Environ Sci Pollut Res (2015) 22:13305–13318
DOI 10.1007/s11356-015-4595-3

RESEARCH ARTICLE

Phytoremediation of cadmium improved with the high production of endogenous phenolics and free proline contents in *Parthenium hysterophorus* plant treated exogenously with plant growth regulator and chelating agent

Nasir Ali[1] · **Fazal Hadi**[1]

Received: 9 November 2014 / Accepted: 23 April 2015 / Published online: 5 May 2015

Abstract Pot experiments were conducted to evaluate the effects of gibberellic acid (GA_3) and ethylenediaminetetraacetic acid (EDTA) on growth parameters, cadmium (Cd) phytoextraction, total phenolics, free proline and chlorophyll content of *Parthenium hysterophorus* plant grown in Cd-contaminated (100 mg/kg) soil. GA_3 was applied as foliar spray (10^{-2}, 10^{-4} and 10^{-6} M) while EDTA (40 mg/kg soil) was added to soil as single and in split doses. Results showed decrease in growth parameters due to Cd stress but *P. hysterophorus* plant demonstrated Cd hyperaccumulator potential based on bioconcentration factor (BCF). Lower concentration of GA_3 (10^{-6} M) showed highest significant increase in the growth parameters while Cd concentration, accumulation (1.97±0.11 mg/DBM) and bioconcentration (9.75±0.34) was significantly higher in the treatment T11 (GA_3 10^{-2}+split doses of EDTA). Cadmium significantly increased the root free proline while total phenolic concentration was significantly high in all parts of the plant. Chlorophyll contents were significantly reduced by Cd. GA_3 showed significant increase in phenolic and chlorophyll contents in plant. Cadmium accumulation in plant tissues showed positive correlation with free proline (R^2= 0.527, R^2=0.630) and total phenolics (R^2=0.554, R^2=0.723) in roots and leaves, respectively. Cd contents negatively correlated with biomass, chlorophyll and total water contents. Proline and phenolic contents showed positive correlation with dry biomass of plant. These findings suggest further investigation to study the role of endogenous phenolics and proline in heavy metal phytoremediation.

Keywords Gibberellic acid · EDTA · Proline · Phenolics · Phytoextraction

Responsible editor: Philippe Garrigues

✉ Fazal Hadi
fazalbiotech@yahoo.com

[1] Department of Biotechnology, Faculty of Biological Sciences, University of Malakand, 18800 Chakdara, Khyber Pakhtunkhwa, Pakistan

Introduction

Cadmium is one of the toxic heavy metals of great environmental concern which enters the agricultural soil mostly through anthropogenic activities such as mining, sewage effluents, pesticides, chemical fertilizer application and industrial waste disposal (Singh et al. 2003; Kidd et al. 2007; Adewole et al. 2010; Mazharia and Homaeeb 2012; Hadi et al. 2014). From soil and water, it can easily be absorbed and accumulated in plant tissues due to its high bioavailability in soil and consequently reaches the human bodies through food chain (Alkorta et al. 2004; Liu et al. 2009; Ambedkar and Muniyan 2013). Crops cultivated in polluted soil may accumulate cadmium (Cd) in different parts, i.e. roots, leaves and fruits. Consumption of Cd-polluted plants may develop a number of Cd-related chronic diseases such as cancer, oxidative stress, tissue necrosis and impairment of the kidney and liver (Ogawa et al. 2004; Liu et al. 2005; Simmons et al. 2005; Åkesson et al. 2006; John et al. 2008; Kafel et al. 2014;). Heavy metals are not biodegradable (by microorganisms and plants) and continuously accumulate in soil, and their presence in soil (especially agricultural soil) is of great concern for both plant and animal health (Mubeen et al. 2010; Okedeyi et al. 2014). For clean and sustainable environment, the removal of toxic heavy metals from soil and water is very important and needs the development of effective, affordable and environment-friendly technologies. Various conventional methods (including both chemical and physical) have been

