Research Paper

Effect of Sulfur Granular, Iron Sulfate, and *Thiobacillus* Bacteria on Biodegradation of Diesel Oil

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ABSTRACT

Background: Remediation of petroleum hydrocarbons from the soil is one of the essential factors. This study was conducted to test the impact of sulfur granular Municipal Soil Waste (sulfur granular MSW), foliar application of iron sulfate on biodegradation of diesel oil in a cadmium (Cd)-polluted soil in the presence of *thiobacillus* bacteria.

Methods: Treatments consisted of applying sulfur granular MSW at the rates of 0 and 15 t/ha and foliar application of iron sulfate (0 and 500 mg/L) in a Cd-polluted (0, 10, and 20 mg Cd/kg) soil that simultaneously contaminated with diesel oil (0%, 4%, and 8% w/w) in the presence of *thiobacillus* bacteria. After 60 days, the corns were harvested, and the plants' Fe and Cd concentration was measured using Atomic Absorption Spectroscopy (AAS). In addition, the biodegradation of diesel oil in the soil was measured.

Results: Soil application of sulfur granular MSW (15 t/ha) significantly increased the biodegradation of diesel oil in the soil by 16.1%. However, soil contamination with Cd had an adverse effect on the biodegradation of diesel oil in the soil. The presence of *thiobacillus* bacteria had a considerable effect on reducing the Fe and Cd concentration of the plant by 16.1% and 17.3%, respectively.

Conclusion: Based on the study results, using sulfur granular MSW and foliar application of iron sulfate has a significant effect on the biodegradation of diesel oil in the heavy metal polluted soil. However, the role of plant physiology, the type, and the amount of pollution on phytoremediation efficiency cannot be ignored.

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1. Introduction

Heavy elements are among the nonpoint pollutants that have become an environmental problem in the world because of their adverse effects on human health [1-3]. After the accumulation of heavy metals in the soil, especially in agricultural lands, they are absorbed by plants, consequently contaminating the food chain of humans and animals [4, 5]. Among the heavy elements, cadmium (Cd) and lead (Pb) are hazardous for humans and animals because of their long half-life [6, 7]. About 95% of the Pb that enters the human body is deposited in the bones as lead phosphates with a half-life of 20-30 years [8, 9]. Accumulation of this element in the body affects the hematopoietic, nervous, gastrointestinal, and urinary systems. Cd mainly accumulates in the liver after entering the body, and the half-life of this element in the human body is about 17 to 30 years [10, 11]. In addition, the accumulation of heavy elements in the soil will disrupt other parts of the ecosystem [12].

On the other hand, environmental and economic problems in the disposal of municipal waste and decreasing the soil organic matter in arid and semi-arid areas have led to the production and use of more fertilizer products from municipal waste [13, 14]. Organic matter is one of the most important factors in soil fertility because of its constructive effects on physical, chemical, and soil properties [15]. The amount of soil organic matter in the wetland soils is naturally high because of the significant return of plant debris to the soil and generally constitutes between 1% to 3% of the soil weight [16, 17]. While the soils of arid and semi-arid areas, which constitute more than 80% of agricultural land in Iran, are poor in terms of organic matter, and their amount in soils is less than 1%. Therefore, increasing the soil organic matter can improve the physical, chemical, and fertility of soils in these lands [18]. However, increasing soil pollution with pollutants such as heavy metals may adversely affect soil quality. Hence the use of phytoremediation methods such as stabilization of heavy metals can decrease the soil and plants heavy metals availability [19, 20]. In this regard, Wang et al. reported that using soil organic amendments is a suitable way of producing safe crops in contaminated soils [21]. However, the role of soil physic-chemical properties such as type of heavy metals or organic matters cannot be ignored.

Municipal waste compost is one of the organic compounds that contain significant amounts of trace and high macro- and micro-elements that can improve soil and plant nutritional conditions [22, 23]. On the other hand, because soil use of iron minerals compounds (due to their low solubility) has little effect on iron deficiency, using foliar or soil organic fertilizers such as municipal waste compost can partially compensate for the lack of iron in the soil [24, 25]. Also, the use of municipal waste compost can help improve soil quality by improving the physical structure of the soil, gradual and continuous releasing nutrients, and increasing water storage capacity in the soil, especially in arid and semi-arid regions of the country that are deficient in the organic compounds [26]. In this regard, Malik et al. investigated the effect of organic amendments on the immobilization of heavy metals in wastewater irrigated soils and concluded that organic amendments have significant effects on immobilizing the heavy metals in the soil [27].

