

The effect of organic chelates and gibberellic acid on petroleum hydrocarbons degradation in the soil co-contaminated with Ni and crude oil under canola cultivation

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Abstract

Background: Soil remediation is one the important problem in environmental studies. Thus, this research was conducted to evaluate the effect of organic chelates and gibberellic acid (GA_3) on the degradation of crude oil in the soil co-contaminated with Ni and crude oil under canola cultivation.

Methods: For treatments, HEDTA and NTA chelates at rates of 0 and 2.5 mmol/kg soil and foliar GA_3 (0 (GA_3 -) and 0.05 (GA_3 +) mM) were used. In addition, the soil was polluted with Ni (0 and 100 mg Ni/kg soil) and crude oil at rates of 0, 2, and 4% (W/W). The plant used in this experiment was canola. The concentration of Ni in soil and plant was measured using atomic absorption spectroscopy (AAS). The concentration of total petroleum hydrocarbon (TPH) was measured using GC-mass. The mean differences were calculated according to the least significant difference (LSD) test.

Results: The greatest degradation of crude oil belonged to the non-Ni-polluted soil under cultivation of GA_3 -treated plant, while the lowest one was observed in the soil received the greatest level of HEDTA and NTA chelates. Applying 0.05 mM GA_3 foliar significantly increased the degradation of crude oil in soil and Ni in plant shoot by 12.1 and 8.3%, respectively. In addition, soil microbial respiration was also increased by 11.3%.

Conclusion: HEDTA, NTA, and GA_3 had a significant effect on the Ni phytoremediation efficiency and degradation of crude oil in soil that is a positive point in environmental pollution. However, the role of soil physico-chemical properties on the phytoremediation efficiency cannot be ignored.

Keywords: Soil pollutants, Biodegradation, Environmental, Petroleum

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Introduction

Petroleum products are the most commonly used materials that are processed from crude oil in an oil refinery. Petroleum products or by-products are generally used in different industries, such as car and airplanes manufacturing (1,2). However, the use of petroleum compounds has been associated with environmental pollution that can be due to its toxicity, and it can cause soil and air pollution (3,4).

Organic pollutants, such as petroleum hydrocarbons, can enter the soil, air, and groundwater through the leakage from pipelines or underground storage tanks, illegal dumping and accidental spills, and as a result, contaminate the soil and air (5). Remediation of soil contaminated by petroleum hydrocarbons, such as crude oil, is generally difficult due to its high hydrophobicity and high sorption capacity. Thus, it is necessary to find a suitable way for the remediation of these components from the soil (6).

However, in the industrialized areas, there is a synchronous effect of heavy metals and petroleum contamination, and these two types of pollution impede the remediation process of the contaminated soils (7).

Various technologies have been applied to remediate petroleum hydrocarbon-contaminated soil, such as bioremediation, volatilization, surfactant flushing, and chemical oxidation (8,9). However, these techniques are generally expensive and can lead to incomplete decomposition of contaminants (10). The efficiency of each method highly depends on the pollution type and soil chemical properties of each area (9). Among above-mentioned remediation methods, biodegradation (phytodegradation) is an environmentally friendly technique applicable for the remediation of soils contaminated by hydrocarbons (11). It should be noted that after bioremediation, the petroleum hydrocarbons convert into water, inorganic compounds, carbon dioxide,



or to other simpler organic compounds (12). On the other hand, applying organic chelates, such as ethylene diamine tetraacetic acid (EDTA), ethylene diamine disuccinate, hydroxyethylene diamine tetraacetic acid (HEDTA), and nitrilotriacetic acid (NTA) greatly contributes to the remediation of heavy metals-contaminated soils (13,14). However, their effect depends on the type of heavy metal, as Meers et al reported that the application of NTA (1.8 mmol/kg soil) chelate did not significantly increase the zinc (Zn), cadmium (Cd), and copper (Cu) concentration in plant compared to the control in sunflower. However, in the case of nickel (Ni), the uptake of Ni increased by 2.5-fold (15). Houshyar et al investigated the effectiveness of DTPA chelate on the Cd availability in the soils treated with sewage sludge and concluded that the application of DTPA chelate had significant effects on increasing the Cd uptake by corn (16). However, they did not consider the interaction effects of heavy metals. The positive role of applying organic chelate on increasing heavy metals uptake by plant was also reported by Baghaie et al (17).