used for the restoration of heavy metal-contaminated soil, but these methods are very costly and laborious and adversely affect both the soil structure and ecosystem. The discovery of some plants having ability to accumulate and tolerate high concentrations of heavy metals led to the development of a new plant-based technology, known as phytoremediation (Reeves and Brooks 1983; Baker and Brooks 1989; Entry et al. 1999). Phytoremediation technology is cost effective, solar driven, aesthetically pleasing and environment friendly (Schwitzguebel et al. 2009; Chai et al. 2012). More than 400 species of plants have been investigated for their heavy metal phytoremediation potential, and most of these plants belong to *Arabidopsis*, *Brassica*, *Sedum* and *Thlaspi* species (Lone et al. 2008). In the present research, *Parthenium hysterophorus* was studied at its reproductive stage for its Cd phytoextraction potential. This plant belongs to Asteraceae family and is native species of America, which invaded Australia, India, Pakistan and some parts of Africa (Parsons and Cuthbertson 1992; Dhawan and Dhawan 1996). It is a fast-growing, stress-tolerant perennial herb, which is unpalatable to herbivores, thus prevents metal entrance into food chain. In previous literature, *Parthenium* has been used for lead (Pb) phytoextraction (Hadi and Bano 2009).

Plants grown on metal-contaminated soil often show slow growth and lower biomass and accumulate lower metal concentration within the biomass (Persans and Salt 2000; Li et al. 2003). For efficient heavy metal phytoremediation, plant should have high biomass and can also tolerate and accumulate high concentration of toxic heavy metal within their tissues. Heavy metal-tolerant plants generally have lower biomass or most plants showing high biomass do not show tolerance to heavy metals in soil. To increase biomass as well as metal phytoextraction potential of plants, several chemical modifications (applied to plant or added to soil/ water) have been done by several scientists, such as the application of hormones and metal chelators (Kamnev and Van Der Lelie 2000; Chen and Cutright 2001; Hadi and Bano 2009; Falkowska et al. 2011). In the present experiment, a plant hormone gibberelic acid (GA_3) and a synthetic chelator ethylenediaminetetraacetic acid (EDTA) were used for increasing the Cd phytoremediation potential of the plant. GA_3 enhances plant growth and biomass while EDTA increases the metal bioavailability in soil by forming complexes with metals (Benjerano and Lips 1970; Broughton and McComb 1971; Chen et al. 2004; Hadi and Bano 2009; Hadi et al. 2010).

Plant under stress conditions produce and accumulate a variety of metabolic products including amino acids (such as proline) and phenolic compounds (Grace and Logan 2000; Diaz et al. 2001; Sakihama and Yamasaki 2002). Many investigators have reported accumulation of free proline under conditions of salinity, drought, intense light and ultraviolet radiation and heavy metals and in response to oxidative stress and biotic stresses (Fabro et al. 2004; Choudhary et al. 2005; Haudecoeur et al. 2009; Yang et al. 2009). Proline not only takes part in protein synthesis but also showed a positive correlation with plant stress tolerance. Proline maintains osmotic or cell turgor pressure, reduces electrolyte leakage by stabilizing membranes and protects plant from oxidative stress by reducing concentration of reactive oxygen species (ROS) (Xu et al. 2009; Hayat et al. 2012). Similarly, phenolic compounds are produced during heavy metal stress and act as antioxidant and directly scavenge ROS (Michalak 2006). ROSs can destroy lipids, DNA, proteins and chlorophyll by producing highly reactive (nascent) oxygen (Ramadevi and Prasad 1998). High concentrations of phenolic compound have been reported in different plants such as wheat in response to nickel toxicity (Diaz et al. 2001), *Phaseolus vulgaris* when exposed to cadmium, *Phyllanthus tenellus* leaves in response to copper sulphate (Diaz et al. 2001) and maize due to aluminium (Winkel-shirley 2002).

The present study was carried out with objectives to evaluate the effect of plant growth regulator (gibberellic acid, GA_3) and a chelating agent (EDTA) either alone or in different combinations (synergistic effect) on (1) growth and biomass of the *P. hysterophorus* plant in Cd-contaminated soil; (2) concentration of proline and phenolics in different parts of the plant; (3) Cd absorption, its translocation into plant shoot and accumulation in different parts of the plant; (4) effect of Cd on contents of chlorophyll in leaves; and (5) correlation of total phenolics and free proline with dry biomass and Cd contents of plant parts.

