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Indole-3-Acetic Acid and Humic Acid Increase the Bio-Degradation of Diesel Oil in Soil Polluted with Pb and Cd

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ABSTRACT

Introduction: Soil remediation is one of the most important fields in environmental studies. This study was conducted to investigate the effect of indole-3-acetic acid (IAA) and humic acid (HA) on increasing the bio-degradation of diesel oil in soil polluted with (lead) Pb and cadmium (Cd).

Materials and Methods: Treatments included foliar application of IAA (0 (control) and 30 ppm) and soil application of HA (0 (control) and 200 mg/kg soil) in the soil contaminated with Cd (0 (control), 10 and 15 mg/kg soil), Pb (0 (control) and 1600 mg/kg soil), and diesel oil (0 (control), and 8% (W/W)). The sunflower was planted in all soil samples. The plants were harvested after 70 days and Pb and Cd concentrations of plants were measured using Atomic Absorption Spectroscopy.

Results: Foliar application of IAA at the rate of 30 mg/l significantly increased the Cd and Pb phytoremediation by 14.8% and 13.4%, respectively. For HA application, it was increased by 11.3% and 10.2%, respectively. A significant increase was found in degradation percentage of diesel oil in soil by 12.6%, when the soil was treated with 200 mg HA/kg soil.

Conclusion: It can be concluded that application of organic amendments such as IAA or HA can be a suitable way for increasing plant growth and increasing plant phytoremediation efficiency, especially in the soil contaminated with diesel oil. However, the phytoremediation efficiency is dependent on the plant physiology and the type of soil pollution that should be considered.

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Introduction

Soil pollution with heavy metals is one of the major environmental problems in human societies. High concentration of heavy metals in the environment can directly damage soil fauna and flora. In addition, the pollutants can enter into the groundwater via leaching processes, reduce crop yield and nutritional quality of foods, and ultimately endanger the health of humans and other organisms [1, 2]. Soil contamination with heavy metals is developing rapidly over the years due to the human activities and industrial processes [3, 4]. The risk of contamination increases since contaminants move in the soil environment, are absorbed by plants, and enter into the surface and groundwater. The soil capacity is limited in receiving different pollutants. If the accumulation of pollutants continues, the soil capacity, as an acceptor capacity, will be significantly reduced or completely lost, in which other components of the environment such as groundwater, plants, animals, and eventually humans will be in serious danger [5, 6].

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Soil pollution has natural and human resources. Natural resources include the activities of volcanoes, the transfer of polluted dust, and the weathering of rocks rich in heavy metals. Meanwhile, some of the main sources of pollutions in agricultural soils include improper application of phosphate fertilizers, sewage sludge, and pesticides. Although heavy metals can accumulate naturally through weathering of rocks and minerals via the soil formation process, the natural pollution sources are less important than artificial pollutants including mining activity and chemical fertilizers [7, 8].

In today's modern world, development of industry and high use of chemical fertilizers have led to increasing concentration of pollutants in soil. So, it is necessary to use appropriate methods to remediate these pollutants [9, 10]. It should be noted that among the methods mentioned to remediate the soil heavy metals, phytoremediation is one of the most important and environmentally friendly strategy. Due to the climatic changes of Iran to arid and semi-arid condition, the growth of plants is difficult and the phytoremediation efficiency is low [11,12]. Moreover, the presence of other pollutants, including petroleum hydrocarbons, has negative effects on plant growth and consequently reduces the phytoremediation process. Therefore, providing a suitable solution to increase plant growth and phytoremediation efficiency seems essential. Meanwhile, application of plant growth regulators such as indole-3-acetic acid (IAA) or organic acids such as humic acid (HA) can be a good way to increase the phytoremediation efficiency [13, 14].

