Co-application of chelate GLDA and French marigold for remediation of Cd contaminated farmlands

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Abstract

The present study, via a 180-days field trial, investigated the potential of chelate tetrasodium glutamate diacetate (GLDA) for the remediation of cadmium (Cd)-contaminated farmlands by French marigold. To do so, five GLDA treatments (e.g., 0, 292.5, 585, 1170, and 2340 kg hm^{-2}) were practiced. For each treatment, the total GLDA was divided into two applications with 15 day intervals (0.25, 0.47, and 0.61 $mg·kg^{-1}$) under French marigold plantation. Compared with the control, our results showed that GLDA application significantly increased the biomass of aerial parts of French marigold by 21.9% ($p < 0.05$). Likewise, Cd content in aboveground and underground parts of French marigold increased by 94.7% and 60.5%, respectively, compared with the control ($p < 0.05$). GLDA application caused significant increases in Cd accumulations in cell soluble fraction and cell wall by 290% and 123%, respectively ($p < 0.05$); Soil pH and DTPA-Cd content increased with the increase of total application of GLDA. Co-application of GLDA (2340 kg hm^{-2}) and French marigold reduced the total soil Cd content by 12.87% compared with the soil background. Altogether, our findings conclude on the efficacy of GLDA application for the remediation of Cd contaminated farmlands under French marigold cultivation.

1. Introduction

Within the last few decades, rapid industrialization and urbanization in China have caused severe environmental soil and wastewater pollution of heavy metals (Liu et al., 2016). These contaminants are insidious, long-lasting, poorly mobile, and difficult to degrade, particularly in soils (Li et al., 2013). At high levels, heavy metals not only cause degradation of soil fertility but also reduce crop productivity/quality. The latter imposes risks to the human health (Liu et al., 2013). Cadmium (Cd) is one of the most migratory and biotoxic heavy metals in the soil, posing serious threats to the agricultural economy and human health (Lin et al., 2017). Currently, most agricultural soils in China are slightly to moderately contaminated with Cd, highlighting the importance of immediate environmental remediation (Pang et al., 2016). Compared with the traditional physical and chemical remediation techniques, phytoremediation is an emerging and efficient approach (Jin and Zhen, 2021). Phytoremediation has received increasing attention due to its high efficacy, low cost, and easy management/operation with no secondary pollution (Salvadora et al., 2021; Thiravetyan et al., 2015). Hence, the international academic community has recognized phytoremediation as an in situ eco-friendly green remediation technology (Garbisu and Alkorta, 2001). Currently, Chelator-enhanced phytoextraction is also known a promising remediation approach for heavy metals' contaminated soils (Li et al., 2019). In recent years, a biodegradable chelating agent (chelating agent tetrasodium glutamate diacetate; hereafter defined as GLDA), mainly derived from plant materials, has been found to have chelating capacity comparable to that of EDTA (AkzoNobel, 2010). Literature review reveals the strong affinity of GLDA for soil Cd, enhancing the Cd uptake/extraction by plants (Wang et al., 2019; Guo et al., 2017).

Only partial indoor and pot experiments have been conducted to demonstrate that GLDA has a good potential for fortifying plants such as Southeast Sedum, seed amaranth and elephant grass (Wei et al., 2015; Wang et al., 2019). However, most of the studied plants have low biomass and poor resistance to

Cd stress (Meng et al., 2018). Alternatively, French marigold is resistant to transplanting and easy to cultivate with rapid growth (Nagashree and Kulkarniet, 2021; Barna et al., 2021). In addition, it has a certain ornamental value and a good prospect as an ornamental flower with high capability for the remediation of Cd-contaminated farmland soil (Garge et al., 2021; Biswojit et al., 2021).

Altogether, this field study was primarily aimed to investigate the Cd content of GLDA and French marigold in different Cd contaminated backgrounds. Furthermore, we explored the Cd distribution in subcellular fractions of French marigold plants. Finally, the efficacy of total Cd and DTPA-Cd in soil when GLDA was applied in different ways. We expect that the results of this study will provide an eco-friendly and inexpensive remediation method for Cd-contaminated sites.

2. Materials And Methods

2.1 Experimental materials and instruments

Site Description: The experimental site was located in Anhui Province, East China, with a north subtropical monsoon climate. The average annual temperature and precipitation in this region are 13°C and 1100 mm, respectively. The basic soil physical and chemical properties are shown in Table 1.

