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ORIGINAL ARTICLE

Effect of Ethylene diamine-N, N'-di-succinic acid (EDDS) on the accumulation of Cadmium and physiological response of Fenugreek plant (*Trigonella foenum-graecum* L.) grown in Cd contaminated soils

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ABSTRACT
The pot experiment was conducted with the aim to determine the effect of EDDS on the growth of plant and accumulation of heavy metal in the Cd contaminated soil using fenugreek (Trigonella foenum-graecum L). This experiment was carried out at Sheila Dhar Institute experimental farm at Prayagraj, Uttar Pradesh, India. The results showed that the application 6 mmol/kg EDDS significantly (P<0.05) utmost decreased the height and dry biomass yield of shoots and roots by 45.63, 71.23 and 49.91, 50.75% respectively, when compared to their control pot at 100 mg/kg Cd contaminated soil. The application of 6 mmol/kg EDDS significantly (P<0.05) enhanced the accumulation of Cd by 172.71, 176.85 and 137.78% respectively, in the root, shoot and leaves of fenugreek plants, compared with that in the control. The treatment of EDDS, the maximum BCF, TF and RF values were observed 13.17±0.17, 1.32±0.12 mg/kg and 0.67±0.05% respectively, compared their control. The addition of EDDS in Cd contaminated soil significantly enhanced the accumulation of Cd however, reduced the growth and dry biomass yield of fenugreek. It may be concluded that EDDS enhanced the clean-up of heavy metal in Cd contaminated soil through fenugreek.
Keywords- *Cadmium, Contaminated soil, EDDS, Fenugreek*

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INTRODUCTION
Heavy metals have been released excessively in farmlands recently due to significant increases in urbanization and industrialization, which have a negative impact on ecosystems [2-25]. One of the most harmful and pervasive environmental issues is the cadmium (Cd) contamination of agricultural soils [1-17]. As a result of direct or indirect inhibition of numerous physiological processes like respiration, transpiration, photosynthesis, oxidative stress, cell elongation, nitrogen metabolism, and mineral nutrition uptake, Cd commonly causes growth retardation, leaf chlorosis and decreased biomass of plant [22-26].
Natural and anthropogenic origins are responsible for heavy metals contamination in subterranean environments. Natural sources like weathering of minerals, anthropogenic sources predominantly rapid industrial effluent and modern agricultural practices including pesticides and fertilizers activities are the foremost contamination sources. Plants growing in heavy metal-contaminated soils commonly accumulate the heavy metals and therefore cadmium to contamination of the food chain through biomagnification [8-10]. Plants having capability to hyper accumulate heavy metals are known as metallophytes and these properties have been utilized by researcher in phytoremediation methods. The effect of heavy metals on plants have been reported by several scientists including oxidative

stress and glutathione depletion in roots of alfalfa [14], chlorosis, loss of yield, lesser nitrogen fixation in leguminous plants [8].

Contamination of heavy metals detrimental effects on ecosystems and human health by direct consumption of contaminated food, physical contact with contaminated soil, through the food chain (soil-plant-human or soil-plant-animal-human), drinking contaminated water, lowering food quality and decreasing the area of land suitable for farming and subsequent food instability [11-35].

Several researches on heavy metals have been conducted for decades and numerous studies have been published. Furthermore, accumulating substantiation showed that phytoremediation of heavy metals has become one of the progressive hotspots [23]. In recent studies, research on the phytoremediation of heavy metal contaminated soils has gradually evolved from the discovery of hyper accumulator plants to the internal growth and developmental processes of plants [4-18]. For instance, researchers have studied the germination of seeds and seedling growth characteristics of a plant that is resistant to heavy metals stress and showed that small proteins metallothioneins and phytochelatins are involved in the mechanisms of resistance to the stress brought by heavy metals [20-32].

