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Research Article

Modulating effect of EDTA and SDS on growth, biochemical parameters and antioxidant defense system of *Dahlia variabilis* grown under cadmium and lead-induced stress

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Abstract

The present study investigated the influence of inorganic amendments *viz*., SDS (sodium dodecyl sulphate) and ethylenediaminetetraacetic acid (EDTA) in enhancing metal tolerance in plants. Seedlings of an important ornamental plant, *Dahlia variabilis* Cav. were grown under cadmium (Cd) and lead (Pb) stress. 30-days old seedlings were transferred to pots containing sterilized sand and supplemented with Hoagland's medium. After 15 days of transplanting, four treatments (0, 10, 25, and 100 mg kg^{-1}) of Cd and four treatments of Pb (0, 100, 500 and 5000 mg kg^{-1}) were used with or without application of 2.0 mM SDS and 2.5 mM EDTA, separately and in combination. Seedlings were further grown for 60 days in culture media. Results revealed that both Cd and Pb significantly reduced plant growth, pigment content, and relative water content. Antioxidant enzymes *viz*., superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) along with protein and total soluble sugar contents showed a declining trend with an increase in Cd and Pb concentrations applied. The Cd and Pb treatment enhanced the production rate of reactive oxygen species (ROS) as depicted by the increased malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) production in leaf. Inorganic amendments *viz.,* EDTA+SDS applied either alone or in combination significantly alleviated Cd and Pb-induced toxic effects. However, a combination of EDTA+SDS showed significant results than used separately. These results revealed that the application of inorganic amendments in combination can enhance the phytoextraction capacity of the species studied. However, the effects of various amendments vary with the nature of the inorganic compound. The study suggests that the application of EDTA and SDS could be a useful strategy for enhancing the phytoextraction capability of *Dahlia variabilis* to remove Cd and Pb from contaminated soils.

Keywords: assisted phytoremediation; cadmium; phytoremediation enhancers; phytoremediation; ROS

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Introduction

The soil is a non-renewable natural source, which is contaminated by heavy metals (HM) coming from different sources *viz.*, waste from smelters, mines, atmospheric deposition, drainage, inorganic fertilizers and other anthropogenic activities (Singh and Kalamdhad, 2011; Pirzadah *et al.*, 2014). These HMs combine with living molecules within the body of an organism, such as proteins and metabolites, to form various toxic biological compounds that hamper the normal functioning of the cellular machinery (Duruibe *et al.*, 2007; Anjum *et al.*, 2014, 2015; Ghori *et al.*, 2019). HM pollution also affects agricultural productivity and causes various diseases in humans such as cancer, brain disorder, gastrointestinal problems etc. (Ozturk *et al.*, 1989; Duruibe *et al.*, 2007; Jaishankar *et al.*, 2014; Hakeem *et al.*, 2015). Cadmium (Cd) and lead (Pb) and are the two most common HMs that are highly toxic to plants as well as humans (Qureshi *et al.*, 2007; Gallego *et al.*, 2012; Rizwan *et al.*, 2016; Saifullah *et al.*, 2016).

Establishing a low cost and eco-friendly remediation plan to decontaminate these HMs from the soil is highly desirable. Many technical methods have been investigated and tried to remediate the contaminated soil viz., cover system, soil washing, stabilization, thermal treatment and disposal landfill (Sabir *et al.*, 2015a; Sarwar *et al.*, 2017). However, phytoremediation is an effective, eco-friendly, low cost and easier technique to clean up the soil from HM contamination (Jabeen *et al.*, 2009; Pirzadah *et al.*, 2014; Saxena *et al.*, 2019). It was observed that large amounts of toxic substances could accumulate in the leaves and stems of many plants without hindering their normal functioning, depending on plant species and environmental factors (Ehsan *et al.*, 2014; Iqbal *et al.*, 2015; Ozturk *et al.*, 2015a,b). Many plant species, known as hyperaccumulators, are used as the viable candidates for performing phytoremediation. Ornamental plants are also known to behave as good phytoremediation agents in HM-contaminated soils (Szczygłowskan *et al.*, 2011; Pirzadah *et al.*, 2014). The idea of using ornamental plants as the candidates of phytoremediation could be economically viable, as they could also serve to boost the local floriculture industry and beautify the land filled with heavy metal pollutants.

While the accomplishments of phytoremediation technique are growing, still various factors such as the complexity, pH, chemical nature and ion-exchange capacity of soil particles, are limiting its application (Quartacci *et al.*, 2000; Aziz *et al.*, 2016). The use of chemical enhancers of phytoremediation is nowadays gaining high attention (Farid *et al.*, 2013; Pirzadah *et al.*, 2014; Hasan *et al.*, 2019). Various chelating agents have been used for the remediation of heavy metals. Ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), ethylene gluataro tetraacetic acid (EGTA) are a few significant chelating agents to sequester the toxicity of Cu, Cd and other heavy metals (Bareen 2012; Chigbo and Batty, 2013; Kambhampati, 2013; Mani *et al.*, 2014). The chemical amendment-induced remediation of heavy metals by plant species is an effective technique because it has high binding capacities toward the majority of the heavy metals (Hasan *et al.*, 2019). However, the combination effects of these chelating inorganic molecules have not been comprehensively investigated, particularly in ornamental plants.

To fill this void, the present study was conducted to determine the enhancing role of EDTA and SDSon the *Dahlia variabilis* growth, biochemical parameters and antioxidant defense system, besides determining its phytoextraction capability and tolerance index against Cd and Pb. This study should be helpful in understanding the role and mechanism of enhancing phytoextraction capabilities of the ornamental plants against under Cd and Pb stress.

