JAEHR
Journal of Advances in Environmental Health Research
J Adv Environ Health Res, 2023; 11(3): 182-188. doi: 10.34172/jaehr.1337
http://jaehr.muk.ac.ir

Original Article

Inorganic and Organic Fe Sources Increased Crude Oil Biodegradation in Soil Under Cultivation of the Canola Plant Inoculated with *Piriformospora indica*

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Article history:
Received: April 15, 2023
Accepted: June 20, 2023
ePublished: September 29, 2023

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Abstract

Background: This study was done to investigate the impact of drought stress, inorganic and organic Fe sources on biodegradation of crude oil in the Cd polluted soil under cultivation of canola inoculated with *Piriformospora indica*.

Methods: Treatments consist of soil application of pure iron from Fe sources ((Fe sulfate and Fe chelate) (0, 60 and 90 kg Fe pure/ha)), and canola plant inoculated with *P. indica* cultivated in the Cd (0, 15 and 20 mg Cd/kg soil)-polluted soil that was naturally polluted with crude oil under drought stress. After 70 days, the plants were harvested and the soil and shoot Cd concentration was determined using atomic absorption spectroscopy (AAS). In addition, the biodegradation of crude oil was measured.

Results: Using 90 kg/ha pure iron from iron chelate significantly improved the biodegradation of crude oil in the soil by 13.1 and 8.9% under normal soul moisture and drought stress, respectively. Plant inoculation with *P. indica* had significant effect on increasing the biodegradation of crude oil in the soil by 12.1%. Furthermore, the soil microbial respiration was also increased. The ascorbate peroxidase (APX) and peroxidase (POX) enzyme activity was significantly increased under heavy metal toxicity.

Conclusion: Using organic and inorganic Fe sources has significant effects in increasing the biodegradation of crude oil in the soil under normal soil moisture regime and drought stress. Regardless of soil moisture regime, plant inoculation with *P. indica* had significant effects on reducing the Cd concentration of the plant and increasing the biodegradation of crude oil in the soil, respectively.

Keywords: Cd, Soil pollution, Biodegradation, Petroleum hydrocarbon

Please cite this article as follows: Baghaie AH. Inorganic and organic Fe sources increased crude oil biodegradation in soil under cultivation of the canola plant inoculated with *Piriformospora indica*. J Adv Environ Health Res. 2023; 11(3):182-188. doi:10.34172/jaehr.1337

Introduction

Soil pollution, which is caused by improper use of hazardous urban and industrial waste, has long been an environmental issue that has caused social concerns.[1] Soil is the substrate on which the life of living organisms depends, and soil is the third major component of the environment. The risk of soil pollution is not less than the risk of air pollution, but because this type of pollution is not tangible.[2,3] it is given less attention. If the contaminated soil is not remediate and the pollutants are not removed from it, the pollutants can penetrate deep into the soil over time and cause it to be transferred to the food chain and thus cause groundwater pollution.[4] This increases the costs of environmental pollution monitoring, including heavy metals or petroleum compounds. Therefore, one of the most important problems of global environmental organizations is the problem of soil pollution removal.[5]

Heavy metals in high concentrations alter the structure of proteins, interfere with enzyme activities and change their specificity, or by substituting an essential element in its binding sites or by ligand interaction, disrupts cell function and cause symptoms. Thereby, cause the lack of element in the plants. On the other hand, since the composition of the soil is heterogeneous and consists of various organic and organic-mineral substances, the mechanisms of pollutant binding and the forms of their occurrence in the soil are complex and change with the composition and physical characteristics of the soil.[6]

One of the important problems related to heavy metals is that they are not metabolized in the human body, in such a way that the heavy metals that have entered the human body are not excreted from the body and accumulate in the muscle and bone tissues. In general, the entry of heavy metals into the body causes harmful effects such as neurological disorders, types of cancers, nutrient deficiency, hormone imbalance, respiratory and cardiovascular disorders, damage to the liver, kidneys, and brain, and in severe cases cause death.[7] On the other hand, the accumulation property of heavy metals and their bio-magnification in plants and their entry into the food chain doubles the risks caused by them. Therefore, pollution caused by heavy metals is a serious and fundamental problem that must be tried to reduce the effects of this pollution.[8]