Trigonella foenum-graecum L. (family: Fabaceae or Leguminosae) is a leguminous annual plant which reaches a height of around 60 cm tall. It is called “methi” in Urdu or Hindi and Fenugreek in English name. Fenugreek is an herb that is frequently used in medicine. It is grown as semi-arid crop all over the world. The young leaves are frequently used in Asian cuisine and can be encompassed in salad mixtures. Conventionally, fenugreek tea produced from the crushed seed has historically been used medicinally for a range of ailments. Animals were fed the herb both tonic and as a valuable source of food. Heavy metal contamination is becoming an increasingly important issue worldwide, particularly in emerging nations. One of the main food chain routes for human exposure is through the consumption of food crops contaminated with heavy metals [31]. The aims of this pot experiment were therefore: i. to evaluate the physico-chemical properties of contaminated soils. ii. to evaluate the effect of EDDS on the performance of fenugreek plant to remediate Cd contaminated soils. iii. to evaluate the effect of EDDS on the physiological parameters of fenugreek plant such as height of plant and dry biomass yield iv. to evaluate the effect of EDDS on the concentration of Cd by using fenugreek plant in the terms of bioconcentration, translocation and remediation factor processes.

MATERIAL AND METHODS

Experimental site and design

The pot experiment was conducted in Cd contaminated soils at Sheila Dhar Institute (SDI) experimental form Mumfordganj situated between latitudes 20°20′N and longitudes 81°52′E with elevation slope of 101m at Sheila Dhar Institute of Soil Science, University of Allahabad, Prayagraj, Uttar Pradesh. The pot experiment was carried out as an overall completely randomized design (CRD) at SDI experimental farm in the Rabi season during the year of 2021-2022. Soil samples were collected from SDI experimental farm and then pots filled with 5 kg soil samples. After the pots had been filled with soil and fenugreek (*Trigonella foenum-graecum*) seeds sown as test crop with 90% germination power of seeds and thinned to some seedlings after 15 days of germination of seeds. The experiment design was as follows: (i) without EDDS and Cd treatment (Control) (ii) EDDS (Four levels- 0, 02, 04, 06 mmol/kg) (iii) $CdCO_3$ (Three levels- 0, 50, 100 mg/kg. The pot experiment work was consisted of 12 treatments including a control pot.

Soil sampling and analysis

Soil samples were collected from SDI experimental farm at depth of 0-20 cm for the analysis of heavy metal contents and physico-chemical properties of soil. The collected soil samples were air dried at room temperature ground to fine in particle size and finally sieved samples to 2 mm sieve. The concentration of total Cd was determined using di-acid digestion method in mixture of concentrated HNO_3 and $HClO_4$ (1:4 by volume). The concentration of Cd in digested final soil samples were determined by Atomic Absorption Spectrophotometer (AAS) (Analyst400, PerkinElmer Inc., MA, USA) at National Botanical Research Institute, Lucknow (U.P.) using their respective Lumin™Lamps [18].

Plant sampling and analysis

The height of plants were measured 60 days after germination and plant root and shoot were also measured after harvesting of plants. Plant samples of root and shoot were washed carefully with tape water, $CdCl_2$ (2%) and finally rinsed with double distillation water (DDW) to remove contaminated particles and then dry biomass of plant root and shoot were obtained by drying thermostatic hot air oven at 70°C for 48 hours. The dried plant samples of root and shoot were ground into fine powder. Then one gram of each sample roots and shoots were digested individually in a tri-acid mixture H_2SO_4, $HClO_4$ and HNO_3 (1:2:5 through volume, respectively) [19]. The total content of Cd in extracted plant roots and shoots samples were analyzed through Atomic Absorption Spectrophotometer (AAS) (AAnalyst400, PerkinElmer Inc., MA, USA) at National Botanical Research Institute, Lucknow (U.P.) to determine Cd by using their respective LuminTH Lamps.

