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Comparison of EDTA, Citric Acid and TiO_2 Nanoparticles to Support Cd Phytoaccumulation in Spiked Soil

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Abstract **-** Chelating agents can increase the bioavailability of cadmium (Cd) which is an important factor in phytoremediation. Current study aimed at comparing potential of organic and inorganic ligands for improving phytoextraction of Cd in soil. The greenhouse experiment was carried out to investigate the impacts of ethylenediaminetetraacetic acid (EDTA), citric acid and titanium dioxide nanoparticles (TiO_2 NPs) on the Cd accumulation and growth of *Pelargonium hortroum*. For this purpose, soil was spiked with different levels of Cd. One month old seedlings of *P. hortorum* were transplanted in each pot and different concentrations of EDTA, citric acid and TiO_2 were applied. After harvesting, plant biomass was measured and plant parts were digested in mixture of acid for the determination of Cd in root and shoot. The results revealed that the dry biomass of *P. hortorum* decreased by 46.5% with increasing levels of Cd and EDTA. However, the dry plant biomass increased with increasing levels of citric acid and TiO_2 NPs. The maximum dry plant biomass was observed in soil amended with TiO_2 NPs followed by citric acid and EDTA. The maximum accumulation of Cd in root (350, 260, 238 mg Cd kg^{-1}) and shoot (997, 650, 450 mg Cd kg^{-1}) was observed at 4 mmol kg^{-1} EDTA followed by 10 mmol kg^{-1} citric acid and 100 mg kg^{-1} TiO_2 NPs, respectively. Our results demonstrated that citric acid and TiO_2 NPs had affirmative effect on the Cd uptake in the artificially Cd-contaminated soil.

Keywords**:** Phytoaccumulation, EDTA, Citric acid, TiO_2 Nanoparticles, Cadmium.

1. Introduction

Cadmium is persistent, non-biodegradable, carcinogenic element and cause toxic effects even at low concentrations (1, 2, 3). Cd enters in environment through various natural and anthropogenic sources such as weathering, metal smelting, nickel-cadmium batteries, use of phosphorous fertilizers and industrial wastewater (4, 5). The Cd affects soil fertility, microorganisms and can easily transfer to plants (6) which cause biochemical and physiological disorder in plants such as lower photosynthetic activity and growth, nutrients uptake, oxidative stress (7, 8) and ultimately affect human through food chain. High intake of Cd by human through food cause severe damage to the organs such as liver and lungs (9). Therefore, the remediation of Cd contaminated soil to reduce toxicity is important for environmental safety. Various *in situ* and *ex situ* techniques have been developed such as soil washing, solidification, stabilization, and phytoremediation (10).

Phytoremediation is eco-friendly and economically feasible technology for the remediation of metals contaminated soil in which plants are used for the removal of pollutant from soil and transfer to the harvestable parts (11). Phytoextraction removes inorganic contaminants primarily heavy metals and concentrate in aerial parts (12). The phytoextraction or phytoaccumualtion depends on different factors including: a) the ability of plants to uptake heavy metals from soil and transfer to the aerial parts, b) the availability of heavy metals for plant uptake. Many species from *Pelargonium* family have been reported as hyper accumulator of heavy metals. Arshad et al. (2008) reported that cultivars of P*elargonium* namely Attar of Roses, Clorinda and Atomic Snowflake accumulated 1467, 1182, 1107 mg Pb kg^{-1} respectively, in shoots. In another study it was reported that *Pelargonium roseum* accumulated 4416 and 1957 mg kg^{-1} of

Pb and Cd respectively, in shoot (14). *Pelargonium hortorum* and *Pelargonium peltatum* showed higher translocation factor (TF >1) for Pb and Cd, respectively (15).

The second important factor in the success of phytoaccumulation is the availability of heavy metals in the soil. For this purpose, different chelating agents such as oxalic acid, citric acid, ethylenediaminedisuccinate (EDDS), ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetate (NTA) have reported in literature (16,17,12). Most of studies have focused on EDTA - assisted phytoextraction of Cd. But, due to low biodegradability of EDTA with the half-life of 120-300 days and high solubility, it adversely affects the environment by increasing metal mobility or solubility. Therefore, the total amount of Cd that plants are able to uptake is much smaller than the amount of Cd mobilized from the soil during EDTA-assisted phytoextraction of Cd (18). Epelde et al. (2008) studied the impact of EDDS and EDTA on the soil microbial community and reported that the addition of EDTA significantly lowered the values of basal respiration, dehydrogenase activity and microbial metabolic quotient. Chen et al. (2004) reported that 20.6% Cd was leached from the soil upon 5 mmol kg^{-1} of EDTA application.