Today, remediation of soils contaminated with petroleum hydrocarbons, such as crude oil, is also one of the major environmental problems (18). Due to the simultaneous effect of heavy metals contamination with petroleum hydrocarbons, finding a suitable way for the degradation of petroleum hydrocarbons with increasing heavy metals remediation seems necessary. Generally, serpentine rocks are the primary sources of Ni metal. In addition, it can be founded in Ni-contaminated areas due to various human activities, such as extraction of nickel ore, burning of fossil fuels, oil residues and sewage sludge and production of Ni-Cd batteries. Today, bioremediation is considered as one of the most successful methods for the degradation of petroleum hydrocarbons and heavy metals remediation (19). However, soil remediation efficiency has been impeded due to the slow growth of plants in these soils. Thus, applying a suitable strategy for enhancing plant growth in these soils can be helpful for the bioremediation process (20).

However, the use of plant growth regulators, such as gibberellic acid (GA_3), could be effective in the remediation of contaminated soils via increasing plant growth. Chouychai et al investigated the effect of plant growth regulators on the phytoremediation of hexachlorocyclohexane-contaminated soil and concluded that the use of GA_3 can increase the phytoremediation efficiency (21). Shafiqh et al investigated the phytoremediation of lead by corn in the Pb-polluted soil and concluded that the use of chelates with plant growth regulators can have an effective role in enhancing the remediation efficiency of the contaminated soils (22). Hence, despite of the positive effect of GA_3 application on increasing remediation efficiency, a practical solution should also be considered to increase the heavy metals availability in soil. Accordingly, the interaction effects of

applying organic chelates and GA_3 may be useful in the remediation of the contaminated soils. Thus, this research was conducted to evaluate the effects of organic chelates and GA_3 on the petroleum hydrocarbons degradation in the soil co-contaminated with Ni and crude oil under canola cultivation.

Materials and Methods

To investigate the effect of HEDTA, NTA, and GA_3 on the petroleum hydrocarbons degradation in the Ni-treated soil, a non-Ni-polluted soil sample with low organic carbon was selected from the surface layer of soil (0–15 cm) around Pakal village, Arak, central of Iran. Physico-chemical properties of the studied soil are shown in Table 1. This research was conducted as a factorial experiment in the layout of randomized complete block design. For treatments, HEDTA and NTA chelates at rates of 0 and 2.5 mmol/kg soil and foliar GA_3 (0 ($GA_3(-)$) and 0.05 ($GA_3(+)$) mM) were used. In addition, the soil was polluted with Ni (0 and 100 mg Ni/kg soil) and crude oil at rates of 0, 2, and 4% (W/W). The plant used in this experiment was canola. The soil used in this experiment was polluted with Ni at rates of 0 and 100 mg Ni/kg soil and incubated for two weeks. Then, crude oil was added to the soil at rates of 0, 2, and 4% (W/W) and incubated for two weeks to reach equilibrium. Canola seedling surface was first sterilized in 15% H_2O_2 , thoroughly washed in distilled water, and pre-germinated on moistened filter paper. After that, two canola seedlings were planted into each pot with five kg soil. GA_3 were sprayed on a canopy as foliar spray method 30 days after sowing (23). After 80 days, plants were harvested and the concentration of Ni in the soil was measured according to the Lindsay method (24). To measure the Ni concentration in the plant, samples were washed with deionized water, and then, dried in an oven at 60°C for 24 hours, and finally, digested in a mixture of $HNO_3/HClO_4$ (85:15%, v/v) to determine the total concentration of Ni in the plant according to the Yang et al method (25). Afterwards, the Ni concentration in the plant was measured using atomic absorption spectroscopy (AAS). The basal soil microbial respiration was measured according to Besalatpour et al (26). For this purpose, 3 replicate soil samples for each treatment were incubated

Table 1. Selected physico-chemical properties of the studied soil

Characteristic	Unit	Amount
Soil texture	-	Sandy loam
pH	-	7.3
EC	dS/m	1.2
Soil Pb availability	mg kg ⁻¹	ND
Soil Ni availability	mg kg ⁻¹	ND
Soil As availability	mg kg ⁻¹	ND
Organic carbon	%	0.1
CaCO ₃	%	9%

ND: Not detectable by atomic absorption spectroscopy.

for three days at 26°C in 250-mL glass containers closed with rubber stoppers. The evolved CO₂ was trapped in a standard NaOH solution, and then, the excess of which in alkali was titrated with HCl (26). The total petroleum hydrocarbons (TPHs) in the soil were extracted from 30 g of the soil subsamples by soxhlet extractor using 150 mL of the mixture of dichloromethane and n-hexane (1:1, v/v) after 24 hours, and then, using the method applied in a study by Baghaie et al (27).

