Original Article

Effect of EDTA chelate, cow manure, elemental sulfur application and Thiobacillus inoculant on Cd, Zn and Fe phytoremediation efficiency in a Cd-polluted soil

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Abstract

***Background and Purpose*:** Phytoremediation efficiency of heavy metals is one of the important points in environmental studies. This research was conducted to investigate the effect of cow manure, elemental sulfur and EDTA on Cd uptake by Indian mustard in a Cd-polluted soil in the presence of Thiobacillus thiooxidans.

***Materials and Methods*:** Treatments consisted of applying cow manure (0, 5 and 10 g/kg soil), soil application of elemental sulfur (2 g/kg soil), and Cd-polluted soil (0 and 20 mg Cd/kg soil) with 1.5 mmol EDTA/kg soil in the presence of Thiobacillus spp. After 90 days, Indian mustard plant was harvested and plant Zn, Fe and Cd concentration was measured using atomic absorption spectroscopy. In addition, the soil microbial respiration was measured.

***Results*:** The use of 2 g/kg soil of elemental sulfur significantly increased the plant Cd concentration in the presence and absence of Thiobacillus by 14.2 and 11.7%, respectively. Adding cow manure to the soil at the rates of 5 and 10 g/kg soil significantly increased the plant Cd concentration by 15.7 and 18.1%, respectively. Also, the application of EDTA chelate at the rate 0f 1.5 mmol/kg soil significantly increased the Cd concentration of the plants grown in the Cd-polluted soil (20 mg Cd/kg soil) by 13.6%.

***Conclusion*:** The results of the present study showed that using elemental sulfur in the Cd-polluted soil can increase the Cd concentration of the plant which was cultivated in the soil amended with cow manure in the presence of Thiobacillus bacteria. However, the role of soil physic-chemical properties on phytoremediation efficiency cannot be ignored.

***Keyword*:** Phytoremediation; Heavy metal; Indian mustard; elemental sulfur; EDTA

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1. *Introduction*

Today, environmental pollution is one of the most important issues faced by different communities and is usually caused by industrial activities, such as the exploitation of metal smelters, the use of fertilizers, pesticides and processing of municipal waste (1, 2). For this reason, there are serious concerns in this area, which makes it necessary to take proper measure for pollution control, which is one of the goals of environmental engineers who are committed to protect humans from environmental pollution (3, 4).

Among heavy metals, Cd is of special importance, because it is easily absorbed by the plant roots and its toxicity is up to 20 times more than other heavy metals. The high Cd bio-availability and possibility of its entering food chain, even at low concentrations, have led to a greater need to investigate the factors that affect the soil Cd bio-availability (5). Cd is considered as an unnecessary element for the plant since it prevents its growth (6, 7). Also, Cd is a mobile element compared to other heavy metals in the soil and can easily be exchanged with other elements in its solution phase. Therefore, knowledge of the chemical status of heavy metals in the soil can play an important role in remediation of soils contaminated with Cd (8, 9).

So far, several methods have been proposed to remediate the soils polluted with heavy metals so as to prevent the spread of these contaminants to other sources. However, many of these methods are not applicable in developing countries due to their high cost or lack of access to the necessary facilities to implement them. One of the main problems in remediation of heavy metals in contaminated soils of arid and semi-arid regions of the country is the low availability of heavy metals, which can make it difficult to clean them. Therefore, a suitable solution should be sought to increase the availability of heavy metals and select plants with high biomass in order for these elements to be absorbed by the plant (10, 11).

One of the most important methods to increase the availability of heavy metals is to reduce the soil pH, which is usually difficult to do in calcareous soils due to the high buffering capacity of the soil. Meanwhile, the use of sulfur compounds can help reduce soil pH and thus increase the availability of heavy metals in the soil. Many researchers reported that the use of sulfur, resulting in increased sulfuric acid production, reduces soil pH and increases the availability of heavy metals. However, due to different soil buffering capacities, the soil pH decrease rate can be different (12). Under aerobic conditions, chemosynthetic bacteria are responsible for oxidation. Some heterotrophic bacteria and fungi have the ability to oxidize hydrogen sulfide. However, oxidation of elemental sulfur is mainly performed by Thiobacillus species (13). Thiobacillus spp. have significant effect on lowering the soil pH due to the production of acid, and as a result, increasing the solubility of heavy metals and facilitating their accessibility by the plant (14). Zhi-Hui et al. pointed to the elemental sulfur oxidation by Thiobacillus spp. and aerobic heterotrophic sulfur-oxidizing bacteria and concluded that using elemental sulfur can increase the plant nutrient concentration via decreasing soil pH (15). However, they did not study the role of decreasing soil pH via using elemental sulfur on soil heavy metals availability (15).

