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The Effect of Montmorillonite Nano-Clay on the Changes in Petroleum Hydrocarbon Degradation and Cd Concentration in Plants Grown in Cd-Polluted Soil

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Abstract
Contamination with heavy metals and petroleum hydrocarbons is considered as an environmental problem. Thus, this research was done to evaluate the effect of montmorillonite nano-clay on the changes in petroleum hydrocarbon degradation and cadmium (Cd) concentration in plant grown in a Cd-polluted soil. Treatments consisted of two levels of montmorillonite nano-clay (0 and 1% W/W) in a Cd-polluted soil (0, 5, 10 mg Cd/kg soil) and crude oil-polluted soil (0, 1 and 2% W/W). The plant used in this study was Tall Fescue (*Festuca arundinacea* L.). After 20 weeks, the concentration of Cd in plants was measured by atomic absorption spectroscopy and the total petroleum hydrocarbon (TPHs) in the soil was determined using the GC-mass spectrometry. Soil respiration was determined according to the method used by Qiao et al. ANOVA was used for statistical analysis of data. The least significant difference (LSD) test was used to determine the differences between the means. The application of 1% (w/w) montmorillonite nano-clay in Cd-polluted soil (10 mg Cd) without crude oil decreased Cd concentration in plant and increased microbial respiration by 18% and 34%, respectively. In addition, the application of 1% montmorillonite nano-clay in soil polluted with 1% crude oil and 10 mg Cd enhanced TPHs degradation by 27%. The use of montmorillonite nano-clay increased Cd adsorption in soil which resulted in an increase in microbial respiration and, hence the degradability of petroleum hydrocarbon in the soil.
Keywords: Petroleum, Hydrocarbons, Cadmium, Adsorption

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1. Introduction

Petroleum hydrocarbons are the most common pollutants in the environment (1). Today, there is a lot of contaminated soil in different parts of the world, which is an environmental catastrophe in many parts of the world, especially in petroleum-rich countries such as Iran. In general, when crude oil tanks are discovered in one place, pollution occurs in that place. This problem is known to be a contaminated site because it can penetrate into groundwater and cause cancerous diseases (2). Petroleum hydrocarbons are poorly soluble or insoluble in water and have lower specific gravity than water. The decomposition rate of organic compounds depends on the environmental conditions such as the availability of oxygen and the presence of appropriate microbial population in addition to the sensitivity and degradability of these materials (3,4). In general, linear chain hydrocarbons are more degradable than non-linear hydrocarbons or cycloalkanes. The presence of heavy metals such as cadmium (Cd) in combination with hydrocarbons in the soil can have a major impact on its various characteristics and functions, which can reduce soil quality and cause soil contamination. In addition, Cd has a negative effect on human health. Houdaji et al reported that there is a significant association between the exposure to absorbable contaminants such as Cd and the prevalence of multiple sclerosis (5).

Remediation of polluted soil with these components is very difficult (6) because these compounds can enter the surface and subsurface waters, be absorbed by plants and enter the human food chain (7,8). Therefore, finding a suitable way to remediate soil is necessary (9,10). Generally, there are several plants that can extract pollution from petroleum-polluted soil (3,11,12). However, the results of previous studies show that grass and legume species are suitable because of their root systems for phytoremediation of soils contaminated with petroleum compounds (13). Alavi Bakhtiarvand et al investigated the phytoremediation efficiency of the *Aeluropus littoralis* for degradation of petroleum hydrocarbons in silty clay soil and concluded that physicochemical conditions of soil could have a significant effect on the plant growth process. In addition, they reported that the high salinity and soil contamination can have a negative effect on plant growth and phytoremediation processes (14). Kechavarzi et al have mentioned the use of the plant as an effective method for removing hydrocarbons from the soil. In this regard, in their study, physicochemical conditions of soil played a significant role in plant growth and subsequent removal of hydrocarbons from soil (15).

Accordingly, phytoremediation is a useful method to reduce petroleum pollutants or heavy metals in soil. However, the high level of contamination reduces the plant biomass and, as a result, decreases the phytoremediation efficiency in some cases. The choice of the method for reducing the availability of heavy metals or petroleum pollutants in the soil depends on several factors including pollution type and pollution concentration (16-18).

