Full Length Research Article

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Research Journal of Agricultural Sciences
Volume 16; Issue 02 (Mar–Apr 2025); pp 128–133

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Phytoremediation Potential of Gladiolus (*Gladiolus grandiflora* L.) Grown in Cd and Pb Contaminated Soils through EDTA

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Received: 28 Jan 2024; Revised accepted: 04 Mar 2025

Abstract

The pot experiment was carried out to investigate the phytoremediation potential of Gladiolus (*Gladiolus grandiflora* L.) in contaminated soils with cadmium and lead by using EDTA. The EDTA was applied @ 0, 3 and 6 mmol kg^{-1} with Cd applied @ 0, 40 and 80 mg kg $^{-1}$ and Pb @ 0, 40 and 80 mg kg^{-1}. The results demonstrated that the applied EDTA (6 mmol kg^{-1}) significantly decreased the dry biomass yield and plant height of corm, stem and flower i.e. Cd (5.38±0.08, 2.48±0.04 and 1.13±0.03 g pot^{-1} and 49.02±1.67 cm) and Pb (7.86±0.19, 3.24±0.11 and 1.26±0.16 g pot^{-1} and 53.18±1.36 cm) respectively, when compared the control pot to (T_6) 40 mg kg^{-1} Cd and Pb contaminated soil. The applied EDTA (6 mmol kg^{-1}) significantly increased the uptake by corm, stem and flower of Gladiolus plants i.e. Cd (25.66±0.63, 15.89±0.29 and 9.96±0.24 mg kg $^{-1}$) and Pb (26.37±1.43, 16.79±0.83 and 10.09±0.27 mg kg $^{-1}$) respectively, with comparison to the control pot (T_9) 80 mg/kg Cd and Pb contaminated soil. When EDTA applied, then the highest TF, BCF and RF values were recorded i.e. Cd (1.357±0.027, 0.0357±0.028 mg kg^{-1} and 0.209±0.017%) and Pb (1.222±0.012, 0.379±0.027 mg kg^{-1} and 0.245±0.018%) in that order. The application of EDTA in Cd and Pb polluted soils considerably enhanced the uptake of these metals (Cadmium and Lead) though, reduced the growth and dry biomass yield of Gladiolus. Thus, it may be accomplished that EDTA played a considerable role in removing of cadmium and lead through Gladiolus plants.

***Key words*:** Bioconcentration, Dry biomass yield, Heavy metals, Plant height, Remediation, Translocation factor

Heavy metals pollution of soil is a global environmental problem because of the persistent, poisonous, and bio-accumulative nature of these elements [21]. The previous few decades have seen an increase in heavy metals, or potentially hazardous elements in soil, as a result of urbanization, industrial growth, and agricultural practices [23]. The main sources of industrial processes that release heavy metals into the soil include mining or refining, waste disposal, sewage discharge, fertilizers and pesticides in the field [22]. While certain metals, like As, Cr, Cd and Pb, can be poisonous even at low concentrations and pose serious risks to plant, animal, and human health throughout the food succession, others, like Fe, Mn, Cu and Zn, are necessary in trace amounts for biological activities [33]. Metal pollutant absorption additionally reduces yield and quality of agricultural goods but also deteriorates soil quality and has a direct impact on chemical and physical characteristics of soil [5].

Higher concentrations of heavy metals in the soil may promote plant uptake of these metals in higher amount [9]. Because lead & cadmium are highly soluble in water, they are more dangerous than other metals viz. Cr, Fe, Cu, Zn and Mn [11]. Furthermore, to their severe toxicity to plants and animals as well as their great solubility in water, cadmium and lead are among the most dangerous contaminants [4]. In which effect plant of cadmium and lead impede the growth of corm, stem and flower promote the leaves turn and yellowing, and ultimately reduce the activity of enzymes involved in photosynthesis, respiration, transpiration, and nutrient intake, plants eventually die [19]. When adding synthetic chelating agents, it can improve the absorption of heavy metals in plants and its ability to dissolve in soil solutions [31]. It has also been suggested that biodegradable agents such nitrilo triacetic acid and ethylene diamine disuccinate be used in place of (EDTA) ethylene diamine tetraacetic acid and other persistent [8]. It has been demonstrated that EDTA is a potent and comparatively bio-stable chelating agent with potential uses in soil remediation [30]. It has been suggested that the chelator assisted phyto-extraction will increase the effectiveness of soil decontamination.