Due to the environmental hazards of heavy elements, using plants for cleaning the

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environment and metallic elements has received much attention in recent decades. Plants that are used to remediate the contaminated soils are called hyper-accumulators (16). Meanwhile, the use of chemical compounds to increase the efficiency of phytoremediation can greatly help to increase the absorption of heavy metals by plants, which is called chemical phytoremediation. In chemical phytoremediation method, compounds, such as citric acid, sulfuric acid and Ethylenediamine tetraacetic acid (EDTA) have been used to increase the solubility of heavy metals in the soil (17).

Turgut et al. investigated the effect of EDTA on Cd uptake by sunflower and concluded that using chemical modifiers, such as EDTA chelate, can increase the soil heavy metal availability and thereby increase the plant Cd availability. In addition, they mentioned that using different rates of EDTA chelate had different effect on phytoremediation efficiency. However, they did not point to the role of soil physic-chemical properties on heavy metals uptake by plants (18).

Among the chemical modifiers, EDTA has been introduced as a substance that increases the solubility of heavy metals in soil and has a sufficient effect on desorption of heavy elements, such as Pb and Cd. However, the physicochemical properties of soil can affect the phytoremediation efficiency, which should be considered separately in different studies. Thus, the current research was conducted to evaluate the effect of EDTA chelate, cow manure, elemental sulfur application, and Thiobacillus inoculant on Cd phytoremediation efficiency in Cd-polluted soil.

2. *Material and Methods*

To investigate the effect of cow manure and elemental sulfur application on Cd heavy metal uptake by Indian mustard (*Brassica juncea L.*) plant, a factorial experiment in the layout of randomized completely block design was conducted. Treatments consisted of applying cow manure at the rates of 0, 5 and 10 g/kg soil, soil application of elemental sulfur (2 g/kg soil), Cd-polluted soil (0 and 20 mg Cd/kg soil) amended with 1.5 mmol EDTA/kg soil in the presence of Thiobacillus spp.

Cd was spiked to the studies soil at the rate of 0 and 20 mg Cd/kg soil and incubated for one month to equilibrium. After that, cow manure was added to the soil at the rates of 0, 5 and 10 g/kg soil. The initial inoculum of *Thiobacillus* (PTCC 1668) was obtained from Water and Soil Research Institute of Iran. Then, the Thiobacillus inoculant was added to the 2 cm depth of top soil according to the procedure described by Heydari et al. (19). Then, 5 kg pots were filled with treated soil. After that, eight Indian mustard seeds were planted in each pot and after germination; the number of seedlings in each pot was reduced to five. During the experiment, the pots were watered every two to three days as needed. After one week, EDTA Chelate was added to the soil at the mentioned rate three weeks after planting. After 90 days, plants were harvested and plant Zn, Fe and Cd concentration (20) was measured using atomic absorption spectroscopy (AAS). Soil Cd concentration was also determined by the method described by Lindsay et al. (21). Soil microbial respiration, on the other hand, was measured using the method described by Zamani et al. (22).

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Statistical analyses were done according to the ANOVA procedure. The differences between means were evaluated using the LSD test. The P value <0.05 was considered as the significance level in the present study.

The bio-concentration factor (BCF) was calculated according to the following equation:

BCF= C shoot/C soil (Eq.1)

C shoot and C soil are the heavy metal concentrations in the plant and soil, respectively.

3. Results

Adding 2 g/kg soil of elemental sulfur significantly decreased the soil pH by 0.3 and 0.2 units (Figure 1-a) in the presence and absence of Thiobacillus bacteria. In addition, soil application of cow manure at the rate of 5 and 10g/kg soil had no significant effect on soil pH. However, a significant increase was observed in soil CEC (cation exchange capacity) by 10.2 and 11.7%, when the soil was amended with cow manure at the rates of 5 and 10 g/kg soil, respectively (Figure 1-b).