Materials and Methods

Plant material and growth conditions

Healthy seeds of *Dahlia variabilis* were procured from the Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, thoroughly washed first with tap water and then with distilled water to remove the debris, and then sown in egg trays containing 5.08 cm^3 axenic sand. The whole experiment was conducted under the environmental controlled conditions (in a greenhouse under randomized completely block design with three replications. The pots were filled up with sandy soil and were kept under greenhouse-controlled conditions light/dark regime about 12/12 h, at 25/15±3 °C and relative humidity (RH) 30-50%, respectively) during November 2017. After 3 weeks, the healthy uniform seedlings were transferred to pots. Hoagland's nutrient solution (Hoagland and Arnon, 1950) containing KNO_3, 10.11; K_2SO_4, 9.75; $MnCl_2.4H_2O$, 0.0178; $MgSO_4.7H_2O$, 2.218; $FeSO_4$, 0.05; EDTA, 0.075; NaH_2PO_4, 1.404; $CaSO_4$, 0.17; H_3BO_3, 0.014; $ZnSO_4$, $7H_2O$, 0.01; $CuSO_4$, $7H_2O$, 0.002 and $NaMoO_4$. $2H_2O$, 0.000725 (all in g/100 ml)was applied to plants and revived after every 7 days interval until the plants were harvested. Cd concentrations (from the cadmium salt $CdCl_2$) (0=Control, Cd1=10, Cd2=25, and Cd3=100 mg kg^{-1} and Pb concentrations (from the Pb salt $PbCl_2$)(0=Control, Pb1=500, Pb2=1000, and Pb3=5000 mg kg^{-1}) were applied in pot with chemical amendments along with the Hoagland solution (2.5 mM EDTA+ 2 mM SDS designated as E 1,2.5 mM EDTA, designated as E2 and 2 mM SDS designated as E3).The plants were harvested after the 2 months.

Morphological parameters

Morphological traits such as as the height of the plant, number of leaves, internode distance and fresh mass, were determined from ten seedlings. Biomass accumulation was quantified after processing the plant following the protocol of Qureshi *et al.* (2005). The remaining harvested leaf samples were kept in sealed vials and immediately frozen in liquid N_2 and then stored at -80 °C for further analysis.

2.2 Tolerance index (TI) and translocation factor (TF)

The TI was determined according to Wilkins's equation (Wilkins, 1957).

$TI_{(percentage)} = ML_{metal}/ML_{control} \times 100.$

ML_{metal} and ML_c represent the average length of the longest roots of Pb-treated and control seedlings, respectively. TF was calculated by the following equation (Marchiol *et al.*, 2004).

TF=Metal concentration in shoots/Metal concentration in roots.

Relative water content (RWC)

For determining the RWC, small fresh leaf discs were weighed (FW) and later floated on the deionized water in a petri dish for 8 h under dark conditions. The surplus water was dried using paper towels and turgid weights (TW)were calculated. Lastly, the samples were kept in the oven for 48 h at 80 °C to measure the dry mass. The RWC was measured by using the following equation:

$RWC (\%) = (FW\text{-}DW/TW\text{-}DW) \times 100$

Chlorophyll content

For determining the chlorophyll content, 500 mg of leaf sample was taken and homogenized with 10 mL of acetone (80% v/v). Later the mixture was centrifuged for 10 min at 5,000×g and the supernatant were used to measure the absorbance at 663, 645 and 470 nm respectively, according to Lichtenthaler and Wellburn (1983).

Measurement of H_2O_2

Hydrogen peroxide (H_2O_2) was determined according to Yu *et al.* (2003) and the absorbance was measured at 410 nm to quantify the concentration of H_2O_2 expressed as nanomoles/gram fresh mass.

Measurement of lipid peroxidation

Lipid peroxidation usually measured in terms of TBARS, chiefly MDA was calculated following the protocol of Heath and Packer (1968) and the concentration of MDA was expressed as nanomoles/gram fresh mass, with molar extinction coefficient as 155mM^{-1} cm^{-1}.

Determination of osmolytes

Proline concentration was assayed according to Bates *et al.* (1973) and expressed as µgg^{-1} FM. The total soluble protein was analyzed following the method of Bradford (1976) using Coomassie brilliant blue G-250 as dye and BSA as standard.

Antioxidant enzymes and total soluble protein content

For enzymatic analysis, both root and leaf samples were ground into powdered form, using liquid nitrogen. The powdered samples were homogenized in 0.05 M phosphate buffer (pH: 7.8) and filtered using a muslin cloth and then centrifuged at for 10 min; temp. 4 °C at 12,000 × g and the filtrate collected was used for the analysis of various enzymes.

To measure activity, the samples were homogenized in a phosphate buffer (50 mM potassium phosphate buffer; pH 7.0) mixture containing; 0.1 mM of EDTA and 1 mM dithiothreitol (DTT), as described by Dixit *et al.* (2011). SOD activity was determined by assessing its ability to inhibit photochemical reduction nitro blue tetrazolium (NBT); POD activity was assayed following the modified protocol of Chance and Maehly (1955), whereas, Catalase (CAT) enzyme assay was done according to Aebi (1984) by monitoring the decrease in the absorbance at 240 nm (ε=39.4 mM^{-1} cm^{-1}).

Statistical analysis

Data generated were subjected to Analysis of variance (ANOVA) and the mean differences of data were tested using Fisher's LSD test by Minitab-17 statistical software. Differences among values at $P \leq 0.05$ were considered as significant.

Results

Growth parameters

Increasing Cd and Pb concentrations reduced plant growth parameters such as plant height, number of leaves / plants; internode distance, and the fresh and dry mass of *Dahlia variabilis* (Table 1). This reduction was more prominent at higher Cd (Cd3) and Pb (Pb3) concentrations. However, due to the application of EDTA as well as SDS, the growth parameters showed significant recovery, which was much more significant under EDTA+SDS [E1] combinations, than when augmented with EDTA and SDS alone concentrations (Table 1). The combined amendment concentrations with HMs (Cd1+E1 and Cd2+E1) have recovered plant height by 80.2% and 79.9% respectively, concerning the control. A similar trend was observed with Pb1+E1 and Pb2+E1, were the increase in plant height was enhanced by 34.7% and 57.9% respectively. However, at higher concentrations of both Cd3+E3 as well as Pb3+E3, the enhancement in growth parameters was insignificant concerning their control (Cd3 and Pb3). This positive correlation with E1 and E2 along with their Cd1, Pb1 and Cd2, Pb2, were observed in all the growth parameters studied (*viz.* number of leaves, internode distance and plant fresh and dry weights). Also, EDTA has shown better performance in enhancing all the growth parameters than SDS along the concentration gradient under both Cd and Pb stresses (Table 1).