Due to the effects of toxicity in polluted places, the plant cultivation is difficult and the remediation of soils contaminated with metals is difficult and is considered a big challenge. Unlike organic pollutants, metals cannot be decomposed and usually, the decontamination of soil contaminated with them requires the separation of toxic metals from such environments. Meanwhile, the cost of soil remediation is very variable and depends on the pollutant concentration, soil properties and environmental conditions.[5,9] According this, the plant rhizosphere is an important intermediate environment between soil and plants and plays a key role in plant removal of heavy metals from soil. In this environment, microorganisms change the ability of heavy metals to be accessible to the plant by creating chelates, acidifying the root environment, solubilizing phosphate and changing the oxidation number of metals, and as a result, affect plant heavy metals sorption. One of the proposed solutions to increase the efficiency of plant treatment is to use the symbiotic relationship of beneficial soil microorganisms with plants, including the process of inoculation with fungi, including *Piriformospora indica*.[10] The difference between *P. indica* and mycorrhizal fungi is its wide host range. This fungus has the ability to increase the growth and performance of different hosts, including legumes and medicinal plants.[11]

Today, there is a problem of simultaneous pollution of heavy metals and petroleum compounds in the industrial soils of the country. On the other hand, due to the change in the country's climate, drought stress is one of the main problems that hinders the growth of plants,[12] especially heavy metal hyper-accumulators, and thereby affect on the efficiency of phytoremediation. On the other hand, there is a problem of lack of essential elements, especially micronutrients (including iron) and adding such compounds may change the process of phytoremediation by increasing plant growth. Although the physic-chemical characteristics of the soil, such as the type of pollutants and drought stress, are effective on the efficiency of phytoremediation. On the other hand, plant inoculation with *P. indica* fungi may also be effective on the process of removing heavy metals, especially in the soils of industrial areas of the country, where there is a problem of simultaneous contamination of heavy metals with petroleum compounds. Therefore, this research was conducted with the aim of investigating the effect of iron sulfate and iron chelate application on the biodegradation rate of crude oil in a soil contaminated with Cd under canola cultivation inoculated with *P. indica* that has been exposed to drought stress.

Materials and Methods

To investigate the role of drought stress and organic and inorganic Fe sources on Cd uptake by canola, a factorial experiment in the layout of randomized block design was selected. Treatments (216 samples) including of soil application of pure iron from Fe sources ((Fe sulfate and Fe chelate) (0, 60 and 90 kg Fe pure/ha)), and canola plant inoculated with *P. indica* cultivated in the Cd (0, 15 and 20 mg Cd/kg soil)-polluted soil that was naturally polluted with crude oil (Total petroleum hydrocarbons (TPHs) = 7000 mg/kg soil) under normal moisture condition (full irrigation) and drought stress (70% water depletion of field capacity). For this research, a non-saline soil (soil texture = Sandy loam) (EC = 1.3 dS/m)) with low organic carbon (OC = 0.2%) and low calcium carbonate (8% (W/W) that was naturally polluted with crude oil was selected. The soil being studied was contaminated with Cd at rates of 0, 15, and 20 mg Cd/kg soil at the beginning of the experiment, and it was incubated for two weeks to reach equilibrium. The soil was then treated with 0, 60, and 90 kg of pure iron (iron sulfate and iron chelate) and allowed to equilibrate for two weeks. Then, treated soil was put in to the 5 kg plastic pots. Following the production of the initial *P. indica* fungus inoculum (Figure 1), some of the fungus was taken away from the culture media surface, stained with fuchsin acid, and its spherical bodies and mycelium were studied under an optical microscope. After that, 10 mL of sterile water containing 0.02% (v/v) tween 20 was applied to the plate surface before being gently scraped with a spatula to collect the chlamydospores. To eliminate the mycelium fragments, spore suspension was filtered. The sample was then centrifuged for 7 minutes at 3000 g, suspended in 0.02% Tween 20, and the quantity of spores was determined using a neobar lam. The presence of 5×10^5 [*P. indica* chlamydospore $(mL)^{-1}$] fungal spores