In this regard, IAA is the major auxin in plants, regulating developmental processes such as tissue differentiation, elongation and cell division, apical dominance and response to light and pathogens. Singh et al. reported that IAA could alleviate Cd toxicity on plant growth, oxidative reactions, and photosynthesis in eggplant seedlings [15]. Najafi et al. investigated the role of IAA on plant growth, protein content as well as catalase and peroxidase activities in soybean plant under aluminum chloride stress and concluded that application of IAA could decrease the negative effects of aluminum chloride stress [16]. In addition, Rong et al. studied the effect of HA on heavy metal uptake by tobacco and reported that using this organic amendment had positive effects on plant growth and increased the heavy metal uptake efficiency. However, they did not mention the role of other pollutants such as petroleum hydrocarbons on plant growth [17]. On the other hand, applying organic amendments such as HA was effective on improving soil quality or fertility and plant growth [17]. Ekin et al. investigated the effect of HA on potato productivity in sustainable agriculture and reported that using this organic amendment had positive effect on plant growth. However, they did not study the effect of HA on plant growth in heavy metal or hydrocarbon polluted soil [18]. In the central regions of the country, a simultaneous contamination of heavy metals with petroleum hydrocarbon compounds was found, which restricts plant growth. Thus, this research was conducted to evaluate the effect of IAA and HA on increasing the bio-degradation of diesel oil in the soil contaminated with Pb and Cd.

Materials and Methods

This research was conducted as a factorial experiment in the layout of randomized completely block design. Treatments consisted of foliar application of IAA at the rates of 0 (control) and 30 ppm and application of HA (0 (control) and 200 mg/kg soil) in the soil contaminated with Cd (0 (control), 10 and 15 mg/kg soil), Pb (0 (control) and 1600 mg/kg soil) and diesel oil (0 (control), and 8% (W/W)) in three replications. Selected soil phyco-chemical properties are shown in table 1.

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Table 1: Selected properties of soil used in this experiment

Soil	Amount
pH	7.5
EC (dS m^{-1})	1.9
Organic Carbon (%)	0.2
Soil Texture	Silty loam
$CaCO_3$ (%)	14
Total Pb (mg kg^{-1})	ND*
Total Cd (mg kg^{-1})	ND*
CEC (C mol/ kg soil)	14.6

ND: Not detectable by Atomic Absorption Spectroscopy (AAS)

The studied soil was contaminated with Cd and Pb at the mentioned rates and incubated for two weeks to equilibrium. After that, the soil was contaminated with diesel oil at the rates of 0% and 8% (W/W) and incubated for one month to equilibrium.

Later, the plastic pots were filled with 5 kg soil polluted by Pb, Cd, and diesel oil. The sunflower seeds were immersed in HCl 0.1N for 5 minutes and washed with distilled water several times to avoid fungal contamination. Seeds of sunflower were sown on treated soil in the pots (3 seeds in each pot) and irrigated with distilled water. After two weeks, the seedlings were thinned to one plant per pot and grew for 8 weeks. Soil and foliar application of HA and IAA at the mentioned rate were applied three weeks before harvest. After the end of experiment, the plants were harvested and the concentration of Pb and Cd were measured in the soil and plant samples [19] using AAS (Perkin-Elmer model 3030).

Soil microbial respiration was measured as evolved CO_2. To this purpose, three replicate soil samples of each treatment were incubated for three days at 26◦C in 250 ml glass containers closed with rubber stoppers. The evolving CO_2 was trapped in NaOH solution and the excess in alkali was titrated with HCl. In the next stage, three glass containers with NaOH and without soil were used as controls [20, 21]. The total petroleum hydrocarbons (TPHs) in soil was extracted from 30 g soil subsamples by Soxhlet using 150 mL of a mixture of dichloromethane and n-hexane (1:1, v/v) after 24 hours. The concentration of TPHs in soil extracts was determined using gas-chromatography (GC) [20] with a Delsi DI 200 chromatograph equipped with a direct injection port and a FID detector at 340˚C (Table 2). 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).

Statistical analyses of data were done using SAS software according to the ANOVA procedure. The least significant difference (LSD test) was used to determine the differences between the means. In addition, 95 percentage (P = 0.05) probability value was considered as the significant difference between the means.

