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The Effects of Biodegradable Chelating Agent GLDA on Phytoremediation Potential of *Solanum nigrum* L. Grown in Cd Contaminated Soils

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The current research work emphasises the effect of tetrasodium glutamate diacetate (GLDA) on growth and phytoextraction capacity of *Solanum nigrum* L. grown in soils contaminated with cadmium (Cd). The application of GLDA at the rate of 3 mmol/kg reduced the root and shoot length

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by 51.02 and 43.20 % respectively, while under 6 mmol/kg application of GLDA reduced the root and shoot length by 56.46 and 50% respectively, as compare to the control pot at 50 mg/kg Cd polluted soils. The applied quantity of GLDA at the rate of 3 mmol/kg reduced the root and shoot dry biomass by 80.55 and 70.45 % respectively, while under 6 mmol/kg application of GLDA reduced the root and shoot dry biomass by 83.33 and 72.15% respectively, as compare to the control pot at 50 mg/kg Cd polluted soils. The applied quantity of 3 mmol/kg GLDA absorbed the 22.72, 26.92% quantity of applied Cd i.e., 25 mg/kg of soil significantly, in the roots and shoots of *Solanum nigrum* L. respectively, while the applied quantity of 6 mmol/kg GLDA significantly absorbed the 33.35, 35.04 % quantity of applied Cd i.e., 50 mg/kg of soil, in the root and shoot of *Solanum nigrum* L. respectively. However, the maximum accumulation of Cd was observed in treatment having combination of 6 mmol GLDA and 25 mg/kg Cd i.e., 34.26 and 36.81 % in root and shoot of *Solanum nigrum* L. respectively. The BAF, TF and RF values of Cd contaminated soils are varied from 0.187±0.001-0.375±0.003, 1.050±0.003-1.244±0.005 and 0.222±0.002-1.253±0.005 respectively. These results showed that GLDA improve the phytoaccumulation capacity of *Solanum nigrum L.* and suitable for remediation of Cd contaminated soil.

Keywords: Cadmium; contaminated soils; GLDA; phytoremediation; Solanum nigrum L.

1. INTRODUCTION

Soil pollution through the heavy metals has become a serious issue related environment which is affecting the entire world (Ma et al., 2021). It is directly related to agro-based ecosystem and possess various detrimental risk to the vegetation and other biological agents. The heavy metal pollution is a serious problem has deteriorated mainly by rapid industrialization and disturbance of natural bio-geo-chemical cycles (Fulke et al., 2024). Naturally Heavy metals exist in certain rocks, but accumulation of theses metals in soils has increased at toxic level through anthropogenic activities. Excessive and indiscriminate use of waste water, sewage sludge, biosolids, manures and agrochemicals are all the chief sources of heavy metals in soils (Figueiredo et al., 2019; Singh et al., 2022; Ullah et al., 2024; Yadav et al., 2023). These sources containing various toxic heavy metals like Cd, Cr, Pb, As, Hg and many more which disturb the whole ecosystem by affecting biotic and abiotic components of the environment. In the soil, there are many adverse and toxic effects of heavy metals on soil microbial diversity, plants, invertebrates and many more which decrease the activity and population of these organism. (Zhao et al., 2020).

Phytoremediation, that mainly utilized hyperaccumulators to detoxify heavy metals from polluted soils, is believed to be foremost promising methods of remediation (Madhav et al., 2024; Sharma et al., 2023; Tao et al., 2020; Wu et al., 2018; Yang et al., 2018). *Solanum nigrum* L. is a well-known hyper accumulator plant belongs to family Solanaceae. There are several hyper accumulator plant species which are used for phytoremediation purpose in which *Solanum nigrum* L. is widely used for remediation of Cd contaminated soils (Han et al., 2021; Li et al., 2021). The plants of *Solanum nigrum* L. has been utilized to phytoextract cadmium from contaminated soils which have capacity of fast growing, high yielding and strong bio-accumulation (Dou et al., 2022; Yu et al., 2015). Chelating agents enhance phytoremediation by forming soluble complexes with soil heavy metals, increasing their availability for plant uptake and translocation to above-ground parts, thereby improving metal removal efficiency and plant biomass.

