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RESEARCH ARTICLE

EDTA ameliorates phytoextraction of lead and plant growth by reducing morphological and biochemical injuries in *Brassica napus* L. under lead stress

Urooj Kanwal · Shafaqat Ali · Muhammad Bilal Shakoor · Mujahid Farid · Sabir Hussain · Tahira Yasmeen · Muhammad Adrees · Saima Aslam Bharwana · Farhat Abbas

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Abstract *Brassica* species are very effective in remediation of heavy metal contaminated sites. Lead (Pb) as a toxic pollutant causes number of morphological and biochemical variations in the plants. Synthetic chelator such as ethylenediaminetetraacetic acid (EDTA) improves the capability of plants to uptake heavy metals from polluted soil. In this regard, the role of EDTA in phytoextraction of lead, the seedlings of *Brassica napus* L. were grown hydroponically. Lead levels (50 and 100 μM) were supplied alone or together with 2.5 mM EDTA in the nutrient culture. After 7 weeks of stress, plants indicated that toxicity of Pb caused negative effects on plants and significantly reduced growth, biomass, chlorophyll content, gas exchange characteristics, and antioxidant enzymes activities such as superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT). Exposure to Pb induced the malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) generation in both shoots and roots. The addition of EDTA alone or in combination with Pb significantly improved the plant growth, biomass, gas exchange characteristics, chlorophyll content, and antioxidant enzymes activities. EDTA also caused substantial improvement in Pb accumulation in *Brassica* plants. It can be deduced that application of EDTA significantly lessened the adverse effects of lead toxicity. Additionally, *B. napus* L. exhibited greater degree of tolerance against Pb toxicity and it also accumulated significant concentration of Pb from media.

Keywords Antioxidant enzymes activities · *Brassica napus* L · EDTA · Phytoextraction · Lead · Toxicity

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U. Kanwal · S. Ali (✉) · M. B. Shakoor · M. Farid · S. Hussain · T. Yasmeen · M. Adrees · S. A. Bharwana · F. Abbas
Department of Environmental Sciences, Government College University, Allama Iqbal Road, 38000 Faisalabad, Pakistan
e-mail: shafaqataligill@yahoo.com

Introduction

Pollution of urban and suburban soils by heavy metals signifies a severe ecological problem (Yang et al. 2010; Vacullk et al. 2009). This is a major environmental concern stemming from unexpected enterprise of industrialized division (Xian 1987). Numerous studies have been accomplished to consider phytoavailability of heavy metals in contaminated soils using metal chronological withdrawal methods (Ahmad et al. 2011).

Heavy metals are nondegradable in nature and have long life to be persistent in soil (Chen et al. 2000). Lead (Pb) is a toxic heavy metal because it is very destructive to animals, plants, and humans (Kirkham 2006). The contamination of agricultural, urban, and suburban soils caused by heavy metals is a severe problem and needs immediate remediation. Such contamination is mainly due to anthropogenic activities such as unsystematic use of pesticides, discharge of untreated industrial wastes and effluents, illegal solid waste disposal and dumping, high rate of burning of fossil fuels, mining, etc. putting huge burden of Pb toxicity (Alihan et al. 2010). Lead could harmfully affect seed germination (Obidzinska 1998), instruct folio chlorosis, stunt root and shoot growth and decrease the process of photosynthesis (Ahmad et al. 2011).

High biomass production and greater tolerance, such as *Brassica napus* L. are highly capable of extracting large quantities of trace metals by depositing sufficient amounts of metal concentrations in their roots and shoots.

One effective remediation method of metal-contaminated soil is phytoremediation that utilizes plants to eradicate harmful contaminants from soil and water. This method has become a corporeal substitute to conventional methodologies. On the whole, very few plants are well known and capable of sequestering, absorbing, and depositing more than one metal effectively (Mohd et al. 2010).

Generally, it is very difficult to decontaminate Pb-polluted fields by using plants alone, because metal is frequently accumulated in surface soil layers and only a small portion is present in soil solutions (Saifullah et al. 2009). To enhance metal bioavailability, chemical chelating agents have been commonly used to assist the phytoextraction of heavy metals from the polluted areas (Nowack et al. 2006), especially of lead (Saifullah et al. 2009). Among chelators, the EDTA is used most widely because it has high efficiency in removing heavy metals (Komarek et al. 2007). Even though, the, effect of EDTA regarding Pb accumulation and uptake through plants has not been clearly described in past. EDTA enhanced metal uptake and root to shoot translocation of Pb was observed in many plants (Ruley et al. 2006). Thus, this study was planned to investigate the effects of EDTA on different morphological, physiological, and biochemical parameters of *B. napus* L. under Pb stress. Pb uptake and accumulations were also investigated in this study.

Materials and methods

Experimental site

The experiment was performed in wire house of Ayub Agricultural Research Institute (AARI) and analytical work was performed in labs of Government College University, Faisalabad.