Figure 1. *Piriformospora indica* Spores

is required to have enough inoculum for *P. indica* to infect roots.[11]

Half of the seedlings' roots were then infected with *P. indica* by dipping them in a suspension of the bacterium for 2 hours containing 5×10^5 [*P. indica* spore (ml suspension)$^{-1}$]. The canola plant was harvested after 70 days and rinsed with deionized water. Atomic absorption spectroscopy (AAS) (Perkin Elmer, model:3030, USA) was used to determine the soil[13] and plants Cd concentration.[14] According to Besalatpour et al, the soil microbial respiration and the degradation of crude oil in the soil were also determined.[15] The ascorbate peroxidase (APX) and peroxidase (POX) enzyme activity was also determined.[16] The biodegradation of crude oil was measured according to the Hatami et al method.[17] According this, the concentration of soil TPHs was measured before the cultivation period (89.5 g kg^{-1} soil). To extract TPHs from contaminated soil, 10 mL of dichloromethane was added to 2 g of soil and shacked for 10 min in 500 rpm. Then the suspension was centrifuged in 3000 rpm for 10 minutes to separate supernatant from the soil. The above step was repeated twice and the supernatant was added to previous one. Then, the supernatant was left in laboratory temperature for 24 hours to evaporate dichloromethane and the remaining was weighed. The concentration of TPHs in soil samples was measured as described above

SAS V. 9.1 was used for the statistical analysis. To determine the variations between the means, the least significant difference (LSD) test was applied. To determine whether there was a significant change, the 95 percent probability value ($P<0.05$) was taken into account.

Results and Discussion

Piriformospora indica plant inoculation showed a considerable impact on lowering the shoot Cd concentration (Table 1), as the results of this study has been shown that the lowest soil Cd concentration has belonged to the soil under plant cultivation that inoculated with *P. indica*. Based on the results of this study, plants inoculated with *P. indica* grown in Cd-polluted soil considerably reduced the soil Cd availability by 11.7%. that may be related to the role of *P. indica* on the plant growth (Data was not shown) and thereby immobilize the Cd in the soil. According this, according to research done by Davodpour et al on the bio-concentration and stabilization potential investigations of arsenic and other heavy metals in *Astragalus gossypinus*, enhancing plant development has an important effect on the immobilization of heavy metals in the soil[18] that is similar to our results. It is noteworthy that the efficiency of inoculation of plants cultivated in the soils contaminated with Cd is lower than in non-contaminated soils, as, the results of our study showed that inoculation of plants cultivated in the Cd-polluted (15 mg Cd/kg soil) and non-pollute soil significantly decrease the Cd availability by 13.2 and 17.1%, respectively. On the other, the soil Cd availability was significantly increased in the soils under cultivation of plants in drought stress.

The biodegradation of crude oil in the soil (Table 2) was significantly affected by the treatments. According this, using inorganic and organic Fe sources in the form of sulfate and chelate had significantly decreased the soil Cd availability, as the results of our study has been showed that using 90 kg/ha Fe from sulfate and chelate

Table 1. The Impact of Treatments on Cd Concentration of the Studied Soil (mg/kg soil)