However, synthetic chelators can also lead to plant phytotoxicity, reduced biomass and issues with leaching into groundwater. Organic and biodegradable chelating agents, as well as plant-based strategies, are being explored as more sustainable alternatives to mitigate these drawbacks. GLDA is a biodegradable chelating agent obtained from a natural source which are safer to environment and has been used for removing Cd, Pb, Cr and Zn from soils in an effective manner (Begum et al., 2012; Thinh et al., 2021; Wei et al., 2015).

The current research work containing some specific objectives, which consists of: (i) studying influence of GLDA on Cadmium uptake in the plants of *Solanum nigrum* L. (ii) evaluating the impact of GLDA with Cd on growths and dry biomass of plants (iii) ascertaining the phytoremediation efficiency of *Solanum nigrum* L. raised in Cd contaminated soil.

2. MATERIALS AND METHODS

2.1 Experimental Site and Design

A pot experiment was conducted at experimental farm lies between 25º28' N latitudes and 81º50' E longitudes, in rabi season at Sheila Dhar Institute of Soil Science, Department of Chemistry, University of Allahabad, Prayagraj, Uttar Pradesh during the year 2023-24. The pots were filled with 5 kg of soil contaminated with Cd (0, 25, 50 mg/kg). The experimental design consisted of 9 treatments including a control pot. Completely randomized design (CRD) was used as experimental design. Seeds of *Solanum nigrum* L. (test crop) having good germination ability were sown in all filled pots and thinned dense growing seedlings 15 days after germination to maintain proper spacing. The experiment involved the following treatment combinations i.e., (i) without any treatment (control) (ii) GLDA (three doses- 0, 3, 6 mmol/Kg) and (iii) $CdCO_3$ (three doses- 0, 25, 50 mg/kg).

2.2 Soil Sampling and Analysis

Representative soil samples were collected from the SDI experimental farm at 0-20 cm depth and analysed for physico-chemical properties and heavy metal content. Collected soils were air-dried in the shade and sieved to pass through a 2 mm fine sieve. Soil extract of prepared samples was obtained by shaking one gram of soil, with 5 ml of each nitric acid (HNO_3) and perchloric acid ($HClO_4$). The mixture was then heated to dryness and added the hot distilled-water finally (Mani et al., 2007). The digested soil samples were analysed for Cd concentration using atomic absorption spectrophotometer (AAS) at BHU, Varanasi.

2.3 Plant Sampling and Analysis

The plants were harvested from all the pots 60 days after germination also measured the root and shoot length. Harvested plants were washed with tap water followed by 2% $CaCl_2$ solution and finally dipped in distilled water 2-4 times to remove external impurities. The whole plant parts were separated into roots and shoots, dried in the shade and subsequently placed in an oven at 60ºC for final drying. The oven dried roots and shoots materials were ground into fine powder. Plant extract of prepared samples was also made; for this, took one gram of grounded plant material, HNO_3, H_2SO_4 and $HClO_4$ in a 5:1:2 ratio. Then, properly mixed and heated to obtain a semi-solid appearance, and brought to desired volume with hot distilled-water (Mani et al., 2013). The digested plant samples (roots and shoots) were analysed for Cd concentrations through AAS at BHU, Varanasi.

2.4 Bioaccumulation (BAF), Translocation (TF) and Remediation Factors (RF)

BAF, TF and RF values were calculated by using following formulas-

$$\mathrm{BAF} = \frac{\mathrm{HM_{shoot}}}{\mathrm{HM_{soil}}}$$

where,

$\mathrm{HM_{shoot}}$ is the concentrations of heavy metals in shoot (mg/kg dry weight), $\mathrm{HM_{soil}}$ is the concentrations of heavy metals in soils in mg/kg (Roca-Perez et al., 2023)

$$\mathrm{TF} = \frac{\mathrm{HM_{shoot}}}{\mathrm{HM_{root}}}$$

Where,

$\mathrm{HM_{shoot}}$ is the heavy metals concentrations in shoot (mg/kg dry weight), M_{root} is the heavy metals concentrations in root in mg/kg (Korzeniowska & Stanislawska-Glubiak, 2019)

$$\mathrm{RF}\ (\%) = \frac{\mathrm{HM_{plant}} \times \mathrm{DW_{plant}}}{\mathrm{HM_{soil}} \times \mathrm{W_{soil}}} \times 100$$