Due to the fact that in industrial cities there is a possibility of simultaneous presence of heavy metals and petroleum products (19,20), the phytoremediation method cannot be a successful approach due to the reduction of plant biomass. In this regard, using stabilizing compounds such as nano-clay (21) with high specific surface area may affect plant growth due to decreasing heavy metal concentration in soil. Merrikhpour and Sobhan Ardakani conducted a study on the effect of applying nano-clay on heavy metal removal and concluded that nano-clay is a suitable material for removing heavy metal from aqueous solutions (22). On the other hand, decreasing heavy metal concentration may affect the activity of micro-organisms, thereby increasing the degradation of petroleum hydrocarbon in the soil. Therefore, the aim of this study was to investigate the effect of montmorillonite nano-clay on the changes in degradation of petroleum hydrocarbon in soil contaminated with petroleum hydrocarbon and Cd.

2. Materials and Methods

This study was done as a factorial experiment in a completely randomized block design. For determining the effect of nano-clay on Cd uptake by the plant, non-polluted soil sample was collected from the fields of the agricultural research station in Rudasht (32°29′N, 52°10′E), located approximately 40 km east of Isfahan, Iran. Treatments (treatments with three replications) consisted of contamination of soil by crude oil at three levels of 0 (P_0), 1 (P_1) and 2 (P_2) %, contamination of soil by Cd at concentrations of 0 (Cd_0), 5 (Cd_5), and 10 (Cd_{10}) mg/kg soil and application of montmorillonite nano-clay at 2 levels of 0 (M_0) and 1 (M_1) % (W/W). Physico-chemical properties of nano-clay are shown in Table 1. It should be noted that the montmorillonite nano-clay produced by Southern Clay Company under the trade name of Cloisite 15A was used in this study, 90% of which had a particle size less than 10 µm.

The non-polluted soil was contaminated with 0, 1 and 2% crude oil and allowed for 3 weeks to reach equilibrium. After 2 weeks, Cd (0, 5, and 10 mg/soil) was added to the soil and incubated for three weeks at 25ºC to reach equilibrium. After this time, the soil samples were mixed with 0 and 1% (w/) montmorillonite nano-clay and incubated for 3 weeks again. Then, 100 seeds of Tall Fescue (*Festuca arundinacea* L) were planted. The plants were irrigated to keep soil moisture at approximately 75% of field capacity. Irrigation rates were based on evapotranspiration data collected at the local weather station of Rudasht. The irrigation water had an electrical conductivity (EC) of 0.8 dS m^{-1}. After 120 days, plants were harvested and Cd concentration in the plants was measured using atomic absorption spectroscopy (Perkin Elmer, model: 3030) (23). Soil pH was measured in a 1:5 solid/liquid aqueous extract (24). Cation exchange capacity (CEC) of soil was measured by $BaCl_2$ method (25). The total petroleum hydrocarbons (TPHs) in soil were 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) (26). Soil microbial respiration was determined by titration with 0.25 N sulfuric acid using the method applied in a study by Qiao et al (27). The soil microbial respiration was measured as evolved CO_2. The statistical analysis was done by SAS software version 9.2. ANOVA was used for statistical analysis of the data. The least significant difference (LSD) test was used to determine the differences between the means

3. Results and Discussion

Based on the results of this study, the use of nano-clay montmorillonite did not have a significant effect

Table 1. Physico-chemical Properties of Montmorillonite Nano-Clay Used in this Study

Characteristic	Unit	Amount	Components	%	Components	%
Density	g/cm³	5.5	Sio_2	50.9	K_20	0.8
Specific area	m²/g	1-2	Al_2O_3	19.6	Cao	1.9
pH	---	6.3	Li_2O	15.4	TiO_2	0.6
CEC	me/100g	48	Fe_2O_3	5.6		
Color	---	Yellow	MgO	3.2		
Humidity	%	1-2	Na_2O	0.9		

on soil pH (Fig. 1a) but significantly increased soil absorption properties such as cation exchange capacity (CEC) (Fig. 1b), which may be related to its high specific surface area (28). The application of 1% (W/W) montmorillonite nano-clay in soil increased soil CEC by 13.2% (Fig. 1b). In addition, applying montmorillonite nano-clay significantly decreased Cd availability in soil (Fig. 1c). Montmorillonite has an octahedral sheet of alumina and a two-sided silica sheet with a net negative charge on its surface that reduces the availability of Cd in the soil (Fig. 1c) based on electrostatic force (29). In an experiment, Wang et al mentioned that the use of organic additives is an effective method for reducing the availability of heavy metals in soil (30). Baghaie investigated the role of organic amendment in decreasing the availability of heavy metals in soil and concluded that application of organic amendments such as sewage sludge increased the soil CEC, thereby reducing the availability of heavy metals in the plants (25). The result of a study by Kwiatkowska-Malina confirms this matter clearly (31). Considering the fact that the solubility of heavy metals such as Cd increases with decreasing pH (25), the use of montmorillonite nano-clay had no significant effect on decreasing soil pH (Fig. 1a) in the current study, which can be considered as an environmentally positive point. In addition, based on the results of this study, the effect of using montmorillonite nano-clay on soil electrical conductivity (EC) was not significant, which is a positive point in environmental pollution because Cd solubility is directly affected by soil salinity.