In the calcareous soils of arid and semi-arid regions with high pH, the availability of nutrients in the soil is low. Using sulfur as an acidogenic agent can help increase the absorption of some nutrients in calcareous and alkaline soils, reclamation of sodic soils, and control or eliminate some plant pathogens. Because of the importance of biological oxidation of sulfur compared to chemical oxidation, the presence of *thiobacillus* bacteria in the soil or with organic fertilizers such as compost is essential [28, 29]. Lack of sulfur fertilizers in previous years, continuous and dense cultivation of agricultural lands, and the presence of sodium saline lands along with sulfur abundance are the most important reasons for paying serious attention to sulfur consumption in agricultural lands. Thus, this research was conducted to investigate the effect of granular Municipal Soil Waste (sulfur granular MSW) and foliar application of iron sulfate on biodegradation of diesel oil in a Cd-polluted soil in the presence of *thiobacillus* bacteria.

2. Materials and Methods

This research investigated the effect of sulfur granular MSW, foliar application of iron sulfate on biodegradation of diesel oil in a non-calcareous soil in the presence of *thiobacillus* bacteria. For this purpose, non-saline soil with low organic carbon (Typic Xerorthents) was collected from the 0–15 cm layer of a wheat field around Arak City, central Iran. Selected physicochemical properties of the studied soil are presented in Table 1.

This research (36 treatments) was done as a factorial experiment in the layout of a completely randomized block design as a pot experiment in three replicates. Treatments included applying sulfur granular MSW at the rates of 0 and 15 t/ha, foliar application of iron sul-

Table 1. Physicochemical properties of the studied soil

Characteristic	Unit	Amount
Soil texture	-----	Sandy loam
pH	------	7.5
Electrical conductivity	dS/m	1.0
Pb availability	mg/kg	ND*
Cd availability	mg/kg	ND
As availability	mg/kg	ND
Ni availability	mg/kg	ND
Organic carbon	%	0.1
$CaCo_3$	%	8
Cation Exchange Capacity (CEC)	Cmol/kg soil	10.9

*ND: Not Detectable by Atomic Absorption Spectroscopy (AAS)

fate (0 and 500 mg/L) in a Cd-polluted soil (0, 10, and 20 mg Cd/kg soil) that is simultaneously contaminated with diesel oil (0%, 4%, and 8% w/w) in the presence or absence of *thiobacillus* bacteria. The studied soil was polluted with Cd (0, 10, and 20 mg Cd/kg soil) and diesel oil (0%, 4%, and 8% w/w) and incubated for one month to equilibrium. After this time, the sulfur granular MSW was added to the soil at 0 and 15 t/ha and incubated again for another month to equilibrium. Then, 5 kg plastic pots were filled with treated soil. The initial inoculum of *thiobacillus* was obtained from the Soil & Water Research Institute of Iran. To have enough bacterial inoculant, we used the Postgate medium. After preparation of the culture media, the bacterial inoculant was proliferated at 25-28°C by gentle shaking (120 rpm) for 2 h to reach the propagation of at least 10^7 CFU/mL. Then, the *thiobacillus* inoculant was added to the soil [30]. After that, the corn (Zea mays L. Single cross 704) was surface sterilized in H_2O_2 15% for 15 min, rinsed with distilled water, germinated in the sterilized sand for 1 week, and then planted in the 5 kg plastic pot. Foliar application of iron sulfate as a Fe source was applied after one month at 0 and 500 mg/L [31]. After 60 days, the plants were harvested, and their Fe and Cd concentration was measured using Atomic Absorption Spectroscopy (AAS) based on the methods described by Yang and Norvell. [32]. In addition, the soil Cd concentration was measured based on the DTPA (Diethylenediamine Pentaacetate)-extractable method according to Lindsay and Norvell's study [33]. Furthermore, diesel biodegradation in the soil was determined based on the Minai-Tehrani et al. method [34].

Statistical analysis

The experimental data were analyzed by ANOVA using SAS software. The significant differences in means were determined by the Least Dignificant Difference (LSD) test. The significant difference was determined based on the $P<0.05$ value.

3. Results and Discussion

The highest biodegradation percentage of diesel oil (Table 2) was found in the soil with the highest level of organic amendments. Our study indicated that soil application of sulfur granular MSW at the rate of 15 t/ha significantly enhanced the diesel oil biodegradation by 14.7%, which can be attributed to the role of sulfur granular MSW as a carbon source for soil micro-organisms which increases the decomposition of petroleum hydrocarbon in the soil. Increasing soil microbial activity (Table 3) with soil application of sulfur granular MSW confirms our results.