Where,

HM_{plant} is the concentrations of heavy metals in plants (mg/kg), DW_{plant} refers dry weight of the plants (g), HM_{soil} refers total heavy metals concentrations in soils (mg/kg) and W_{soil} is the weight (g) of soils in the pots (Saraswat & Rai, 2011)

2.5 Statistical Analysis

All research data in current study was analysed in triplicate and presented as mean ± SD (standard deviation) and ANOVA (analysis of variance) at level of significance at P<0.05. GraphPad Prism was used as graphical tool.

3.RESULTS AND DISCUSSION

3.1 Physico-Chemical Properties of Soils

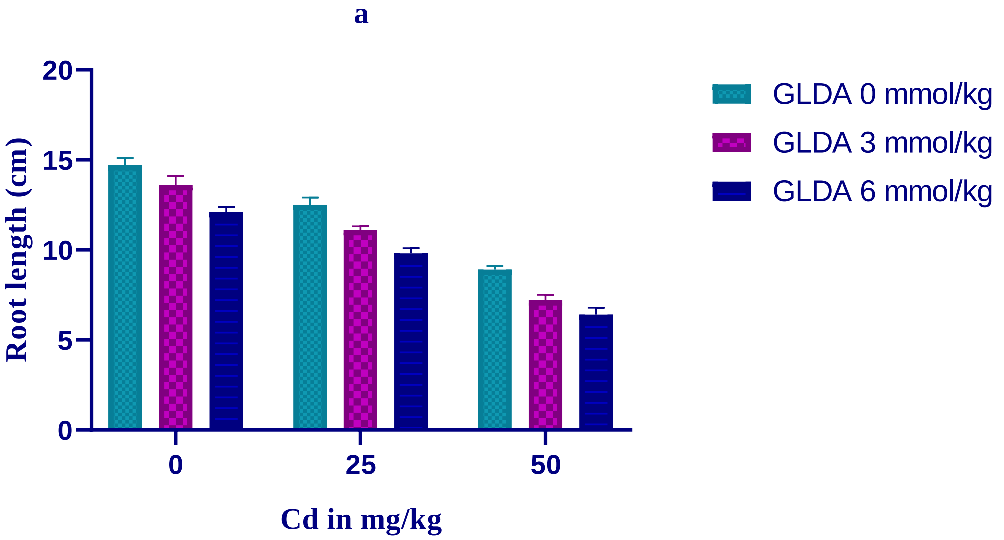
The soils physico-chemical properties are shown in Table 1. The values of textural soil parameters i.e., sand, silt and clay were 56.7±4.7,20.4±3.5 and 22.9±5.1% respectively. The pH value i.e., 8.1±0.2 represented saline soil and value of EC was 0.33±0.06 dS/m. The values of organic carbon (OC) content and cation exchange capacity (CEC) of soils was 0.61±0.10 % and 20.95±01.58 cmol (P^+)/kg respectively. Total nitrogen, phosphate values were 0.09±0.02, 0.10±0.03 respectively. The concentration of total Cadmium was 0.05±0.02 mg/kg.

3.2 Effects of GLDA on the Growth and Dry-Biomass yields of *Solanum nigrum* L.

The GLDA application has reduced the dry biomass yields by decreasing the growth of *Solanum nigrum* L. in Cd polluted soils at varying rate compared to control pot. The effect of GLDA can be clearly seen in fig.1. The application of GLDA at the rate of 3 mmol/kg reduced the root and shoot length by 51.02 and 43.20 % respectively, while under 6 mmol/kg application of GLDA reduced the root and shoot length by 56.46 and 50.00 % respectively, as compared to the control treatment at 50 mg/kg Cadmium polluted soils (Fig. 1a and b). Moreover, the application of GLDA at the rate of 3 mmol/kg reduced the root and shoot dry biomass by 80.55 and 70.45 % respectively, while under 6 mmol/kg application of GLDA reduced the root and shoot dry biomass by 83.33 and 72.15% respectively, as compare to the control treatment at 50 mg/kg cadmium polluted soils (Fig. 1c & d).