Tolerance index (TI) and translocation factor (Tf)

With an increase in Cd and Pb concentration in the media, their accumulation in root and shoot was significantly decreased, with a higher content in roots. At higher concentrations of both HMs (Cd3 and Pb3), stress seemed to be saturated and further accumulation and translocation of HMs from root to shoot declined as evident from the data of Table 2. However, on chemical amendments, a significant increase in both tolerance index ratio (TI) and translocation factor (Tf) was observed (Table 2). Combined chemical amendment (E3) increased the shoot tolerance index (STI) by 15.9-50.8% and 23.2-60.4% under the corresponding and Pb concentrations, respectively. Similarly, Tf were corresponding to the concentrations of the chemical amendments (E1, E2 and E3) along with their concentration gradient (Table 2). However, both TI and Tf levels were recorded higher in plants when under E1 amendment in comparison to E2 and E3.

Table 1. Plant growth parameters of *Dahlia Variabilis* including plant height, number of leaves per plant; internode distance, fresh and dry weights of the plant under various Cd and Pb concentrations and influenced by the inorganic amendments viz. (EDTA+SDS= E3), EDTA (E2) and SDS (E3)

Treatments	Plant height	Leaf number	Internode distance	Fresh weight	Dry weight
Control	19.27 ± 1.2^{a}	13.00 ± 1.1^{a}	2.36 ± 0.2^{a}	1.38 ± 0.1^{a}	0.24 ± 0.01^{a}
Cd1+E0	$12.92\pm1.1^{b,g}$	12.50 ± 1.2^{b}	1.76 ± 0.2^{b}	0.73 ± 0.1^{b}	0.12 ± 0.02^{b}
Cd2+E0	11.15 ± 1.3^{c}	10.00 ± 1.0^{c}	1.53 ± 0.1^{b}	0.65 ± 0.1^{c}	0.10 ± 0.01^{b}
Cd3+E0	10.97 ± 1.0^{d}	09.00 ± 1.2^{d}	1.30 ± 0.3^{c}	0.57 ± 0.2^{c}	0.09 ± 0.04^{c}
Cd1+E1	$20.90\pm1.2^{a,e}$	13.00 ± 1.0^{a}	1.98 ± 0.1^{a}	1.01 ± 0.1^{a}	0.20 ± 0.01^{a}
Cd2+E1	$19.25\pm1.4^{e,f}$	13.50 ± 1.5^{a}	1.90 ± 0.1^{a}	0.99 ± 0.3^{a}	0.17 ± 0.02^{a}
Cd3+E1	15.95 ± 1.0^{g}	10.50 ± 1.0^{c}	1.89 ± 0.2^{a}	0.83 ± 0.1^{b}	0.14 ± 0.02^{b}
Cd1+E2	$15.35\pm2.1^{g,}$	13.25 ± 1.2^{a}	1.86 ± 0.1^{a}	0.98 ± 0.4^{a}	0.11 ± 0.04^{b}
Cd2+E2	$17.02\pm1.9^{a,e,h}$	10.50 ± 1.4^{c}	1.68 ± 0.3^{b}	0.77 ± 0.1^{b}	0.11 ± 0.01^{b}
Cd3+E2	13.35 ± 1.0^{g}	09.75 ± 1.2^{d}	1.40 ± 0.3^{b}	0.61 ± 0.1^{c}	0.09 ± 0.01^{c}
Cd1+E3	$15.87\pm1.3^{g,i}$	13.50 ± 1.1^{a}	1.81 ± 0.1^{a}	0.95 ± 0.5^{a}	0.17 ± 0.01^{a}
Cd2+E3	$17.15\pm1.3^{a,e,h}$	11.50 ± 1.5^{a}	1.49 ± 0.1^{b}	0.74 ± 0.5^{b}	0.11 ± 0.01^{b}
Cd3+E3	13.85 ± 1.4^{g}	11.00 ± 1.5^{a}	1.38 ± 0.3^{b}	0.58 ± 0.1^{c}	0.10 ± 0.01^{b}
Control	21.92 ± 1.2^{a}	12.25 ± 1.5^{a}	2.53 ± 0.3^{a}	1.29 ± 0.1^{a}	0.24 ± 0.01^{a}
Pb1+E0	$17.00\pm1.8^{i,}$	09.75 ± 1.4^{d}	1.63 ± 0.1^{b}	0.84 ± 0.1^{a}	0.09 ± 0.02^{b}
Pb2+E0	$11.37\pm1.3^{c,j}$	08.50 ± 1.5^{d}	1.48 ± 0.1^{c}	0.56 ± 0.1^{c}	0.08 ± 0.01^{b}
Pb3+E0	07.95 ± 1.0^{k}	06.75 ± 1.0^{e}	1.31 ± 0.3^{d}	0.48 ± 0.1^{c}	0.09 ± 0.03^{c}
Pb1+E1	$22.30\pm1.3^{e,f}$	11.50 ± 1.5^{a}	1.96 ± 0.2^{a}	0.91 ± 0.1^{a}	0.20 ± 0.01^{a}
Pb2+E1	$18.50\pm1.2^{I,h}$	11.00 ± 1.3^{a}	1.81 ± 0.1^{a}	0.74 ± 0.1^{b}	0.17 ± 0.01^{a}
Pb3+E1	15.57 ± 1.5^{g}	07.25 ± 1.4^{d}	1.68 ± 0.2^{b}	0.64 ± 0.1^{c}	0.15 ± 0.01^{a}
Pb1+E2	$17.50\pm1.4^{i,h}$	10.50 ± 1.4^{a}	1.80 ± 0.4^{a}	0.89 ± 0.1^{a}	0.14 ± 0.01^{a}
Pb2+E2	$17.72\pm1.1^{i,h}$	09.75 ± 1.3^{b}	1.78 ± 0.2^{b}	0.69 ± 0.2^{b}	0.09 ± 0.01^{b}
Pb3+E2	10.80 ± 1.4^{d}	07.25 ± 1.2^{d}	1.44 ± 0.4^{c}	0.52 ± 0.1^{c}	0.08 ± 0.01^{b}
Pb1+E3	$16.72\pm1.6^{g,i}$	11.50 ± 1.4^{a}	1.86 ± 0.1^{a}	0.86 ± 0.2^{a}	0.08 ± 0.01^{b}
Pb2+E3	11.75 ± 1.2^{g}	08.75 ± 1.3^{b}	1.53 ± 0.1^{c}	0.69 ± 0.1^{c}	0.07 ± 0.01^{a}
Pb3+E3	10.65 ± 1.5^{g}	07.25 ± 1.7^{e}	1.32 ± 0.1^{c}	0.49 ± 0.1^{c}	0.06 ± 0.01^{b}