Growth conditions

Seeds of *B. napus* L. (Faisal canola) were obtained from Ayub Agricultural Research Gene Bank, Faisalabad. The healthy seeds were rinsed with distilled water thoroughly and sown in trays containing 2 in. of layers of sterilized quartz sand and were put in growth chamber with temperature 20–22 °C. After 2 weeks, the uniform seedlings were wrapped with foam at root shoot junction and translocated in thermopore sheets having holes in them and floating on 40 l capacity of water iron tub, lined with polythene sheet, containing modified Hoagland's solution. Hoagland's nutrient solution that contain $K(NO_3)_2$ 3,000 μM; $Ca(NO_3)$ 2,000 μM; $KH_2(PO_4)$ 100 μM; $MgSO_4$ 1,000 μM; H_3BO_3 50 μM; $MnCl_2.4H_2O$ 0.05 μM; $ZnSO_4.7H_2O$ 0.8 μM; $CuSO_4.5H_2O$ 0.3 μM; $H_2MO_4.H_2O$ 0.10 μM; and FeNa-EDTA 12.5 μM. With an air pump, aeration was supplied constantly. The solution renewed every 7 days. The design of the experiment was complete randomized design (CRD).

After 2 weeks of transplantation, uniform plants were treated with lead nitrate ($Pb(NO_3)_2$) and EDTA as T1: control (CK), T2: Pb (50 μM), T3: Pb (100 μM), T4: EDTA (2.5 mM), T5: Pb(50 μM)+EDTA (2.5 mM), and T6: Pb (100 μM)+EDTA (2.5 mM) with three replications, whereas in control, no $Pb(NO_3)_2$ and EDTA were applied. The pH was maintained at 6.0±0.1 during the experiment by adding 1 M sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) at alternate days.

Measurements

Plants were harvested after 7 weeks of Pb stress, and data regarding plant height, root length, number of leaves per plant, and fresh and dry weights of leaf, stem roots were collected.

Leaf area

Leaf area meter was used for measurements of leaf area.

Gas exchange characteristics

The gas exchange characteristics of *B. napus* L. were observed with the help of infrared gas analyzer (IRGA, CI-340, Analytical Development Company, Hoddesdon, England) which was used for measurement of photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and water use efficiency (A/E).

SPAD value

For the determination of SPAD value, SPAD-502 meter was used.

Determination of chlorophyll contents

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were determined spectrophotometrically (Halo DB-20/DB-20S, Dynamica Company, London, UK) (Metzner et al. 1965). Top most fully expanded leaves were taken to extract the pigments. The photosynthetic pigment contents were extracted from a known fresh weight of leaves in 85 % (*v*/*v*) aqueous acetone. The extract was centrifuged at 4,000 rpm for 10 min; the supernatant was then obtained and diluted with 85 % aqueous acetone to the

appropriate concentration for spectrophotometric analysis. The extinction was evaluated against a blank of a pure 85 % aqueous acetone at wavelengths of 663, 644, and 452.5 nm for chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively. Total chlorophyll and total carotenoids were calculated by using the following equations:

$$\text{Chlorophyll } a \ (\mu g\ ml^{-1}) = 10.3 * E_{663} - 0.98 * E_{644}$$
$$\text{Chlorophyll } b \ (\mu g\ ml^{-1}) = 19.7 * E_{644} - 3.87 * E_{663}$$
$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b$$
$$\text{Total carotenoids} (\mu g\ ml^{-1}) = 4.2 * E_{452.5} - \{(0.0264 * \text{chl } a) + (0.426 * \text{chl } b)\}$$

At the end, those pigment fractions were calculated as milligrams per mg g^{-1} fresh weight.

Assay of antioxidant enzymes

Antioxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) in roots and leaves were determined by spectrophotometer.

After 7 weeks of treatment, fully stretched leaves of the plants and roots samples were taken for enzymatic analysis. Leaves and roots were firstly chomped with mortar and pestle under chilled condition with liquid nitrogen. This pattern were standardized in 0.05 M phosphate buffer (maintaining pH at 7.8) and filtered through four layers of muslin cloth and for 10 min at 4 °C centrifuged at 12,000×*g*. Finally, this enzyme extract were used for quantification of SOD, POD activities following to (Zhang 1992).

Catalase (CAT, EC 1.11.1.6) activity was measured by the method described by (Aebi 1984). The assay mixture (3.0 ml) consisted of 100 μl enzyme extract, 100 μl H_2O_2 (300 mM), and 2.8 ml 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was determined by measuring the reduction in the absorbance at 240 nm as a result of H_2O_2 disappearance (ε=39.4 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was analyzed according to the method of (Nakano and Asada 1981). The reaction mixture contained 100 μl enzyme extract, 100 μl ascorbate (7.5 mM), 100 μl H_2O_2 (300 mM), and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation activity of ascorbate was monitored by the change in absorbance at 290 nm (ε=2.8 mM^{-1} cm^{-1}).

Determination of electrolyte leakage, MDA, and H_2O_2

Electrolyte outflow was checked through method described by Dionisio-sese and Tobita (1998) using pH/conductivity Model 720, Inco Lab Company, Kuwait. After treatment of 7 weeks, the uppermost completely extended leaves were cut in small parts of 5 mm length and positioned in test tubes in which there was 8 ml deionized and distilled water. The tubes was processed in incubator in water bath at 32 °C for 2 h then electrical conductivity of initial medium (EC1) was assessed. All of the samples were placed in autoclave at 121 °C for 20 min so that all electrolytes expel, then these samples were cooled to 25 °C then again electrical conductivity (EC2) was noticed and computed with formula.

$$EL = (EC1/EC2) \times 100.$$

The level of lipid peroxidation in leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using the method of Heath and Packer (1968), with minor modifications as described by Dhindsa et al. (1981) and Zhang and Kirkham (1994). A 0.25-g leaf sample was homogenized in 5 ml 0.1 % TCA. The homogenate was centrifuged at 10,000×*g* for 5 min. To 1-ml aliquot of the supernatant, 4 ml of 20 % TCA containing 0.5 % TBA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000*g* for 10 min, the absorbance of the supernatant at 532 nm was read and the value for the nonspecific absorption at 600 nm was subtracted. The MDA content was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Hydrogen peroxide (H_2O_2) was extracted by homogenizing 50 mg leaf or root tissues with 3 ml of phosphate buffer (50 mM, pH 6.5). Then, the homogenate was centrifuged at 6,000*g* for 25 min.