Evaluation of bioconcentration (BCF), translocation (TF) and remediation factors (RF)

There are several parameters which used to assess the phytoextration efficiency. BCF provides an index of the capacity of plant shoot and root to accumulate the Cd with respect to the contaminated soil. TF represents the translocation of Cd from the root to shoot. RF is the ratio of accumulation of heavy metal in shoots to in the Cd contaminated soil. The following equations are used [13].

$$\text{Bioconcentration factor} = \text{Mroot}/\text{Msoil}$$

Where, Mroot is the Cd content (mg/kg dry biomass) in roots, Msoil is the total Cd content in the soil. Msoil was calculated by total Cd content in contaminated soils.

$$\text{Translocation factor} = \text{Mshoot}/\text{Mroot}$$

Where, Mshoot is the Cd concentration (mg/kg dry biomass) and Mroot is the Cd concentration (mg/kg dry biomass).

$$\text{Remediation Factor (\%)} = (M_{shoot} \times W_{shoot}) / (M_{soil} \times W_{soil}) \times 100$$

Where, Mshoot is concentration of heavy metal in the plant shoots (mg/kg), Wshoot is the dry biomass of the plant shoot (g), Msoil is the total heavy metal content in the soil (mg/kg) and Wsoil is the amount of soil in every pot (gram) [21-30].

Statistical analysis

Statistical analysis was performed using ICARGOA-1.0, Goa, India. Data was presented by mean±SD and analysis of variance (ANOVA) in thrice replicates and level of significance at $P < 0.05$. The graphical work was plotted with using Graph Pad Prism 9.3.1.471 (MSI, Version 2.0, USA).

RESULTS AND DISCUSSION

Physico-chemical characteristics of soil used in the pot experiment

The results of physico-chemical properties of soil samples are shown in Table 1. The studied sand, silt and clay percentage of soil samples were ranged from 56.86±4.40, 23.38± 2.40 and 19.76± 3.20% respectively. The pH of the soil in the experimented area varied from 7.9± 0.60, its pH indicating saline nature of the soil. The high pH of soil contributes to lesser heavy metals solubility while values of low pH enhance the solubility of heavy metals in the soil [28]. The electrical conductivity (EC), organic carbon (OC) and cation exchange capacity (CEC) of experimented soil samples were found ranging from 0.32± 0.04 dS/m, 0.58± 0.07% and 20.78± 1.70 cmol (p^+)/kg respectively. The total nitrogen and phosphorus content in the study area of soil samples ranged from 0.12± 0.03 and 0.10± 0.04% respectively. The results of cadmium (Cd) content in the experimented soil samples varied from 2.58± 0.55 mg/kg. The physico-chemical properties of soil including pH, EC and organic matter (OM) may affect the solubility capacity of heavy metals [6].

Table 1. Mean(±SD) characteristics of physico-chemical properties of soil used in this pot experiment

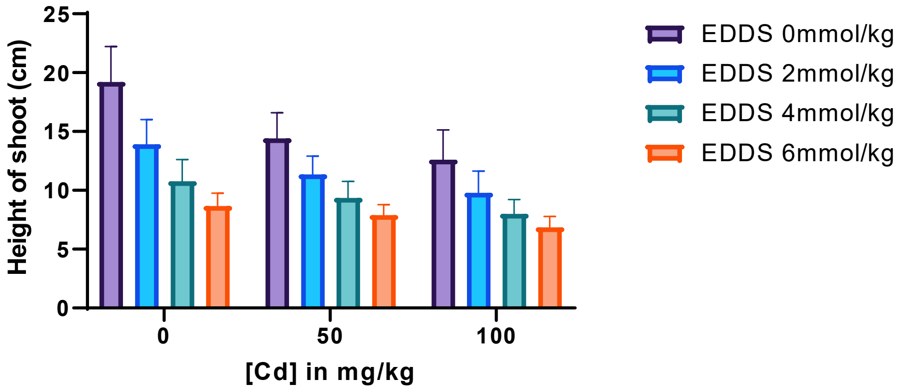
Parameters	Unit	Soil
Sand	%	56.86±4.40
Silt	%	23.38±2.40
Clay	%	19.76±3.20
pH		7.90±0.60
EC at 25°C	dS/m	0.32±0.04
Organic carbon	%	0.58±0.07
CEC	cmol(p^+)/kg	20.78±1.70
Total nitrogen	%	0.12±0.03
Total phosphate	%	0.10±0.04
Total Cd	mg/kg	2.58±0.55