Dissimilar letters within mean and between columns are significantly different at $p \leq 0.05$ level of significance by applying Fisher's LSD Test. Cadmium (Cd) concentrations of (0= Control, Cd1=10, Cd2=25, and Cd3=100 mg kg-1 and Lead (Pb) concentrations of (0= Control, Pb1=500, Pb2=1000, and Pb3=5000 mg kg^{-1}) were applied in the pot with the chemical amendments, 2.5 mM EDTA + 2 mM SDS [E1], 2.5 mM EDTA [E2] and 2 mM SDS [E3]. [E0]= Without any amendment

Relative water content

The effect of Cd and Pb stresses over relative water content (RCW) under the influence of inorganic chemical amendments is shown in Figure 1. With the increase in Cd stress, *D. variabilis* seedlings showed 10%-20.5% reduction in RWC with respect to the control. Similarly, under Pb Stress the RWC was observed to be declined from 10%-23% in the plants with respect to the control (Figure 1). However, the inorganic amendments increased RCW by 70.5-96.1% with E1 under Cd stress and by 80.4%-100% with E1 under Pb stress. A similar trend was shown when augmented with E2 and E2 concentrations alone. Nevertheless, recovery was much significant under E1 than E2 and E3.

Table 2. Effect of increasing concentration of Cd and Pb in the growth medium and the influence of inorganic amendments viz. EDTA+SDS in combination [E1], EDTA [E2] and SDS [E3] alone, on the root tolerance index (root TI%), shoot tolerance index (shoot TI%) and translocation factor values (TF-values)

Treatments	Root tolerance index (root TI %)	Shoot tolerance index (Shoot TI %)	TF value
Cd1+E0	40.4	62.41	0.552
Cd2+E0	34.26	40.61	0.513
Cd3+E0	29.91	20.11	0.390
Cd1+E1	85.21	90.21	0.791
Cd2+E1	80.32	85.91	0.781
Cd3+E1	90.13	82.61	0.803
Cd1+E2	56.21	79.94	0.725
Cd2+E2	60.10	77.26	0.723
Cd3+E2	64.50	70.46	0.672
Cd1+E3	51.10	80.26	0.710
Cd2+E3	54.60	77.10	0.683
Cd3+E3	60.01	77.01	0.603
Pb1+E0	50.50	61.64	0.503
Pb2+E0	39.16	30.26	0.452
Pb3+E0	33.69	78.15	0.313
Pb1+E1	81.22	106.21	0.690
Pb2+E1	75.30	102.96	0.791
Pb3+E1	74.31	100.76	0.881
Pb1+E2	74.51	97.64	0.603
Pb2+E2	80.16	89.26	0.752
Pb3+E2	81.51	87.64	0.713
Pb1+E3	61.16	90.26	0.527
Pb2+E3	78.69	90.15	0.688
Pb3+E3	80.01	89.01	0.703

Dissimilar letters within mean and between columns are significantly different at $p \leq 0.05$ level of significance by applying Fisher's LSD Test. Data represent the mean ± SE of three different experiments (n=10).Cadmium (Cd) concentrations of (0= Control, Cd1=10, Cd2=25, and Cd3=100 mg kg^{-1} and Lead (Pb) concentrations of (0= Control, Pb1=500, Pb2=1000, and Pb3=5000 mg kg–1) were applied in the pot with the chemical amendments, 2.5 mM EDTA + 2 mM SDS [E1], 2.5 mM EDTA [E2] and 2 mM SDS [E3]. [E0] = Without any amendment.

Chlorophyll, total soluble protein and proline contents

A significant decline in chlorophyll a (Chl-a), chlorophyll b (Chl-b) and carotenoid concentrations were found in the leaf samples of *D. variabilis* with a concomitant increase in Cd and Pb concentration in soil culture with respect to control (Figure 2 A,B,C). However, enrichment within organic amendments at E1, E2 and E3 significantly enhances the pigment content under Cd and Pbtreated conditions. Higher chlorophyll and carotenoid concentrations were observed in leaves under Cd and Pb stress when augmented with E1treatment along their concentration gradient.

The soluble protein and proline contents in leaves increased in a dose-dependent manner with an increase in the Cd and Pb concentration of the soil culture (Figure 3A, B). Application of inorganic amendments (E1, E2 and E3) also enhanced the total soluble protein content significantly in leaves compared to the respective Cd and Pb treated samples. The proline content also showed significant increase under Cd and Pb stresses (Figure 3B). However, it declined markedly by the addition of E1.

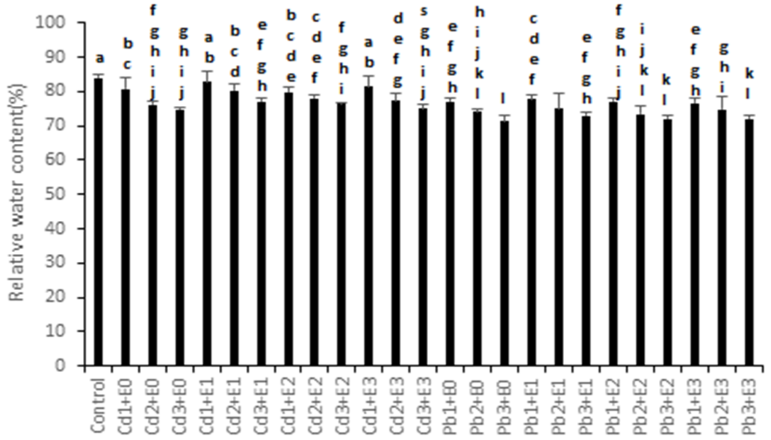

Figure 1. Effect of EDTA and SDS on relative water content (RWC) in leaves of *Dahlia variabilis* seedlings under Cd and Pb stress. Dissimilar letters within mean and between columns are significantly different at p ≤0.05 level of significance by applying Fisher's LSD test