Cd Pollution	Plant Inoculation	Normal						Drought Stress					
		Iron Slag (kg/ha)			Iron Sulfate (kg/ha)			Iron Slag (kg/ha)			Iron Sulfate (kg/ha)		
		0	60	90	0	60	90	0	60	90	0	60	90
0		ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	+ *P. indica*	14.3r**	13.9v	13.5x	14.3r	14.1t	13.7w	14.5q	14.2s	14.0u	14.5q	14.3r	14.1t
20		18.4g	17.3l	17.0m	18.4g	17.8j	17.5k	19.1d	18.2h	17.5k	19.1d	18.7f	17.8j
0		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	- *P. indica*	14.7o	14.2s	13.9v	14.7o	14.5q	14.1t	14.8n	14.5q	14.2s	14.8n	14.7o	14.6p
20		18.9e	18.4g	18.1i	18.9e	18.7f	18.2h	19.6a	19.3c	19.1d	19.6a	19.5b	19.3c

*ND: Not detectable by AAS, ** Data with similar letters do not indicate significant differences ($P>0.05$).

Table 2. The Impact of Treatments on Biodegradation of Diesel Oil in the Soil (%)

Cd Pollution	Plant Inoculation	Normal						Drought Stress					
		Iron Slag (kg/ha)			Iron Sulfate (kg/ha)			Iron Slag (kg/ha)			Iron Sulfate (kg/ha)		
		0	60	90	0	60	90	0	60	90	0	60	90
0		55.3f*	58.4c	61.2a	55.3f	57.3d	59.4b	51.7j	55.2f	57.4d	51.7j	53.4h	55.7f
15	+ *P. indica*	50.7k	55.4	57.4d	50.7k	53.2h	54.6g	48.2l	50.3k	54.4g	48.2l	48.2l	50.2k
20		45.7o	47.8m	50.1k	45.7o	46.2n	48.9l	41.4s	44.7p	48.2l	41.4s	43.1q	45.8o
0		53.1h	55.2f	58.4c	53.1h	54.7g	56.4e	50.1k	52.7i	55.1f	50.1k	51.5j	53.9h
15	- *P. indica*	47.6m	48.2l	50.2k	47.6m	45.2o	47.9m	44.3p	47.2m	48.1l	44.3p	45.2o	46.9n
20		40.4t	42.3r	44.1p	40.4t	41.3s	42.5r	37.8v	40.1t	42.2r	37.8v	39.2u	41.5s

* Data with similar letters do not indicate significant differences ($P>0.05$).

significantly decreased the Cd availability by 13.1% and 19.4%, respectively that can be related to the role of iron nutrient on decreasing the Cd toxicity and its effect on increasing the biodegradation of crude oil in the soil. The antagonistic effects between nutrient elements and heavy metal availability are mentioned by researchers.[19-21]

The biodegradation of crude oil in the soil was significantly higher under normal moisture condition relative to drought stress. Our results showed a significant increasing in biodegradation of crude oil in the soil by 14.2%, when the soil was amended with 90 kg Fe/ha from iron chelate source under normal moisture condition relative to drought stress. It can be concluded that the soil microbial activity (soil microbial respiration (Table 3) can be decrease under drought stress and thereby decrease the biodegradation of crude oil in the soil. In addition, soil pollution with Cd has significantly decline the biodegradation of crude oil in the soil. A significant decreasing by 17.3% in biodegradation of crude oil in the soil was observed, when the soil pollution with Cd was increase from 0 to 15 mg Cd/kg soil. In the central regions of the country, where there is a simultaneous problem of soil contamination with heavy metals and petroleum compounds, the decrease in plant growth and the availability of nutrient elements in the soil due to drought stress has reduced the efficiency of phytoremediation.