Table 1
Physical and chemical properties of the soil.

	pH	Total soil Cd mg·kg^{-1}	DTPA-Cd mg·kg^{-1}	Organic matter/g·kg^{-1}	Alkali-hydrolyzable nitrogen/ mg·kg^{-1}	Olsen-P mg·kg^{-1}
D disposal	6.02	0.25	0.05	7.48	56.33	24.62
Z disposal	6.53	0.47	0.09	30.71	253.06	53.51
G disposal	5.84	0.61	0.11	18.22	37.18	33.84

Plant: Yellow variety of French marigold.

Materials: Tetrasodium glutamate diacetate (GLDA) was purchased from Anhui Cool Biological Engineering Co. Other reagents (HCl, HNO_3, $HClO_4$, etc.) were guaranteed grade reagents, and could be used without further purification.

Instrument: Acidimeter (STARTER 3100) for soil pH; Coolable thermostatic shaker (IS-RDD3, USA) for temperature regulation and control during DTPA-Cd extraction; Graphite furnace-flame spectrophotometer (iCE 3500 Thermo, Thermo Fisher Scientific Ltd.) for determination of Cd concentration.

2.2 Experimental design

Overall, 45 plots (2m×2m) were selected for this study. Experimental plots were categorized in three different groups with low, medium, and high classes of Cd contamination, 15 plots for each group. A ditch (20 cm width and 30 cm depth) was dug between the plots. To eliminate surface runoff, plastic plates were inserted into ditches. For individual Cd contamination classes, five different treatments were practiced including control (CK), total GLDA application of 292.5kg hm^{-2} (D1, Z1, G1), total GLDA application of 585kg hm^{-2} (D2, Z2, G2), total GLDA application of 1170kg hm^{-2} (D3, Z3, G3), and total GLDA application of 2340kg hm^{-2} (D4, Z4, G4). In GLDA amended plots, the total GLDA was divided in two applications with 15 days interval. All treatments were replicated three times. In each plot, 12 French marigold seedlings were transplanted evenly on May 10, 2020. The first part of GLDA was applied after 60 days of cultivation, and the second application took place 15 days later. To do so, GLDA was dissolved in deionized water and uniformly applied to the inter-root soil with 25-liter buckets. French marigold and soil samples were collected 180 days after seedlings' transplantation. We applied identical fertilization scheme for all plots, i.e., compound fertilizer (25:10:16 $N:P_2O_5:K_2O$) was applied simultaneously with seedlings' transplantation at a rate of 200 kg hm^{-2}, and urea (total N ≥ 46.0%) was added 30 days after seedlings' transplantation at a rate of 1100kg hm^{-2}. Compound fertilizer and urea were applied by burrowing and spreading, respectively.

2.3 Sample collection and analysis

Plant sample collection and analysis: After sample collection, we measured the plant height. The plant samples were thoroughly washed with ultrapure water and air-dried, and then oven dried at 105°C to constant weight. Afterwards, the aboveground/underground plants were sieved (1mm) for further analyses. To measure the Cd content, air-dried plant samples were digested by nitric acid - hydrochloric acid - perchloric acid, and Cd concentration was determined by graphite furnace-flame spectrophotometer via the quality control of national standard plant sample of celery (GBW 10048).

Subcellular distribution of Cd in French marigold: We prepared 2 g of fresh plant in pre-cooled extraction buffer [0.25M sucrose, 1.0 mM dithioerythritol, and 50 mM Tris-HCl (pH 7.5)]. We divided the cells into three fractions as follows (Sheet al. 2013): cell wall (F1) is the first precipitate after centrifugation of the tissue at 3000 g for 15 min; organelles (F2) and soluble fractions (F3) are the second precipitate and supernatant, respectively, after the first supernatant was centrifuged at 12,000 g for 30 min. All these steps were performed at 4°C. All fractions were extracted and digested with $HNO_3:HClO_4$ (9:1, v/v) as described above. A graphite furnace atomic absorption spectrophotometer (iCE 3500 Thermo, Thermo Fisher Scientific Ltd.) was used to determine the concentration of subcellular Cd.

Soil sample collection and analysis: Composite soil samples were collected to the depth of plant roots. Prior to analyses, plant residues were removed, soil samples were air-dried and sieved through 1 mm and 0.149 mm, respectively, and then stored in self-sealing bags. Soil total Cd and DTPA-Cd were extracted by acid digestion with aqua regia-perchloric acid mixture and leaching with DTPA extractant, respectively.

Finally, their concentrations were determined using a graphite furnace-flame spectrophotometer (iCE 3500 Thermo, Thermo Fisher).

2.4 Data Analysis

In this study, the correlation coefficients were calculated as follows.