To measure H_2O_2 content, 3 ml of extracted solution was mixed with 1 ml of 0.1 % titanium sulfate in 20 % (*v*/*v*) H_2SO_4 and the mixture was then centrifuged at 6,000*g* for 15 min. The intensity of the yellow color of the supernatant was measured at 410 nm. H_2O_2 content was computed by using the extinction coefficient of 0.28 μmol^{-1} cm^{-1}.

Estimation of lead concentration

Known weight of sample (0.5 g) was taken in a flask of 100 ml and then added 15 ml of concentrated HNO_3 in flask through pipette. After mixing, the sample flasks were put on hot plate

which temperature was gradually increased up to 275 °C, which cause dense yellow fumes from flask. When quantity of dense yellow fumes became low, then we added hydrogen peroxide until dens yellow fumes disappeared. When samples became colorless, the flasks were removed from hot plate and shifted to lab where its volume was made up to 25 ml by using distilled water and Pb contents in root, stem, and leaf was determined by using flame atomic absorption spectrometry (Nov Aa 400 Analytik Jena, Germany) by using the method described by Ehsan et al. (2013) with some modifications.

Statistical analysis

All values described in this study are mean of three replicates. Analysis of variance (ANOVA) was done by using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL) followed by Tukey's test between the means of treatments to determine the significant difference.

Results

Plant growth characteristics

The response of *B. napus* L. in term of growth parameters like plant height, root length, number of leaves per plant, and leaf area in applied conditions is shown in Table 1. The *B. napus* L. showed significant and visible symptoms of toxicity when exposed to Pb stress in term of reduction in growth parameters as compared to control one. Furthermore, the reduction was clearer at higher Pb concentration (100 μM).

The application of 2.5 mM EDTA in solution medium significantly decreased Pb induced growth inhibition features. The application of EDTA improved the growth characteristics by reducing the inhibitory effects at both levels of Pb stress. Alone, EDTA application presented significant increase; moreover, the positive effects of EDTA were more obvious at Pb 50 μM.

Plant biomass

The reaction of plant biomass parameters such as fresh and dry weight of leaf, stem, and root is shown in Table 1. Application of Pb produced a major reduction in plant biomass parameters, and this reduction was dose dependent. The addition of EDTA expressively increased fresh and dry weights of leaf, stem, and root under both concentrations of Pb (50 and 100 μM) treatment. Addition of Pb alone under different dose levels (50 and 100 μM) decreased the fresh and dry weights of different parts of the plant, and EDTA increased them significantly by increasing the plant tolerance. The correlation between Pb concentration and biomass of *B. napus* L. is given in Table 2. The results showed that the

Table 1 Effect of different concentrations of lead and EDTA on growth and biomass of *B. napus* L

Treatments	Root length (cm)	Plant height (cm)	Leaf area (cm^2)	No. of leaves $plant^{-1}$	Root fresh weight (g)	Root dry weight (g)	Stem fresh weight (g)	Stem dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)
CK	36.10±0.91b	62.49±1.23b	314.66±6.1a	19.66±0.57a	19.76±0.80a	2.69±0.02a	17.93±0.07a	2.72±0.02a	51.23±1.06a	5.23±0.15b
EDTA	40.37±1.29a	69.40±2.44a	319.26±7.0a	21.33±0.61a	20.67±0.75a	2.77±0.02a	17.731±0.30a	2.85±0.03b	55.41±2.12a	5.82±0.08a
Pb50	28.70±0.43d	48.50±0.92d	270.07±4.9c	16.16±0.28c	15.91±0.29c	1.54±0.08c	14.41±0.45c	1.72±0.02d	35.28±1.99c	3.77±0.07d
Pb50+EDTA	32.63±1.08c	54.91±1.11c	296.12±5.3b	17.63±0.41b	18.24±0.69b	1.81±0.05b	16.26±0.30b	2.06±0.08c	42.86±0.73b	3.66±0.05c
Pb100	23.21±0.70f	33.83±0.99f	204.56±4e.	13.43±0.40d	11.63±0.37e	0.99±0.05e	10.46±0.41e	1.34±0.03f	25.10±0.91e	2.46±0.10f
Pb 100+EDTA	25.95±0.47e	41.29±1.49e	241.82±7.7d	14.66±0.12e	12.83±0.54d	1.28±0.06d	12.46±0.39d	1.57±0.03e	30.33±0.82d	3.12±0.03e

Values are the means of three replicates±S.D. Means followed by similar *small letters* are not significantly different at $P \leq 0.05$

Table 2 Pearson correlation among Pb concentration in the leaf, stem, and root and biomass of *B. napus* L

	Pb concentration in roots	Pb concentration in stems	Pb concentration in leaves	Root fresh weight	Root dry weight	Stem dry weight	Stem fresh weight	Fresh weight leaf
Pb concentration in stems	0.992**							
Pb concentration in leaves	0.995**	0.999**						
Root fresh weight	0.998**	0.998**	0.999**					
Root dry weight	0.996**	1.000**	1.000**	0.999**				
Stem dry weight	0.997**	0.999**	1.000**	1.000**	1.000**			
Stem fresh weight	0.997**	0.999**	0.999**	1.000**	1.000**	1.000**		
fresh weight leaf	0.996**	0.999**	1.000**	1.000**	1.000**	1.000**	1.000**	
Dry weight leaf	0.997**	0.999**	0.999**	1.000**	1.000**	1.000**	1.000**	1.000**

**Correlation is significant at 0.01 level (two-tailed)

Pb concentrations in all three parts of plants viz root, stem ,and leaves significantly affected the fresh and dry biomass of plant at different treatments.