Inoculation of plant with *P. indica* has significantly increased soil microbial respiration (Table 3) and thereby has increased the biodegradation of crude oil in the which can be attributed to the role of *P. indica* in the secretion of organic compounds and thus increasing the biodegradation of crude oil in the soil. According this, a significant increasing in soil microbial respiration and biodegradation of crude oil by 11.8 and 13.7%, respectively, was observed when the plants have been inoculated with *P. indica*. In this regard, Dianat Maharluei et al investigated the effect of rice husk biochar and *P. indica* on corn yield in Zn polluted soil and conclude that corn inoculation with *P. indica* had significantly affect the soil microbial activity and that caused a significant increasing the plant growth.[22] However, they did not mention the soil physic-chemical properties such as the type of pollutants. Today, the use of microorganisms capable of symbiosis with plants such as plant growth promoting bacteria and endophytic fungi to increase the tolerance of plants to environmental stresses such as salinity, drought and soil contamination with heavy metals has given researchers hope in achieving sustainable agriculture. By colonizing the roots of different host plants, *P. indica* fungus increases their growth and increases their resistance to biotic and abiotic stresses. Unlike arbuscular mycorrhizal fungi, *P. indica* is a facultative symbiont and can easily grow in artificial culture environments. Therefore, it is very important to take advantage of the ability of this fungus to increase the plant's resistance to abiotic stresses, including water or heavy metal stresses.[23,24] In this line, Tsai et al mentioned that rice's ability to tolerate water stress is increased by *P. indica* symbiosis through control of stomata behavior and ROS scavenging mechanisms.[25] Furthermore, Zhang et al stated that *P. indica* can reduce the stress response to drought in maize via producing special genes in plant roots to control drought stress.[26]

Regardless of soil moisture regime, soil addition of organic and inorganic iron sources had significant effect on increasing the soil microbial respiration. Accordingly, using 90 kg/ha Fe pure from sulfate and chelate iron sources in the Cd-polluted soil (15 mg Cd/kg soil) significantly increased the soil microbial respiration by 13.1% and 18.6%, respectively which can be attributed to iron as a nutritional element and its role in increasing the activity of the microbial population and as a result increasing the microbial respiration. However, this increasing was higher in normal moisture condition relative to drought stress. Increasing the biodegradation of crude oil with increasing the soil microbial respiration confirms our results clearly.

The lowest shoot Cd concentration (Table 4) has belonged to the plants cultivated in the soil amended with highest application rate of iron chelate (90 kg Fe/ha) which can be related to antagonistic effects of nutrient elements with heavy metals.[27] Ratié et al reported the antagonistic effect of heavy metals with nutrient elements [28] that is in line with our results. In addition, Halajnia et al mentioned that using Pb and Mn sources can diminish the Cd uptake by sunflower[29] that confirms our results. The results of our study has been shown that the Cd sorption in plants cultivate under normal moisture normal was lower than drought stress that maybe related to the role of drought stress on decreasing the plant growth and thereby

Table 3. The Impact of Treatments on Soil Microbial Respiration (mg C-CO_2/kg soil) in the Soil

Cd Pollution	Plant Inoculation	Normal						Drought Stress					
		Iron Slag (kg/ha)			Iron Sulfate (kg/ha)			Iron Slag (kg/ha)			Iron Sulfate (kg/ha)		
		0	60	90	0	60	90	0	60	90	0	60	90
0		11.4c´*	14.8g	17.3a	11.4c´	13.2p	14.5i	10.8h´	13.1q	15.2f	10.8h´	11.3d´	13.2p
15	+*P. indica*	10.8h´	13.5o	16.2c	10.8h´	12.7u	13.8m	10.5k´	12.5v	14.7h	10.5k´	11.0g´	12.5v
20		10.2m´	12.5v	15.3e	10.2m´	11.8a´	12.5v	10.0n´	12.1x	13.9l	10.0n´	10.7i´	12.0y
0		11.2e´	13.7n	17.0b	11.2e´	13.0r	14.1k	10.5k´	12.8t	14.8g	10.5k´	11.0g´	12.9s
15	- *P. indica*	10.5k´	12.4w	15.4d	10.5k´	12.0y	13.5o	10.0n´	12.1x	14.2j	10.0n´	10.4l´	11.5b´
20		9.9o´	11.3d´	11.9z	9.9o´	11.0g´	11.4c´	9.0p´	10.4l´	10.6j	9.0p´	10.0n´	11.1f´

* Data with similar letters do not indicate significant differences ($P>0.05$).

increasing the Cd uptake by plants (dilution effect). Considering the climate change in recent years and the simultaneous contamination of soil with heavy metals and petroleum compounds, it seems necessary to find methods to reduce the sorption of heavy trace elements.