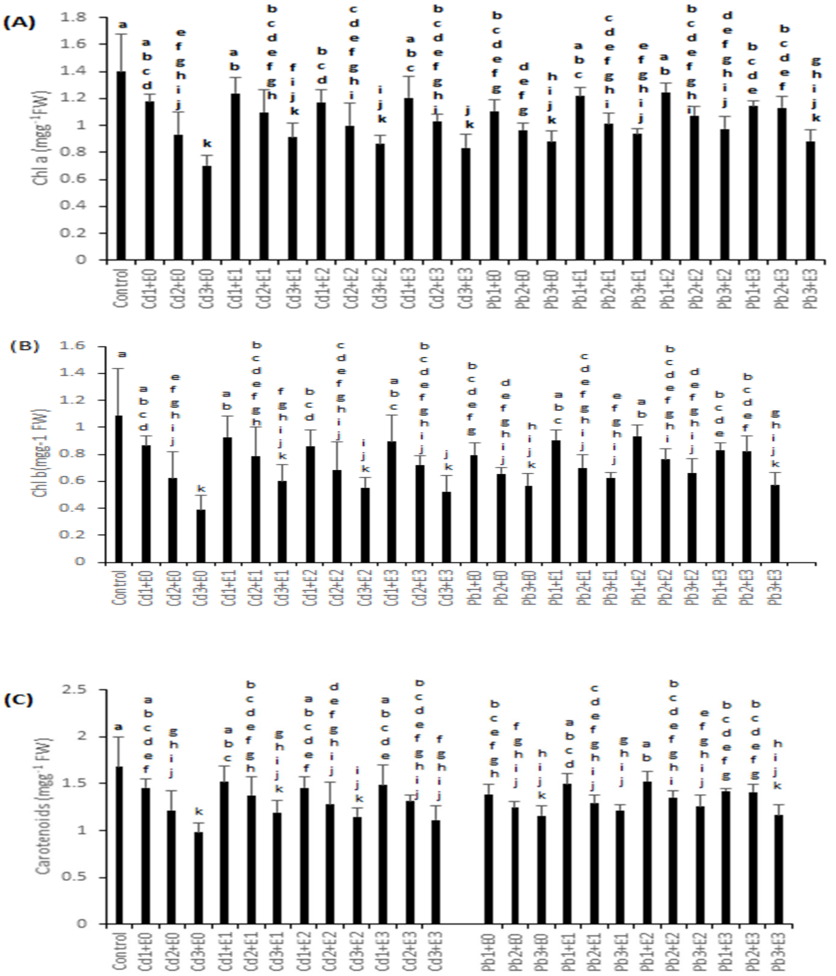

Figure 2. Effect of EDTA and SDS on Chl a (A), Chl b(B), carotenoids (C) content in leaves of *Dahlia variabilis* seedlings under Cd and Pb stress. Dissimilar letters within mean and between columns are significantly different at p ≤0.05 level of significance by applying Fisher's LSD test

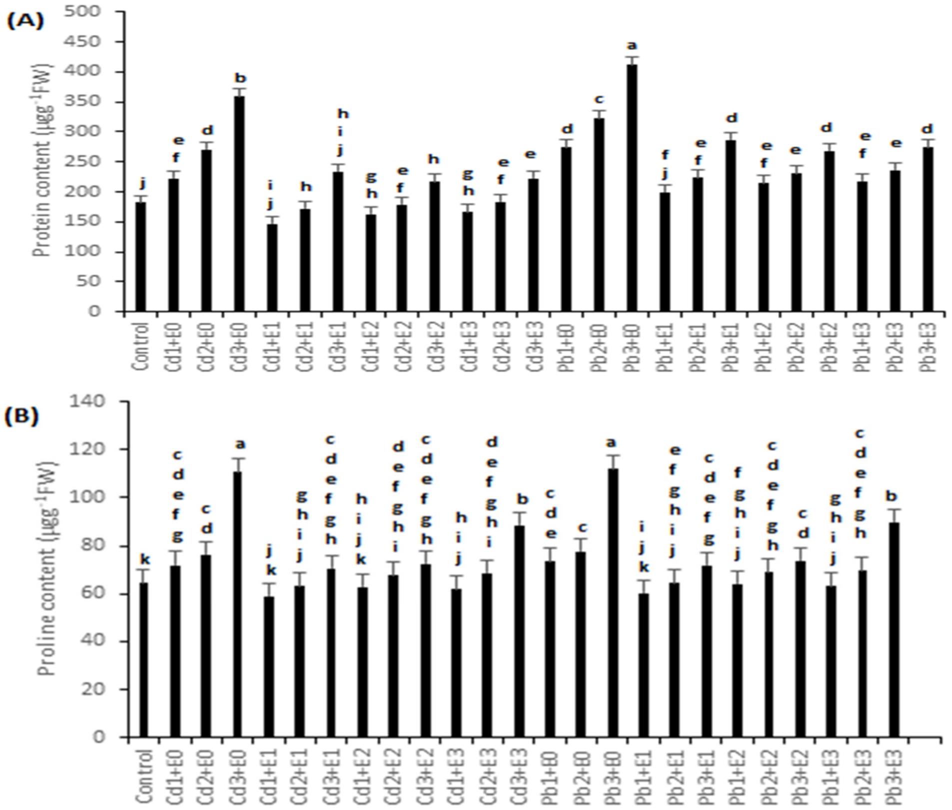

Figure 3. Effect of EDTA and SDS on soluble protein (A), proline (B) content in leaves of *Dahlia variabilis* seedlings under Cd and Pb stress. Dissimilar letters within mean and between columns are significantly different at p ≤0.05 level of significance by applying Fisher's LSD test

MDA and H_2O_2 content

The H_2O_2 and MDA levels were significantly increased, with respect to control, in leaves of *D. variabilis* when subjected to different Cd and Pb concentrations (Figure 4). Nevertheless, extraneous applications of EDTA (E2) and SDS (E3) reduced the H_2O_2 as well as MDA contents. However, application of E1 under Cd and Pb stresses caused a significant decline in H_2O_2 and MDA compared to E2 and E3 treatments treatment reduced the H_2O_2 and MDA by 20.2-36.7% under Cd stress and by 35.7-62.1% under Pb stress along the concentration gradient.