Materials and methods

Preparation of soil and addition of cadmium

Soil was collected from fields nearby the University of Malakand at Chakdara, Pakistan. The soil was grounded into powdered form after drying in sunlight. Water holding capacity (300 ml water/kg soil±3) and pH (6.5±0.3) of the soil was calculated. The dried soil was then added into plastic pots (18 cm height×15 cm diameter) at the rate of 1 kg soil/ pot. Cadmium (100 mg/kg soil) was added to each pot as cadmium acetate dihydrate $(CH_3COO)_2Cd{\cdot}2H_2O$ (Merck, Germany) solution and allowed for 2 months. No cadmium was added to the control (C) pots.

Transplantation of seedlings and plant growth

Each pot was watered a day before transplantation of plantlets. Seedlings (*P. hysterophorus* plantlets) of uniform size were selected and single plantlet was transferred into each pot. Three replicate pots were used for each treatment and controls. Two controls were used, one without cadmium (C) and the other with cadmium only (C1). C and C1 were compared for

the effect of Cd on growth. Plants were watered, at 3-day interval. Plants were grown under natural condition of light and temperature (35/25 °C).

Treatments used

The plants were treated as given in Table 1.

Exogenous application of GA_3

Three different concentrations (10^{-2}, 10^{-4} and 10^{-6} M) of GA_3 were applied to the plants in the form of foliar spray (10 ml/plant) in four doses (each dose at 10-day intervals). First treatment was made 10 days after transplantation. Polythene bags were used to cover soil in pots during application of GA_3 so that hormone droplets will not reach into the root zone.

EDTA addition into soil

A total of 40 mg of EDTA was added per kilogramme soil (i.e. single pot) in the form of aqueous solution in two different ways, i.e. single dose of 40 mg EDTA/pot (kg soil) and in four split doses, each of 10 mg EDTA/dose, at 10-day interval. First treatment of EDTA (single or split dose) was made 10 days after transplantation.

Combination treatments of GA_3 and EDTA

Some plants were treated with both GA_3 and EDTA in combination. Three different concentrations of GA_3 (10^{-6}, 10^{-4} and 10^{-2} M) and two different ways of EDTA (40 mg/pot) application (single and split doses) were used in six types of different combinations (3×2). In the combination treatments, both the GA_3 and EDTA applications were made as mentioned earlier.

Plant growth parameter analysis

Plant shoot length was measured on weekly basis. After two and a half months from transplantation (at flowering stage), the plants were harvested and lengths of the plants root and shoot were measured using a centimetre ruler. Plants were washed with a 5 mM solution of EDTA and Tris-HCl (pH 6.0) and then rinsed with distilled water to remove any surface-bounded metal (Genrich et al. 2000). After washing, each plant was cut into three parts, i.e. roots, stem and leaves, and fresh biomass of each part was measured with the help of analytical balance. The parts of each plant were packed in separate paper envelopes and then dried in oven for 48 h at 80 °C. The dry biomass of each part was measured by analytical balance and then grinded into powdered form.

Analysis of free proline in plant root and leaves

Bates et al. (1973) method was used for the quantification of free proline within different parts (root and leaves) of three replicate plants. Fresh plant tissue (100 mg from each part) was homogenized/crushed in 2-ml tubes containing 1.5 ml of 3 % sulfosalicylic acid. The homogenate was then centrifuged for 5 min at 13,000 rpm. The supernatant (only 300 μl) was transferred into new tube and then 2 ml each of acid ninhydrin (containing 1.25 g of ninhydrin heated in 20 ml of phosphoric acid (6 M) and 30 ml of glacial acetic acid until dissolved completely) and glacial acetic acid were added to it. The mixture was kept in water bath (100 °C) for 1 h. The tubes were immediately dipped into ice after removing from water bath. Toluene (1 ml per tube) was added to the reaction mixture and then vigorously mixed for 10–30 s. Toluene containing chromophore layer was removed from the aqueous phase with the help of micropipette and warmed to the room temperature. Spectrophotometer was used (250-nm wavelength) to measure the absorbance of each sample. Toluene was used as a blank (control). The standard curve was used to calculate the concentration of proline in samples. Three replicates were used for each sample.