According to the results of our study, using 15 t/ha in the soil that was simultaneously contaminated with Cd (20 mg Cd/kg soil) and petroleum hydrocarbons (8% w/w) significantly increased the soil microbial respiration (Table 3) by 17.7%. It is noteworthy that applying this amount of organic fertilizer has reduced the soil Cd availability by 21.3%. Thus, sulfur granular MSW significantly increased the soil absorption property via increasing soil Cation Exchange Capacity (CEC) (data was not shown) and thereby decreasing the Cd toxicity

Table 2. Effect of treatments on bio-degradation of diesel oil in the presence of *thiobacillus* bacteria

Sulfur Granular MSW (t/ha)	Iron Sulfate (mg/L)	Soil Cd Concentration (mg/kg soil)	With *Thiobacillus*			Without *Thiobacillus*		
			Diesel Oil (% w/w)					
			0	4	8	0	4	8
0	0	0	70.2k*	71.3	73.7h	65.2p	67.8n	68.3m
	500		72.4i	73.7h	76.2e	70.1k	71.5	74.2g
	0	10	65.3p	69.5l	71.2j	60.4u	61.5t	64.7q
	500		67.2n	70.5k	72.8i	63.1r	64.8q	66.8o
	0	20	60.7u	62.7s	65.1p	55.1y	57.8x	59.2v
	500		62.6s	65.4p	69.1l	58.7w	61.5t	64.2q
15	0	0	75.8f	77.8d	78.2c	71.7j	73.9h	77.5d
	500		77.8d	79.2b	80.6a	74.2g	75.9f	79.5b
	0	10	72.4i	73.5h	75.8f	69.8l	72.5i	74.1g
	500		70.7k	72.8i	73.7h	66.8o	68.9m	72.4i
	0	20	67.8n	69.2l	72.4i	64.1q	67.2n	68.2m
	500		64.2q	67.2n	70.5k	62.8s	65.1p	66.4o

* Similar letters show the non-significant differences.

(soil Cd availability, Table 4) that can help increase the soil microbial community.

In this regard, Wang et al. investigated the role of organic amendments in decreasing the soil's heavy metals availability and concluded that using organic amendments such as cow manure can directly decrease the heavy metals availability and their potential for safe crop production [21]. Today, healthy food production, especially in industrial areas of the country, is one of the most important food security issues and provides appropriate solutions to reduce the availability of heavy metals or petroleum compounds in the soil. Providing a healthy food chain is one of the most important environmental missions. Natural organic additives such as manure can reduce the availability of heavy metals in the soil [35, 36] to some extent. However, the physicochemical properties of the soil in each region can affect the availability of heavy metals in the soil that should be investigated in different research studies.

On the other hand, using sulfur granular MSW had a significant effect on increasing the plant biomass (data were not shown) via decreasing the plant Cd concentration (Table 5) and increasing the plant Fe concentration (Table 6). This condition can help increase the plant resistance against the abiotic stresses. Accordingly, increasing the plant growth may increase the plant root exudate, a carbon source to increase the soil microbial respiration (activity) and then increase the biodegradation of diesel oil in the soil. Similarly, Phillips et al. reported that plant root exudates impacted the hydrocarbon degradation potential as they can be used as a carbon source for microorganism activity [37].

Foliar application of iron sulfate at the rate of 500 mg/L also significantly increases the plant Fe concentration (Table 6), which can help decrease the heavy metal uptake by plants via the interaction of heavy metals with nutrient elements [38]. According to the results of our study, foliar application of iron sulfate at the rate of 500 mg/L significantly increased and decreased the plant Fe and Cd concentration by 15.3% and 17.1%, respectively, and consequently increased the biodegradation of diesel oil in the soil that is a positive point in environmental studies. However, soil pollution to Cd had adverse effects on plant Fe concentration and decreased the biodegradation of diesel oil in the soil. According to our study, foliar application of iron sulfate at the rate of 500 mg/L significantly increased the Fe concentration of the

Table 3. Effect of treatments on microbial respiration of soil (mg C-CO_2/kg soil) in the presence of *thiobacillus* bacteria