The highest Cd concentration in plant belonged to $P_2Cd_{10}M_0$ treatment (Table 2), while the lowest concentration was observed in $P_0Cd_5M_1$ treatment. The application of 1% (W/W) montmorillonite nano-clay in soil contaminated with 10 mg Cd decreased Cd concentration in plants by 18% which may be related to the role of nano-clay in decreasing Cd solubility in soil (32). This matter was also presented by Monajjem et al (33). In their research, they mentioned that 2:clays had a high specific surface area and CEC which increases sorption properties of soil, thereby decreasing the availability of Cd in soil (33). However, the adsorption rate of heavy metals on the exchange sites of soil varies depending on their concentration and type (34). Based on the results of this study, contamination of soil by crude oil can increase Cd concentration in plant which is similar to the results of a study conducted by Askary Mehrabadi et al (35). It should be noted that contamination of soil by crude oil had a significant effect on Cd concentration in plant, as Cd concentration was 2.2 times greater in $P_2Cd_5M_1$ compared to $P_0Cd_5M_1$ treatment.

The contamination of soil by crude oil or Cd had a significant effect on microbial respiration. The lowest and highest microbial respiration belonged to the $P_0cd_{10}M_0$ and $P_2Cd_0M_1$, respectively (Table 3). Adding montmorillonite nano-clay to the non-polluted soil did not have any significant effect on the soil microbial respiration, however, it was effective in polluted soil. The application of 1% montmorillonite nano-clay in soil polluted with crude oil (2% (W/W)) and Cd (5 mg/kg soil) significantly increased microbial respiration in soil (0.2 units). On the other hand, increasing Cd pollution decreased microbial respiration in soil, as increasing the Cd level from 5 to 10 mg in a crude oil-polluted soil (2% (W/W)) decreased microbial respiration in soil by 0.9% (Table 3).

According to the results of this study, increasing crude oil pollution increased microbial respiration in soil

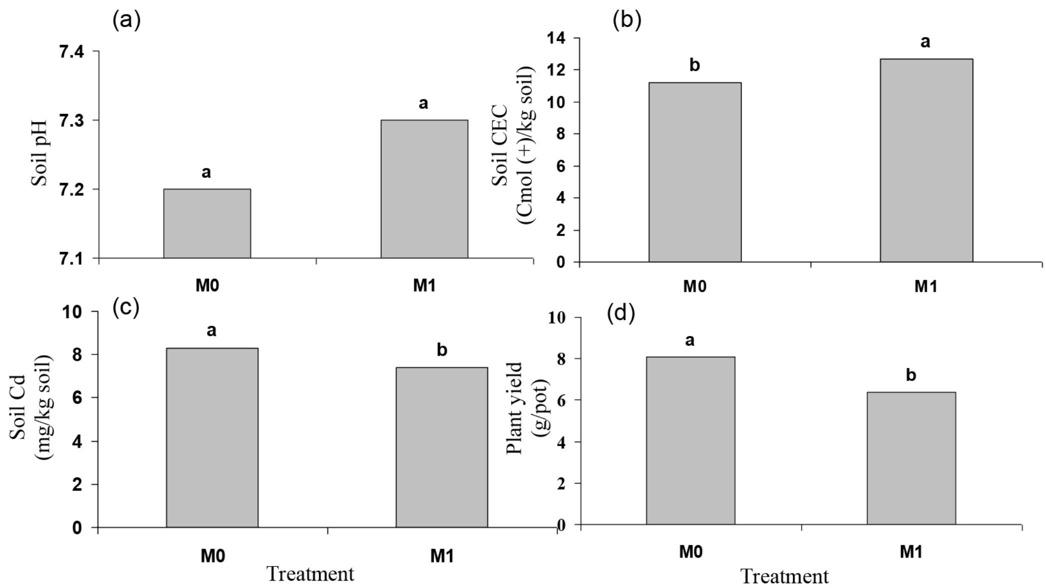

Figure 1. Effect of Montmorillonite Nano-clay on Soil pH (a), Soil CEC (b), Availability of Cd in Soil (c) and Plant Yield (d).