Gas exchange attributes

Figure 1a–d demonstrates the variations in gas exchange attributes of *B. napus* L. driven by Pb or EDTA either in combination or alone in culture medium. A significant decrease was observed in gas exchange characteristics such as net photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency of *B. napus* L. at both level of Pb stress as compared to control, and the reduction was dose dependent.

Application of EDTA alone had a progressive effect on gas exchange characteristics of *B. napus* L. Chelating properties of EDTA appeared to be more subsequent and tangible in reducing the inhibitory effect of Pb and also enhanced the gas exchange attributes. EDTA has an individual positive effect under Pb stress, and its effects were more clear and visible when added in combination with Pb.

Chlorophyll contents and SPAD value

The effects of different treatments of Pb and EDTA on chlorophyll contents and SPAD values are also demonstrated in Fig. 1e–i. A significant decrease was detected under both levels of Pb (50 and 100 μM) stress as related to control one having no Pb and EDTA.

Addition of EDTA alone boosted chlorophyll *a*, *b*, total chlorophyll, and carotenoid contents significantly in the leaves of *B. napus* L. associated to those of controls. The supreme chlorophyll values were detected at EDTA alone but no clear variation was observed from controls one. SPAD values also followed the same trend in the leaves of *B. napus* L.

Activities of antioxidant enzymes

The activities of SOD, CAT, POD, and APX in the leaves and roots of *B. napus* L. exposed to Pb stress and EDTA are shown in Fig. 2. Lead has considerable effect on the activities of antioxidant enzymes in leaves and roots of *B. napus* L. at both stress levels of Pb. The additions of Pb 50 μM ominously boosted the activities of antioxidant enzymes as associated to Pb 100 μM and control one. Application of EDTA significantly increased the activities of antioxidant enzymes and exhibited a synergetic effect. The application of EDTA and Pb alone had no severe effects on the contents of POD and APX in both roots and leaves while higher effects were noted in SOD activities. The combination of EDTA into the Pb treatments improved the activity of antioxidant enzymes as compared to Pb alone.

Table 3 describes the correlation among antioxidant enzymes activities and Pb concentration. The increase in Pb concentration significantly affected the antioxidant enzymes activities.

MDA, H_2O_2, and electrolyte leakage

A significant effect on electrolyte leakage, H_2O_2 and MDA content in leaves and roots of *Brassica napus* L was observed under Pb stress as shown in Fig 3 respectively. Under both levels of Pb stress (50 and 100 μM) an increase was observed in Electrolyte leakage, H_2O_2 and MDA content and the increase was dose dependent. The exogenous application of EDTA caused a significant reduction at both level of Pb stress as compared to alone one.

Lead contents

Lead contents in shoot (leaf, stem) and root of *B. napus* L. is given in Fig. 4a. The degree of increase in uptake