EC, electrical conductivity, CEC, cation exchange capacity, Cd, cadmium

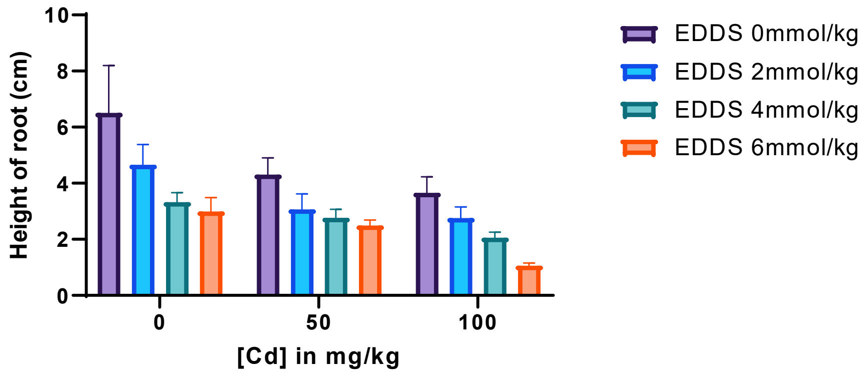
Effect of EDDS on growth and dry biomass yield of Fenugreek

The application of EDDS to soil contaminated with cadmium (Cd) significantly ($P < 0.05$) decreased the growth and dry biomass of shoot and root of fenugreek plants compared to their control (Fig. 1). The application of 2 mmol/kg EDDS significantly ($P < 0.05$) decreased height of shoots and roots by 22.30 and 24.65% respectively. However, under the application of 6 mmol/kg significantly ($P < 0.05$) utmost decreased height of shoots and roots by 45.63 and 71.23% respectively, when compared to the control pot at the 100 mg/kg Cd contaminated soil (Fig. 1a & b). Furthermore, the addition of 2 mmol/kg EDDS significantly (P <0.05) reduced dry biomass yield of shoots and roots by 24.70 and 27.13% respectively. While the under treatment of 6 mmol/kg EDDS significantly (P <0.05) utmost reduced dry biomass yield of shoot and root of fenugreek plants by 49.91 and 50.75% respectively, when compared to their control pot at the level of 100 mg/kg Cd contaminated soil (Fig. 1c & d). In addition, the dry biomass yield of shoot and root of fenugreek plants decreased with increasing amounts of EDDS (Fig. 1c & d). The application of EDDS showed the deleterious effects on fenugreek plant vitality which is evidenced by growth and dry biomass yield of shoot and root of fenugreek plants [33].