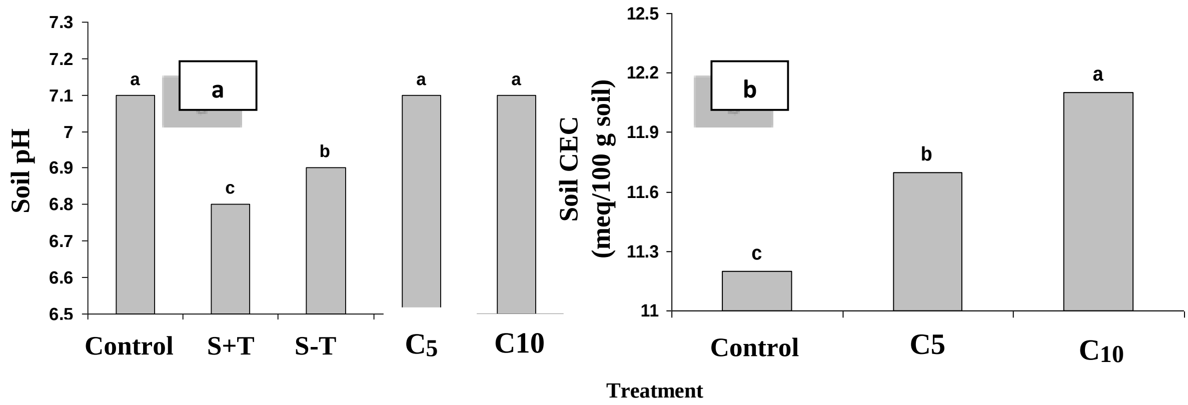

Figure 1. Effect of treatments on soil pH (a) and soil CEC (b), S+T, S-T, C_5 ,C_{10} and control are elemental sulfur in the presence (S+T) and absence (S-T) of Thiobacillus bacteria, soil application of 5 (C_5) and 10 (C_{10}) g/kg soil of cow manure and control soil, respectively.

The greatest soil Cd availability (Table 1) belonged to the soil receiving 2 g/kg soil elemental sulfur fertilizer, while the lowest amount of that belonged to the non-polluted soil. Increasing soil pollution with Cd from 0 t0 20 mg Cd/kg soil significantly increased the soil Cd availability by 10.6%. At this time, using 2 g/kg soil of elemental fertilizer significantly increased the soil Cd availability in the presence and absence of Thiobacillus by 16.2 and 12.3%, respectively. Adding cow manure to the soil significantly increased the soil availability, and as the results of this study showed, with increasing the soil application of cow manure from 0 to 5 and 10 g/kg soil, the soil Cd availability significantly increased by 13.7 and 16.1%, respectively. In addition, using 1.5 mmol/EDTA/ kg soil chelate in the Cd-polluted soil (20 mg Cd/kg soil) significantly increased the soil Cd availability by 13.7%.

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Table 1. Effect of cow manure, EDTA chelate and elemental sulfur on soil and plant Cd concentration (mg/kg soil) in the presence of Thiobacillus

EDTA (mmol/kg soil)	Cd concentration (mg/kg soil)	With Thiobacillus						Without Thiobacillus					
		Elemental sulfur (g/kg soil)											
		0			2			0			2		
		Cow manure (g/kg soil)											
		0	5	10	0	5	10	0	5	10	0	5	10
		Soil Cd concentration (mg/kg soil)											
0	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		17.0j	17.2h	17.5f	17.4g	17.5f	17.9c	16.1p	16.4o	16.9k	16.5n	16.8l	17.1i
1.5		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		17.4g	17.6r	18.0b	17.8d	17.9c	18.2a	16.7m	17.0j	17.4g	16.9k	17.2h	17.5f
		Plant Cd concentration (mg/kg DW)											
0	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		11.1o *	11.9l	12.8g	12.0k	12.6j	13.4d	9.8r	10.5q	11.7m	10.7p	11.9l	12.7h
1.5		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		12.2	12.7h	13.9b	13.0f	13.8c	14.7a	11.2n	11.9l	13.1e	12.0k	12.7h	13.8c

***ND: Not detectable by AAS**

Soil application of EDTA had significant effect on increasing the plant Cd concentration (Table 1). Accordingly, our results showed that application of EDTA chelate at the rate of 1.5 mmol/kg soil in the Cd-polluted soil significantly increased the plant Cd concentration by 14.1%. Increasing soil pollution with Cd from 0 t0 20 mg Cd/kg soil significantly increased the plant Cd concentration by 11.1%. The presence of Thiobacillus had positive effect on increasing the plant Cd concentration. Thus, using 1.5 mmol/kg soil of EDTA chelate in the Cd polluted soil significantly increased the plant Cd concentration by 11.7 and 14.3% in the absence and presence of Thiobacillus, respectively. In addition, using 5 and 10 g/kg soil of cow manure in the Cd polluted soil (20 mg Cd/kg soil) significantly increased the plant Cd concentration by 11.8 and 14.1%, respectively.