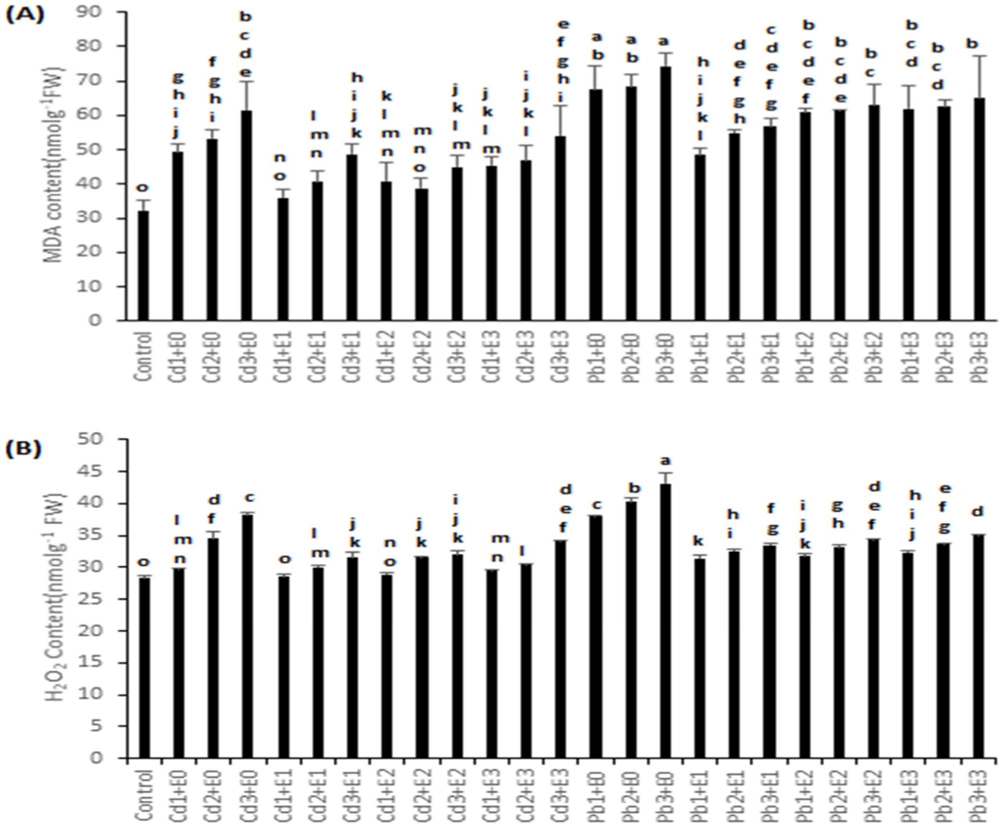

Figure 4. Effect of EDTA and SDS on MDA (A), H_2O_2 (B) content in leaves of *Dahlia variabilis* seedlings under Cd and Pb stress. Dissimilar letters within mean and between columns are significantly different at p ≤0.05 level of significance by applying Fisher's LSD test

Antioxidant defense mechanism (SOD, POD, CAT and APX)

The activity of antioxidant enzymes*viz.*, superoxide dismutase, guaiacol peroxidase, catalase and ascorbate peroxidase (SOD, POD, CAT and APX) in the leaves of *D. variabilis* exposed to Cd and Pb treatments either alone or in combination with chemical enhancers (EDTA+SDS) is depicted in Figure 5. The enzyme activities showed a concomitant decrease with each increment level of Cd and Pb treatment, as compared to control. However, under EDTA and SDS augmentation, SOD, POD, CAT, and APX activity was significantly enhanced at all levels of Cd and Pb treatments with respect to control. Nevertheless, the combined concentrations of E1 generated signifycantly greater activity of these antioxidant defense enzymes.

Further, a negative correlation was observed between H_2O_2 and MDA content and enzyme activities, which may indicate the role of enzyme activities in lowering the oxidative damage in *D. variabilis* under Cd and Pb toxicity**.**

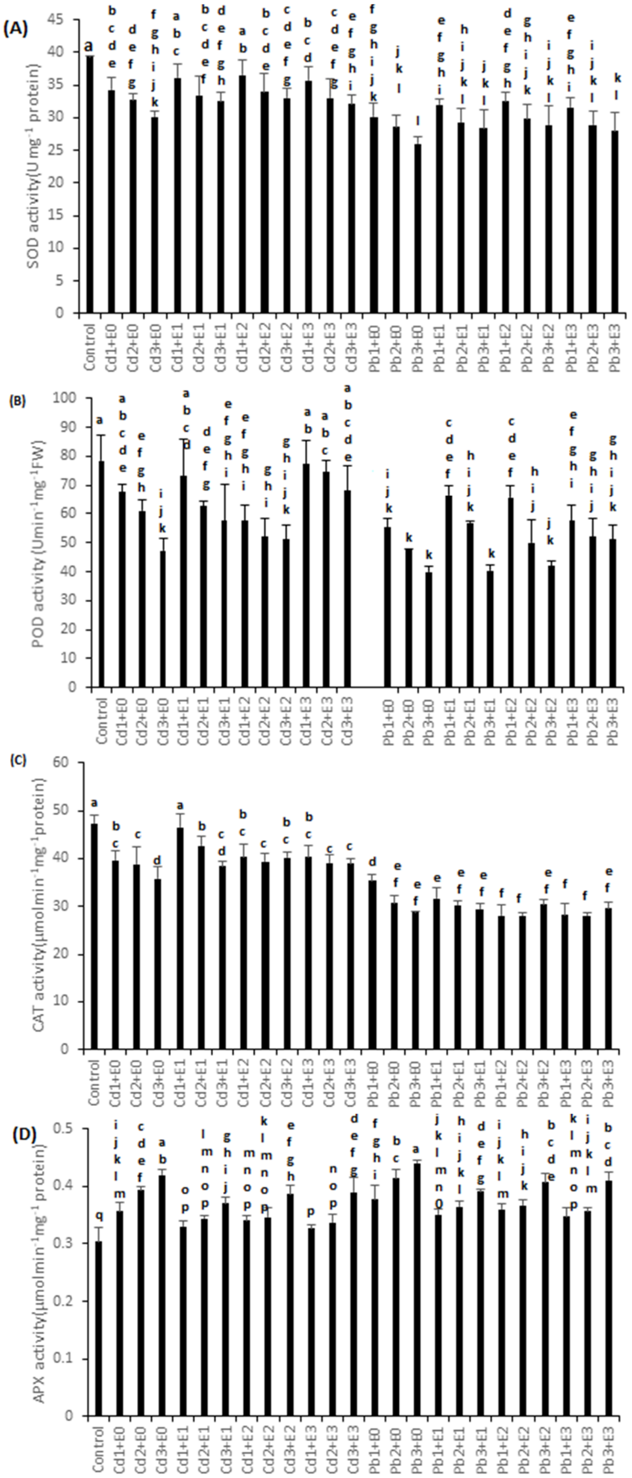

Figure 5. Effect of EDTA and SDS on SOD (A), POD (B), CAT (C), and APX (D) activity in leaves of *Dahlia variabilis* seedlings under Cd and Pb stress. Dissimilar letters within mean and between columns are significantly different at p ≤0.05 level of significance by applying Fisher's LSD test