BCF = C_{ds}/C_t (1)

TI = C_{ds}/C_g (2)

where BCF is the enrichment factor, indicating the ability of aboveground plant to enrich soil Cd; TI is the transfer factor, indicating the ability of aboveground plant to transfer Cd from the roots; C_{ds} is the Cd content (mg kg^{-1}) of aboveground plant; C_t is the total Cd content in the soil (mg kg^{-1}); and C_g is the root Cd content (mg kg^{-1}).

The mean and standard deviation of the data were calculated using Excel 2010. The significance of differences, correlation analyses, and linear fitting were carried out using SPSS 20.0. The data were plotted using Origin 2017C.

3. Results And Discussion

3.1 Effect of GLDA application on the aboveground biomass of French marigold

The plant biomass not only affect the economic efficiency, but also it is an important indicator of plants' ability to extract soil heavy metals (Gang and Liu, 2019). Figure 1 shows the biomass (fresh weight) of aboveground plants of different treatments. As can be seen, the biomass contents of DCK, ZCK, and GCK were 0.32, 0.31, and 0.25 kg m^{-2}, respectively. Compared with control, D1 and Z3 had significantly higher biomass ($P < 0.05$). The highest biomass (0.40 kg m^{-2}) occurred in D1, 21.9% higher than that of control. In accordance, the biomass of these treatments were first increased from 0.32(DCK), 0.31(ZCK), and 0.25 kg(GCK) m^{-2} to 0.39(D1), 0.40(Z3), and 0.34(G3) kg m^{-2}, respectively, and then decreased to 0.27, 0.28, and 0.23 kg m^{-2}, respectively. Overall, at low application rate (585kg hm^{-2}), GLDA increased the yield of French marigold by 6%(D2), 16%(Z2), and 33%(G2) compared with control. This may be due to the effect of GLDA on the chlorophyll content of French marigold, influencing the biomass (Sali et al., 2018; Sarwat et al., 2021). This is consistent with the results of Wang et al. (Wang et al., 2017) who found that high GLDA application (12 mmol kg^{-1}) caused a significant impact on tall fescue biomass, while low GLDA had no significant effect on chlorophyll content of tall fescue. Another possible explanation is that GLDA application affects soil enzyme activity. Zhang et al. (2013) found that low application (5 mmol kg^{-1}) of ethylenediaminetetraacetic acid (EDTA) significantly increased the activity of soil enzymes such as urease and sucrase, promoting the uptake of nutrients and increasing the tall fescue biomass. While, high

EDTA application (15 mmol kg^{-1}) reduced the activity of these soil enzymes (Zhang et al., 2013). It is also worth mentioning that GLDA degradation provides C and N for the growth of French marigold. GLDA-induced excess DTPA-Cd stress also contribute to this biomass response (Pan, 2017).

3.2 Effect of GLDA application on the enrichment and transfer of Cd by French marigold

The enrichment and transfer coefficients can reflect the enrichment and the transfer characteristics of Cd in soil-French marigold system (Gong et al., 2019). As shown in Table 2, the Cd contents in the aboveground French marigold of D, Z, and G treatments ranged from 0.14 to 0.23, 0.24 to 0.37, and 0.51 to 0.78 mg kg^{-1}, respectively. BCF was significantly higher (ranging from 0.50, 0.43, and 0.72 to 1.32, 0.88, and 1.36) in all GLDA amended treatments compared with the control ($p < 0.05$). This indicates that even at low application, GLDA significantly increased the aboveground enrichment capacity of soil Cd ($p < 0.05$). The TI values of DCK, ZCK, and GCK were 0.25, 0.35, and 0.35, respectively. The mean TI values of D1 ~ D4, Z1 ~ Z4, and, G1 ~ G4 were 0.39, 0.44, and 0.43, respectively, all of which increased compared with control. These results suggest that GLDA application promoted the Cd transfer from roots to the aboveground plants.

Table 2
Cd content and enrichment/transport coefficients of different treatments after GLDA application.