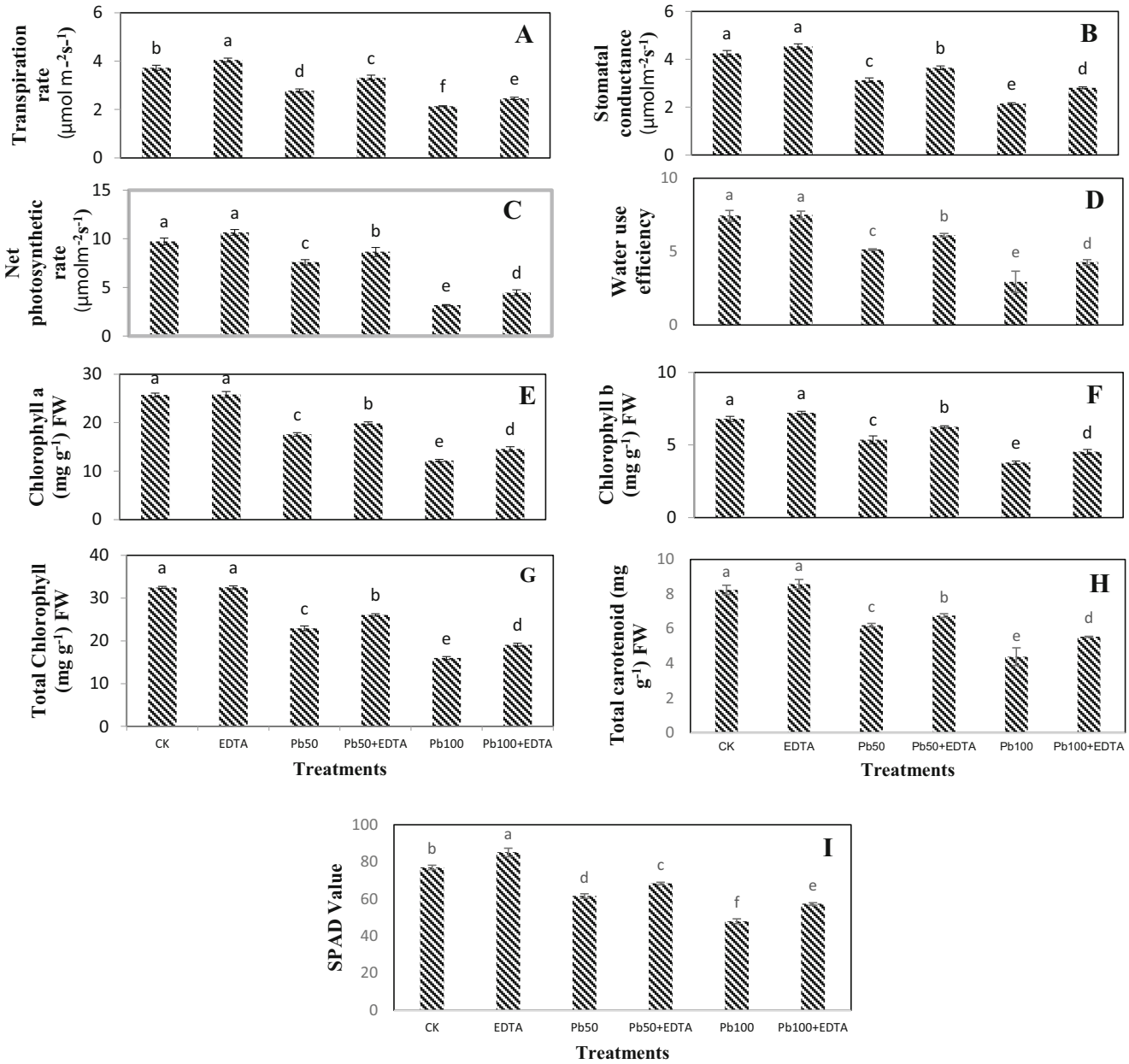

Fig. 1 Effects of different concentrations of lead (Pb) (0, 50, and 100 μM) and EDTA (0 and 2.5 mM) on transpiration rat (**a**) stomatal conductance (**b**), net photosynthetic rate (**c**), water use efficiency (**d**), chlorophyll *a*, *b* (**e**, **f**), total chlorophyll and total carotenides (**g**, **h**), and SPAD value (**i**) in *B. napus* L. Values shows the mean of three replicate± SE. Means followed by same *small letters* are not significantly different at $P \leq 0.05$

of lead in all three plant parts viz. root, stem, and leaves was dose dependent. At higher Pb level (100 μM) the lead concentration significantly increased in root regardless of lead levels followed by stem and leaf. EDTA application significantly increased Pb concentrations in root, stem, and leaf of plants at both Pb levels. Furthermore, the use of EDTA also improved the translocation of Pb from roots to aboveground parts of *Brassica*.

The bioconcentration factor (BCF) of Pb regarding leaf, stem, and root is given in Fig 4b. The maximum concentration of Pb was found in roots followed by stem and leaf.

Discussion

Lead (Pb) is considered as one of the toxic heavy metals with unidentified biological function, and its concentration