Ni translocation factor (TF) was calculated using the following formula (28):

$$TF = H_{\text{shoot}} / H_{\text{root}} \quad (1)$$

Where H_{shoot} and H_{root} are heavy metals concentrations in the plant shoot and root, respectively.

Statistical analyses were calculated according to the analysis of variance (ANOVA) procedure. The mean differences were calculated according to the least significant difference (LSD) test. Statistical significant value was considered at $P=0.05$.

Results

The simple effect of applying HEDTA, NTA, and GA₃ on increasing the soil Ni availability was significant ($P=0.05$).

The greatest soil Ni availability belonged to the Ni- and crude oil-polluted soil, which received the greatest level of HEDTA, NTA with the GA₃ foliar application, and the lowest one was observed in the soil received no pollutant (Table 2). Application of HEDTA in the Ni-polluted soil significantly ($P=0.05$) increased the soil Ni availability by 15.2%. Foliar GA₃ application had significant effect on increasing the soil Ni availability, as the application of 0.05 mM GA₃ had significantly increased the soil Ni availability (Table 2), which was contaminated with crude oil (4% W/W). Increasing soil contamination with crude oil had significant effect on increasing the soil Ni availability, as the greatest soil Ni availability belonged to the soil polluted with 4% (W/W) crude oil (Table 2).

The greatest root Ni concentration belonged to the plants cultivated in the Ni-polluted soil, which received the greatest level of organic chelate, such as HEDTA, simultaneous application of NTA with the GA₃ foliar, while the lowest one was observed in the soil received no organic chelate (Table 3). Applying 2.5 mmol/kg soil HEDTA and NTA chelate in the Ni-polluted soil significantly increased the root Ni concentration by 18.4 and 12.1%, respectively.

The greatest shoot Ni concentration has belonged to the plants sprayed with GA₃ and cultivated in the soil that

Table 2. Effects of the treatments on the soil Ni availability

Ni Pollution (mg/kg soil)	Chelate (mmol/kg soil)		Crude oil (%)			Crude oil (%)		
			0	2	4	0	2	4
	HEDTA	NTA	GA ₃ (+)			GA ₃ (-)		
0	0	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
	2.5	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
100	0	0	18.3p	22.1l	24.6i	17.8q	19.2o	21.9m
		2.5	21.9m	25.2h	27.4e	20.4n	23.1k	24.8i
	2.5	0	24.2j	27.6e	29.4c	22.4l	25.2h	28.1d
		2.5	27.1f	30.2b	31.3a	25.8g	27.4	29.4c

ND: Not detectable by the atomic absorption spectroscopy.

Note: Means with similar letters are not significant

Table 3. Effects of the treatments on root Ni concentration

Ni Pollution (mg/kg soil)	Chelate (mmol/kg soil)		Crude oil (%)			Crude oil (%)		
			0	2	4	0	2	4
	HEDTA	NTA	GA ₃ (+)			GA ₃ (-)		
0	0	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
	2.5	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
100	0	0	82.8l	85.1i	87.9g	78.1n	81.6m	83.1k
		2.5	84.6j	87.2g	90.4d	81.1m	83.2k	85.6i
	2.5	0	87.3g	91.3c	93.5b	84.4j	86.2h	88.1e
		2.5	91.4c	93.2b	95.1a	88.2e	90.4d	91.2c

ND: Not detectable by the atomic absorption spectroscopy.

Note: Means with similar letters are not significant

received the greatest level of HEDTA and NTA chelates (Table 4), while the lowest that was observed in the soil received no organic chelate. Foliar application of GA₃ significantly increased the shoot Ni concentration that maybe related to the positive role of GA₃ on increasing plant resistance to abiotic stress such as Ni, and as a result, increasing Ni concentration in the plant. Based on the results of this study, applying 0.05 mM GA₃ as a plant growth regulator significantly increased plant biomass (data was not shown) and shoot Ni concentration by 8.3 and 12.2%, respectively. Similar results were observed for the positive effects of GA₃ application on increasing Ni TF value, as the greatest Ni TF value belonged to the GA₃-treated plants (Table 5).

NTA, can help increase the Ni TF value, as applying 2.5 mmol/kg soil HEDTA significantly increased the Ni TF value by 0.04 units in the soil under cultivation of GA₃-treated plant. Similar results for NTA chelate confirm the results of the present study clearly.