	Cd content/mg kg^{-1}		BCF	TI
	above-ground plant parts	foot end		
DCK	0.13 ± 0.02b	0.38 ± 0.01c	0.50 ± 0.07c	0.25 ± 0.01c
D1	0.14 ± 0.03b	0.44 ± 0.04bc	0.63 ± 0.15c	0.33 ± 0.02bc
D2	0.17 ± 0.01ab	0.59 ± 0.04ab	0.83 ± 0.06bc	0.36 ± 0.01b
D3	0.20 ± 0.03ab	0.60 ± 0.08ab	1.06 ± 0.14ab	0.48 ± 0.06ab
D4	0.23 ± 0.01a	0.61 ± 0.02a	1.32 ± 0.05a	0.38 ± 0.02a
ZCK	0.19 ± 0.02d	0.54 ± 0.01e	0.43 ± 0.03d	0.35 ± 0.02c
Z1	0.24 ± 0.01c	0.63 ± 0.01d	0.55 ± 0.01c	0.39 ± 0.01bc
Z2	0.31 ± 0.02b	0.64 ± 0.01c	0.72 ± 0.05b	0.48 ± 0.03ab
Z3	0.35 ± 0.01ab	0.78 ± 0.01b	0.84 ± 0.03a	0.45 ± 0.02ab
Z4	0.37 ± 0.02a	0.84 ± 0.01a	0.88 ± 0.03a	0.44 ± 0.01a
GCK	0.44 ± 0.01b	1.23 ± 0.01c	0.72 ± 0.02b	0.35 ± 0.01b
G1	0.51 ± 0.06b	1.31 ± 0.02b	0.84 ± 0.10b	0.39 ± 0.04b
G2	0.54 ± 0.01b	1.34 ± 0.01b	0.90 ± 0.01b	0.41 ± 0.01b
G3	0.57 ± 0.08b	1.43 ± 0.01a	0.97 ± 0.14b	0.40 ± 0.06b
G4	0.78 ± 0.06a	1.49 ± 0.01a	1.36 ± 0.12a	0.54 ± 0.04a

3.3 Effect of GLDA application on subcellular distribution of Cd in aboveground parts of French marigold

Figure 2 shows the effects of different rates of GLDA application on Cd subcellular distribution in aboveground part of French marigold. Herein, soluble(F3), organelles(F2), and cell wall(F1) Cd were the Cd fractions in french marigold. Cell wall(F1)counted for 2–30% of total up-taken Cd; organelles(F2) counted for 25–38% ; and soluble(F3)counted for 50–59%. These findings suggested that Cd compartmentalization in organelles and soluble fractions enable French marigold to tolerate Cd toxicity and fix the Cd in the plant (Fu et al., 2011; Xu et al., 2018). A large number of negatively charged ligands with strong Cd^{2+} adsorption capacity on the cell wall might cause a reduction in the percentage of Cd in the organelle and an increase in the Cd content on the cell wall (Zhang et al., 2019; Shi et al., 2016). At high Cd concentrations (30μmol L^{-1}), the synthesis of nucleic acids and protein transporters are inhibited in plants, limiting Cd transport (Zhang et al., 2016), Therefore the percentage of Cd in soluble (F3) starts to decrease. Altogether, our findings highlight the fixation of Cd in French marigold after the application of GLDA .

3.4 Effect of GLDA application on soil pH and organic matter

Soil pH and organic matter (OM) are of major importance for both plant growth and bioavailability of heavy metals including Cd (Dong et al., 2014; Dai et al., 2018; Hou et al., 2018). Figure 3 shows the soil pH in different treatments. As can be seen, soil pH in DCK, ZCK, and GCK were 6.13, 6.84, and 5.95, respectively. GLDA amended plots had all significantly higher soil than the control. The highest pH appeared in Z4 (7.2), 0.36 units higher than control. The average soil pH of D1 ~ D4, Z1 ~ Z4, and G1 ~ G4 treatments were 6.64, 7.08, and 6.51, respectively, indicating increases in soil pH with the enhancement of total GLDA application. This is consistent with the results of Wei et al. (2014), although these authors found no significant differences among the treatments. This might attribute to the strong buffering capacity and the hydrolysis and degradation process of GLDA in the soils (He et al., 2020). The hydrolysis process of -COO- content of GLDA is as follows: -COO- + H_2O = -COOH + OH-, thereby increasing the OH^- ions in soil solution and subsequently solution pH (Jin et al., 2016). Furthermore, OH- competes with the solution Cd for the adsorption sites, reducing the sorption capacity of soil Cd (Guo et al., 2017).

As shown in Fig. 4, soil OM contents of D1 to D4, Z1 to Z4, and G1 to G4 with the mean values of 9.17, 32, and 17.4 g kg^{-1}, respectively, were slightly lower than that of control (10.45, 33.56, and 19.46 g kg^{-1}, respectively), suggesting reductions in soil OM content with the increasing total GLDA application. GLDA promotes the extraction of Cd from the soil by French marigold. The decrease in Cd content led to an increase in soil microbial activity that, in turn, promote the activation of organic matter by soil microorganisms. In addition, GLDA promotes the secretion of soluble organic acids by plant roots, which also helps to enhance the activation of organic matter by microorganisms, resulting in lower soil OM content. (Kopáček et al., 2018; Anwar et al., 2017).

Our findings reveal that GLDA application cause slight reduction in soil OM content. Hence, it is essential to apply GLDA together with organic amendments/fertilizers.