The greatest BCF value (Table 2) was calculated for the plants cultivated in the Cd polluted soil that was amended with 1.5 mmol EDTA /kg soil, while the lowest amount of that belonged to the plants grown in the non Cd-polluted soil. Soil inoculation with Thiobacillus significantly increased the BCF and plant Cd concentration. Accordingly, a significant increase was observed in BCF value by 13.5%, while growing in the presence of Thiobacillus bacteria: The interaction effects of cow manure and Thiobacillus spp. was also significant, so that soil application of cow manure at the rate of 38 g/pot significantly increased the plant Cd concentration by 16.2% in the presence of Thiobacillus bacteria.

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Table 2. Effect of cow manure, EDTA chelate and elemental sulfur on BCF value in the presence of Thiobacillus

EDTA (mmol/kg soil)	**Cd concentration (mg/kg soil)**	**With Thiobacillus**						**Without Thiobacillus**					
		Elemental sulfur (g/kg soil)											
		0			2			0			2		
		Cow manure (g/kg soil)											
		0	5	10	0	5	10	0	5	10	0	5	10
0	0	NC*	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	20	0.65l	0.69j	0.73f	0.69j	0.72g	0.75d	0.61n	0.64m	0.69j	0.65l	0.71h	0.74e
1.5	0	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	20	0.70i	0.72g	0.77c	0.73f	0.77c	0.81a	0.67k	0.70i	0.75d	0.71h	0.74e	0.79b

***NC: Not calculated**

As shown in the table, soil application of cow manure at the rate of 5 and 10 g/kg soil significantly increased the plant Fe and Zn concentration (Table 3), thus the application of cow manure at the rates of 5 and 10 g/kg soil significantly increased the plant Fe and Zn concentration by 11.7 and 13.4%, respectively. However, it was found that soil pollution with Cd had negative effect on plant Fe and Zn concentration. As a result, increasing soil pollution with Cd significantly decreased the Fe and Zn concentration of the plants grown in the soil which did not receive any organic amendments. Soil application of EDTA had also adverse effect on plant Zn and Fe concentration.

Table 3. Effect of cow manure, EDTA chelate and elemental sulfur on plant Fe and Zn concentration (mg/kg) in the presence of Thiobacillus

EDTA (mmol/kg soil)	**Cd concentration (mg/kg soil)**	**With Thiobacillus**						**Without Thiobacillus**					
		Elemental sulfur (g/kg soil)											
		0			2			0			2		
		Cow manure (g/kg soil)											
		0	5	10	0	5	10	0	5	10	0	5	10
		Plant Fe concentration (mg/kg DW)											
0	0	62.4a'	68.3l	75.3b	68.4k	72.4f	78.3a	60.4e'	65.4q	71.4g	65.7p	68.4k	74.2c
	20	60.3f'	65.4q	70.2i	63.5y	70.2i	73.6e	58.1j'	60.3f'	64.9s	61.4c'	64.2v	70.3h
1.5	0	60.2g'	63.5y	66.3m	65.2r	68.9j	73.7d	58.4i'	60.3f'	64.3u	61.4c'	63.8x	65.9o
	20	58.1j'	60.2g'	64.1w	62.8z	66.1n	70.2i	55.3l'	57.8k'	61.7b'	58.7h'	61.2d'	64.5t
		Plant Zn concentration (mg/kg DW)											
0	0	15.9w	17.8k	19.4f	16.2t	19.1g	22.8a	13.1i'	16.2t	18.4i	15.2y	18.1j	20.3d
	20	15.1z	16.5r	18.4i	15.4w	18.1j	21.6b	12.5k'	14.7b'	17.3n	14.3c'	17.2o	19.4f
1.5	0	13.7g'	14.2d'	16.9p	15.3x	17.4m	20.9c	12.1l'	13.1i'	15.2y	14.1e'	17.7l	18.9h
	20	12.6j'	13.8f'	15.5v	14.9a'	16.8q	19.8e	10.5n'	11.9m'	13.7g'	13.6h'	15.5v	16.3s

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The greatest soil microbial respiration (Table 4) was measured in the soil amended with 38 g/pot cow manure, while the lowest amount of that was observed in the Cd-polluted soil that was not amended with cow manure. Increasing soil application of cow manure from 0 to 5 and 10 g/kg soil significantly increased the soil microbial respiration by 12.5 and 14.9%, respectively. For soil polluted with 20 mg Cd/kg soil, it increased by 10.1 and 11.2%, respectively. Using 1.5 mmol EDTA/kg soil significantly decreased the soil microbial respiration by 15.1%. At the same time, the soil Cd availability increased by 13.1%. Soil application of elemental sulfur significantly increased the soil Cd concentration and decreased the soil microbial respiration by 10.2 and 7.8%, respectively in the presence of Thiobacillus bacteria.