The greatest soil microbial respiration belonged to the soil treated with 4% (W/W) crude oil in non-Ni-polluted soil without receiving any organic chelates (Table 6), while the lowest one was observed in the soil received no petroleum hydrocarbons. Applying organic chelates had adverse effect on the soil microbial respiration, as applying 2.5

mmol/kg soil HEDTA in the Ni-polluted soil (100 mmol Ni/kg soil) significantly decreased the soil microbial respiration by 8.4%. The crude oil degradation in the soil showed a similar trend with the changes in soil microbial respiration (Table 7).

Discussion

Applying organic chelates had a significant effect on increasing the heavy metal availability in the soil that maybe due to the role of organic chelate on the heavy metal solubility in the soil. Chen et al investigated the effects of HEDTA chelate on the heavy metals availability in the soil and concluded that applying this chelate has a positive role on increasing the availability of heavy metals in the soil, such as Ni, which is consistent with the results of this study (29). However, they did not investigate the role of soil chemical properties on the changes in the heavy metals availability in the soil. The results of a study by Evangelou et al about the role of organic chelate on increasing the heavy metal availability in soil confirm the results of this study clearly (30).

The positive role of foliar GA₃ application on increasing the soil Ni availability may be related to the role of plant growth regulators on increasing plant biomass (data were not shown) and may be increasing plant root exudate. The

Table 4. Effects of treatments on shoot Ni concentration

Ni Pollution (mg/kg soil)	Chelate (mmol/kg soil)		Crude oil (%)			Crude oil (%)		
			0	2	4	0	2	4
	HEDTA	NTA	GA ₃ (+)			GA ₃ (-)		
0	0	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
	2.5	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
100	0	0	33.9q	37.4o	43.1l	28.1s	32.6r	36.6p
		2.5	38.0n	41.9m	49.7i	34.1q	37.4o	42.8l
	2.5	0	47.1k	56.6e	63.6b	42.2m	47.4k	54.6g
		2.5	53.0h	59.6d	68.5a	48.5j	55.1f	62.0c

ND: Not detectable by the atomic absorption spectroscopy.

Note: Means with similar letters are not significant

Table 5. Effects of treatments on the Ni TF value

Ni Pollution (mg/kg soil)	Chelate (mmol/kg soil)		Crude oil (%)			Crude oil (%)		
			0	2	4	0	2	4
	HEDTA	NTA	GA ₃ (+)			GA ₃ (-)		
0	0	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
	2.5	0	ND	ND	ND	ND	ND	ND
		2.5	ND	ND	ND	ND	ND	ND
100	0	0	0.41o	0.44m	0.49k	0.36q	0.40p	0.44m
		2.5	0.45l	0.48k	0.55g	0.42n	0.45l	0.50i
	2.5	0	0.54h	0.62d	0.68b	0.50i	0.55g	0.62d
		2.5	0.58f	0.64c	0.72a	0.55g	0.61	0.68b

ND: Not detectable by the atomic absorption spectroscopy.

Note: Means with similar letters are not significant

Table 6. Effects of treatments on the soil microbial respiration

Ni Pollution (mg/kg soil)	Chelate (mmol/kg soil)		Crude oil (%)			Crude oil (%)		
			0	2	4	0	2	4
	HEDTA	NTA	GA ₃ (+)			GA ₃ (-)		
0	0	0	54.1c	55.4b	57.1a	50.1h	52.2f	54.2c
		2.5	51.9g	53.2e	55.3b	47.8k	49.2i	51.6g
	2.5	0	52.2f	53.9d	55.3b	45.1m	47.6k	49.3i
		2.5	48.7j	51.4g	53.8d	40.4r	42.9p	44.8n
100	0	0	50.1h	52.2f	53.9d	44.8n	48.6j	51.4g
		2.5	48.6j	50.1h	51.9g	41.6q	43.2o	46.8l
	2.5	0	45.1m	46.8l	48.1j	39.2s	42.1p	44.8n
		2.5	42.1p	44.8n	46.8l	34.1v	35.1u	37.4t

Note: Means with similar letters are not significant.