In the present study, TiO_2 nanoparticles (TiO_2 NPs) and citric acid, were studied and compared with EDTA to enhance phytoaccumulation of Cd by *Pelargonium hortorum*. The citric acid is usually used to enhance the crop growth and yield and is helpful in the uptake of metals from the contaminated sites (Chhab et al. 2016). Recently researchers focused on the use of TiO_2 NPs to enhance the nutrient uptake and improve the crop yield. But the impact of TiO_2 NPs on the uptake of heavy metals in not studied. Keeping in view this background the specific objectives of the present study were a) Investigating the effect of EDTA, citric acid and TiO_2 NPs on plant growth b) Comparison of EDTA, citric acid and TiO_2 NPs for Cd phytoaccumulation

2. Material and Methods

2.1. Preparation and Characterization of TiO_2 Nanoparticles

TiO_2 nanoparticles were prepared by using protocol of Mehrizad et al. (2009). Briefly, titanium isopropoxide, ethanol, distilled water and hydrochloric acid in a ratio1:15:60:0.2 were used as starting material. Titanium isopropoxide was added slowly in the mixture of distilled water, ethanol and hydrochloric acid and stirred continuously to get white slurry. The obtained slurry was stirred on the magnetic stirrer for 48 h at room temperature. The solution after drying in oven at 90 ℃ for 48 h was converted into golden yellow crystals. The obtained crystals were powdered in mortar and pestle and then calcinated at 400 ℃ for 2 h in the muffle furnace (NEYO M-525 SERIES II). X ray Diffraction (XRD, JEOL JDXII, X-Ray) was used for the estimation of crystalline size and identification of crystallite phase. The prepared TiO_2 nanoparticles were in anatase phase with average size between 10-15 nm and were spherical in shape.

2.2. Pot Experiment

For pot experiments, soil was air dried and pulverized through Ball mill and sieved through 2mm mesh. The physicochemical characteristics of prepared soil are shown in table 1. The prepared soil was spiked with salt of cadmium sulphate [$CdSO_4$] and mixed regularly to obtain desired concentrations of Cd i.e. 25, 50, 100, 150 mg Cd kg^{-1}. Pots were filled with 1 kg of Cd-spiked and control (without Cd) soils. One month old seedlings of *P. hortorum* were transplanted in each pot. After one month of seedlings transplantation, EDTA (0, 1, 2, 3, 4, 5 mmol kg^{-1}), citric acid (0, 2, 4, 6, 8, 10 mmol kg^{-1}) and TiO_2 nanoparticles (0, 20, 40, 60, 80, 100 mg kg^{-1}) were applied in solution forms. Plants were exposed till flowering stage and were watered regularly and kept in green house with natural light illumination.

Table 1: Physicochemical characteristics of soil.

Parameters	**Values**
Soil pH	7.38
EC (dS m^{-1})	0.44
Organic content (%)	0.41
Soil texture	Clay loam
Cd (mg kg^{-1})	BD*

BD = below detection

2.3. Plant Harvesting and Analysis

After exposure duration, plants were carefully harvested. The plants were separated into root and shoot, washed with deionized water and rinsed with 0.01M HCl solution to remove any external Cd. The plants were dried in oven at 65 ℃ for 48 h and dry plant biomass was measured. For the analysis of Cd, 0.5g of root and shoot was digested in the acid mixture of HNO_3 and $HClO_4$ in a ratio of 3:1 on hot plate until the clear aliquot was obtained. The deionized water was added in digested samples and filtered. The filtrate was used for the analysis of Cd through atomic absorption spectrophotometer (AAS; Perkinelmer 900T).

2.3. Statistical Analysis

All results were statistically analyzed for significant difference ($P < 0.05$) by performing Analysis of Variance (ANOVA) and Least Significant Difference (LSD) in Statistix 8 software.