Results

The greatest plant Cd concentration (Table 2) belonged to the soil polluted with the greatest levels of Cd, Pb, and diesel oil, while the lowest level was found in plants cultivated in the soil contaminated with the lowest rate of Cd, Pb and diesel oil. Plant Cd concentration in non-polluted soil was not detectable by AAS. Increased soil pollution with Pb decreased the plant Cd concentration significantly. According to the results of this study, increasing soil Pb pollution from 0 to 1600 mg/kg significantly decreased the plant Cd concentration by 11.9%. In contrast, increasing soil pollution to Cd from 0 to 15 mg Cd/kg significantly increased the plant Cd concentration by 11.8%.

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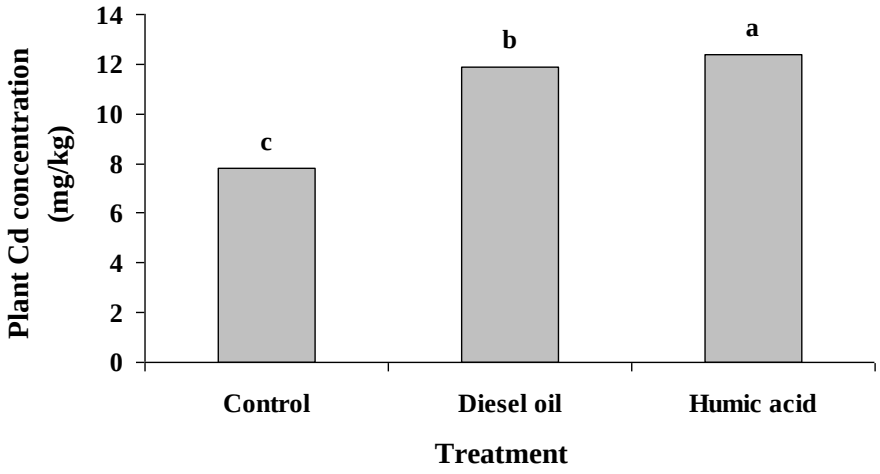


Figure 1: The simple effect of diesel oil and humic acid on plant Cd concentration, columns with different letters are significant ($p < 0.05$)

Table 2: Effect of treatments on plant Cd concentration (mg/kg soil)

Diesel oil (% (W/W))	Pb concentration (mg Pb/kg soil)	Cd concentration (mg Cd/kg soil)											
		0				10				15			
		IAA concentration (mg/l)											
		0		30		0		30		0		30	
		HA concentration (mg/kg soil)											
		0	200	0	200	0	200	0	200	0	200	0	200
0	0	ND**	ND	ND	ND	7.8v*	9.1o	8.1u	9.3n	10.6i	14.2d	12.4g	14.8a
	1600	ND	ND	ND	ND	7.5w	8.7q	7.8v	9.0p	10.3k	14.0e	10.6i	14.3c
8	0	ND	ND	ND	ND	7.3x	8.5s	7.5w	9.0p	10.2l	14.0e	12.0h	14.5b
	1600	ND	ND	ND	ND	7.2y	8.2t	7.2y	8.6r	10.0m	13.2f	10.4j	14.0e

*Data with the same letters are not significant ($P < 0.05$), ** ND: Not detectable by AAS.

Plant Cd (Table 2) and Pb (Table 3) concentration was affected by plant growth regulators of IAA or organic acids of HA. Soil and foliar application of HA and IAA increased the plant Pb and Cd concentration significantly. However, increase of soil pollution to petroleum hydrocarbons had adverse effects on plant phytoremediation efficiency. For instance, application of HA at the rate of 200 ppm in the soil contaminated with Cd (10 mg Cd/kg soil) significantly increased the plant Pb and Cd concentration by 11.2% and 14.8%, respectively. Regardless of soil pollution to heavy metals or diesel oil, foliar application of IAA increased the plant heavy metal uptake significantly. However, the amount of plant heavy metals uptake depended on the amount of soil contamination with heavy metals or petroleum hydrocarbons. For example, foliar application of IAA at the rate of 30 ppm significantly increased Cd and Pb concentrations of the plant cultivated in soil polluted with Cd (10 mg Cd/kg soil) and Pb (1600 mg Pb/kg soil) by 12.2 and 10.8%, respectively. However, in the same polluted soil contaminated with diesel oil (8 % (W/W)), Cd and Pb concentrations increased by 11.1% and 8.1%, respectively.