Inoculation of plants with *P. indica* had significant effects on decreasing the Cd sorption by plants, as, the results of our study showed that the lowest shoot Cd concentration had belonged to the plants inoculated with *P. indica*. Although, this reduction has been more effective in the normal moisture regime relative to drought stress. Accordingly, a significant decreasing in shoot Cd concentration by 11.8 and 19.7% was observed when the plants inoculated with *P. indica* under normal and drought stress condition. In this regard, Su reported that *P. indica* can assist the alleviation of Cd in plant.[10] Since hyper-accumulator plants have many disadvantages such as low growth rate and small biomass, plant inoculation suitable maybe a suitable way to immobilize the heavy metals in the soil and thus reduce heavy metal sorption by plants. Accordingly, Ghaffari et al mentioned that *P. indica* can improve drought stress adaptation in plants by metabolic and proteomic reprogramming.[30] Although *P. indica* is an advantageous endophytic fungus that can colonize with a variety of host plant species, but the processes by which *P. indica* symbioses with other plants may differ and require additional investigation.[23,31]

The greatest plant enzyme activity (Table 5) has belonged to the plants cultivate in the Cd-polluted soil (20 mg Cd/kg soil) without receiving any Fe fertilizer. Using 90 kg/ha Fe pure from iron sulfate and iron chelate significantly decreased the APX enzyme activity by 13.4% and 17.6%, respectively. For POX enzyme activity, it was decreased by 17.7% and 19.2%, respectively that can be relate to the role of iron sources on decreasing the shoot Cd availability and thereby decline the APX or POX enzyme activity. Based on the results of other studies, under the Fe, Zn, Cu, Cd, Mn, Ni, Pb, Hg, Cr, and As stresses, the activity of antioxidants such as superoxide dismutase (SOD), POD, APX, catalase (CAT), and hydrogen peroxidase can increase in plant.[32,33] Therefore, due to the lack of iron availability in the central regions of the country, using inorganic and organic iron compounds can help to increase the plant's resistance to abiotic stresses and thus reduce the activity of plant

Table 4. The Impact of Treatments on Shoot Cd Concentration (mg/kg)

Cd Pollution	Plant Inoculation	Normal						Drought Stress					
		Iron Slag (kg/ha)			Iron Sulfate (kg/ha)			Iron Slag (kg/ha)			Iron Sulfate (kg/ha)		
		0	60	90	0	60	90	0	60	90	0	60	90
0		ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	+*P. indica*	11.1b´**	10.8d´	10.4f´	11.1b´	10.9c´	10.7e´	11.8w	11.5x	11.2a´	11.8w	11.4y	11.3z
20		15.8l	15.3n	15.1o	15.8l	15.6m	15.3n	16.8d	16.5g	16.1k	16.8d	16.6f	16.3i
0		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	- *P. indica*	12.1u	11.8w	11.5x	12.1u	12.0v	11.8w	14.5p	13.9r	13.2t	14.5p	14.1q	13.8s
20		17.1b	16.2j	15.8l	17.1b	16.7e	16.3i	17.8a	16.9c	16.4h	17.8a	17.1b	16.8d

*ND: Not detectable by AAS, ** Data with similar letters do not indicate significant differences ($P>0.05$).