Table 1. Physico-chemical properties of soils

Soil parameters	Unit	Values
Sand	%	56.7±4.7
Silt	%	20.4±3.5
Clay	%	22.9±5.1
pH	-	8.1±0.2
Electrical conductivity (EC) at 25ºC	dS/m	0.33±0.06
Organic Carbon (OC)	%	0.61±0.10
Cation exchange capacity (CEC)	cmol (P^+)/kg	20.95±1.58
Total nitrogen	%	0.09±0.02
Total phosphate	%	0.10±0.03
Total Cadmium	Mg/kg	0.05±0.02



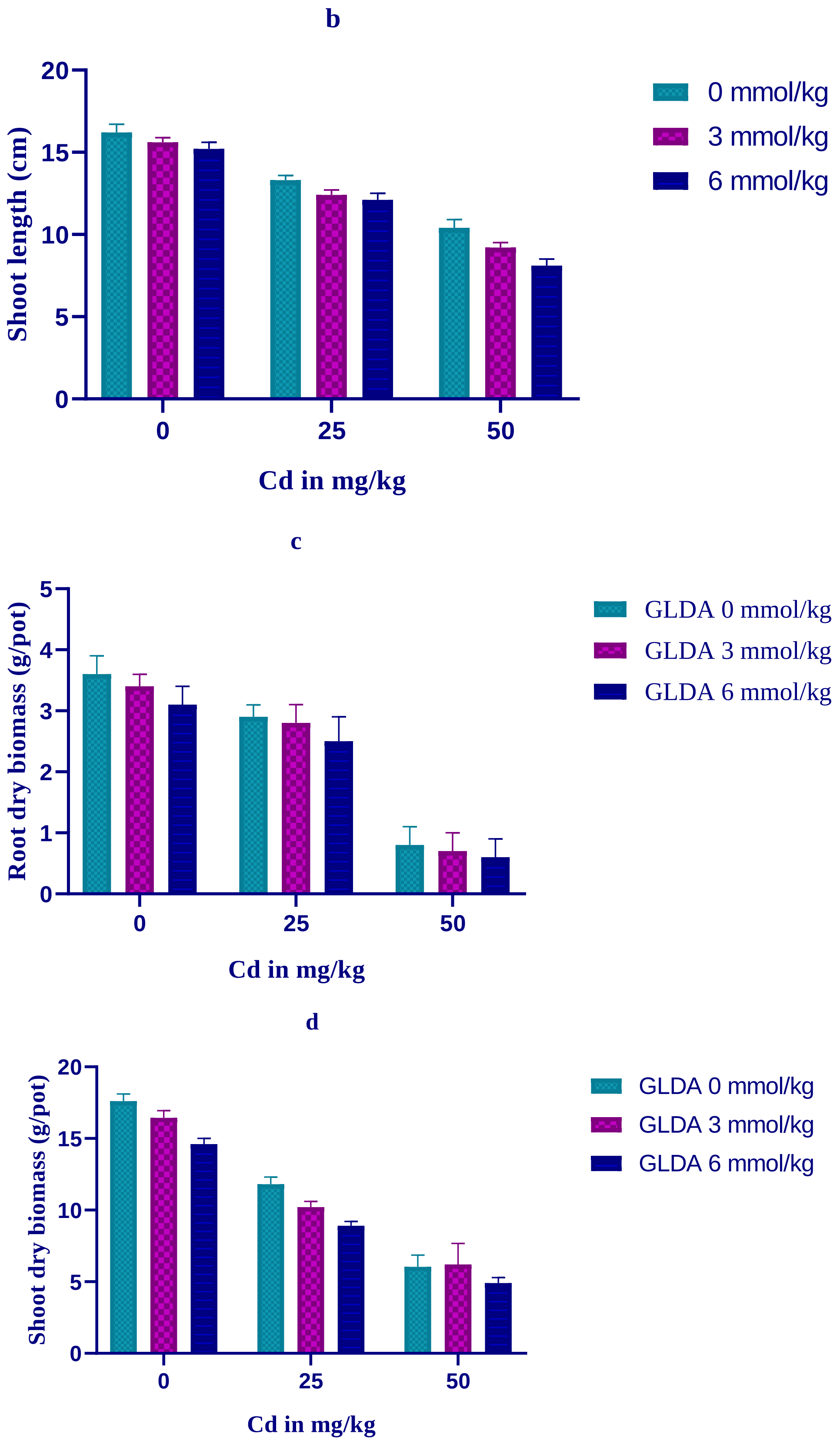
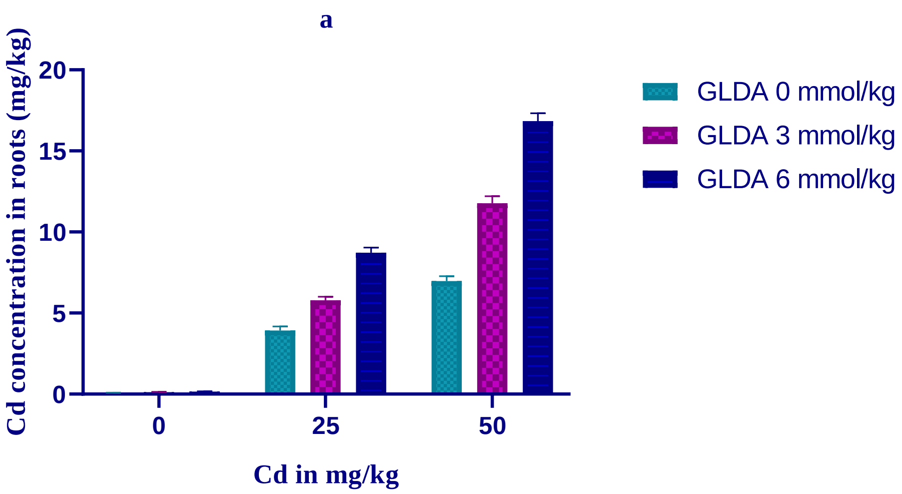