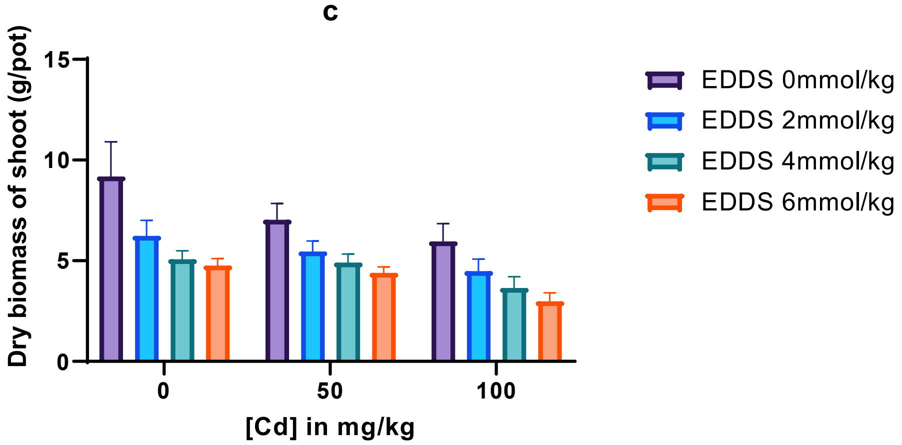
a



b



c



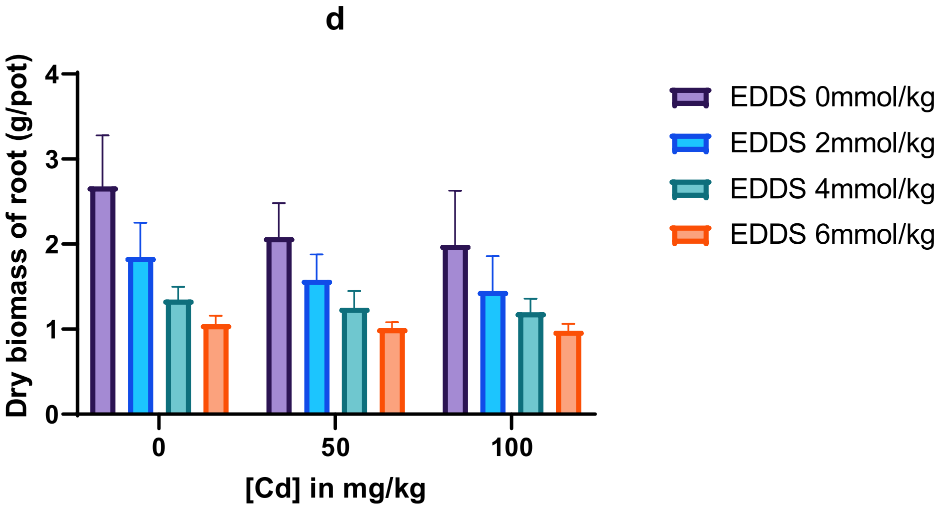
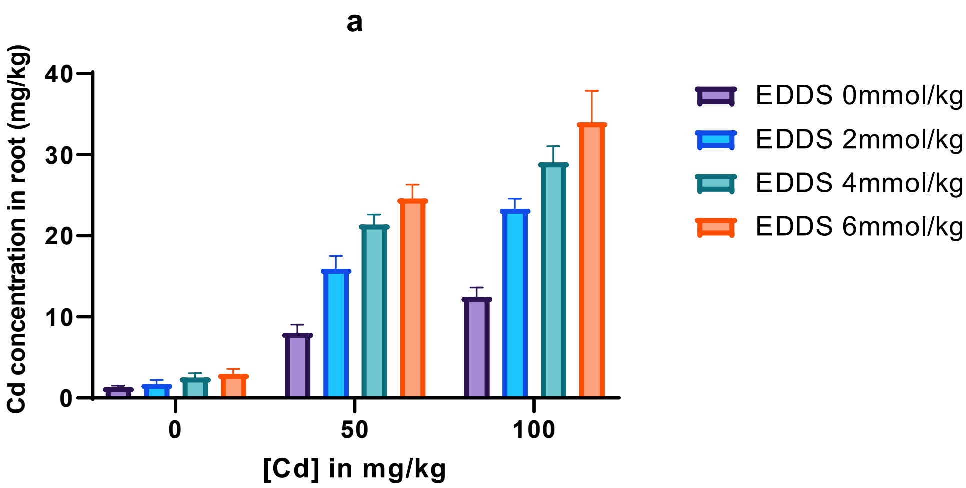
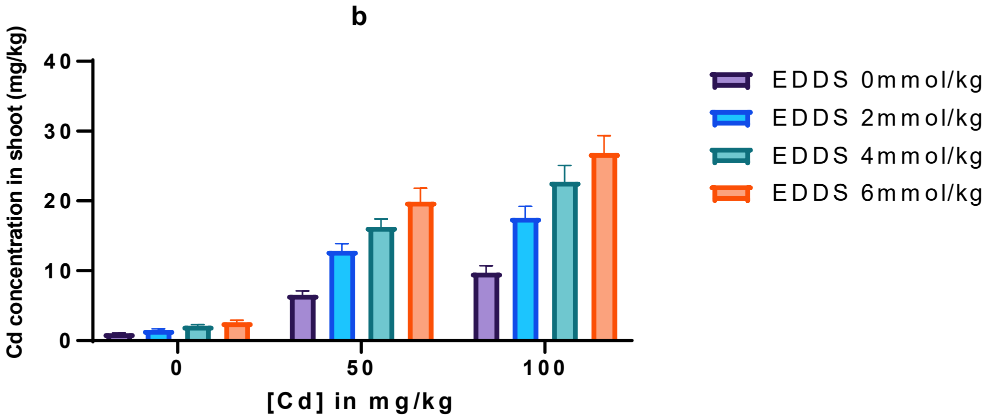