3.5 Effect of GLDA application on soil total Cd and DTPA-Cd content

Chelating agents induce the release of Cd from the soil solid phase into the soil solution and increase the DTPA-Cd content that, in turn, increases its enrichment and accumulation by plants and decreases soil total content of Cd (Beygi and Jalali, 2019; Chen et al., 2019; Gul et al., 2019). As appeared from Fig. 5, the total Cd content in the soils of each treatment decreased significantly with increasing GLDA addition to the minimum levels of 0.20(D4), 0.41(Z4), and 0.87(G4) mg kg^{-1} at 2340 kg m^{-2} treatment. The highest reduction (12.9%) occurred in D4 treatment.

Figure 6 shows that GLDA significantly increased the effective Cd content by 31%(D4), 35%(Z4), and 28% (G4) at 2340 kg m^{-2} compared with control, although no significant changes took place in the effective Cd concentrations with increasing the GLDA application. This is consistent with the findings of Wang et al. (2008) who studied the effect of EDTA application on Cd enrichment in foliar red beet. Although large GLDA application increased the soil DTPA-Cd content, its effect on DTPA-Cd content may be limited by the soil Cd background value.

In summary, GLDA application revealed to have an activation effect on heavy metals, promote the uptake of Cd in French marigold, and improve the restoration efficiency, among which the best activation effect appeared at the application rate of 2340 kg m^{-2}.

3.6 Correlation analysis of Cd enrichment in French marigold

The results of correlation analysis show that soil physiochemical properties and chelator concentration had positive correlations with Cd of French marigold (Table 3). These findings suggest that there is an intrinsic correlation between soil physiochemical properties and GLDA application rate on the enrichment characteristics of Cd in French marigold, i.e., the enrichment efficiency of Cd in French marigold increases with increasing GLDA concentration, and higher soil pH values stimulates the Cd migration from roots to the above-ground plant. Slight reduction in soil OM content with the increasing GLDA application rate had insignificant correlation with the enrichment of French marigold .

Table 3
Pearson correlation coefficients among the GLDA application methods, soil physiochemical properties, and biomass, and Cd uptake and accumulation in aboveground French marigold.

	pH	organic matter	DTPA-Cd	GLDA application	Above-ground biomass	Above-ground Cd content	Soil total Cd content
pH	1	0.482	0.099	0.614*	0.134	-0.001	-0.143
organic matter		1	0.42	-0.113	0.098	0.078	0.437
DTPA-Cd			1	0.233	-0.323	0.919**	0.939**
GLDA application				1	-0.321	0.378	-0.077
Above-ground biomass					1	-0.459	-0.348
Above-ground Cd content						1	0.826**
Soil total Cd content							1

4. Conclusion

Herein, we carried out a field experiment to investigate the effects of tetrasodium glutamate diacetate (GLDA) on the biomass/growth and cadmium (Cd) accumulation/subcellular distribution of French marigold under Cd stress. Our results indicated that:

(1)Overall, the total GLDA application was the key factor influencing the biomass and Cd removal of French marigold. In particular, under mild Cd contaminated soil conditions, GLDA significantly increased the biomass and Cd content of aboveground French marigold ($p < 0.05$). GLDA application significantly increased the soil DTPA-Cd content.

(2)Although GLDA application slightly decreased soil OM content, this properties had no significant correlation with the aboveground biomass and Cd content of French marigold. Unlike, negative correlations appeared among soil pH, aboveground Cd content, and soil total Cd, the main factors affecting Cd bioavailability.

(3)Compared with the soil background, reductions took place in total content of all GLDA amended soils by maximum 12.9% after 120 d of cultivation of French marigold, suggesting that GLDA promoted the remediation capability of French marigold in moderate to mild Cd contaminated agricultural soils.

Declarations

Ethical Approval

Not applicable.

Consent to Participate

All authors consent to participate.

Consent to Publish

All authors consent to publish.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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Acknowledgments

Not applicable.

Supplementary Materials

Not applicable.

Authors' contributions

Hongchuan Li proposed the experimental design, carried out the experimental work, wrote the article, and carried out the later modification. Rui Jin anf Zhaowen Xu helped with the analysis of experimental data. Hongxiang Hu and Yusef Giampur Karkaje proposed revisions to the paper. Yingying Zhao proposed an improvement to the experimental process. Linchuan Zhan assisted in the experimental work. All authors approved the publication.