Fig. 1. Effects of GLDA on plant hight (a, b) and dry biomass yield (c, d) of *Solanum nigrum L.*



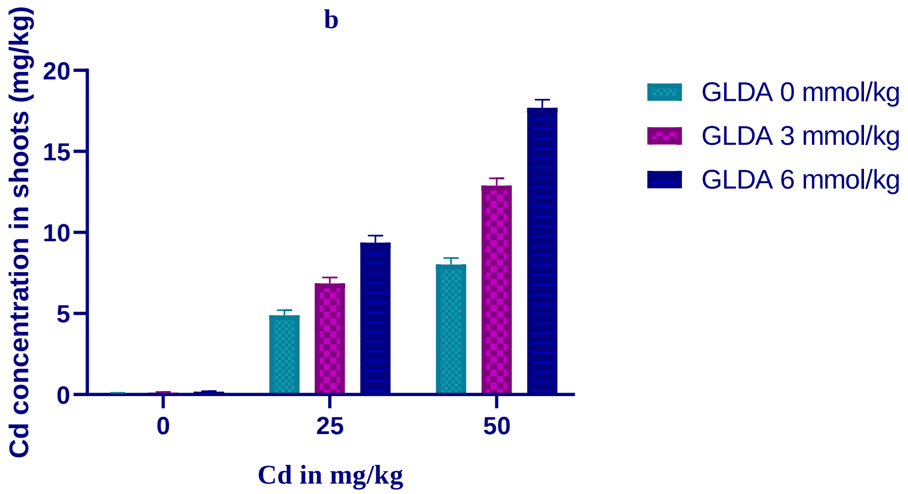


Fig. 2. Effects of GLDA on Cd accumulation in the roots (a) and shoots (b), of *Solanum nigrum* L.