3. Results and Discussion

3.1. Plant Biomass

Dry plant biomass of *P. hortorum* in Cd-spiked soil upon amendment's application is presented in figure 1. Significant decrease in dry plant biomass was observed with increasing Cd-concentration without application of amendments. At 150 mg Cd kg^{-1} treatment, 22.5% decrease in the dry plant biomass was observed as compared to control (without Cd). The decrease in dry plant biomass was more pronounced upon EDTA application. The dry plant biomass in Cd-spiked and EDTA amended soil ranged from 1.52-2.68 g. The maximum dry plant biomass was observed at 25 mg Cd kg^{-1} treatment without application of EDTA and minimum dry plant biomass was observed in combined treatment of 150 mg Cd kg^{-1} and 5 mmol kg^{-1} EDTA. At higher Cd and EDTA levels, 46.5% decrease in the dry plant biomass was observed as compared to control. Cay et al. (2016) found that the dry plant biomass of *Lonicera japonica*, *Althaea rosea*, *Dahlia hybrida* and *Salvia virgate* were significantly affected upon EDTA and Cd application. The dry plant biomass of *L. japonica*, *A. rosea*, *D. hybrid* and *S. virgate* reduced by 48.0, 47.7, 38.9 and 28.1%, respectively as compared to control.

The plant dry biomass in control group (without Cd) increased by 3.5% and 2.8% at 10 mmol kg^{-1} citric acid and 80 mg kg^{-1} TiO_2 NPs application, respectively as compared to plant grown without amendments application. By comparing plant dry biomass in term of similar citric acid and TiO_2 NPs concentrations and different Cd levels, decrease in plant biomass was observed. By application of 10 mmol kg^{-1} citric acid and 80 mg kg^{-1} TiO_2 NPs at different levels of Cd, the dry plant biomass of *P. hortorum* decreased by 18.0% and 17.8%, respectively. By comparing the combinations of amendments and Cd levels, 15.1% and 12.6% decrease in dry plant biomass was observed in 150 mg kg^{-1} Cd treatment upon 10 mmol kg^{-1} citric acid and 80 mg kg^{-1} TiO_2 NPs application, respectively. The maximum dry plant biomass was observed in TiO_2 NPs amended soil followed by citric acid and EDTA.