Total phenolic estimation in roots and leaves

Total phenolics were calculated in root, stem and leaves of three replicate plants. Dried sample (200 mg each) was mixed with 10 ml of methanol (80 %) and then shake for at least 30 min in close vessel (flask) to prevent evaporation of solvent. From each extract, 2 ml was taken in separate tubes and

Table 1 Treatments done during the whole experiment

Treatment	Treatment code	Treatment	Treatment code
Control (without Cd)	C	Cd+GA_3 10^{-6} M+EDTA 40 mg	T6
Control (Cd only)	C1	Cd+GA_3 10^{-6} M+EDTA 10 mg	T7
Cd+GA_3 10^{-2} M	T1	Cd+GA_3 10^{-4} M+EDTA 40 mg	T8
Cd+GA_3 10^{-4} M	T2	Cd+GA_3 10^{-4} M+EDTA 10 mg	T9
Cd+GA_3 10^{-6} M	T3	Cd+GA_3 10^{-2} M+EDTA 40 mg	T10
Cd+EDTA 40 mg single dose	T4	Cd+GA_3 10^{-2} M+EDTA 10 mg	T11
Cd+EDTA 10 mg split doses	T5		

centrifuged at 13,000 rpm for 3 to 5 min. Singleton and Rossi (1965) method with slight modifications was used for analysis of total phenolics in extract. Folin-Ciocalteau (FC) reagent (250 μl) was mixed with 100 μl gallic acid standard solutions or methanolic extract, and the mixture was kept in dark (at room temperature) for 3–5 min. Then 7 % (500 μl) sodium carbonate (Na_2CO_3) solution was added to the mixture, and dH_2O was used and raised the net volume up to 5 ml. The mixture was kept in dark at room temperature for 2 h. Spectrophotometer was used to measure the absorbance of the samples at 760 nm. Different standard solutions (0, 10, 30, 50, 100, 150 mg/l) of gallic acid were prepared in methanol (80 %), and their absorbances were used as standard for measuring total phenolics in each sample. Methanol solution with a concentration of 80 % was used as blank (control). Three replicates were used for each sample.

Chlorophyll estimation in leaves

Concentration of chlorophyll a and b was measured by using the method of Arnon (1949). First of all, fresh leaves were obtained from the plants (both control and treated plants). Then 2 ml of acetone (80 %) was mixed with 200 mg of fresh leaves and properly grinded. After grinding, the mixtures were shifted into Ependorf tubes and then centrifuged for 5 min at 10,000 rpm. The supernatant (after centrifugation) was poured into clean test tubes and 6 ml of acetone (80 %) was added to it. The samples were then analysed for absorbance at 645 and 663 nm in spectrophotometer. The following formulas were used for calculating the concentration of chlorophyll a and b:

$$\text{Chlorophyll}\,\underline{a}(\mu g/ml) = 12.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll}\,\underline{b}(\mu g/ml) = 22.9(A_{645}) - 4.68(A_{663})$$

Cd analysis in different plant parts

Oven-dried samples (root, stem and leaves) were first grounded into powdered form and then subjected to acid digestion using Allen's (1974) method. Dried powder (0.25 g) from each sample was taken into separate flasks (50 ml). Three-acid mixture (6.5 ml) containing sulfuric acid, nitric acid and perchloric acid (1, 5 and 0.5 ml, respectively) was added to each flask. For complete digestion, each flask (sample) was kept on electric hot plates until completely digested. The digested samples were then filtered into another volumetric flask (50 ml), and with the help of dH_2O, the volume was raised up to 50 ml. Each filtrate sample was then stored in small plastic bottles. The samples were then analysed for Cd concentration with the help of atomic absorption/flame spectrophotometer (model Hitachi Z-8000, Japan). Three replicate plants per treatment were analysed for Cd content.