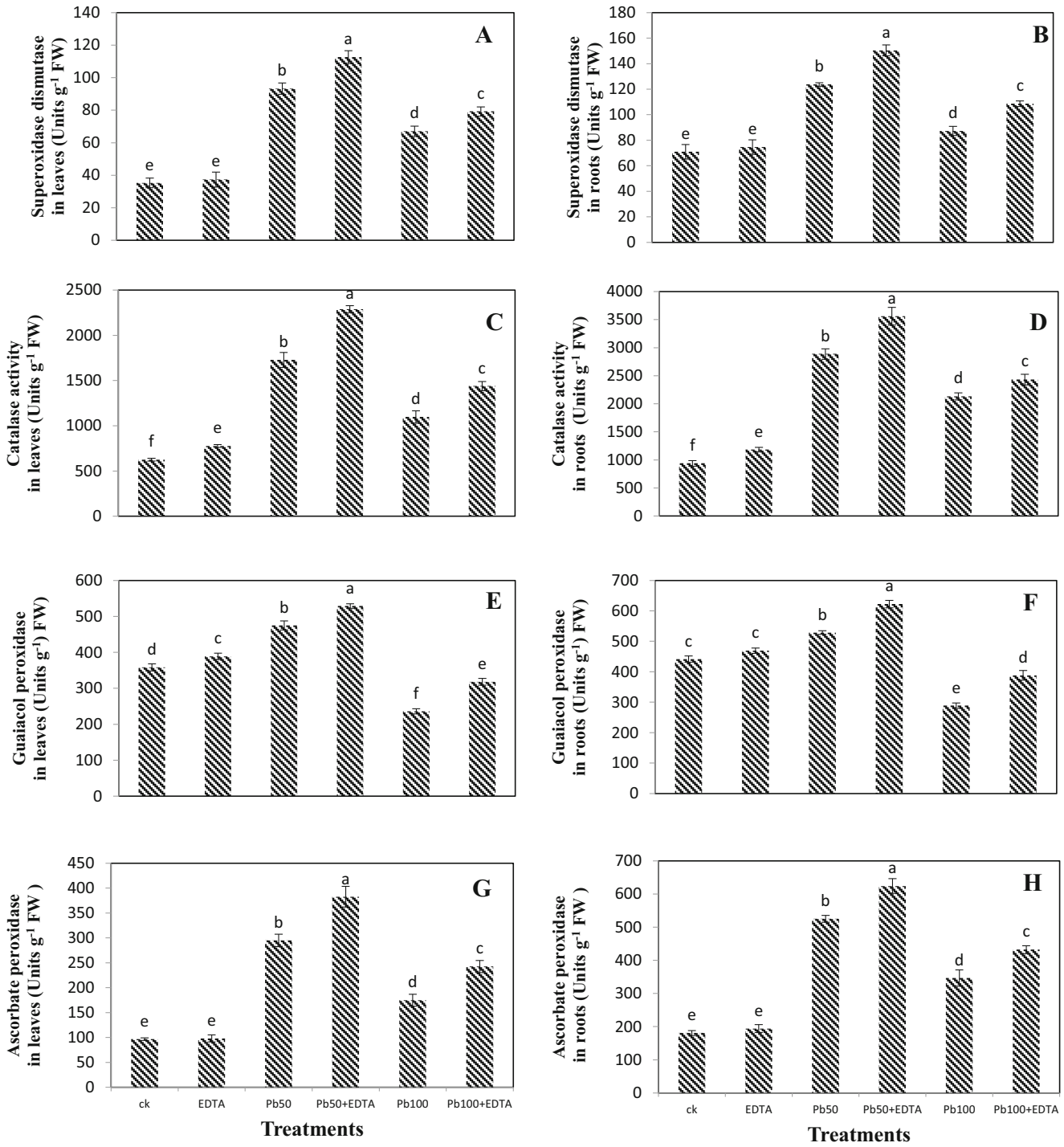

Fig. 2 Effects of different concentrations of lead (Pb) (0, 50, and 100 μM) and EDTA (0 and 2.5 mM) on SOD (**a**, **b**), CAT (**c**, **d**) POD (**e**, **f**), and APX (**g**, **h**) in the leaves and roots of *B. napus* L. Values shows the mean of three replicate±SE. Means followed by same *small letters* are not significantly different at $P \leq 0.05$

is being increased rapidly in agricultural soils due to its extensive use in industries (Hamid et al. 2010). It has been investigated that accumulation of heavy metals including Pb may cause many physiological, biochemical,

Table 3 Pearson correlation among Pb concentration in the leaf, stem, and root and antioxidant enzymes activities in the leaf and roots of *B. napus* L

	Pb root	Pb stem	Pb leaf	APX root	APX leaf	POD root	POD leaf	CAT root	CAT leaf	SOD root
Pb stem	0.992**									
Pb leaf	0.995**	0.999**								
APX root	0.502	0.472	0.499							
APX leaf	0.588	0.552	0.576	0.989**						
POD root	0.55	0.53	0.551	0.993**	0.990**					
POD leaf	0.496	0.465	0.489	0.996**	0.992**	0.994**				
CAT root	0.536	0.494	0.521	0.991**	0.992**	0.980**	0.992**			
CAT leaf	0.588	0.553	0.579	0.993**	0.998**	0.990**	0.991**	0.994**		
SOD root	0.599	0.564	0.59	0.989**	0.993**	0.985**	0.988**	0.996**	0.997**	
SOD leaf	0.608	0.565	0.592	0.983**	0.993**	0.974**	0.981**	0.995**	0.996**	0.997**

**Correlation is significant at 0.01 level (two-tailed)

and morphological alterations in exposed plants like reduction in chlorophyll contents, biomass, photosynthetic rate and uptake of necessary elements (Ali et al. 2013), root and shoot growth inhibition, chlorosis, and decline in water potential and production of plant hormones (Sharma and Dubey 2005). In this study, we tried to evaluate how exogenous application of EDTA controls Pb-induced variations in the *Brassica* plant growth and biochemical modifications. According to the results, we noticed that Pb stress causes significant toxic effects on plant growth and biomass of *B. napus* L. with respect to control. A remarkable decrease in growth and biomass of different plant parts was observed that might be caused by adverse effects of Pb toxicity on the roots, and consequently, plants were unable to uptake nutrients and continue to perform their normal activity. Lead toxicity has been reported to inhibit the growth of different plant species (Gopal and Rizvi 2008; Sharma and Dubey 2005), which is partially in accordance with the results of our present experiment. However, application of EDTA along with two levels of lead significantly enhanced all plant growth and biomass parameters. This was also confirmed by Ruley et al. (2006) who analyzed the effects of Pb and chelates on the growth and photosynthetic activity in *Sesbania drummondii* in a soil polluted with 7.5 g kg^{-1} of $Pb(NO_3)_2$. They further investigated that application of EDTA mitigated the negative effects caused by Pb. A significant increase was observed in plant shoot and root weights when EDTA was applied in combination with Pb.

The results showed that Pb toxicity significantly decreased the chlorophyll contents and gas exchange parameters in the leaves of *B. napus* L. as compared to control plants but exogenous application of EDTA enhanced the chlorophyll contents and gas exchange parameters under lead stress. Decline in chlorophyll contents under metal toxicity may be the reaction of plants to metal stress, ultimately resulted in chlorophyll reduction and inhibition of photosynthetic characteristics (Gajewska et al. 2006). Many researchers have examined that heavy metals can disturb chlorophyll contents, gas exchange parameters, and stomatal conductance, subsequently photosynthetic rate decreased in plants exposed to metal stress (Wahid et al. 2007; Balakhnina et al. 2005).