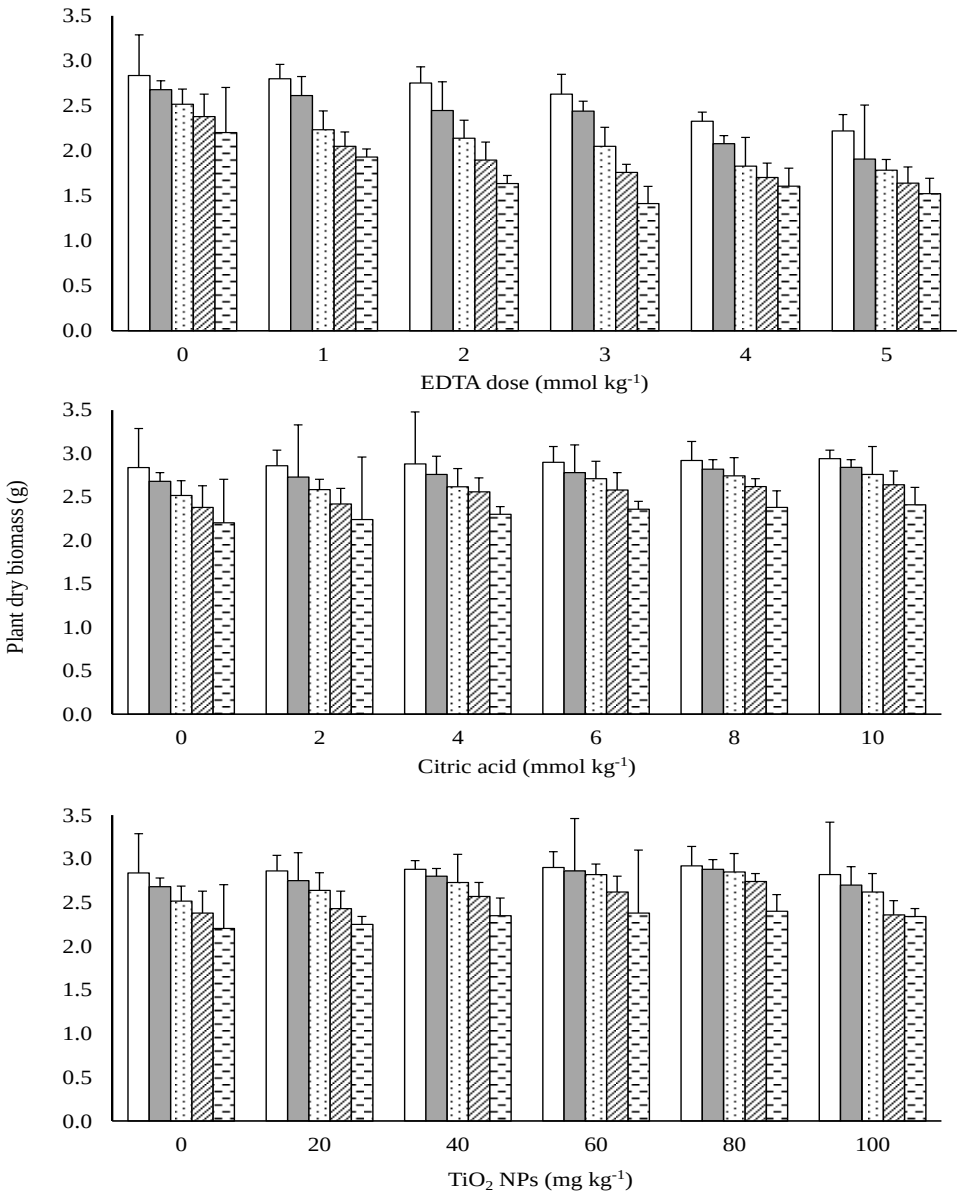

Fig. 1: Plant dry biomass of *P. hortorum* in Cd spiked and amendments mediated soil □ = 0; ■ = 25; ⊡ = 50; ▨ = 100; ◘ = 150 mg Cd kg^{-1}.

3.2. Cd Concentration in Root

Figure 2 illustrates the Cd accumulation in root of *P. hortorum* upon amendments application. The Cd concentration in root of plant was not observed in control groups upon amendments application. The Cd accumulation in treated groups increased with increasing levels of Cd and soil amendments i.e. EDTA, citric acid and TiO_2 NPs. The minimum accumulation of Cd in roots of *P. hortorum* was observed at 25 mg Cd kg^{-1} treatment without application of any amendments. The maximum accumulation was observed in 150 mg Cd kg^{-1} treatment upon 4 mmol kg^{-1} of EDTA application followed by 10 mmol kg^{-1} citric acid and 100 mg kg^{-1} TiO_2 NPs. At higher Cd treatment 2.3-fold, 1.47-fold and 1.2-fold increase in the Cd accumulation in root was observed upon application of EDTA, citric acid and TiO_2 NPs, respectively. Cay et al. (2016) investigated the impact of EDTA on Cd accumulation in different parts of four ornamental plants i.e. *Lonicera japonica* Thunb, *Althaea rosea* Cavan, *Dahlia hybrid* and *Saliva Virgata* Jacq. It was found that the application of EDTA significantly enhanced the accumulation of Cd in root and *L. japonica* showed

maximum concentration of Cd. Wang et al (2017) indicated that the accumulation of Cd in root of *Festuca arundinacea* and *Poa pratensis* was increased by 3-fold and 1.66-fold, respectively upon citric acid application. The EDTA and citric acid significantly enhanced the bioavailable Cd in soil and citric acid also increased the antioxidant defence and phytoremediation of Cd.