Statistical analysis

The data was subjected to analysis of variance (ANOVA) and correlations between parameters. Tukey's HSD test ($p \leq 0.05$) was used for checking significant differences between means. SPSS 16 and MS Excel 2007 were used.

Results

Effect of GA_3 and EDTA treatments on length (root and shoot), biomass (fresh and dry) and water contents of *P. hysterophorus* plant under Cd stress

Plant length, biomass and water content were significantly reduced by Cd addition into soil (100 mg Cd/kg) when control C (without Cd) and C1 (with Cd only) were compared, except the dry biomass (DBM) of stem and total water content (TWC) of leaves (Table 2). The effect of all treatments (except EDTA-treated plants T4, T5) significantly increased the root and shoot length on Cd-contaminated soil when compared to C1 (Cd only) (Fig. 1). The highest significant root length (24.33±1.00 cm) and stem length (44.33±4.73 cm) was demonstrated by the treatment T3 (GA_3 10^{-6} M, foliar spray). Fresh biomass (FBM) and TWC of root significantly increased in all treatments (except T10 and T11), while FBM of stem and leaves were significantly higher only in GA_3-alone treatments (T1, T2 and T3) as compared to C1 (Table 2). All treatments significantly increased DBM of root, stem, leaves and entire plant, and the highest significant DBM in roots (1.65±0.02 g), stem (2.40±0.05 g), leaves (2.40±0.05 g) and entire plant (6.45±0.12 g) was recorded in treatment T3 (Cd+GA_3 10^{-6} M) as given in Table 2.

Effect of different treatments of GA_3 and EDTA on Cd phytoaccumulation

Root cadmium concentration of the plant was increased significantly in all the treatments when compared with C1 (control with Cd only), and the highest significant root Cd concentration (1267.00±12.60 and 1245±16.20 ppm) was recorded for the EDTA treatments T4 and T5, respectively (Table 3). The treatment T11 (Cd+GA_3 10^{-2} M+EDTA split doses) significantly increased the highest Cd concentration in stem and leaf (166.33±18.00 and 570.00±23.45 ppm, respectively). Accumulation of Cd (mg/DBM) in different parts of the plant was high significantly in all treatments (except in T1 plant stem) as compared to C1 (Table 3). The high significant Cd accumulation in roots was found in GA_3 and EDTA combination treatments T9, T10 and T11, respectively, 0.84±0.04, 0.86±0.03 and 0.87±0.04 mg Cd/DBM. The treatment T11 showed the highest Cd accumulation in stem (0.26±0.03 mg Cd/DBM), leaves (0.83±0.04 mg Cd/DBM) and entire plant

Table 2 Effect of different treatments of GA_3 and EDTA on different growth parameters (length, biomass and water content) of *Parthenium hysterophorus* plant in cadmium-contaminated soil (100 mg Cd/kg soil)