Discussion

Plant growth, metal accumulation and translocation factor (TF)

Cd and Pb are generally present in the soil in high concentration due to agricultural malpractices (Chen *et al.*, 2009). These metals are readily available to plants and cause serious biochemical as well as physiological disturbances in their cellular mechanism (Hameed *et al.*, 2017). In the present study, the significant decline recorded in growth parameters of Cd and Pb treated *Dahlia variabilis* seedlings was possibly due to the disturbed homeostasis, inhibited mitotic index and restrained aquaporins as well as high accumulation of these metals in various plant organs (Gajewska and Skłodowska, 2010; Hakeem *et al.*, 2019). Inorganic chemical amendments (EDTA and SDS) have significantly improved the growth parameters, tolerance index (TI) and translocation factor (TF) and the overall growth of plants was higher in plants treated with chelating agents, compared to the control (Table 1). EDTA, as well as SDS-assisted escalation in growth and biomass, has been reported in sudangrass, sweet sorghum and *Calendula officinalis* (Mani *et al.*, 2014; Székely *et al.*, 2011; Liu *et al.*, 2010). Liu *et al.* (2010) described that the use of SDS and EGTA could not only enhance the growth of *Calendula officinalis* but also increased its Cd-accumulation capacity. In the current study, combined concentrations of EDTA + SDS were more effective in enhancing the growth and recovery processes. Our study confirms the positive correlation of tolerance index as well as translocation factor with both EDTA alone as well as in combination with SDS. This counters to the observations of Liu *et al.* (2010) about poor growth of *Calendula officinalis* under EDTA, particularly at higher (100 mg/kg) levels in Cd-spiked soils. This might be due to species-specific effect because it is known that EDTA increases both mobility of soil Pb as well as Pb concentrations in plants (Wang *et al.*, 2016; Hasan *et al.*, 2019). Our study has also shown that EDTA application enhances Cd and Pb shoot to root ratio, as compared to Cd and Pb-treatments (Table 1). This is in line with the study of Székely *et al.* (2011), who reported that EDTA chelating effect in sweet sorghum and Sudan grass plants under Cu stress (Székely *et al.*, 2011).

The uptake and translocation of heavy metals in plants differ with plant species and genotype (Gill and Tuteja, 2010; Ansari *et al.*, 2012; Sabir *et al.*, 2015b). In the present study, accumulation of Cd and Pb in root and shoot of *D. variabilis* increases with increasing treatment. TF characterizes the plant's ability to translocate metals from root to shoot (Placek *et al.*, 2016). Cd and Pb concentration in shoots of *D. variabilis* were lower as compared to roots. Therefore, TF was observed be < 1, (Table 2), indicating that large amounts of Cd and Pbwererestricted in roots and insignificant amount is translocated to the above-ground parts for that *D. variabilis* could be interestingly considered for phyto-stabilization (Nouri *et al.*, 2011). Our study revealed that *D. variabilis* accumulates more Cd and Pb under combined EDTA + SDS treatment due to the secretion and chelation of Cd and Pb using organic acids, *viz*. oxalic acid or citric acid (Ma *et al.*, 1997). In the absence of these inorganic amendments, retention of Cd and Pb in the root cells might be due to insolubilization at the root surface or compartmentation in cells, avoiding the release of these metals to the xylem. It is also reported that the organic acids, phytochelatins and other ligands secreted by root cells lead to the formation of complex compounds that are important for the retention of toxic metals in roots (Ma *et al.*, 1997).In some studies, it was shown that EDTA form complexes in solution, then enter plants (Hasan *et al.*, 2019). Recently Souza *et al.* (2009) reported that the heavy metal behaviour of a non-hyperaccumulator plant is relatively slow, but the metal uptake increases quickly after the application of chemical amendments (EDTA) along with metals

In the current investigation, both EDTA and SDS, particularly when used in combination, increased Cd and Pb contents in both roots and shoots of *D. variabilis,* as their dosage was raised in soil culture media (Table 2). Similar results were recorded by Marschner (1995) under Cu levels in leaves that were above the threshold for Cu toxicity in plants. The reason is that EDTA and SDS act as chelating agents to reduce the toxic effects of Cu and improve metal availability (Evangelou and Marsi, 2001; Zhang *et al.*, 2008). The enhancement in Cd and Pb uptake with EDTA application might be because roots can liberate trace metals from dissociated organometallic compounds, as recorded by Nor and Cheng (1986), and this enhances metal uptake by plants (Han *et al.*, 2005; Hasan *et al.*, 2019).

The total Cd and Pb contents were found to be more in shoot than in roots of *D. variabilis* under all levels of EDTA + SDS concentration. Therefore, this phenomenon could be used to rejuvenate the health of soil contaminated with Cd and Pb. Similar observations were reported by Liu *et al.* (2010) in *Calendula officinalis* under the influence of SDS + EGTA combination.

Relative water content, pigment content and other biochemical parameters

Relative water content (RWC) in *D. variabilis* was found to decrease under Cd and Pb treatments (Figure 1), which corroborates our previous findings (Qureshi *et al.*, 2005; Hakeem *et al.*, 2019) in Pb and Cd-stressed plants. This might be because HM stress stimulates the closure of stomata (Brunet *et al.*, 2008). However, under EDTA and SDS combined treatments, the RWC of Cd and Pb-stressed plants showed a significant enhancement with respect to the control.

Photosynthetic pigments represent as biomarkers of metal stress and thus, play a pivotal role in studying plant-metal dynamic interaction (Khudsar *et al.*, 2001). Plant chlorophyll and carotenoid contents were progressively decreased under Cd and Pb stress (Figure 2)possibly due to the substitution of metal ions and inhibition of mineral nutrients *viz.*, Mn, K, Mg, which retard the rate of photosynthesis by interfering with photosystems especially with PS-II (Kupper *et al.*, 1998; Bashir *et al.*, 2015). It also interferes with pigment synthesis by interacting with the enzyme namely δ-aminolevulenic acid dehydratase (Prasad and Prasad, 1987). Besides, the decline in pigment may also be associated with peroxidation of chloroplast membranes due to enhanced ROS production. This aligns with an increased level of H_2O_2 and MDA production in *D. variabilis* plants subjected to Pb stress. The level of carotenoids does not exhibit a significant increase with each increment in Pb treatment (Figure 3B). Carotenoids play an essential role in to protect Chl from ROS by quenching the triplet Chl. Carotenoids are produced as adjunct pigments in plants subjected to metal stresses and play a vital role to maintain the equilibrium state (Hakeem *et al.*, 2019). Our results corroborate those of Habiba *et al.* (2015) that EDTA enrichment reduced the oxidative damage as indicated by the declining trend of H_2O_2 and MDA contents (Figure 4).