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Figures

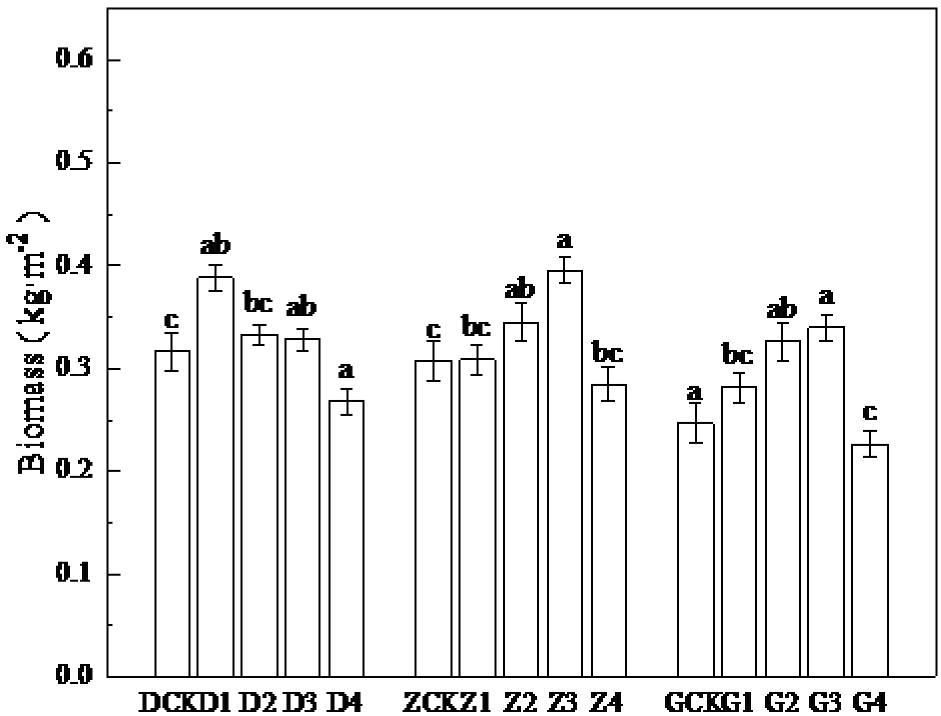

Figure 1

Biomass of aboveground parts of different treatments after GLDA application.

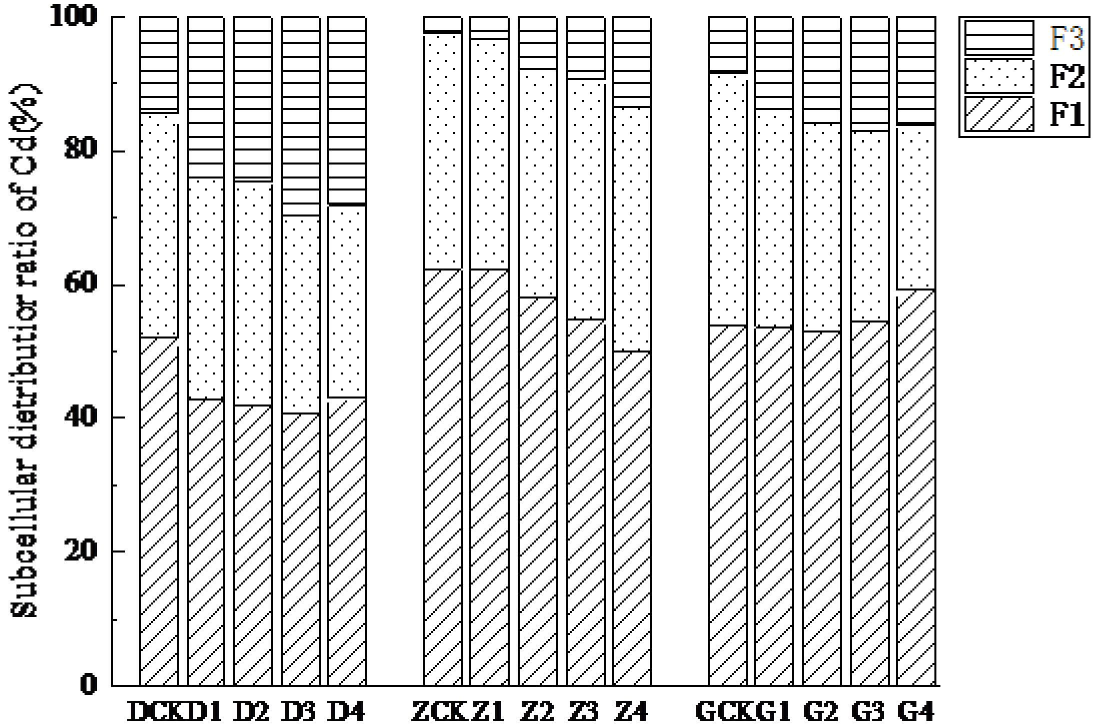

Figure 2

Subcellular distribution of Cd in different treatments after GLDA application.