Phytoremediation efficiency, cost-effectiveness and eco-friendly nature is capable for soil remediation that has gained attention from various experts. This technique uses plants to remove or break down pollutants into soil [3]. The Gladiolus plants used phytoremediation to remove toxic metals from soil and water. In the presents an opportunity to increase farmers' profitability in addition to the simples and affordable reclamation of metal-affected soils, such as those contaminated with Cd and Pb through phytoremediation [25], [20]. In the global cut flower trade, gladiolus (*Gladiolus grandiflora* L., family Iridaceae) is a highly desirable ornament plant species

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Citation: Verma J, Mani D, Sahu V, Vishwakarma H, Kumar M, Ojha RK, Patel S. 2025. Phytoremediation potential of gladiolus (*Gladiolus grandiflora* L.) grown in Cd and Pb contaminated soils through EDTA. *Res. Jr. Agril. Sci*. 16(2): 128-133.

that comes in a wide variety of shapes and colors with exceptional preservation qualities. High level of metal tolerance is found in this species. This commercial plant has the capacity to remediate high level of Cd and Pb in moderately contaminated soils [14]. The use of ornamental plants on global level that are not edible in contaminated soils reduces possibility in food chain with heavy metal contamination [17].

These are the fundamental objectives of the recent study i.e., to evaluate the phytoremediation efficiency of Gladiolus (*Gladiolus grandiflora* L.) plants grown in Cd & Pb contaminated soils., to study the effects of EDTA on physiological characteristics (Plant height & dry biomass) of Gladiolus plants., to study the effects of EDTA on the accumulation of heavy metals (Cd & Pb) by Gladiolus plants grown in contaminated soils.

MATERIALS AND METHODS

The experimental layout and location

The pot experiment was conducted in Cd and Pb contaminated soils at Sheila Dhar Institute of Soil Science experimental form situated between latitudes 20° 20´N and longitudes 81° 52´E, with an elevation slope of 101m at University of Allahabad, Prayagraj (Uttar Pradesh). The pot experiment was carried out as an overall completely randomized design (CRD) method and then pots filled with 5 kg soil. After these various doses of EDTA (ethylene diamine tetraacetic acid) were mixed with soil and Gladiolus (*Gladiolus grandiflora* L.) corm sown as test plant in rabi season 2022-23 (Table 1).

Table 1 Treatments combinations of cadmium and lead with EDTA

Treatments	Combinations of Cd	Combinations of Pb
T_1:	Control	Control
T_2:	Cd 0 mg kg^{-1} + EDTA 3 mmol kg^{-1}	Pb 0 mg kg^{-1} + EDTA 3 mmol kg^{-1}
T_3:	Cd 0 mg kg^{-1} + EDTA 6 mmol kg^{-1}	Pb 0 mg kg^{-1} + EDTA 6 mmol kg^{-1}
T_4:	Cd 40 mg kg^{-1} + EDTA 0 mmol kg^{-1}	Pb 40 mg kg^{-1} + EDTA 0 mmol kg^{-1}
T_5:	Cd 40 mg kg^{-1} + EDTA 3 mmol kg^{-1}	Pb 40 mg kg^{-1} + EDTA 3 mmol kg^{-1}
T_6:	Cd 40 mg kg^{-1} + EDTA 6 mmol kg^{-1}	Pb 40 mg kg^{-1} + EDTA 6 mmol kg^{-1}
T_7:	Cd 80 mg kg^{-1} + EDTA 0 mmol kg^{-1}	Pb 80 mg kg^{-1} + EDTA 0 mmol kg^{-1}
T_8:	Cd 80 mg kg^{-1} + EDTA 3 mmol kg^{-1}	Pb 80 mg kg^{-1} + EDTA 3 mmol kg^{-1}
T_9:	Cd 80 mg kg^{-1} + EDTA 6 mmol kg^{-1}	Pb 80 mg kg^{-1} + EDTA 6 mmol kg^{-1}