Table 4. Effect of cow manure, EDTA chelate and elemental sulfur on soil microbial respiration (mg C-CO_2/kg soil) in the presence of Thiobacillus

EDTA (mmol/kg soil)	Cd concentration (mg/kg soil)	With Thiobacillus						Without Thiobacillus					
		Elemental sulfur (g/kg soil)											
		0			2			0			2		
		Cow manure (g/kg soil)											
		0	5	10	0	5	10	0	5	10	0	5	10
0	0	17.1k	18.0d	18.1c	17.3i	18.5b	18.9a	16.4r	16.7o	17.1k	16.9m	17.0l	17.4h
	20	16.8n	17.1k	17.6f	17.2j	17.5g	17.8e	16.1u	16.5q	16.7o	16.4r	16.8n	17.0l
1.5	0	16.2t	16.5q	16.7o	16.6p	17.0l	17.4h	16.3s	16.5q	17.0l	16.5q	16.8n	17.0l
	20	15.2y	15.5x	15.9v	15.5x	15.9v	16.6p	15.6w	16.2t	16.7o	15.9v	16.4r	16.9m

4. *Discussion*

The results of the present study showed that using 2 g/kg soil of elemental sulfur had significant effect on soil pH which can influence the soil Cd availability and thereby increase the Cd uptake by plant (Table 1), and the greatest soil Cd availability belonged to the soil amended with the greatest receiving of elemental sulfur. Increasing soil heavy metals availability via decreasing soil pH has been previously reported by researchers (23, 24). Kasraian et al. investigated the role of sulfur application on spinach phytoremediation process of Cd in contaminated calcareous soils, and concluded that soil application of sulfur had significant effect on plant Cd uptake. However, they found that Sulfur oxidation can also improve the phytoremediation efficiency, which was in line with our results (25). Farzanegan1 et al. reported that using plant cultivar had an important role in phytoremediation efficiency, as the results of their study showed that canola has higher potential for remediation of Cd and Pb from contaminated soil in comparison with sorghum. However, they did not refer to the role of sulfur oxidizing bacteria in increasing the phytoremediation efficiency of heavy metals in soil (26). Based on the results of the current study, the greatest plant and soil Cd concentration was measured in the soil that amended with 2 g/kg soil of elemental sulfur in the presence of Thiobacillus spp. that can be related to the role of Thiobacillus bacteria on sulfur oxidation and consequently the reduction of soil pH which can increase the soil Cd availability (27). Since soil pH is one of the main factors controlling the mobility of heavy metals in calcareous soils, the use of

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sulfur compounds can play an effective role in increasing the availability of heavy metals in the soil and thus increase the phytoremediation efficiency (28). This finding was consistent with the results of the present study in that, according to our findings, using 2 g/kg soil of elemental sulfur in Cd-polluted soil (20 mg Cd/kg soil) significantly increased the plant biomass by 14.7% (date was not shown) which can be related to the role of elemental sulfur in decreasing soil pH and increasing the plant nutrient status that can help to improve the plants growth. A significant increase in plant Fe and Zn concentration (Table 3) while using elemental sulfur confirms our results clearly. On the other hand, the plant Zn and Fe concentration more significantly increased in the presence of Thiobacillus bacteria, a result which can be attributed to the role of Thiobacillus bacteria in oxidizing sulfur compounds and thus increasing the availability of nutrient elements for the plant. The results of the study of Besharati were also in line with the findings of the current research in that they investigated the effects of soil application of sulfur and Thiobacillus inoculation on soil and plant nutrient concentration in a non-polluted calcareous soil, and concluded that soil elemental sulfur application and the presence of Thiobacilus bacteria significantly increased the plant biomass and soil and plant nutrient concentration (29). Consequently, our results showed that soil application of sulfur at the rate 0f 2 g/kg soil significantly increased the plant Fe and Zn concentration in the presence of Thiobacillus spp. by 12.7 and 15.4%, respectively which can be attributed to the role of elemental sulfur fertilizer and Thiobacillus inoculation on decreasing soil pH and thereby increasing soil nutrient availability. Increasing soil nutrient availability due to decreasing soil pH has been mentioned by researchers (30, 31). However, increasing soil pollution to Cd has significantly decreased the plant biomass that has an important role in phytoremediation efficiency. Therefore, based on the findings, the use of organic amendments, such as cow manure, which are not contaminated with heavy metals can help to increase the phytoremediation efficiency by increasing the plant biomass. The findings of the present study revealed that using 5 and 10 g/kg soil of cow manure can increase the phytoremediation efficiency of the plant cultivated in the Cd-polluted soil (20 mg Cd/kg soil) by 14.1 and 16.2%, respectively, which can be related to the role of applying cow manure in increasing plant biomass via increasing the plant nutrient status and thereby increasing the Cd phytoremediation efficiency. However, the interaction effect of applying cow manure, elemental sulfur and Thiobacillus inoculation on increasing the phytoremediation efficiency was positive.