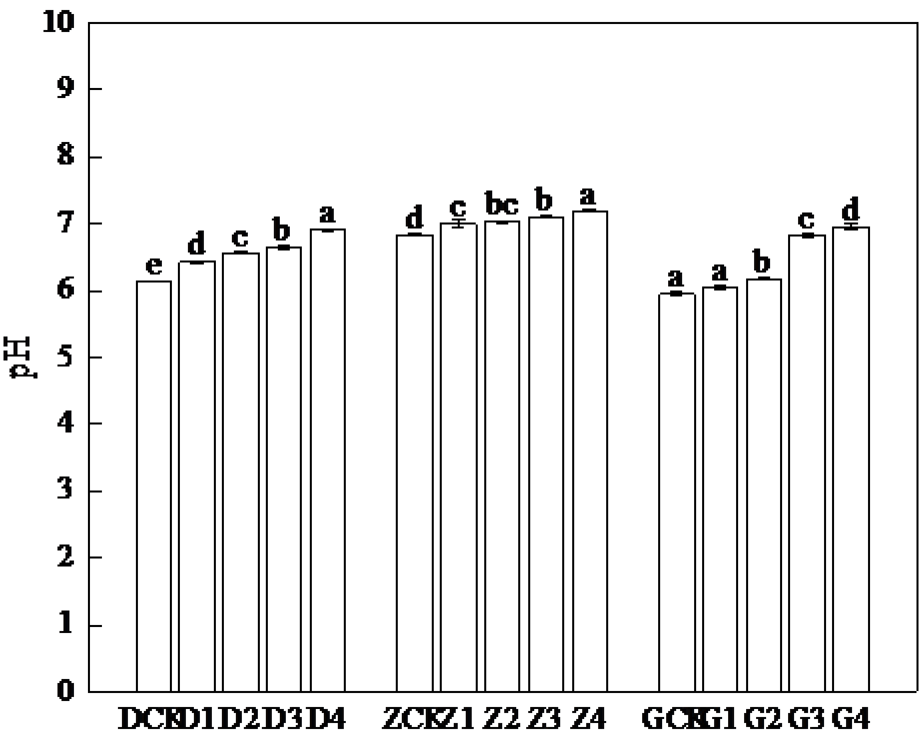

Figure 3

Soil pH of different treatments after GLDA application.

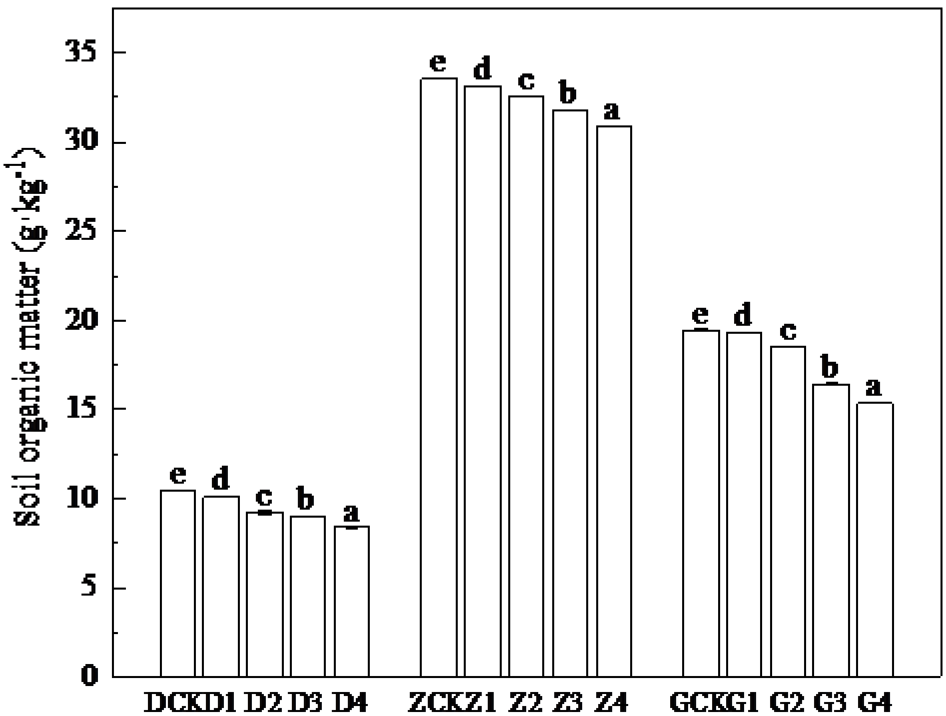

Figure 4

Soil organic matter of different treatments after GLDA application.