Note- Cd: cadmium, Pb: lead, EDTA: ethylene diamine tetraacetic acid

Soil sampling and analysis

The physicochemical properties and heavy metal concentrations in soil sample were carried out from SDI experimental farm at depth 0-15cm. The gathered soil samples were first allowed to air dry at normal temperatures before being ground into small particles and then sieved with a 2 mm sieve. The content of total Cd & Pb was determined by the di-acid digestion method using a mixture of concentrated HNO_3 and $HClO_4$ (1:4 by volume). The heavy metals (Cd and Pb) were analyzed by Atomic Absorption Spectrophotometer at NBRI, Lucknow, U. P. [13].

Plant sampling and analysis

Plant height was measured 75 days after germination and measurements of the corm, stem, and flower were taken when the plants were harvested. After thoroughly cleaning the plant samples of the corm, stem and flower with tape water and $CdCl_2$ (2%) and rinsing them with double distillation water (DDW) to get rid of any remaining contaminants, the dry biomass of the plant corm, stem, and flower was obtained by drying them in thermostatic hot air oven for 48 hours at 70°C. The corm, stem & flower dry plant samples were crushed into a fine powder. Then one gram of each sample corm, stem and flower were digested separately in a tri-acid mixture H_2SO_4, $HClO_4$ and HNO_3 (1:2:5 through volume, respectively) [16]. The analyses of Cd & Pb content extracted plant corm, stem and flower samples were determined through the Atomic Absorption Spectrophotometer (AAS) at National Botanical Research Institute, Lucknow (U.P.).

Translocation factor

The assessment amount of heavy metals that is transferred from the underground (corm) component to the shoot (stem + flower) depends mainly on the translocation factor (TF). This also helps to characterize the phytostabilization and phytoremediation efficiency of the plants under study. TF has been determined using the equation below [15].

$$TF = M_{shoot\ (stem\ +\ flower)}\ M_{corm}$$

Where, $M_{shoot\ (stem\ +\ flower)}$ in content the metals (mg kg^{-1}), M_{Corm} in content the metals (mg kg^{-1}).

Bioconcentration factor

Bioconcentration factor is used to calculate a plants ability to collect heavy metals in its corms from contaminated soils [28], calculated based on the following equations,

$$BCF = M_{corm}\ M_{soil}$$

Where, M_{Corm} metal content in corm (mg kg^{-1}), M_{soil} total metal content in soils.

Remediation factor

The ratio of heavy metals uptake in stem & flower from the polluted soil is known as remediation factor [18], [27], calculated based on the following equations,

$$RF\% = M_{shoot}\ W_{shoot}\ M_{soil}\ W_{soil} \times 100$$

Where, the metal content in $M_{shoot\ (stem\ +\ flower)}$ is expressed as mg kg^{-1}, $W_{shoot\ (stem\ +\ flower)}$ is the yield of dry biomass plant shoot (stem + flower) in gram, M_{soil} was calculated by total metal content in contaminated soils and W soil every pot soil weight in grams.

Statistical analysis

The statistical study was performed on the ICARGOA (WASP 2.0) in Goa, India. An analysis of variance (ANOVA) with three repetitions and significant level of $P < 0.05$ was used to present the data. Graph Pad Prism 8.0.1.244 (MSI, Version 2.0, USA) w1as used to prepare the graphical work.

RESULTS AND DISCUSSION

Physical and chemical properties of soil

The calculated sand, silt and clay percentage is varied from 55.36±2.12, 24.45±1.68 and 20.19±1.26% respectively. The pH value of the soil is 7.8±0.08, which indicates that the soil is moderately saline. In soil samples' electrical conductivity, cation exchange capacity, and organic carbon ranged from 0.34±0.02 dSm^{-1}, 21.86±1.44 cmol (p+) kg^{-1} and 0.56±0.05%, respectively. The soil samples' total nitrogen and phosphorus contents varied from 0.14±0.02 to 0.11±0.01%, respectively. In soil samples' amounts of Pb and Cd ranged from 0.42±0.03 & 0.58±0.04 mg kg^{-1}, respectively (Table 2). The heavy metals solubility capacity may be influenced by physic-chemical properties of soil, like pH, EC, and organic matter (OM). These are most important harmful of contaminated soils were related to the decrease in the level of physiological properties from contaminated soils as a nutrients collection and improved nutrient cycling. In this way similar results have also been found by [1], [7].