Based on the results of this study, the interaction effects of cow manure, elemental sulfur fertilizer and inoculation of Thiobacillus bacteria could reduce the negative effects of heavy metals on phytoremediation process. It was also revealed that increasing soil pollution with Cd from 0 to 20 mg Cd/kg soil significantly decreased the plant biomass by 16.5 %, while it increased by 15.2%, when the soil was amended with 10 g/kg of cow manure and 2 g/kg of elemental sulfur fertilizer. In another study, Tabak et al. investigated the effect of amending soil with elemental sulfur on soil heavy metals availability, and concluded that using waste elemental sulfur had significant effect on decreasing soil pH and thereby increasing the soil heavy metal availability. However, their results showed that the content of heavy metal availability depends on soil physicochemical properties such as soil (28). For instance, they showed that decreasing the soil pH via using the waste elemental sulfur in the heavy soil texture was lower than the light soil (28).

Generally, soils of arid and semi-arid regions have high buffering capacity and the use of sole sulfur cannot change or slightly change the pH and heavy metal availability of calcareous soil. Hence, using organic chelate such as EDTA may help in increasing soil heavy metal availability and thereby increasing the phytoremediation efficiency. According to our results, adding 1.5 mmol/kg soil EDTA chelate to the soil significantly increased the soil Cd availability in the soil treated with 5 and 10 g/kg of cow manure by 13.3 and 15.4% which is similar to the results of Hoshyar et al. (32). Mirkhani et al. in a study with similar results investigated the effect of EDTA on heavy metal uptake by canola and concluded that EDTA application was able to increase Pb and Cd solubility in soil solution and thereby increase the plant heavy metals concentration (33). In addition, Li et al. reported that applying organic chelate such as EDTA can increase the phytoremediation efficiency of heavy metals via increasing the solubility of heavy metals in the soil (34). Basically, chelating agents remove metals from the soil environment with the minimal effect on the soil physicochemical properties. It was also found that Chelate reagents increase the availability of heavy metals by plants in two ways, first by preventing the deposition of metals and then by adding metals to the soil solution through desorption of adsorbed species, decomposition of iron and manganese oxides, and decomposition of precipitated compounds. There are many differences in the stimulating effect of chelates on the accumulation of heavy metals in the shoots of different plant species, thus it is necessary to identify and use plant species with high biomass that are able to accumulate large amounts of metals in the presence of chelate treatments. In general, due to the fact that heavy metals have negative effects on plant biomass, the simultaneous application of cow manure with organic chelates such as EDTA can help increase the efficiency of phytoremediation via increasing the plant biomass in heavy metal-polluted soil.

5. Conclusion

Based on the results of the present study, using 2 g/kg soil of elemental sulfur fertilizer had significant effect on increasing the soil and plant Cd concentration that can be related to the role of elemental sulfur on decreasing soil pH and thereby increasing the soil and plant Cd concentration. A significant decrease in soil pH by 0.3 units clearly confirms our results. In addition, soil application of cow manure at the rates of 5 and 10 g/kg soil significantly increased the plant Cd concentration by 11.3 and 17.1%, respectively, which can be related to the role of cow manure in increasing plant biomass and consequently increasing the plant Cd concentration. It was also found that using EDTA chelate had positive effect on increasing the soil and plant Cd concentration which can be attributed to the role of EDTA chelate on increasing the Cd solubility in the soil. In addition, soil application of elemental sulfur decreased soil pH and consequently increased the nutrient uptake by plant, the action that had a positive effect on improving the plant biomass.

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