Fig. 1. Effect of EDDS application on the plant height (a, b) and dry biomass yield (c, d) of shoots and roots fenugreek plant grown in Cd contaminated soil. All the values are in three replicates (n=3, mean±SD) with significantly different at P<0.05.

Effect of EDDS on the accumulation of cadmium (Cd) in the root, shoot and leaves of Fenugreek

The accumulation of cadmium (Cd) in the root, shoot and leaves of fenugreek plants grown in Cd contaminated soil (Fig. 2). The results of the pot experiments showed that the application of EDDS significantly (P <0.05) enhanced the Cd concentrations in the root, shoot and leaves of fenugreek plants respect to their control pot for every Cd contaminated soil. The application of 2 mmol/kg EDDS significantly (P <0.05) enhanced the concentration of Cd by 87.15, 81.19 and 69.33% respectively, in the root, shoot and leaves of fenugreek plants, when compared with control group of pot experiments at the level of 100 mg/kg Cd contaminated soil (Fig. 2a, b & c). However, when the treatment of the 6 mmol/kg EDDS significantly (P <0.05) increased the concentration of Cd by 172.71, 176.85 and 137.78% respectively, in the root, shoot and leaves of fenugreek plants, when compared their control pot experiments at 100 mg/kg Cd contaminated soil (Fig. 2a, b & c). The treatment with chelating agent EDDS could increase the plant's capacity to removal of heavy metals from the contaminated soils [7-22]. Chelators can increase the absorption, translocation and accumulation of heavy metals in the plants, possibly due to chelators may be able to enhance desorption of heavy metals from the soil matrix into the soil solution. Change the forms of heavy metals in the soil, enhance the concentrations of accessible heavy metals in the soil, accelerate metal transport into the xylem, and enhance the heavy metals translocation from the roots to shoots by chelating insoluble heavy metals to water soluble in the soil. [5-9].





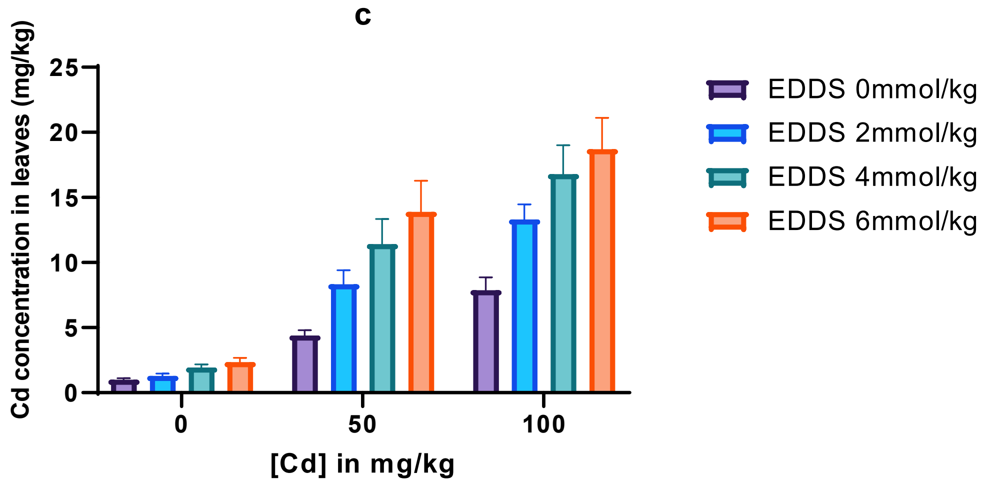


Fig. 2. Effect of EDDS application on the accumulation of cadmium in the root, shoot and leaves (a, b, c) of fenugreek plants grown in Cd contaminated soil. All the values are in three replicates (n=3, mean±SD) with significantly different at $P<0.05$.