Table 2 Physical and chemical properties of soil

Parameters	Unit	Value
Sand	%	55.36 ± 2.12
Silt	%	24.45 ± 1.68
Clay	%	20.19 ± 1.26
pH	–	7.8 ± 0.08
EC at 25 °C	dSm^{-1}	0.34 ± 0.02
CEC	Cmol(p^+) kg^{-1}	21.86 ± 1.44
Organic carbon	%	0.56 ±0.05
Total N_2	%	0.14 ± 0.02
Total P_2O_5	%	0.11 ± 0.01
Total cadmium	Mg kg^{-1}	0.42 ± 0.03
Total lead	Mg kg^{-1}	0.49 ± 0.04

Note- EC (electrical conductivity), CEC (cation exchange capacity), OC (organic carbon), Pb (lead) & Cd (cadmium), each value of the three replicates (n=3, mean±SD)

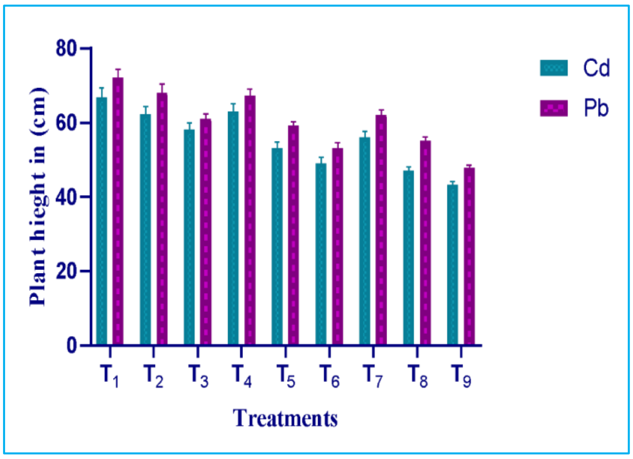

Fig 1 The effect of EDTA application on Gladiolus plant height in Cd and Pb-contaminated soil. For every value in the three replicates (n = 3, mean±SD), there is significantly difference at P<0.05

The effect of EDTA on the height of Gladiolus plants

The application of Cd and Pb with EDTA significantly decreased the plant height of Gladiolus plants as compared to the control pot. The results showed (Fig 1) that when a lower dose of EDTA (3 mmol kg^{-1}) with Cd and Pb significantly less decreased the plant height by (53.09±1.72 cm) and (59.14±1.16 cm) respectively, when compared the control pot to (T_5) 40 mg kg^{-1} of Cd and Pb contaminated soils. However, under the applied maximum dose of EDTA (6 mmol kg^{-1}) with Cd and Pb significantly utmost decreased the plant height by (43.22±0.86 cm) and (48.03±0.93 cm) respectively, when compared the control pot to (T_9) 80 mg kg^{-1} of Cd & Pb contaminated soils. The highest plant height was in control pot, while lowest plant height in (T_9) treatment. The effect in Gladiolus plants of Cd is greater than that of Pb because of its highly toxic nature. The applied of EDTA showed the harmful effects on Gladiolus plants vitality which is evidenced by plant height of corm, stem and flower of Gladiolus plants. Furthermore, showed no discernible change from the control, indicating that gladiolus were tolerant of Cd and Pb stress. Conversely, higher Cd and Pb concentrations were found to considerably lower plant height indices. These types of results have also been found by [2], [32].