Table 7. Effects of treatments on the crude oil degradation in the soil

Ni Pollution (mg/kg soil)	Chelate (mmol/kg soil)		Crude oil (%)			Crude oil (%)		
			0	2	4	0	2	4
	HEDTA	NTA	GA ₃ (+)			GA ₃ (-)		
0	0	0	65.4d	67.2c	71.5a	62.3g	65.4d	68.1b
		2.5	63.3f	64.4e	67.4c	61.2h	63.1f	65.2
	2.5	0	63.1f	65.2d	68.4b	60.3i	62.4g	65.1d
		2.5	61.2h	62.6g	64.1e	58.1j	61.2h	63.5f
100	0	0	55.4k	58.1j	65.9d	50.1m	55.3k	58.2j
		2.5	50.2m	52.9l	55.4k	47.5p	49.4n	50.3m
	2.5	0	50.1m	55.3k	58.1j	45.1r	47.2p	48.6o
		2.5	47.6p	50.1m	52.9l	41.2t	43.4s	46.1q

Note: Means with similar letters are not significant.

positive effects of plant root exudate on decreasing the soil pH, and thereby, increase the heavy metal availability in soil has been mentioned by researchers (31,32). Azimzadeh et al investigated the remediation of some heavy metals in the soil by corn and canola in single and mixed culture system and concluded that plant root exudate can increase the heavy metal availability in the soil (33). Generally, plant root exudate increases the availability of heavy metals in the soil, although heavy metals solubility varies depending on the type of the metal (31). A study by Hadi et al showed that applying GA₃ and synthetic chelates had significant effect on increasing heavy metal uptake by plant, which is consistent with the results of this study (34).

A significant increase in the soil Ni availability was observed when the soil was polluted with petroleum hydrocarbon, which may be related to the role of organic carbon on increasing the heavy metal availability in the soil. Askary Mehrabadi et al investigated the phytoremediation of petroleum hydrocarbon and heavy metals using *Catharanthus roseus* and concluded that increasing the soil pollution by petroleum hydrocarbon could increase the heavy metal availability in the soil. In addition, they mentioned that the simultaneous contamination of soils with petroleum compounds and heavy metals had a negative effect on the plant growth (35). However, they introduced *Catharanthus roseus* as the phytoremediator

of the petroleum-contaminated soil at low concentrations. Based on the results of this study, applying organic chelates had a positive effect on increasing the soil Ni availability, and thereby, increased Ni uptake by plant. Naghipour et al investigated the effect of EDTA and NTA on the heavy metal extraction from the sandy-loam-contaminated soils and concluded that applying these chelates had significant effects on heavy metal extraction from the soil, and thereby, heavy metal sorption by plants (36). In addition, they mentioned that using EDTA has higher efficiency in remediating the contaminated soils, in comparison with NTA, which is consistent with the results of the present study. Accordingly, applying 2.5 mmol/kg soil HEDTA compared to NTA chelate, significantly increased the Ni root concentration by 6.3% in Ni-polluted soil. It is mentioned that in order to decide whether artificial chelating agents can be applied, several important factors should be taken into account (36).

Soil polluted to crude oil pollution significantly increasing soil microbial respiration (Table 6). The remarkable point in this study is that the soil hydrocarbons act as a carbon source rather than pollutant factor. Based on the results of our studies, the greatest soil hydrocarbon degradation (Table 7) has belonged to the same treatment. Besaltpour et al reported the significant increases in soil microbial respiration in petroleum-contaminated soil and they

concluded that the petroleum hydrocarbon can be used as a carbon source rather than toxicity factor. However, they reported that the high concentration of petroleum can decrease the microbial activation (26). Based on the results of this study, increasing the crude oil pollution of soil to 4% (W/W) did not decrease the degradation of crude oil in the soil. However, the role of higher levels of crude oil pollution of soil on the petroleum hydrocarbon degradation in the soil needs to be investigated in the future studies. Franco et al reported a significant increase in microbial biomass in the crude oil-contaminated soil and concluded that this might be due to the use of oil hydrocarbon compounds by microorganisms as a carbon source (37), which is consistent with the results of this study.