Treatment		Length (cm)±SD		Fresh biomass (g)±SD				Dry biomass (g)±SD				Total water content (g)±SD			
		R	S	R	S	L	EP	R	S	L	EP	R	S	L	EP
C	Without Cd	24.00±1.00^{ab}	33.00±1.00^{cd}	8.70±0.44^{a}	10.00±1.00^{bc}	8.50±0.50^{bc}	27.20±1.61^{bc}	1.92±0.07^{a}	1.67±0.06^{ef}	2.40±0.10^{a}	5.99±0.23^{b}	6.78±0.37^{ab}	8.33±0.94^{bcd}	6.10±0.46^{c}	21.21±1.39^{bcd}
C1	With Cd	12.00±0.98^{hi}	24.10±0.85^{ef}	4.23±0.20^{f}	6.40±0.98^{def}	6.50±0.60^{de}	17.13±1.78^{ef}	0.68±0.03^{ij}	1.53±0.03^{f}	1.40±0.03^{e}	3.75±0.09^{f}	3.55±0.17^{e}	4.73±0.95^{efg}	5.10±0.57^{cde}	13.38±1.69^{fgh}
T1	Cd+GA_3 10^{-2} M	21.37±1.09^{bc}	33.50±1.50^{cd}	6.50±0.19^{c}	11.50±1.09^{ab}	10.10±0.56^{ab}	28.10±1.84^{b}	1.30±0.03^{d}	2.20±0.03^{b}	2.27±0.03^{a}	5.77±0.09^{b}	5.20±0.16^{c}	9.30±1.06^{abc}	7.83±0.53^{ab}	22.33±1.75^{bc}
T2	Cd+GA_3 10^{-4} M	22.00±0.78^{abc}	40.30±4.59^{ab}	7.80±0.19^{b}	12.67±0.78^{a}	10.40±0.56^{a}	30.87±1.53^{ab}	1.45±0.01^{c}	2.29±0.05^{ab}	2.34±0.05^{a}	6.08±0.11^{b}	6.35±0.18^{b}	10.38±0.73^{ab}	8.06±0.51^{a}	24.79±1.42^{ab}
T3	Cd+GA_3 10^{-6} M	24.33±1.00^{a}	44.33±4.73^{a}	8.70±0.25^{a}	13.53±1.00^{a}	11.20±0.75^{a}	33.43±2.00^{a}	1.65±0.02^{b}	2.40±0.05^{a}	2.40±0.05^{a}	6.45±0.12^{a}	7.05±0.23^{a}	11.13±0.95^{a}	8.80±0.70^{a}	26.98±1.88^{a}
T4	Cd+EDTA 40 mg	10.00±0.97^{i}	18.00±1.00^{fg}	3.40±0.32^{g}	5.20±0.97^{ef}	5.30±0.96^{e}	13.90±2.24^{f}	0.43±0.02^{k}	1.32±0.02^{g}	1.10±0.02^{f}	2.85±0.06^{g}	2.97 0.30^{ef}	3.88±0.95^{fg}	4.20±0.94^{de}	11.05±2.19^{gh}
T5	Cd+EDTA 10 mg	10.50±1.05^{i}	15.00±1.32^{g}	3.33±0.17^{g}	4.70±1.05^{f}	5.20±0.50^{e}	13.23±1.72^{f}	0.50±0.01^{k}	1.43±0.05^{g}	1.20±0.05^{f}	3.13±0.11^{g}	2.83±0.16^{f}	3.27±1.00^{g}	4.00±0.45^{e}	10.10±1.61^{h}
T6	Cd+GA_3 10^{-6} M+EDTA 40 mg	20.00±0.99^{cd}	35.00±1.00^{bc}	5.40±0.09^{d}	8.90±0.99^{bcd}	8.20±0.26^{cd}	22.50±1.34^{cd}	0.67±0.01^{j}	1.96±0.04^{c}	1.95±0.04^{b}	4.58±0.09^{c}	4.73±0.08^{cd}	6.94±0.95^{cde}	6.25±0.22^{bc}	17.92±1.25^{cde}
T7	Cd+GA_3 10^{-6} M+EDTA 10 mg	18.00±1.00^{de}	31.07±2.11^{cd}	5.32±0.02^{d}	8.70±1.00^{cd}	8.00±0.06^{cd}	22.02±1.08^{d}	0.76±0.03^{hi}	1.87±0.03^{cd}	1.89±0.03^{bc}	4.52±0.09^{c}	4.56±0.01^{d}	6.83±0.97^{cde}	6.11±0.03^{c}	17.50±0.99^{def}
T8	Cd+GA_3 10^{-4} M+EDTA 40 mg	17.60±0.87^{def}	32.50±1.32^{cd}	5.20±0.10^{d}	8.43±0.87^{cd}	7.50±0.30^{cd}	21.13±1.27^{de}	0.84±0.03^{gh}	1.77±0.03^{de}	1.84±0.03^{bc}	4.45±0.09^{cd}	4.36±0.07^{d}	6.66±0.84^{de}	5.66±0.27^{cde}	16.68±1.18^{def}
T9	Cd+GA_3 10^{-4} M+EDTA 10 mg	16.30±0.65^{efg}	28.33±1.15^{de}	5.16±0.14^{de}	8.10±0.65^{cd}	7.34±0.43^{cd}	20.60±1.22^{de}	0.87±0.03^{fg}	1.72±0.03^{e}	1.78±0.03^{c}	4.37±0.09^{cd}	4.29±0.11^{d}	6.38±0.62^{def}	5.56±0.40^{cde}	16.23±1.13^{ef}
T10	Cd+GA_3 10^{-2} M+EDTA 40 mg	15.00±0.45^{fg}	29.67±1.53^{cde}	4.50±0.33^{ef}	7.87±0.45^{cd}	7.26±0.98^{cd}	19.63±1.76^{de}	0.95±0.02^{ef}	1.66±0.06^{ef}	1.56±0.06^{d}	4.17±0.14^{de}	3.55±0.31^{e}	6.21±0.39^{def}	5.70±0.92^{cde}	15.46±1.62^{efg}
T11	Cd+GA_3 10^{-2} M+EDTA 10 mg	14.20±0.43^{gh}	23.67±1.53^{ef}	4.43±0.25^{f}	7.76±0.43^{cde}	7.21±0.75^{cd}	19.40±1.43^{de}	0.99±0.03^{e}	1.58±0.03^{f}	1.46±0.03^{de}	4.03±0.09^{ef}	3.44±0.22^{ef}	6.18±0.40^{def}	5.75±0.72^{cd}	15.37±1.34^{efg}