The effect of dry biomass yield on Gladiolus plants

The application of EDTA with Cd and Pb significantly decreased dry biomass yield in the Gladiolus plants (corm, stem and flower) as compared to the control treatment pot. The results showed in (Fig 2-3) the applied lower dose of EDTA (3 mmol kg^{-1}) significantly less reduced dry biomass yield of Gladiolus plants (corm, stem and flower) i.e. Cd (7.13±0.09, 2.86±0.06 and 1.29±0.07 g pot^{-1}) and Pb (8.79±0.14, 3.76±0.19 and 1.67±0.09 g pot^{-1}) respectively, when compared the control pot to (T_5) 40 mg kg^{-1} of Cd and Pb contaminated soils. While the under applied higher dose of EDTA (6 mmol kg^{-1}) significantly reduced dry biomass yield of Gladiolus plants (corm, stem and flower) i.e. Cd (3.87±0.04, 2.19±0.09 and 1.03±0.03 g pot^{-1}) and Pb (4.73±0.09, 2.49±0.08 and 1.12±0.06 g pot^{-1}) respectively, when compared the control pot to (T_9) 80 mg kg^{-1} of Cd and Pb contaminated soils. The maximum dry biomass yield in the control pot, while the minimum dry biomass yield in the (T_9) treatment. Because EDTA concentration increased, the Gladiolus plants dry biomass yield of the corm, stem and flower decreased. The applied of EDTA showed the deleterious effects on Gladiolus plants vitality which is evidenced by dry biomass yield of corm, stem &flower of Gladiolus plants. It also demonstrates that Gladiolus is more affected than Gladiolus by the total and dissolved soil Cd & Pb. However, the regression model indicates that Gladiolus shows a less pronounced decrease in dry matter. Therefore, it may be said that this plant is more resilient to pollution from Cd and Pb. This type of experiment was done by [24] and [6] and found similar result.

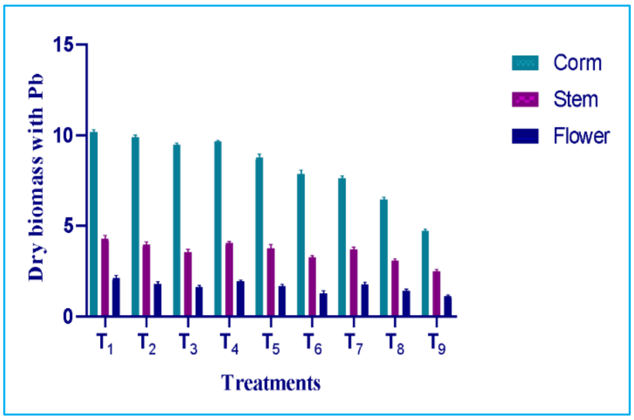

Fig 2 The effect of EDTA on the dry biomass yield by Gladiolus plants grown in soil contaminated with cadmium. For every value in the three replicates (n = 3, mean±SD), there is significantly difference at P<0.05

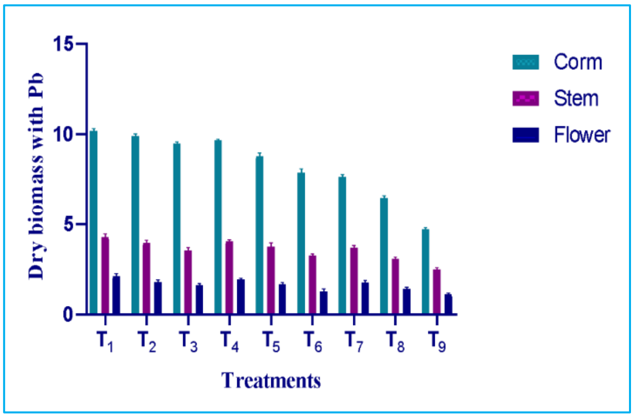

Fig 3 The effect of EDTA on the dry biomass yield by Gladiolus plants grown in soil contaminated with cadmium. For every value in the three replicates (n = 3, mean±SD), there is significantly difference at P<0.05

The effect of EDTA on the uptake of Cd and Pb in the corm, stem and flower of Gladiolus plants