In response to metal stress including Pb stress, plants cells have developed antioxidant defense mechanism to decrease oxidative damage. The antioxidant enzymes comprises of SOD, POD, CAT, APX, and GR which regulate the cellular superoxide (O_2^-) and hydrogen peroxide (H_2O_2), concentration, thus inhibiting the production of ^{-}OH radicals (Rucinska-Sobkowiak and Pukacki 2006). SOD and CAT play a key role in removal of oxidative stress (Gomes-Junior et al. 2006). In our present experiment, activities of antioxidants like SOD, POD, APX, and CAT significantly decreased under the Pb stress. Meanwhile, application of EDTA remarkably improved these activities in leaves and roots of *B. napus* L. under the Pb stress conditions. In present study, the antioxidant enzymes increased their activities under the combined treatment of EDTA and Pb at different concentrations. Our results are in similarity with the findings of Najeeb et al. (2009), who observed similar increase in activities of antioxidants under EDTA applications along with metal stress.

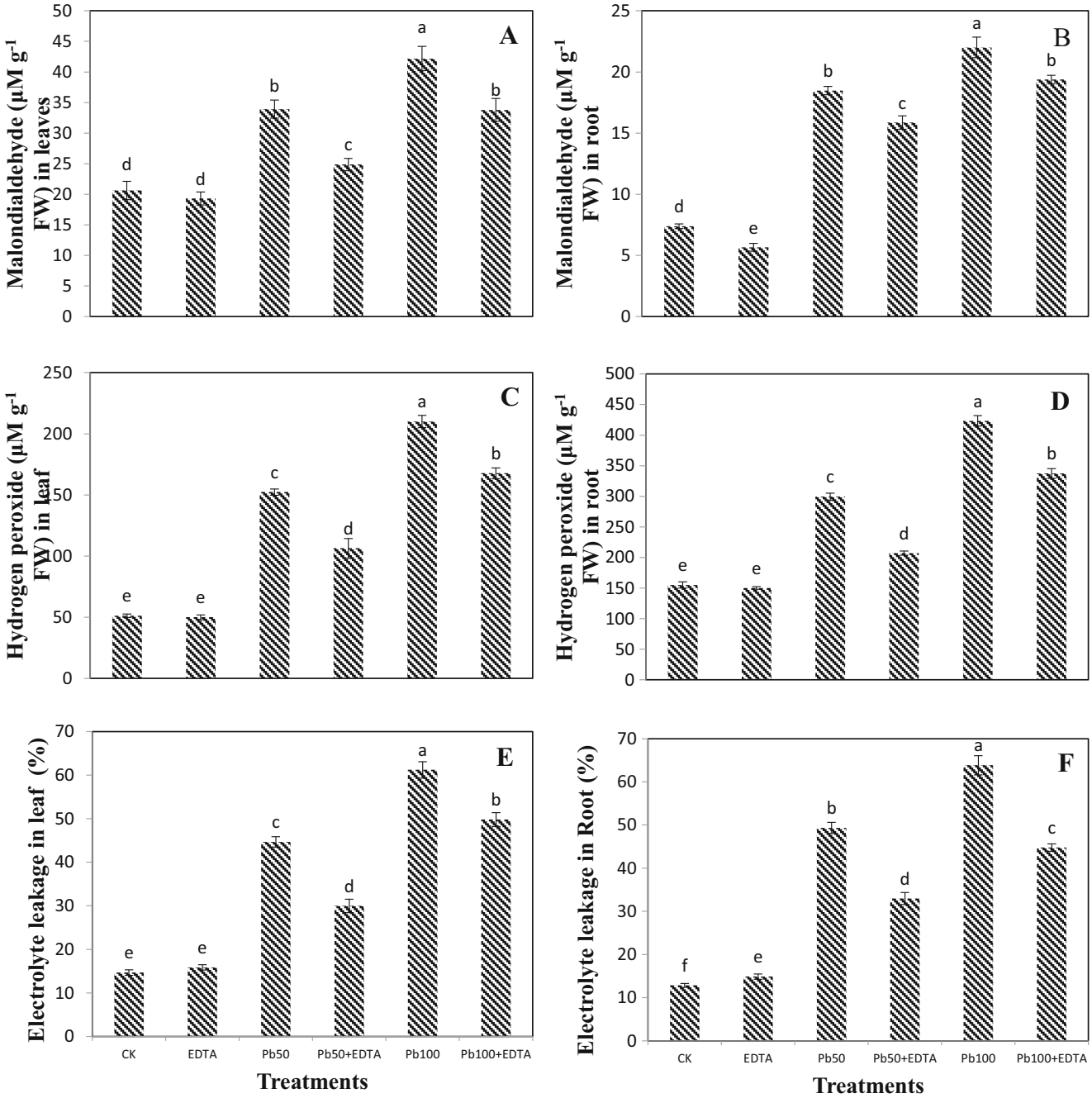

Fig. 3 Effects of different concentrations of lead (Pb) (0, 50, and 100 µM) and EDTA (0 and 2.5 mM) on MDA (**a**, **b**), H_2O_2 (**c**, **d**), and electrolyte leakage (**e**, **f**) in the leaf and root of *B. napus* L. Values shows the mean of three replicate±SE. Means followed by same *small letters* are not significantly different at $P \leq 0.05$

It was also noticed that Pb decreased soluble protein in both roots and leaves of *B. napus* L. It is probably due to more oxidative injury that decreased protein contents (Gupta et al. 2009). Enhanced reactive oxygen species (ROS) was observed under metal toxicity as shown by increased electrolyte leakage. Plants normally face the oxidative stress when exposed to heavy metals (Erdei et al. 2002; Macfarlane 2003). Oxidative injury observed to be involved in Pb stress as indicated by decrease in some antioxidants and rise in ROS (O_2^-, H_2O_2) activities. It

