metal uptake by corn.

However, plant abiotic stresses had negative effects on heavy metal uptake by plants or biodegradation of diesel oil in the soil that is a negative point in environmental studies is in line with our results. Accordingly, the present research showed that corn cultivation in monoculture relative to intercropping system significantly decreased the plant Pb and Cd concentration by 12.2 and 10.8 % under salinity and drought stress, respectively. For biodegradation of diesel oil in the soil, it was decreased by 14.2 and 11.7% indicating the more negative role of salinity than drought stress on heavy metal uptake or biodegradation of petroleum hydrocarbon in the soil. Generally, soil salinity can increase the solubility of heavy metals in the soil that has toxic effects on soil micro-organism or plant growth and thereby decrease the biodegradation of heavy metals in the soil [41]. The remarkable point of this research is that increasing the ratio of corn to white clover can moderate the negative effects of drought or salinity stress on the sorption of heavy metals or the biodegradation of petroleum hydrocarbon in the soil. According to our results, increasing the corn to white clover ratio from 1:10 to 1:30 in the intercropping system significantly increased the plant Pb and bio-degrading of diesel oil in the soil by 13.7 and 14.3% under salinity and drought stress, respectively. For plant Cd concentration, it was increased by 15.2 %.

Table 6: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on plant biomass (g)

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	4.55k	4.50p	4.45s	4.47q
		4	4.60j	4.51o	4.52n	5.41a
	1:20	0	4.71d	4.65g	4.68e	4.61i
		4	4.65g	4.60j	4.61i	4.51o
	1:30	0	4.75b	4.71d	4.71d	4.65g
		4	4.72c	4.68e	4.67f	4.63h
D_1	1:0	0	4.45s	4.40v	4.39w	4.34x
		4	4.39w	4.32y	4.32y	4.25a´
	1:20	0	4.55k	4.50p	4.51o	4.46r
		4	4.50p	4.44t	4.42u	4.29z
	1:30	0	4.60j	4.53m	4.54l	4.50p
		4	4.32y	4.22b´	4.21c´	4.17d´

*Data with similar letters are not significant ($P < 0.05$), D0 and D1 are normal and intensive drought stress, respectively.

Table 7: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on plant Cd concentration (mg/kg plant)

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	8.8y*	9.4u	9.3v	9.8q
		4	10.1o	10.5l	10.5l	10.9i
	1:20	0	9.5t	10.1o	10.5l	10.8j
		4	11.8g	12.5e	12.4f	13.7d
	1:30	0	10.1o	10.4m	9.5t	9.9p
		4	13.9c	14.2a	13.7d	14.1b
D_1	1:0	0	7.6e´	7.9d´	7.9d´	8.3b´
		4	8.9x	9.4u	9.5t	9.9p
	1:20	0	8.1c´	8.5a´	8.6z	9.1w
		4	9.3v	9.7r	10.2n	10.6k
	1:30	0	9.5t	9.6s	10.1o	10.6k
		4	10.1o	10.6	10.9i	11.3h

*Data with similar letters are not significant ($P < 0.05$), D0 and D1 are normal and intensive drought stress, respectively.

Table 8: Effect of corn and white clover intercropping, soil salinity, drought stress, and diesel oil on plant Pb concentration (mg/kg plant)

Drought stress	Corn to white clover ratio	Diesel oil (% W/W)	*P.indica (+)*		*P.indica (-)*	
			Soil salinity (dS/m)			
			Initial salinity	6	Initial salinity	6
D_0	1:0	0	140.3n*	142.7l	137.6q	141.3m
		4	143.7	148.2i	141.2m	145.4j
	1:20	0	141.7m	145.8j	140.2n	142.1l
		4	151.2h	155.4e	148.2i	152.7g
	1:30	0	155.6e	159.2d	151.6h	153.6f
		4	165.8b	169.1a	162.8c	165.3b
D_1	1:0	0	131.2v	133.6t	127.5x	131.4v
		4	135.2s	139.3o	130.1w	132.8u
	1:20	0	133.6t	137.2q	130.1w	132.5u
		4	138.7p	141.4m	135.2s	136.1r
	1:30	0	135.1s	138.3p	131.3v	133.7t
		4	139.1o	141.2m	135.1s	136.2r

*Data with similar letters are not significant ($P < 0.05$), D0 and D1 are normal and intensive drought stress, respectively.

4. Conclusion

Based on the results of this study, plant inoculation with *P.indica* had a significant effect on increasing biodegradation percentage of diesel oil in the soil which can be related to the positive role of *P.indica* fungus on increasing the plant biomass and may increase the plant root exudate which is an important carbon source for soil microbial activates. Therefore, increasing soil microbial activities can increase the biodegradation percentage of diesel oil in the soil. However, salinity and drought stress had an adverse effect on decreasing the diesel oil biodegradation in the soil. Increasing the soil salinity from the initial level (for control soil) to 6 dS/m significantly increased the APX and POX enzyme activities which indicate that plants can resist to plant abiotic stress via increasing the plant enzyme activities. In addition, the corn intercropping system had significant effect on increasing the diesel oil biodegradation in the soil. However, the plant physiology of the main crop, intercrop plant type, and soil physic-chemical properties have different effect on diesel oil biodegradation in the soil which should be considered in different studies.

Authors' Contributions

Amir Hossein Baghaie: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Writing-original draft; Writing-review and Editing.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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