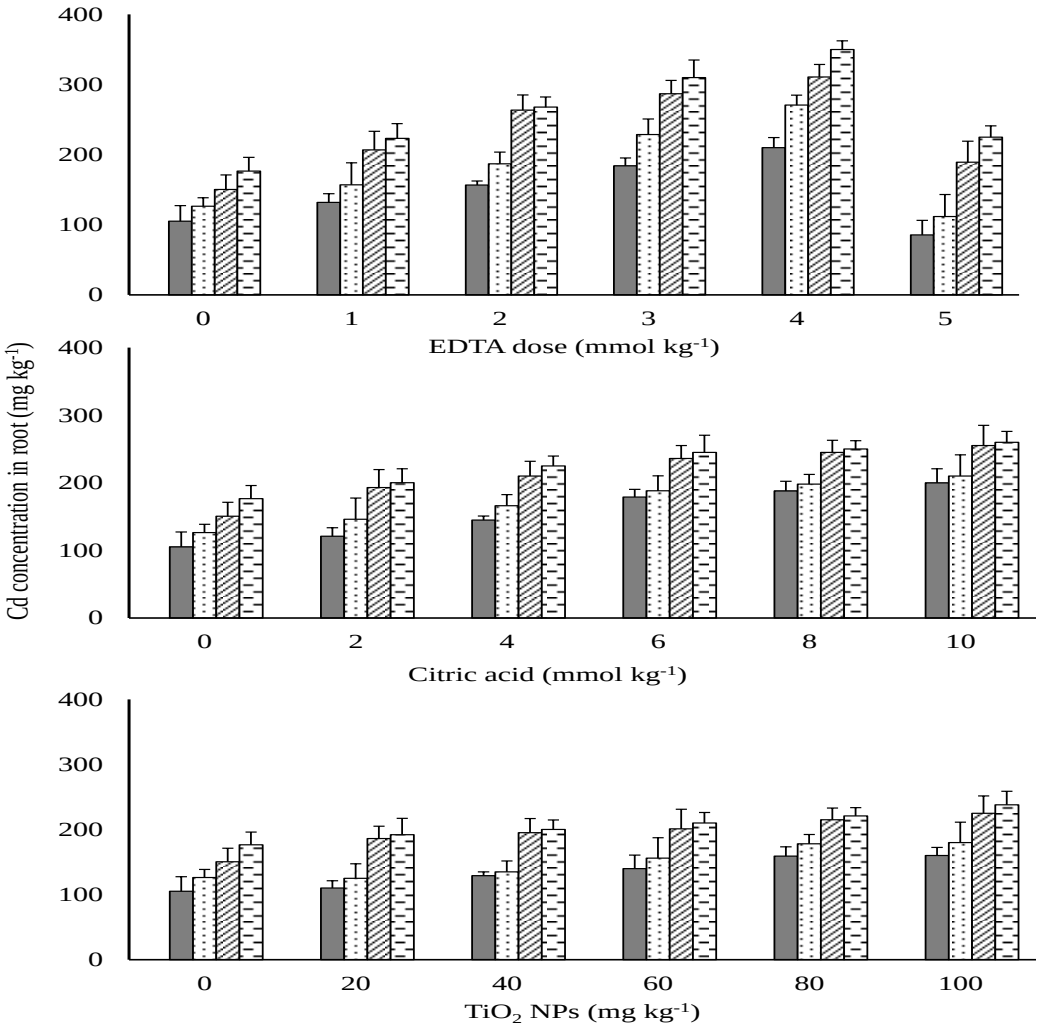

Fig. 2: Cadmium concentrations in root of *P. hortorum* in Cd spiked and amendments mediated soil ■ = 25; ⊡ = 50; ▨ = 100; ⊟ = 150 mg Cd kg^{-1}.

3.3. Cd Concentration in Shoot

The concentration of Cd in shoot of *P. hortorum* grown on Cd-spiked and amendments mediated soil is shown in figure 3. The Cd accumulation in shoot of *P. hortorum* grown in Cd spiked soil ranged from 190.3 – 262.8 mg Cd kg^{-1} without application of amendments. The minimum and maximum accumulation of Cd in shoot was observed at 25 and 150 mg kg^{-1} Cd treatments, respectively. At higher treatment (150 mg Cd kg^{-1}), 38.1% increase in Cd accumulation was observed as compared to lower treatment (25 mg Cd kg^{-1}). Mahdeih et al. (2013) reported that the scented geranium, *P. roseum* accumulated 1957 mg Cd kg^{-1} of shoot dry weight in fourteen days of exposure. In the present study, applications of soil amendments significantly increased the accumulation of Cd in shoot. At 150 mg kg^{-1} Cd treatment, 997 mg kg^{-1}, 645 mg kg^{-1}, 450 mg kg^{-1} of Cd accumulation was observed in shoot of *P. hortorum* upon 4 mmol kg^{-1} of EDTA, 10 mmol kg^{-1} citric acid and 100 mg kg^{-1} TiO_2 NPs application. In a recent study, the marigold (*Tagetes* sp.) has been reported to accumulate 2.4 and 3.4-folds more Cd in shoots at 2 and 4 g EDTA kg^{-1} application as compared to control (Ali et al.,

2016). Plants grown in 150 mg Cd kg^{-1} treatment, showed 4.2, 2.3, and 1.3-folds increase in the Cd accumulation upon 4 mmol kg^{-1} of EDTA, 10 mmol kg^{-1} citric acid and 100 mg kg^{-1} TiO_2 NPs application, respectively as compared to lower treatment i.e. 25 mg Cd kg^{-1} without application of amendments. By comparing all amendments, EDTA application showed maximum accumulation of Cd in shoot followed by citric acid and TiO_2 NPs.