Table 5. Effect of Treatments on Plant Enzyme Activity (unit/mg protein)

Cd Pollution	Plant Inoculation	Normal						Drought Stress					
		Iron Slag (kg/ha)			Iron Sulfate (kg/ha)			Iron Slag (kg/ha)			Iron Sulfate (kg/ha)		
		0	60	90	0	60	90	0	60	90	0	60	90
								APX Enzyme Activity					
0		11.1o´*	10.8q´	10.5r´	11.1o´	10.9p´	10.8q´	11.5l´	11.3n´	11.1o´	11.5l´	11.4m´	11.3n´
15	+*P. indica*	13.7c´	12.6j´	12.4k´	13.7c´	12.9h´	12.6j´	13.9a´	13.1g´	12.7i´	13.9a´	13.4e´	13.2f´
20		14.4w	13.8b´	13.5d´	14.4w	14.1z	13.7c´	14.9r	14.5v	14.2y	14.9r	14.8s	14.1z
0		14.7t	14.4w	14.3x	14.7t	14.6u	14.4w	14.9r	14.8s	14.5v	14.9r	14.7t	14.5v
15	- *P. indica*	15.6n	15.3p	15.2q	15.6n	15.5o	15.3p	16.4i	16.2k	15.9m	16.4i	16.3j	16.1l
20		17.8d	17.5f	17.1h	17.8d	17.6e	17.4g	18.2a	18.0c	17.5f	18.2a	18.1b	17.8d
								APX Enzyme Activity					
0		13.3s´*	13.1u´	12.8w´	13.3s´	13.2t´	13.0v´	14.7o´	14.4q´	14.2r´	14.7o´	14.5p´	14.4q´
15	+*P. indica*	17.4b´	17.1c´	16.5g´	17.4b´	17.4b´	16.9d´	18.3u	18.1w	17.8z	18.3u	18.2v	18.0x
20		21.3o	20.8s	20.2t	21.3o	21.2p	21.1q	21.9j	21.6m	21.0r	21.9j	21.7l	21.5n
0		16.1j´	15.8l´	15.2n´	16.1j´	16.0k´	15.7m´	16.8e´	16.4h´	16.3i´	16.8e´	16.7f´	16.5g´
15	- *P. indica*	17.9y	17.4b´	17.1c´	17.9y	17.8z	17.5a´	18.3u	18.0x	17.8z	18.3u	18.2v	18.0x
20		22.9f	22.4h	21.8k	22.9f	22.7g	22.3i	23.8a	23.5c	23.1e	23.8a	23.7b	23.4d

* Data with similar letters do not indicate significant differences ($P>0.05$).

enzymes that confirms our results.

Drought stress had significant effect on increasing the plant enzyme activity, as, the results of our study has been shown that a significant increasing by 15.6 and 18.3%, respectively, was observed when the plant was exposed to drought stress. However, inoculation of plant with *P. indica* can diminish the negative effect of abiotic stresses and there by decreased the plant enzyme activities such as APX or POX. As a result, our findings demonstrate that the APX and POX activity were significantly reduced by 11.8 and 15.4%, respectively, when plants were inoculated with *P. indica* under drought stress. In this regard, Xu et al stated that by increased antioxidant activity and the expression of genes relevant to drought, *P. indica* gives drought tolerance to *Zea mays* L.[34] that is in line with our results.

Conclusion

Based on the results of this study, using 90 kg/ha pure Fe from Fe chelate significantly decreased the shoot Cd concentration that can be related to the antagonistic effects of Fe and Cd and thereby decreasing the shoot Cd concentration. However, this was more effective in plants under normal moisture condition relative to drought stress. Regardless of soil moisture regime, the biodegradation of crude oil in the soil was significantly increase by using iron sulfate and iron chelate. However, increasing soil pollution with Cd has significant effect on decreasing the biodegradation of crude oil in the soil. Inoculation of plants with *P. indica* had significant effect on increasing the biodegradation of crude oil in the soil under normal soil moisture and drought stress. However, the role of soil physic-chemical properties (such as type of pollutant) on biodegradation of crude oil in the soil cannot sider and should be consider in the future researches.

Acknowledgements

The Islamic Azad University, Arak Branch, provided support to the author in studying the research, and for that the author is grateful.

Competing Interests

The author declared no conflict of interest.

Ethical Approval

This article has no human or animal samples, so no ethical issues were considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

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