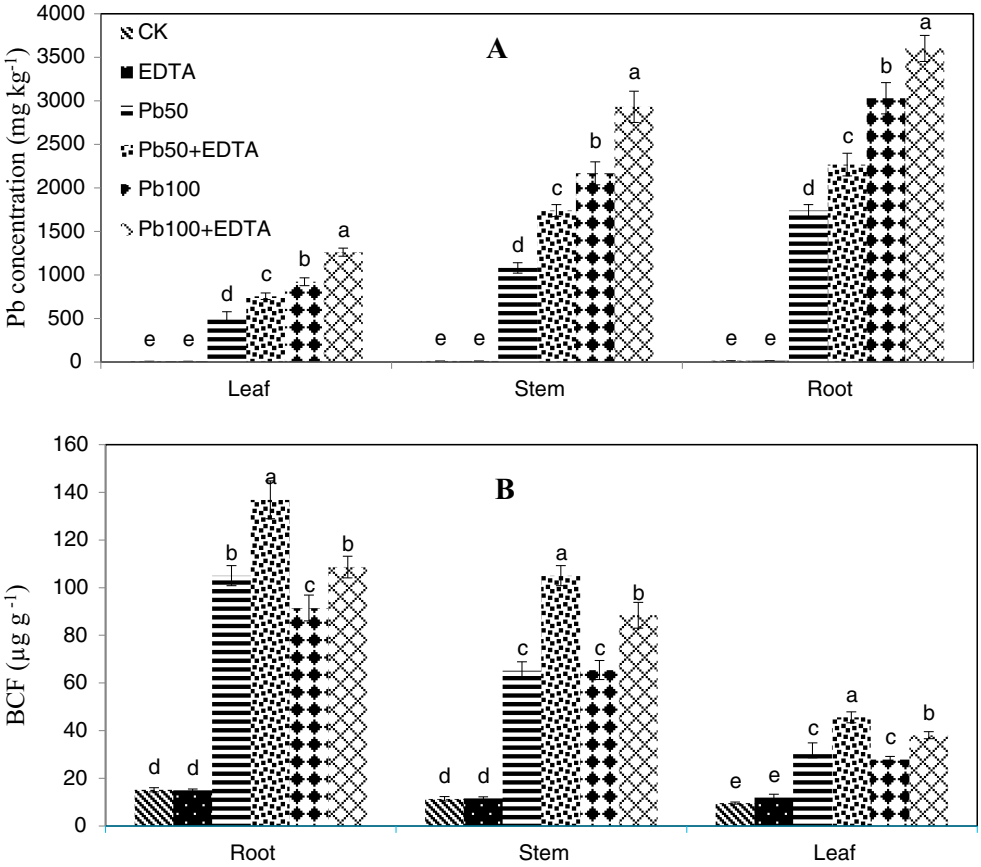

Fig. 4 Effects of different concentrations of lead (Pb) (0, 50, and 100 μM) and EDTA (0 and 2.5 mM) on uptake of Pb in the leaf, stem, and root (**a**) and bioconcentration factor BCF (**b**) of *B. napus* L. Values shows the mean of three replicate±SE. Means followed by same *small letters* are not significantly different at $P \leq 0.05$

was also documented that peroxidases are vital components of the plant defense mechanism against Pb by H_2O_2 scavenging (Singh et al. 2006) as in *Phaseolus vulgaris* (Smeets et al. 2005).

Phytoextraction efficiency of a plant strongly based on the transpiration rate of the plant and can be significantly increased by enhancing transpiration rate (Grifferty and Barrington 2000). In our current study, it was proved that lead contents in all three parts of plant were increased as we enhanced lead concentrations in media. EDTA application further ameliorated uptake of lead and a significant improvement was observed in lead contents with the application of lead as compared to lead alone treated plants. The increase in Pb uptake with EDTA can be clarified by its effect on increasing the absorption and solubility of Pb-EDTA complex by plants (Santos et al. 2006; Wang et al. 1995). The increased Pb-uptake with the amendment of EDTA was not as high as investigations stated by other investigators (Schmidt 2003). However, Huang et al. (1997) documented more than a 100-fold rise in Pb accumulation in plant shoots with the application of EDTA. Similarly Blaylock et al. (1997) documented that the concentration of Pb in shoots of Indian mustard enhanced from <100 to 15,000 mg kg^{-1} of lead, when the plants were grown in soil having Pb amended with EDTA.

Conclusion

It can be deduced that EDTA plays a vital role to enhance growth and development of *B. napus* L. exposed to abiotic stress. During our current research work, we determined that toxicity of lead can cause reduction in the growth, biomass, pigments, photosynthetic characteristics, and antioxidant enzyme capacity. However, EDTA addition significantly improves the morphology, photosynthetic attributes, and antioxidant enzyme capacity. Therefore, in view of these results, it can be concluded that EDTA has favorable role on the *Brassica* plants grown under Pb toxicity. Our results also demonstrate that *B. napus* L. might uptake a substantial amount of toxicant like Pb and it also considered as hyper accumulator plant. Additionally, our experiment is carried out in hydroponic conditions; so as to evaluate the improving role of EDTA more effectively in phytoextraction of lead from polluted soils, more soil-based environment study is necessary.

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