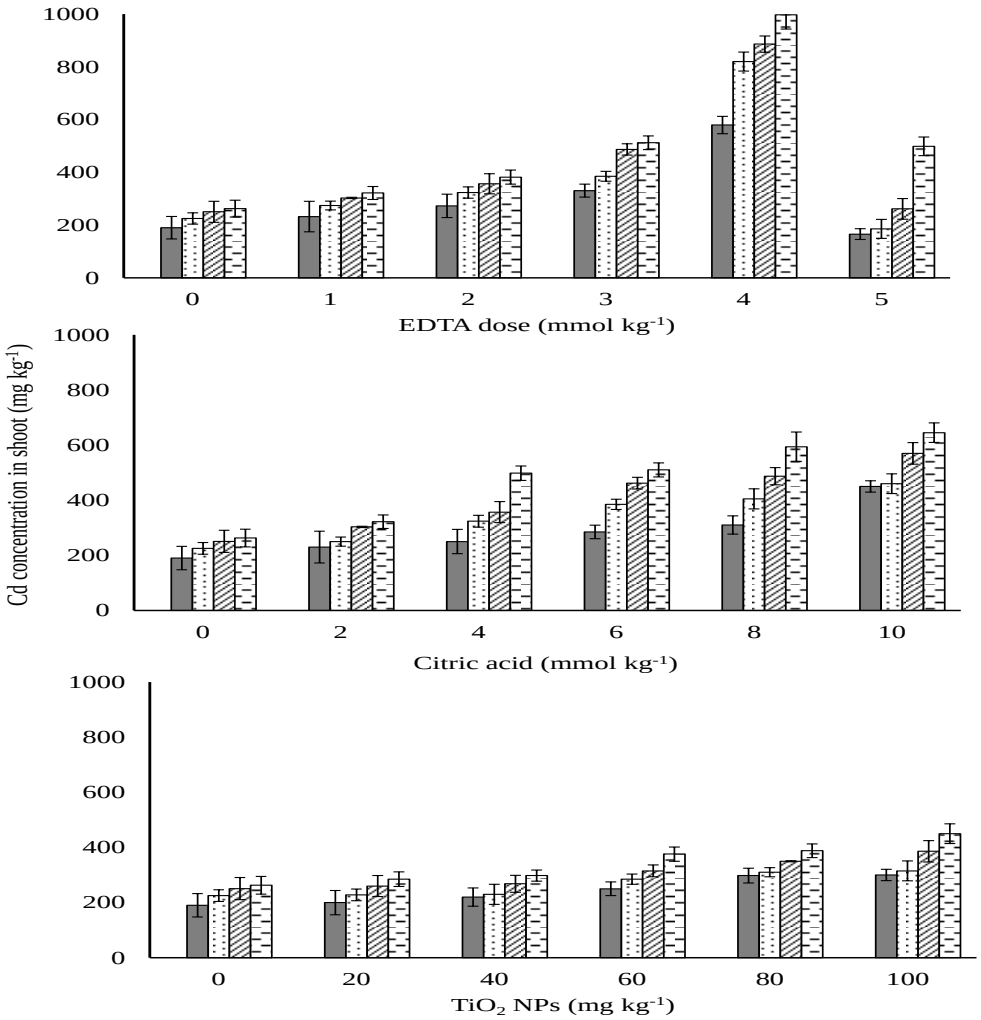

Fig. 3: Cadmium concentrations in shoot of *P. hortorum* in Cd spiked and amendments mediated soil ■ = 25; ⊡ = 50; ▨ = 100; ⊡ = 150 mg Cd kg^{-1}.

4. Conclusions

The application of EDTA, citric acid and TiO_2 NPs efficiently improved Cd accumulation in *P. hortorum*. The biomass was decreased by 46.5%, 15.1% and 12.6% upon EDTA, citric acid and TiO_2 NPs application. The maximum dry plant biomass was observed in TiO_2 NPs amended soil followed by citric acid and EDTA. However, the maximum accumulation of Cd in shoot and root was observed in EDTA amended soil followed by citric acid and TiO_2 NPs. The plants accumulated 2.3, 1.47, 1.20-folds Cd in roots and 4.2, 2.3, 1.3-folds in shoot upon EDTA, citric acid and TiO_2 NPs application, respectively as compared to 25 mg Cd kg^{-1} treatment without application of amendments. Overall, all amendments were efficient in supporting the Cd accumulation and *P. hortorum* accumulated > 100 mg Cd kg^{-1} in shoot.

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