The uptake of Cd & Pb through Gladiolus plants (corm, stem, and flower) grown in contaminated soil. The pot investigations have presented (Fig 4-5) in comparison with the control pot containing the polluted soils; the use of ethylene diamine disuccinate be used in place of (EDTA) significantly increased the Cd and Pb contents in Gladiolus plants (corm, stem, and flower). The applied lower dose of EDTA (3 mmol kg^{-1}) significantly enhanced the lower concentration of corm, stem and flower i.e. Cd (9.36±0.31, 6.14±0.12 and 3.78±0.09 mg kg^{-1}) and Pb (10.56±1.03, 6.87±0.33 and 4.09±0.16 mg kg^{-1}) respectively, when compared with control pot to (T_5) 40 mg kg^{-1} of Cd and Pb contaminated soils. While the higher dose of EDTA (6 mmol kg^{-1}) is applied significantly enhanced the higher concentration of corm, stem and flower i.e. Cd (25.66±0.63, 15.89±0.29 and 9.96±0.24 mg kg^{-1}) and Pb (26.37±1.43, 16.79±0.83 and 10.09±0.27 mg kg^{-1}) respectively, when compared with control pot to (T_9) 80 mg/kg of Cd and Pb contaminated soils. Whereas the maximum concentration found in T_6 of Gladiolus plants (corm, stem and flower) i.e. Cd (14.47±0.43, 8.79±0.06 and 5.96±0.11 mg kg^{-1}) and Pb (15.39±1.19, 9.09±0.52 and 6.67±0.24 mg kg^{-1}) respectively, when compared to all treatments. Therefore, uptake of both Cd and Pb in descending order, corm > stem > flower of Gladiolus plants. The plants improved their ability to remove the metals from contaminated soil may be treating with the chelating agent ethylene diamine disuccinate be used in place of (EDTA). Heavy metals can be changed in form, their concentrations in the soil can be increased, metal transport into the xylem can be accelerated, and the translocation of heavy metals from the corm into the stem and flower can be enhanced by chelating insoluble heavy metals into dissolved in water instances. Similar results have also been found by [29], [26].

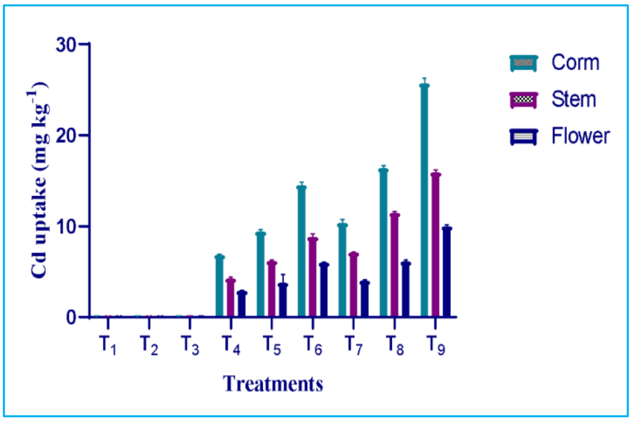

Fig 4 Cadmium concentration at various parts of Gladiolus plants (Corm, stem and flower). For every value in the three replicates (n = 3, mean±SD), there is significantly difference at P<0.05

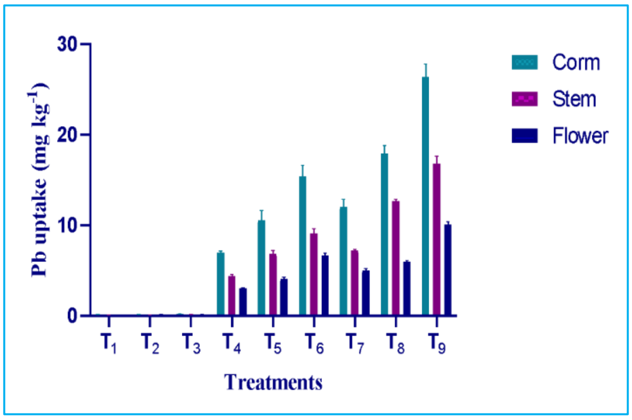

Fig 5 Lead concentration at various parts of Gladiolus plants (Corm, stem and flower). For every value in the three replicates (n = 3, mean±SD), there is significantly difference at P<0.05