Protein is another important biomarker in stress biology (Plata *et al.*, 2009). In the present investigation, the total soluble protein in *D. variabilis* increased with increase in Cd and Pb concentration (Figure 2). This could be due to stimulation of stress proteins under heavy metal exposure or due to the increased activity of metal sequestration pathways to detoxify toxic metals (Bagheri *et al.*, 2015; Hakeem *et al.*, 2019). The concentration of soluble protein content was progressively increasing under the influence of EDTA and SDS. The total soluble sugar content may be closely associated with tolerance to heavy metal stress (Zhang *et al.*, 2015). Herein, found that soluble sugar content of *D. variabilis* showed a concomitant increase with each increment of Pb stress possibly due to degradation of starch (Figure 5C). It is believed that under Cd and Pb stress, accumulation of sugars along with other compatible solutes contribute to osmotic balance (Bohnert *et al.*, 1995). Besides, soluble sugars play an important role in various metabolomic pathways as a signaling molecule and are also involved in structural components of cells (Rosa *et al.*, 2009).

Proline acts as a source of nitrogen besides being used as a signaling molecule, and also acts as an osmo-protectant under stressful conditions (Hayat *et al.*, 2012). In the present study, we found that the proline content of *D.variabilis* exhibited a dose-dependent increase with increasing Cd and Pb stress (Figure 2). This increase is indicative of a correlation between ROS generation and ROS scavenging by proline. Similar results have been reported in tea leaves and roots treated with Al-stress (Hajiboland *et al.*, 2013). However, under the influence of chemical amendments, proline content declined significantly, indicating the release of stabilization of the plant under stressful condition by the influence of these chelating inorganic agents, as noted earlier by Wirosoedarmo *et al.* (2018).

Antioxidant enzymes

Generation of ROS due to heavy metal stress disrupts the various physiological pathways in plants and thus, weakens the defense system by suppressing the activities of osmolytes and antioxidant enzymes (Liu et al. 2010; Ahmad et al., 2012). Stimulation of antioxidant enzymes (SOD, POD, CAT and APX) is the foremost step to reduce the oxidative stress in plants growing at polluted sites. SOD causes reduction of $O_2^{\cdot-}$into H_2O_2 and O_2 and thus maintains $O_2^{\cdot-}$ radicle in an equilibrium state (Ashraf *et al.*, 2010 a, b; Ashraf *et al.*, 2015; Chen *et al.*, 2015). In our study, SOD level increased significantly in a dose-dependent manner when plants were subjected to Cd and Pb stress (Figure 5) and this could be due to over-production ROS or over-expression of SOD-encoding genes (Feng-Tao *et al.*, 2013; Shu *et al.*, 2015). However, in higher concentrations of both Cd and Pb, there was a significant decline in SOD activity, possibly due to enzyme inactivation because of feedback inhibition process. A similar situation was found due to Cd, Co and Pb stress in *Aeluropus littoralis* (Rastgoo and Alemzadeh, 2011). However, under the influence of inorganic amendments, the SOD concentration is enhanced, which causes suppression of oxy free radicles. Similarly, H_2O_2 generated as a byproduct of SOD is toxic at higher levels, and needs are removed by conversion to H_2O through biocatalytic, including CAT, APX and POD. Data on enzyme activities in response to the augmentation of chemical amendments (EDTA and SDS) are depicted in Figure 5. Similar observations were made in *Jatropha curcas* L. (Shu *et al.*, 2015). The decline observed in CAT activity might be due to enzyme inactivation by excess ROS production or inhibition of enzyme synthesis (Sahu *et al.*, 2012). APX also catalyzes H_2O_2 to H_2O by employing ascorbic acid as a reducing agent and has a higher affinity for H_2O_2 than CAT. The present results revealed that APX is increasing in the plants, depending on the concentration ofCd and Pb and exposure time (Figure 5). The increased APX activity could be correlated to an adaptive mechanism to an enhanced level of ROS content generated during Pb treatment. POD also caused a reduction of H_2O_2 (Hu *et al.*, 2012); previous studies have shown a decline, rise or no change in POD activity with respect to heavy metal stress (Schützendübel and Polle, 2002; Dong *et al.*, 2006; Wang *et al.*, 2016). In the present investigation, POD activity increased significantly at E1 (Figure 5) substantiating the findings of Malar *et al.* (2015) with *Eichhornia crassipes* and of Wang *et al.* (2016) with *Dimocarpus longan* grown under Pb stress. It has been observed that POD activity in metal-tolerant plants is adequately high to assist plants in protecting themselves against oxidative damage. Our results suggest that EDTA application sequesters oxidative stress in *D. variabilis* plants by increasing the activity of antioxidant enzymes. In this study, enhanced plant growth and photosynthetic parameters of *D. variabilis* might have a correlation with elevated activities of antioxidant enzymes due to application of EDTA under Cd stress (Figure 5) which decreased the production of H_2O_2 and MDA contents (Figure 5).

Metal phytoremediation enhanced by EDTA and SDS can be affected depending on various biogeochemical processes found in plants, metal, and the soil. Chemical amendment capacity is an important aspect, in order to reduce the time and cost needed for heavy metal contaminated soil. This can be done by increasing the heavy metal bioaccumulation index in plants (Hasan *et al.*, 2019).

Conclusions

Dahlia variabilis appears to be an ideal candidate for chelator-assisted phytoextraction. Extraneous application of EDTA and SDS can significantly improve plant growth, biomass and photosynthetic parameters by increasing the antioxidant enzymes activity and better accumulation of metals. The species studied have a better tolerance to Cd and Pb stress. The EDTA and SDS-assisted phytoextraction with *D. variabilis* could be a sustainable and promising approach to rejuvenate soil health contaminated with these metals.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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