Applying organic chelates, such as HEDTA and NTA, had adverse effects on the degradation of crude oil in the Ni-polluted soil that maybe due to the negative effect of heavy metals on the soil microbial activities (38). In fact, chelate application increased the heavy metals availability in the soil and reduced the activity of microorganisms to decompose petroleum hydrocarbons in the soil, although the Ni phytoremediation efficiency increased. Accordingly, application of 2.5 mmol/kg soil HEDTA chelate significantly decreased the soil microbial respiration and crude oil degradation by 11.6 and 8.9%, respectively, while the Ni TF value increased from 0.41 to 0.58 in the Ni-polluted soil (Table 5). Similar results were obtained for NTA chelate. Generally, soil microorganisms are among the first things that experience the negative impacts of pollutants, and their population and diversity can be used as an index to assess the degree of pollution in the environment (39). Although, the timing of the soil contamination with heavy metals can be a significant factor in the resistance of soil microorganisms to heavy metals, which are needed to be investigated in the future studies. Accordingly, Chander and Joergensen reported that the microbial population in long-term contaminated soils has better tolerance towards the application of organic chelate, compared to the short-term Zn-contaminated soil (40). Applying plant growth regulators such as GA_3 (+), had significant effects on the petroleum hydrocarbons degradation in soil. Regardless of the amount of the Ni pollution in soil, the greatest and lowest degradation rates of crude-oil in soil belonged to the GA_3 (+) and GA_3 (-) treatments, respectively. The results of soil microbial activities showed the similar trends. The foliar application of GA_3 (mmol/kg) significantly increased the petroleum hydrocarbons degradation in the Ni-polluted soil (100 mg/kg soil) by 9.6%. However, increasing the soil contamination with Ni significantly decreased the crude oil degradation in soil. Regardless of the amount of crude oil in soil and GA_3 application, with increasing soil contamination with Ni from 0 to 100 mg Ni/kg soil, a significant decrease in the crude oil degradation in the soil and soil microbial activities was observed.

On the other hand, plant growth-promoting rhizobacteria such (PGPR) as GA_3 can increase plant biomass (data were not shown). The positive effect of PGPR on plant growth is providing the condition for plant growth via compounds that is synthesized by the microorganisms, for example phyto-hormones, or increase plant nutrients uptake (41). Shafigh et al investigated the effect of GA_3 on the Ni phytoremediation in the Ni-polluted soil and concluded that the application of GA_3 had a significant effect on increasing corn biomass, and thereby, increased Ni phytoremediation efficiency (42). Therefore, increasing plant biomass can increase plant resistance to heavy metals toxicity. On the other hand, increasing plant biomass can increase plant root exudate, and thereby, influence increasing soil microbial activities or petroleum hydrocarbon degradation. Generally, rhizoremediation has a great potential for the remediation of the soil contaminated with organic pollutants, such as petroleum hydrocarbons (43). The important point of this study is that although the greater Ni availability was observed in the soil under the cultivation of the GA_3 -treated plants, but due to the increased resistance of plant to abiotic stress (increased plant biomass), the microbial activities were also significantly increased, and as a result, the soil microbial respiration increased. Therefore, the use of plant growth hormone not only increased the Ni phytoremediation efficiency, but also increased the degradation of petroleum hydrocarbons in the soil, which is a positive point in environmental studies. However, the role of soil microbial activities on the heavy metal immobilization in soil cannot be ignored. Microorganisms, which are mostly prokaryotic, participate in redox reactions and change the valance of heavy metals, and thereby, change their activity, which can affect their mobility or toxicity (37). For example, microorganisms such as *Rhizopus* can absorb heavy metal ions in the soil and *Thiobacillus* can absorb heavy metal ions as well as inorganic ions, such as S, which combine with the metal ions to form a precipitate that can be separated from the soil (44).

Conclusion

According to the results of this study, applying HEDTA and NTA chelates significantly increased the soil Ni availability, which has a negative effect on the degradation of crude oil in soil. However, using these chelates significantly increased the Ni uptake by plant. On the other hand, GA_3 foliar application significantly increased the degradation of crude oil in soil that can be due to the positive role of microbial activities (microbial respiration) on the degradation of petroleum hydrocarbons in soil. Accordingly, it was concluded that in order to increase the degradation efficiency of petroleum hydrocarbons in soil, environmental management should be considered to increase the plant resistance to environmental pollutants, such as heavy metals. However, application of plant growth regulators, such as GA_3 , can increase plant resistance to

heavy metals toxicity via increasing plant biomass, which has a positive effect on increasing soil microbial activities, and thereby, increases the degradation of petroleum hydrocarbons in soil. However, the plant physiology, soil chemical properties, and type and amount of organic components has significant effects on the amount of petroleum hydrocarbon degradation, which should be considered in the future studies.

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Ethical issues

The authors hereby certify that all data collected during the research are as expressed in the manuscript, and no data from the study has been or will be published elsewhere separately.

Competing interests

The authors have declared that they have no conflict of interests.

Authors' contributions

All authors contributed in the data collection, analysis, and interpretation. All authors reviewed and approved the manuscript.

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