The effect of EDTA with Cd and Pb on TF, BCF and RF in Gladiolus plants

It is conventional to use the BCF, TF, and RF factors as suitable methods to determine the growing Gladiolus plants' capacity for heavy metal uptake in these pot tests. The values of the TF, BCF, and RF factors of Cd and Pb are shown in the data in (Fig 6-7). A phytoextraction of translocation factor (TF) > 1 will efficiently transport heavy metals from the corm to the stem and flower. The TF and BCF of maximum concentration i.e. Cd (1.357±0.027 and 0.357±0.028 mg kg^{-1}) and Pb (1.222±0.012 and 0.379±0.027 mg kg^{-1}) respectively, whereas the TF and BCF of minimum concentration i.e. Cd (1.007±0.019 and 0.128±0.019 mg kg^{-1}) and Pb (1.019±0.009 and 1.48±0.011 mg kg^{-1}) in that order. It is significantly uptake of Cd & Pb in value was greater than 1of TF concentration and less than 1 of BCF. However, the RF maximum value of Cd (0.209±0.017%) and Pb (0.245±0.018%) and minimum value of Cd (0.063±0.009%) and Pb (0.082±0.013%), Whereas RF values is less than 1. The capacity of various plant species to absorb and transport heavy metals, such as Cd and Pb, in contaminated soils may also result from their genetic variety. The chelating agent significantly increased the RF values for Cd compared to Pb. This increases their solubility and bioavailability in the soils allowing the plant to absorb these metals more efficiently influencing TF, BCF and RF. This type of experiment also conducted by [10], [12] and found similar results.

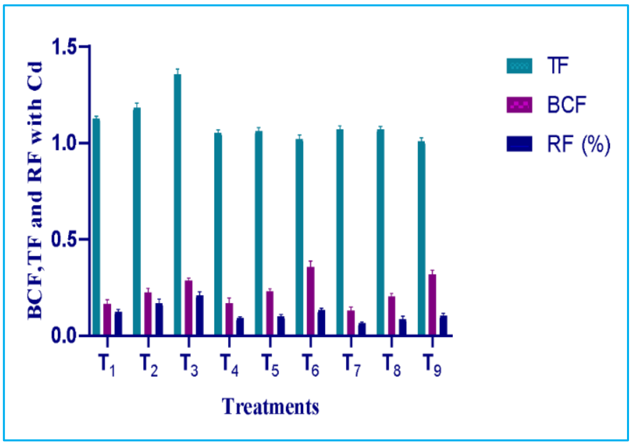

Fig 6 TF, BCF and RF (%) of cadmium in Gladiolus plants

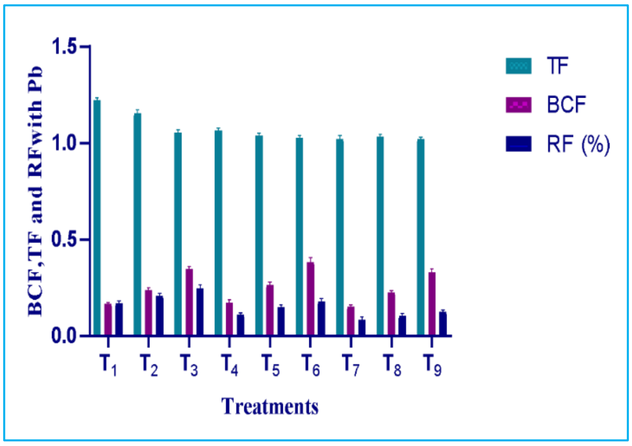

Fig 7 TF, BCF and RF (%) of lead in Gladiolus plants

CONCLUSION

The present study represents the phytoremediation potential of Gladiolus plants (*Gladiolus grandiflora* L.) raised in different soils which are contaminated with toxic heavy metals (Cd & Pb). The strong chelating agent EDTA was used to solubilize the heavy metals. The Gladiolus has good accumulation capacity, and with stand well in heavy metals contaminated soils. Gladiolus is a flower plant and does not a component of human and animal food chain, the values of BCF, TF & RF clearly showed that Gladiolus has been efficient in reducing the amount of heavy metals in contaminated soils. Considering the current level of pollution, the usefulness of this research increases even more. So, the current study recommends that phytoremediation is the best and most eco-friendly approach to mitigate the metal pollutant found in contaminated soils.

Acknowledgments

The authors are thankful to Dr. Alok Lehri, (Principal Scientist), National Botanical Research Institute, Lucknow, India for facilitating the laboratory work especially for heavy metals analysis by AAS (AAnalyst600, Perkin Elmer Inc., MA, USA). The contribution of University Grant Commission, India is also acknowledged for providing the Ph.D. Scholarship with grant (220510147687) during the research period.

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