Sulfur Granular MSW (t/ha)	Iron Sulfate (mg/L)	Soil Cd Concentration (mg/kg soil)	With *Thiobacillus*			Without *Thiobacillus*		
			Diesel Oil (% w/w)					
			0	4	8	0	4	8
0	0	0	10.7q*	10.8p	11.2l	10.3u	10.5s	10.8p
	500		11.3k	11.7g	12.1d	11.0n	11.2l	11.5i
	0	10	10.4t	10.6r	10.9	10.0w	10.3u	10.7q
	500		10.9o	11.1m	11.7g	10.5s	10.7q	11.0n
	0	20	10.0w	10.3u	10.6r	9.7x	10.0w	10.4t
	500		10.4t	10.7q	10.9o	10.0w	10.3u	10.8
15	0	0	11.0n	11.4j	11.8f	10.7q	10.9o	11.5i
	500		11.9e	12.5b	12.8a	11.7g	11.8f	12.4c
	0	10	10.8p	11.0n	11.4j	10.5s	10.8p	11.0n
	500		11.3k	11.6h	11.9e	11.0n	11.4j	11.3k
	0	20	10.4t	10.7q	11.0n	10.0w	10.3u	10.8p
	500		10.8p	11.0n	11.4j	10.2v	10.5s	11.0n

* Similar letters show the non-significant differences.

plants cultivated in non-Cd and Cd polluted soil (20 mg Cd/kg soil) by 14.6% and 11.1%, respectively. Also, it increased the biodegradation of diesel oil in the soil by 16.1% and 14.2%, respectively.

Today, in the country's central regions, because of the lack of organic matter and high pressure, there is a problem of unavailability of plant nutrients in the soil, and soil application of iron compounds such as iron sulfate does not increase the availability of iron in the soil. However, the foliar application of such compounds can increase the plant's resistance to abiotic stresses by improving the plant's nutritional condition. Of course, its efficiency depends on the soil physicochemical properties, as the results of this study showed that with increasing the soil pollution with diesel oil or heavy metal, the plant Fe concentration significantly decreased. Tabarteh et al. investigated the effect of enriched cow manure with converter sludge on Fe bioavailability in the lead polluted soil and concluded that using convertor sludge cow manure had a significant effect on increasing and decreasing the plant Fe and Pb concentration, respectively [39]. However, they did not consider the role of organic iron sources on Fe and heavy metals uptake by plants that were cultivated in the soils that were simultaneously polluted with heavy metals and petroleum hydrocarbons.

The presence of *thiobacillus* bacteria also significantly affected the biodegradation of diesel oil in the soil. The greatest biodegradation of diesel oil in the soil belonged to the non-Cd polluted soil under the cultivation of plants inoculated with *thiobacillus* bacteria. Based on our study, plant inoculation with *thiobacillus* bacteria had a significant effect on increasing the plant Fe concentration, which can be attributed to the role of *thiobacillus* bacteria in oxidizing sulfur compounds and consequently reducing the soil rhizosphere environment that can increase the soil Fe availability. Increasing the soil and plant nutrient availability via decreasing soil pH is mentioned by researchers [40, 41]. Mousavi et al. investigated the effect of sulfur application and *thiobacillus* bacteria on improving wheat morphological characteristics in Khuzestan lands. They concluded that using these sulfur elements and plant inoculation with *thiobacillus* bacteria significantly increases plant biomass via improving the plant nutrient status [42], which is similar to our results. According to our results, with increasing the plant Fe concentration, the biodegradation of diesel oil in the soil has significantly increased, and the plant Cd

Table 4. Effect of treatments on soil Cd concentration (mg/kg) in the presence of *thiobacillus* bacteria

Sulfur Granular MSW (t/ha)	Iron Sulfate (mg/L)	Soil Cd Concentration (mg/kg soil)	With *Thiobacillus*			Without *Thiobacillus*		
			Diesel oil (% w/w)					
			0	4	8	0	4	8
0	0	0	ND*	ND	ND	ND	ND	ND
	500		ND	ND	ND	ND	ND	ND
	0	10	41.0v**	41.9q	42.5	41.4t	41.3u	41.9q
	500		37.3h�	38.9c�	41.7r	38.3e�	39.1b�	39.8z
	0	20	48.6f	49.2e	50.1c	50.1c	52.4b	53.7a
	500		45.7k	46.8j	48.3h	47.8i	48.5g	49.9d
15	0	0	ND	ND	ND	ND	ND	ND
	500		ND	ND	ND	ND	ND	ND
	0	10	37.7g�	38.9c�	40.2y	38.7d�	39.4a�	40.8w
	500		35.2l�	36.2j�	38.1f�	37.1i�	38.9c�	39.8z
	0	20	40.4x	42.3p	43.1m	41.6s	42.9n	44.1l
	500		35.5k�	37.1i�	38.9c�	37.1i�	39.1b�	41.7r

*ND: Not Detectable by AAS, **Similar letters show the non-significant differences.