GA_3 was applied in four split doses, and 40 mg EDTA was added in a single dose while four doses of 10 mg EDTA added to a pot. Different letters show significant difference among values of different parameters.

SD standard deviation, *R* roots, *S* stem, *L* leaf, *EP* entire plant

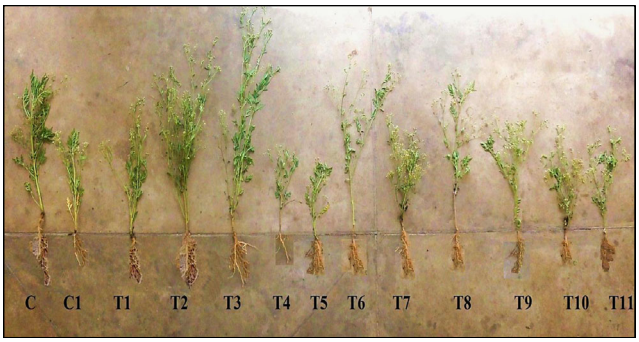

Fig. 1 Effect of different treatments of GA_3 and EDTA on root and shoot length of *Parthenium hysterophorus* plant, in cadmium-contaminated soil (100 mg Cd/kg soil). *C* (without Cd), *C1* (with Cd), *T1* (Cd+GA_3 10^{-2} M), *T2* (Cd+GA_3 10^{-4} M), *T3* (Cd+GA_3 10^{-6} M), *T4* (Cd+EDTA 40 mg), *T5* (Cd+EDTA 10 mg), *T6* (Cd+GA_3 10^{-6} M+EDTA 40 mg), *T7* (Cd+GA_3 10^{-6} M+EDTA 10 mg), *T8* (Cd+GA_3 10^{-4} M+EDTA 40 mg), *T9* (Cd+GA_3 10^{-4} M+EDTA 10 mg), *T10* (Cd+GA_3 10^{-2} M+EDTA 40 mg), *T11* (Cd+GA_3 10^{-2} M+EDTA 10 mg)

(1.97±0.11 mg Cd/DBM). The treatments showed an increase of 4.07–9.79-fold Cd contents in roots, 1.61–3.21 in stem, 1.86–4.71 in leaves and 2.37–5.65-fold in entire plant Cd accumulation compared to C1 as given in Table 3. The highest increase in Cd accumulation within roots (9.79 times), stem (3.21 times), leaves (4.71 times) and entire plant (5.65 times) was demonstrated by the treatment T11 (Cd+GA_3 10^{-2} M+EDTA split doses). The results showed that the highest Cd accumulation percentage was found within roots followed by leaves of the plant while the lowest Cd accumulation percentage was noted in the plant stem (Table 3). Cadmium bioconcentration factor (BCF) of the plant being higher than the one (i.e. 1.85±0.22) in the control C1 plants shows that *P. hysterophorus* is a hyperaccumulator of Cd. The treatment further increased the Cd BCF and the increase was found statistically significant as compared to the control C1. The highest significant Cd BCF (9.75±0.34) was demonstrated by the treatment T11 (Cd+GA_3 10^{-2} M+EDTA split doses).