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Table 3: Effect of treatments on plant Pb concentration (mg/kg soil)

Diesel oil (% (W/W))	Pb concentration (mg Pb/kg soil)	Soil Cd concentration (mg Cd/kg soil)											
		0				10				15			
		IAA concentration (mg/lit)											
		0		30		0		30		0		30	
		HA concentration (mg/kg soil)											
		0	200	0	200	0	200	0	200	0	200	0	200
0	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1600	84.3e**	85.6b	85.4c	87.9a	83.2g	84.1f	84.1f	85.5b	82.0k	82.5i	82.5i	83.3g
8	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1600	83.1h	84.2e	84.5d	85.4c	81.2l	83.2g	82.3j	84.5d	78.4o	80.6m	79.7n	81.3l

*ND: Not detectable by AAS, **data with the same letters are not significant (P < 0.05).

The greatest soil microbial respiration was measured in non-polluted soil under cultivation of sunflower, while the lowest levels of contamination were observed in the soil with the highest contamination levels of Pb and Cd. Increase of the soil pollution to diesel soil increased the soil microbial respiration significantly. Increase of the soil pollution to heavy metals decreased the soil microbial respiration significantly (Table 4). According to the present findings, increasing soil pollution with Cd from 0 to 15 mg Cd/kg significantly decreased the soil microbial respiration by 14.3%. In soil polluted with Pb (1600 mg Pb/kg soil), the contamination decreased by 11.6%. Simultaneous contamination of soil with Pb and Cd had additive effect on decreasing soil microbial respiration. A significant decrease of soil microbial respiration by 19.4% was observed when the soil was simultaneously contaminated with Pb (1600 mg Pb/kg) and Cd (15 mg Cd/kg soil). It can be concluded that increasing soil pollution with Pb and Cd had a negative effect on soil microbial respiration. At the same time, concentration of Pb and Cd as well as the growth rate of the plant (Table 5) also decreased.

Table 4: Effect of treatments on soil microbial respiration (mg C-CO_2/kg soil)

Diesel oil (% (W/W))	Pb concentration (mg Pb/kg soil)	Cd concentration (mg Cd/kg soil)											
		0				10				15			
		IAA concentration (mg/li)											
		0		30		0		30		0		30	
		HA concentration (mg/kg soil)											
		0	200	0	200	0	200	0	200	0	200	0	200
0	0	10.3n*	10.5m	10.6l	11.1k	10.0p	10.2o	10.2o	10.5m	9.6r	9.9p	9.9p	10.3n
	1600	10.0p	10.2o	10.3n	10.7l	9.6r	9.9p	9.8q	9.6r	9.3s	9.6r	9.6r	9.9p
8	0	14.6d	14.8c	14.8c	15.9a	14.2f	14.5d	13.9h	14.1g	14.0g	14.2f	13.5i	13.8h
	1600	14.4e	14.6d	14.6d	15.6b	13.9h	14.2f	13.5i	13.9h	13.8h	13.9h	13.4i	13.0j

*Data with the same letters are not significant (P < 0.05).