Table 2. The Effect of Montmorillonite Nano-Clay on Cd Concentration in Plants Grown in Soil Contaminated with Cd and Crude Oil

Treatment	Cd_0		Cd_5		Cd_{10}	
	M0	M1	M0	M1	M0	M1
P0	Nd*	Nd	2.2j	1.7k	6.4c	5.3e*
P1	Nd	Nd	3.2h	2.6i	7.2b	6.1d
P2	Nd	Nd	4.1f	3.8g	7.8a	7.1b

*ND: not detected by atomic absorption spectroscopy; means with the similar letters are not significant (P=0.05)

Table 3. The Effect of Montmorillonite Nano-clay on Microbial Respiration (mg $C\text{-}CO_2$/kg soil) in Soil Contaminated with Cd and Crude Oil

Treatment	Cd_0		Cd_5		Cd_{10}	
	M0	M1	M0	M1	M0	M1
P0	3.4j	3.4j	2.8m	3.3k	2.3n	3.1l*
P1	7.3bc	7.2c	6.3f	6.7d	6.2i	5.8h
P2	8.6a	9.7a	7.2c	7.4b	6.1g	6.5e

*Means with the similar letters are not significant (P=0.05)

(Table 3), indicating that the hydrocarbon composition is probably used as a source of carbon substrate for microorganisms and it is not toxic to them. However, higher levels of crude oil may reduce the population of microorganisms (24, 36). Kianpour Barjoei et al reported that the gram-positive bacteria of *Streptomyces* were able to decompose petroleum components and use it as the only source of carbon. On the other hand, based on their results, contamination of the soil by Cd caused a significant decrease in microbial respiration due to its toxicity to microorganisms (37). The results of a study by Zhang et al on the effect of heavy metal toxicity on the reduction of microbial population confirm this matter clearly (8).

The highest and lowest TPHs degradation rates were observed in $P_2Cd_0M_0$ and $P_1Cd_{10}M_0$, respectively (Table 4). Application of 1% montmorillonite nano-clay did not have a significant effect on TPHs degradation in soil without Cd, however, using the same amount of nano-clay in Cd-polluted soil (10 mg/kg soil) showed a significant increase in TPHs degradation, as the application of 1% montmorillonite nano-clay in Cd (10 mg/kg soil) and crude oil (2% (W/W)) polluted soil increased TPHs degradation by 17%. On the other hand, with increasing crude oil pollution from 1 to 2% in the soil without Cd-pollution, THPs degradation rate increased by 10.1% (Table 4).

The use of montmorillonite nano-clay decreased available Cd in soil (Fig. 1c) which can increase microbial activity and microbial respiration. Reducing the microbial respiration with increasing Cd-pollution (3) in the soil in this research confirms this matter clearly. Based on the results of this study, the decrease of microbial respiration in soil was simultaneous with increasing Cd concentration in plant and decrease of plant yield (Fig. 1d), indicating that the reduction of microbial respiration in soil was directly affected by Cd availability. The results of a study by Hussain et al confirm our results clearly (38).

Based on the results of this study, the highest TPHs degradation rate (Table 4) and microbial respiration (Table 3) belonged to the $P_2Cd_0M_1$ treatment suggesting that soil microorganisms can use hydrocarbons as a carbon source for decomposition of petroleum hydrocarbons, which is similar to the results of a study by Melali et al. (39). Besalatpour et al studied the reclamation of petroleum-contaminated soils using combined landfarming-phytoremediation and concluded that microorganisms in soils contaminated with petroleum hydrocarbons adapted to pollution in less than two days and their microbial respiration increased (40).

4. Conclusion

According to the results of this study, applying 1% (W/W) nano-clay montmorillonite has an important role in increasing the soil CEC which decreased Cd concentration in soil and plant. Decreasing Cd concentration of soil could affect TPHs degradation. On the other hand, using montmorillonite nano-clay had a significant effect on increasing microbial respiration in Cd-polluted soil. It is noteworthy that due to the different chemical properties of petroleum hydrocarbons, it is necessary to study the role of different nano-clays (with different sorption characteristics) on remediation efficiency of polluted soils. In other words, the role of

Table 4. Effect of Montmorillonite Nano-clay on TPHs Degradation in Soil Contaminated with Cd and Crude Oil

Treatment	Cd_0		Cd_5		Cd_{10}	
	M0	M1	M0	M1	M0	M1
P0	-	-	-	-	-	-
P1	60.1c	61.1c	47.3e	52.4d	32.8g	*44.7f
P2	70.2a	70.3a	60.4c	64.1b	45.1f	53.2d

*Means with the similar letters are not significant (P=0.05) different physico-chemical properties of soil such as soil texture and effect of other heavy metals on the degradation of petroleum hydrocarbon and concentration of heavy metal in the plant should be considered in future studies.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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