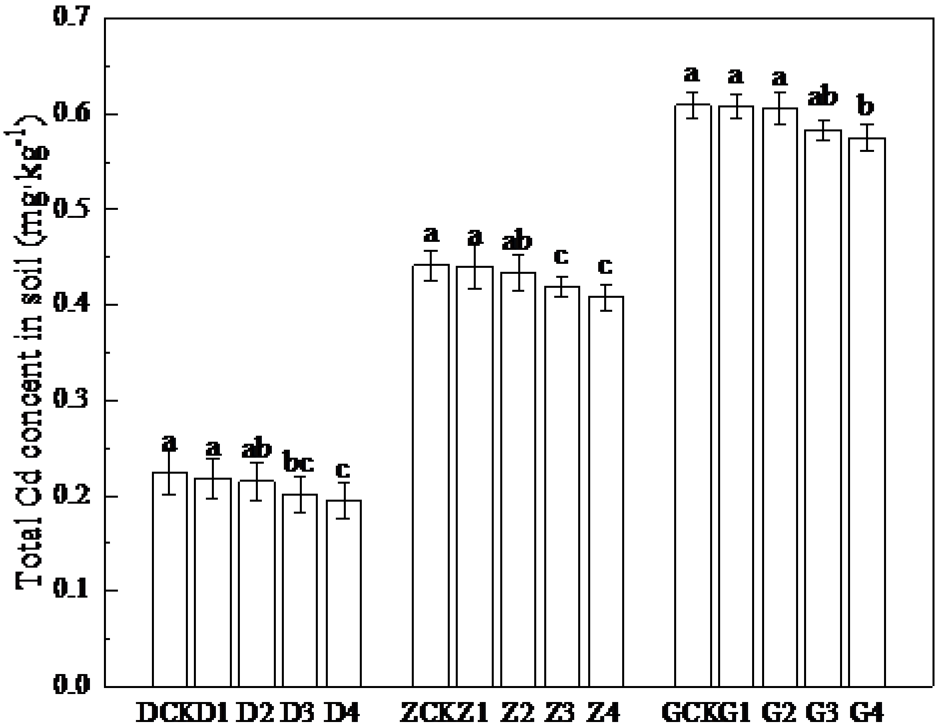

Figure 5

Soil total Cd content of different treatments after GLDA application.

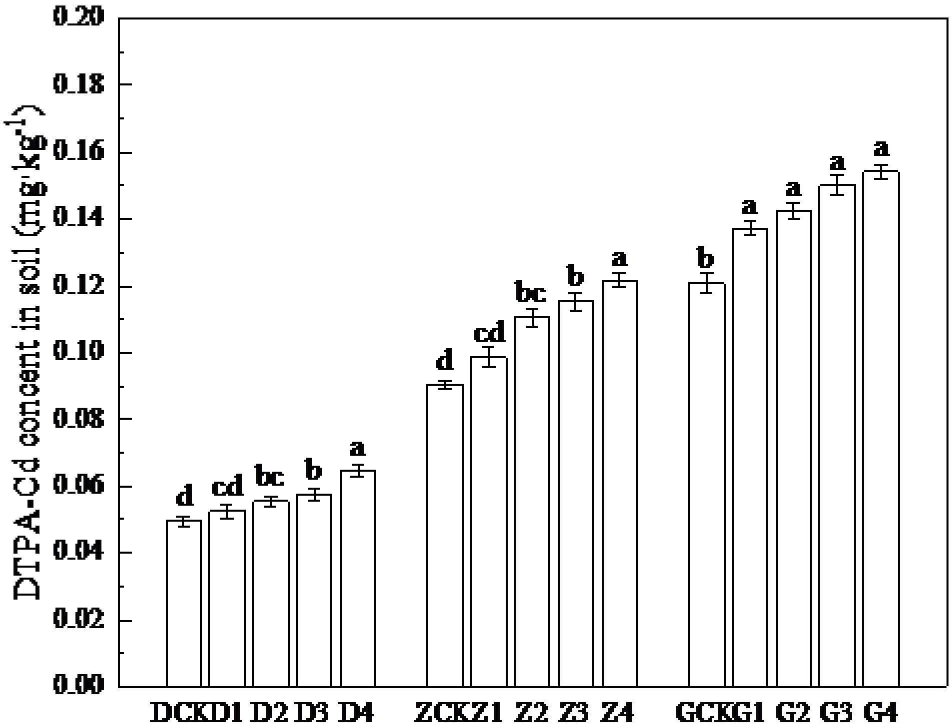

Figure 6

Soil DTPA-Cd content of different treatments after GLDA application.