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Table 5: Effect of treatments on plant biomass (g)

Diesel oil (% (W/W))	Pb concentration (mg Pb/kg soil)	Cd concentration (mg Cd/kg soil)											
		0				10				15			
		IAA concentration (mg/li)											
		0		30		0		30		0		30	
		HA concentration (mg/kg soil)											
		0	200	0	200	0	200	0	200	0	200	0	200
0	0	4.54o*	4.81g	4.60m	4.94a	4.50p	4.78h	4.58n	4.90c	4.45r	4.73j	4.41s	4.86e
	1600	4.50p	4.78h	4.54o	4.92b	4.47q	4.74j	4.55o	4.88d	4.41s	4.70k	4.37u	4.83f
8	0	4.51p	4.79h	4.55o	4.86e	4.42s	4.75i	4.51p	4.83f	4.38u	4.70k	4.34v	4.81g
	1600	4.44r	4.76i	4.50p	4.83f	4.40t	4.73j	4.46q	4.79h	4.34v	4.64l	4.29w	4.74j

*Data with the same letters are not significant (P < 0.05).

The greatest degradation percentage of diesel oil (Table 6) in the soil was measured in the non-heavy metal contaminated soil treated with the highest level of petroleum hydrocarbons, while the lowest degradation percentage belonged to the soil with the highest rate of heavy metals. Increase of soil pollution to heavy metals decreased the rate of diesel oil degradation significantly. According to our results, a significant increase of 12.6% was observed, when the rates of soil pollution with Cd and Pb were increased from 0 to 10 and 1600 mg/kg, respectively.

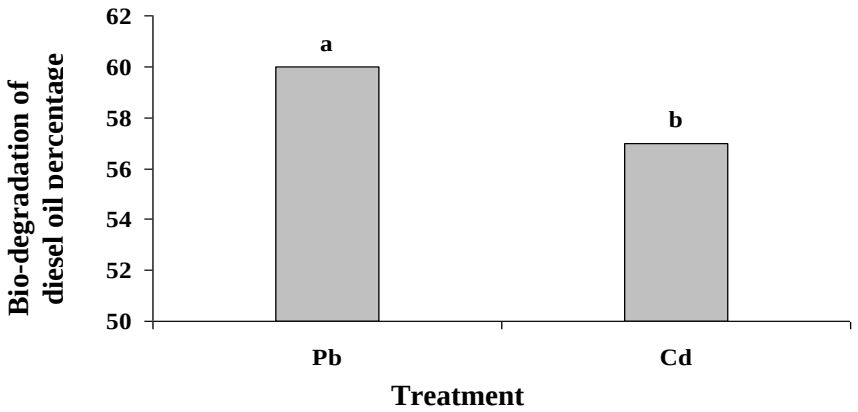


Figure 2: The effect of Cd and Pb treatment on Bio-degradation of diesel oil in the soil

Table 6: Effect of treatments on bio-degradation of diesel oil in the soil (%)

Diesel oil (% (W/W))	Pb concentration (mg Pb/kg soil)	Cd concentration (mg Cd/kg soil)											
		0				10				15			
		IAA concentration (mg/l)											
		0		30		0		30		0		30	
		HA concentration (mg/kg soil)											
		0	200	0	200	0	200	0	200	0	200	0	200
0	0	NC**	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	1600	68.4e*	71.3b	67.3f	72.8a	65.4h	70.6c	63.7i	71.4b	63.1j	68.4e	61.9k	69.2d
8	0	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	1600	67.3f	69.2d	65.4h	67.4f	63.1j	65.9g	60.2l	61.8k	60.2l	58.7m	57.3n	55.4o

*Data with the same letters are not significant (P < 0.05), ** NC: Not calculated

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Soil application of HA and foliar application of IAA significantly affected the increase of diesel oil degradation in the soil. Accordingly, the greatest degradation of diesel oil in the soil belonged to the soil under cultivation of the plants that received foliar and soil application of IAA and HA, respectively. At the same time, plant growth increased significantly. In addition, the lowest degradation rate of diesel oil was observed in the soil under cultivation of plants that did not receive IAA or HA. Foliar application of IAA at the rate of 200 ppm significantly increased the degradation percentage of diesel oil in the non-heavy metal polluted soil by 14.3%.