Table 2. The effects of GLDA on BAF, TF and RF of *Solanum nigrum* L. under Cd treatments

Treatments / GLDA (mmol/kg)	Cd (mg/kg)								
	BAF (Bioaccumulation factor)			TF (Translocation factor)			RF (Remediation factor)		
	0	25	50	0	25	50	0	25	50
0	0.187 ±0.001	0.191 ±0.002	0.159 ±0.002	1.125 ±0.004	1.244 ±0.005	1.152 ±0.003	0.750 ±0.005	0.508 ±0.003	0.222 ±0.002
3	0.270 ±0.002	0.269 ±0.003	0.255 ±0.002	1.083 ±0.002	1.184 ±0.002	1.094 ±0.002	1.020 ±0.006	0.645 ±0.004	0.288 ±0.003
6	0.375 ±0.003	0.368 ±0.004	0.350 ±0.003	1.125 ±0.003	1.074 ±0.003	1.050 ±0.003	1.253 ±0.005	0.810 ±0.006	0.376 ±0.002

GLDA- Tetrasodium glutamate diacetate, Cd- Cadmium

3.3 Effects of GLDA on Cd Accumulation in the Roots and Shoots of *Solanum nigrum* L.

The accumulation of Cd in root and shoot of *Solanum nigrum* L. can be seen in Fig. 2. The applied quantity of GLDA enhanced the phytoaccumulation of cadmium significantly in *Solanum nigrum* L. at varying rate from contaminated soils. The application of 3 mmol/kg GLDA absorbed in significant manner 22.72, 26.92% quantity of applied Cd i.e., 25 mg/kg of soil, in the roots and shoots of *Solanum nigrum* L. respectively, while the applied quantity of 6 mmol/kg GLDA absorbed in significant manner 33.35, 35.04 % quantity of applied Cd i.e., 50 mg/kg of soil, in the root and shoot of *Solanum nigrum* L. respectively. However, the maximum accumulation of Cd was observed in treatment having combination of 6 mmol GLDA and 25 mg/kg Cd i.e., 34.26 and 36.81 % in root and shoot of *Solanum nigrum* L. respectively (Fig. 2 a & b). At first, the phenomenon of active as well as passive transportation of cadmium in plant roots are both considered as active mechanisms. Subsequently cadmium enters into the cells of plant roots via translocation of metal into harvestable parts through the transportation by hyperaccumulators (Bian et al., 2020).

3.4 Effects of GLDA on Bioaccumulation, Translocation and Remediation Factor of *Solanum nigrum* L.

The values of BAF, TF and RF are indicated the potential capabilities of *Solanum nigrum* L. with respect to phytoremediation of Cd contaminated soil. Table 2., Represents the calculated values of BAF, TF and RF for various treatment combinations. The BAF, TF and RF values of Cadmium contaminated soil are varied from 0.187±0.001-0.375±0.003, 1.050±0.003-1.244±0.005 and 0.222±0.002-1.253±0.005 respectively. The criteria for hyper-accumulative plants which having TF at least 1 are considered as Cadmium hyper-accumulator plants (Li et al. 2016). The values of TF for all treatment were >1 which indicates that *Solanum nigrum* L. is a hyper accumulator plant and has better phytoremediation potential.

4. CONCLUSION

The current work mainly focused on phytoremediation potential of *Solanum nigrum* L. enhanced by biodegradable chelating agent to detoxify Cd polluted soil. GLDA enhanced the phytoaccumulation capacity by solubilizing heavy metals of soil complexes. *Solanum nigrum* L. is a strong metals accumulator and thrive in the soils contaminated with Cd. Strong absorption of heavy metal was resulted in the reduction of plants growth. The strongest accumulation of Cadmium was observed in shoots followed by roots of plants. The TF values for all treatments was >1 which proven that *Solanum nigrum* L. is a hyperaccumulator plant and suitable for phytoremediation of Cd contaminated soil. Looking at the challenging situation of today’s chemical era, it can be expected that phytoremediation can prove to be effective in avoiding soil heavy metal pollution in the future. Therefore, future research needs to consider field-scale phytoremediation of contaminated soil. To prevent unwanted side-effects, careful management of phytoremediation methods seems necessary.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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