Table 5. Effect of treatments on plant Cd concentration (mg/kg) in the presence of *thiobacillus* bacteria

Sulfur Granular MSW (t/ha)	Iron Sulfate (mg/L)	Soil Cd Concentration (mg/kg soil)	With *Thiobacillus*			Without *Thiobacillus*		
			Diesel Oil (% w/w)					
			0	4	8	0	4	8
0	0	0	ND*	ND	ND	ND	ND	ND
	500		ND	ND	ND	ND	ND	ND
	0	10	16.1**	16.3p	16.4n	16.7l	16.8k	17.3h
	500		15.9	16.1r	16.2q	16.3p	16.5m	17.1
	0	20	17.5f	17.8e	18.1c	17.9d	18.2b	18.6a
	500		16.1r	16.7l	17.3h	16.9	17.4g	17.9d
15	0	0	ND	ND	ND	ND	ND	ND
	500		ND	ND	ND	ND	ND	ND
	0	10	15.1z	15.4x	15.9t	16.0s	16.3p	16.8k
	500		14.1d�	14.7b�	14.8a	14.4c�	14.7b�	15.3y
	0	20	16.0s	16.4n	16.8k	16.3p	16.8k	17.3h
	500		15.1z	15.5w	15.8u	15.7v	15.9t	16.4n

* ND: Not Detectable by atomic absorption spectroscopy; ** Similar letters show the non-significant differences.

Table 6. Effect of treatments on plant Fe concentration (mg/kg) in the presence of *thiobacillus* bacteria

Sulfur Granular MSW (t/ha)	Iron Sulfate (mg/L)	Soil Cd Concentration (mg/kg soil)	With *Thiobacillus*			Without *Thiobacillus*		
			Diesel Oil (% w/w)					
			0	4	8	0	4	8
0	0	0	14.1n□*	13.7o□	13.4p□	13.7o□	13.1q□	12.7r□
	500		33.7d□	34.1c□	33.5e□	33.1f□	32.5h□	31.7l□
	0	10	12.6s□	12.1t□	11.8u□	12.1t□	11.7v□	11.2y□
	500		12.1t□	11.7v□	11.3	11.5w□	11.2y□	11.0a□
	0	20	11.1z□	10.6c□	10.3e□	10.7b□	10.4d□	10.0g□
	500		10.6c□	10.2f□	9.8h□	10.2f□	10.0g□	9.5i□
15	0	0	51.7g	50.4h	48.6k	50.2i	48.4l	45.8
	500		61.3a	58.4c	55.5d	60.3b	55.1e	53.7f
	0	10	41.7t	40.5w	39.8z	40.2y	38.2a□	36.4b□
	500		49.2j	48.3m	47.2o	45.3p	44.2q	42.8r
	0	20	33.7d□	32.1j□	31.5m□	33.0g□	32.4i□	32.0k□
	500		42.4q	41.5u	40.6v	41.7t	40.4x	38.2a□

*Similar letters show the non-significant differences.

concentration decreased, which can be related to the interaction effects of Fe with heavy metals and thereby increasing the biodegradation of diesel oil in the soil. It can be concluded that using sulfur granular MSW in arid and semi-arid regions can improve the plant nutrient status and thus increase the plant resistance to abiotic stresses such as heavy metals toxicity, which is a positive point in environmental studies.

4. Conclusion

Using 15 t/ha sulfur granular MSW significantly increases the biodegradation of diesel oil in the Cd and non-Cd polluted soil by 15.1% and 18.1%, respectively. However, increasing soil pollution to diesel oil had significant effects on increasing the biodegradation of diesel oil in the soil, which can be related to the role of diesel oil as a carbon source for soil micro-organisms and thereby increasing the biodegradation of diesel oil in the soil. Increasing the soil microbial respiration with increasing the soil pollution to diesel oil confirms our results. However, the greater soil pollution with diesel oil or heavy metals may negatively affect soil microbial activity and biodegradation of diesel oil in the soil, which should be considered in future research studies. In addition, the interaction effects of heavy metals with nutrient elements and their role in phytoremediation efficiency cannot be ignored.

Ethical Considerations

Compliance with ethical guidelines

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

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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