Discussion

Increase of soil pollution to heavy metals had significant adverse effects on the plant growth and consequently decreased the plant phytoremediation efficiency. However, the soil pollution rate and the plant type showed different effects. Hussain et al. investigated the role of different doses of heavy metals on corn plant growth and reported that increasing soil pollution to heavy metals could decrease the plant growth and thereby decrease the phytoremediation efficiency. In addition, they mentioned that decreasing the heavy metals' uptake due to the increase of soil pollution may damage various physiological and biochemical processes of the plants. However, they did not mention the additive effects of heavy metals on the plant growth [22]. Unfortunately, a simultaneous contamination with heavy metals and petroleum compounds was observed in the central regions of Iran, which can affect the phytoremediation efficiency of heavy metals. So, finding a suitable solution for better plant growth may help to increase the phytoremediation efficiency of heavy metals, which is similar to our findings. Increase of plant growth due to the application of HA was mentioned by researchers[23, 24].

Based on our findings, foliar and soil application of IAA and HA had significant effects on increasing heavy metal phytoremediation of plants cultivated in the soil polluted with petroleum hydrocarbons. This can be related to the positive role of IAA or HA on increasing plant growth and remediation of heavy metals from soil. However, greater pollution of soil to petroleum hydrocarbons may show different effect needed to be investigated in the future researches.

The remarkable point of this research is that using organic compounds such as IAA or HA could increase plant growth and soil microbial population, which can bring you a positive point in environmental studies. Accordingly, soil application of HA at the rate of 200 ppm significantly increased the plant growth and degradation of diesel oil that can be related to the role of plant growth regulators such as HA or IAA on increasing soil microbial respiration. Consequently, degradation of petroleum hydrocarbon can increase in the soil. As a result, soil microbial population can use the petroleum hydrocarbon as a carbon source that has positive effect on increasing soil microbial respiration. However, the higher levels of diesel oil may have adverse effects on the soil microbial respiration or plant growth and thereby decrease the phytoremediation efficiency [25, 26]. Heidari et al. investigated the effect of drought stress and application of humic on quantitative yield, photosynthetic pigments, and mineral nutrients content in sunflower seeds and concluded that soil application of HA had significant effects on increasing mineral nutrients content and plant growth. However, they did not consider the soil chemical properties such as soil pollutions and their roles on the plant growth [23]. In addition, Sheyni et al. investigated isolation and identification of oil sludge degrading bacteria from production tank in Masjed Soleiman and concluded that the petroleum hydrocarbon degrading bacteria can use oil sludge as a carbon source and increase the soil microbial respiration [27] that is similar to our results. Nwachukwu et al. investigated the effect of heavy metal toxicity on soil microbial respiration in organic and inorganic amended soil and reported that heavy metals had toxicity effects on soil microbial activity and decreased the soil microbial respiration [28], which confirms our

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results. However, the effect of contaminates can be extremely different via indirect effects on biochemical processes and direct effects on soil micro-organisms.

It can be concluded that soil and foliar application of organic amendments had positive effects on the plant growth, that can increase the soil microbial respiration and diesel oil degradation, which is a positive point in the environmental studies. Increase of the soil microbial respiration and plant growth due to application of organic amendments confirms our findings. Park et al. investigated the effects of HA on phyto-degradation of petroleum hydrocarbons in a soil simultaneously contaminated with heavy metals and concluded that HA could act as an enhancing agent for phyto-degradation of petroleum hydrocarbons in the soil contaminated with diesel fuel and heavy metals[29]. This finding supports our results.

Conclusion

Foliar application of IAA at the rate of 30 mg/l significantly increased the plant Pb and Cd concentrations by 11.3% and 14.5%, respectively. However, soil pollution to diesel oil had a negative effect on heavy metal phytoremediation efficiency. In addition, soil pollution to heavy metal showed a significant decrease on soil microbial respiration that has a negative effect on decreasing diesel oil degradation. Due to different solubility of heavy metals and different properties of petroleum hydrocarbon compounds, it is necessary to study other heavy metals and petroleum hydrocarbons type in the field study.

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Conflict of interest

No conflict of interest

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