Effect of EDDS on BCF, TF and RF of Cd in the different treatments of Fenugreek plants

The use of BCF, TF and RF factors have proven to be suitable tools for the identifying the potential of the growing fenugreek plants for the heavy metal accumulation in this pot experiments. Data presented in Table 2. shows the values of BCF, TF and RF factors of Cd. The application of EDDS enhanced the BCF and TF but reduced the RF for the Cd contaminated soil. The effect of EDDS in the terms of increasing the Cd BCF and TF index parameters were more marked than that for the Cd RF in the compared to their respective control. The addition of 6 mmol/kg EDDS, the BCF observed a maximum of 13.17±0.17 at 100 mg/kg Cd contaminated soil. The BCF is a standard index parameter to quantify the potential of plants to pump the heavy metals from the substrate and to analogize their phytoextraction efficiency [28]. Furthermore, the TF values for the Cd in the fenugreek varied between 1.14±0.07-1.32±0.12 mg/kg under the 50-100 mg/kg Cd contamination level with the treatments of 2-6 mmol/kg EDDS with respective to their control pot. TF determines the efficiency of the plants for the translocation of heavy metals from the roots to the shoots and it is defined as the ratio of heavy metal concentration in the shoots to that in the roots [33]. However, the RF values ranged from 0.07±0.01-0.67±0.05% at the 50-100 mg/kg Cd contamination level with the application of 2-6 mmol/kg EDDS in the soil, when compared to their control pot. The chelator agents induced applications the RF values for Cd were much higher than for the Pb [29]. BCF and TF maximum values were observed by several other authors with such combinatorial PGPE- chelator treatments [3-14].

Table 2. Calculated the values for BCF, TF and RF of Cd in Fenugreek with different treatment in this pot experiments

Treatments EDDS (mmol/kg)	Cd (mg/kg) BCF			TF			RF (%)		
	0	50	100	0	50	100	0	50	100
0	0.51±0.03	3.11±0.11	4.82±0.32	1.28±0.08	1.23±0.08	1.29±0.09	0.08±0.02	0.37±0.07	0.44±0.03
2	0.66± 0.05	6.17±0.14	9.03±0.28	1.17±0.06	1.24±0.04	1.32±0.12	0.07±0.01	0.54±0.05	0.60±0.10
4	0.97± 0.07	8.28±0.20	11.25±0.25	1.21±0.05	1.31±0.10	1.28±0.14	0.09±0.03	0.63±0.07	0.65±0.07
6	1.14±0.10	9.49±0.40	13.17±0.67	1.14±0.07	1.24±0.03	1.27±0.12	0.10±0.04	0.67±0.05	0.61±0.11

BCF, bioconcentration factor, TF, translocation factor, RF, remediation factor, EDDS, Ethylenediamine-N, N'-disuccinic acid

CONCLUSION

The results of the current study indicate that fenugreek plants showed differential behavior in accumulation, BCF, TF and RF of Cd and subsequent growth and dry biomass yield parameters, when plants are grown in Cd contaminated soil. The application of EDDS significantly ($P<0.05$) decreased the growth and dry biomass yield of shoot and root of fenugreek plants. The accumulation of Cd significantly ($P<0.05$) enhanced in the root, shoot and leaves of fenugreek plants increased with EDDS application in Cd contaminated soil, when compared with control. The Cd accumulation was observed maximum in root followed by shoot and leaves of fenugreek plants. EDDS proved that BCF and TF index increased with the addition of EDDS but less effect was observed on RF respect to control. Thus, current findings obtained from the experiment revealed that the addition of EDDS in Cd contaminated soil significantly enhanced the clean-up of Cd through fenugreek plant in a sustainable way.

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