Effect of exogenous GA_3 and EDTA on total phenolics, free proline and chlorophyll (a/b) under Cd stress

Free proline and total phenolics in roots, stem and leaves while chlorophyll (a/b) contents in leaves of the plant are presented in Table 4. Cadmium added to the soil significantly increased free proline contents in plant roots while total phenolics were significantly higher in all parts (root, stem and leaves) of the plant (comparing C with C1). Free proline in stem increased significantly in T3 (GA_3 treatment) and in leaves of combination treatments (T10 and T11) when compared with control C1 (Table 4). The total phenolics in roots significantly increased with GA_3 treatments while EDTA treatments (T4 and T5) showed non-significant increase in phenolics (compared to control C1) as given in Table 4. In leaves, all the treatments showed significant increase in total phenolics (compared to C1). The highest significant total phenolic content in roots (79.00±3.94 ppm) was found in treatment T7 while in stem (34.00±2.50 ppm) and leaves (156.00 ±13.20 ppm), it was recorded in the treatment T11 (Table 4). The chlorophyll (a/b) was reduced significantly in plant on cadmium-polluted soil (comparing C with C1). The treatments containing GA_3 foliar spray significantly increased chlorophyll (a/b) concentration in leaves of the plant as compared to C1, except the chlorophyll a contents of T9, T10 and T11 (where the increase is non-significant compared to C1). EDTA-alone treatments showed significant decrease in chlorophyll content as compared to C1 (Table 4).

Correlations among different parameters measured

Correlations among different parameters measured in roots of the plant are presented in Table 5. The table shows significantly positive correlations among certain parameters (length, FBM, DBM and TWC). The correlation of root Cd concentration with length (R^2=−0.452), FBM (R^2=−0.588), DBM (R^2=−0.674) and TWC (R^2=−0.546) of the plant roots was found negative. Cadmium concentration in roots showed highly significant positive correlation (R^2=0.661) with the accumulation of Cd in roots. The concentration of free proline and total phenolics in roots showed a positive significant correlations (R^2=0.527 and R^2=0.554, respectively) with Cd accumulation, but their correlation with the concentration of Cd in roots was non-significant. Total phenolics of roots showed positive significant correlations with length (R^2=0.728), FBM (R^2=0.537) and TWC (R^2=0.590) while its correlation with roots DBM was positive but non-significant. Proline contents of roots demonstrated positive correlation with the growth parameters but were non-significant. Table 6 shows positive and significant correlations among length, FBM, DBM and TWC of the stem, while the correlations of these parameters with stem Cd concentration were negative. Stem Cd accumulation showed negative correlations with growth parameters. Free proline and total phenolic concentration of stem showed positive correlation with all the parameters measured in plant stem, but the correlations of proline concentration was statistically significant only with phenolic content (R^2=0.577) and Cd accumulation (R^2=0.867). The correlations of total phenolics were significant with all the parameters except Cd concentration and accumulation in stem. Table 7 demonstrates correlations among different parameters measured in plant leaves. Like roots and stem, the leaves of plant showed positive significant correlations among the growth parameters (FBM, DBM and TWC). Cadmium concentration and accumulation of leaf demonstrated negative correlations with the growth parameters. Correlation between free proline and total phenolics of leaves was positive and significant. The correlations of chlorophyll with growth parameters were positive and were negative with Cd concentration.

Table 3 Effect of different treatments of GA_3 and EDTA on Cd concentration, accumulation, translocation and bioconcentration of *Parthenium hysterophorus* plant in cadmium-contaminated soil (100 mg Cd/kg soil)

Treatment		Cd concentration (ppm)±SD			Cd accumulation (mg/DBM)±SD				Fold increase in Cd accumulation compared to C1*				Cd accumulation %			Cadmium TF		Cadmium BCF
		R	S	L	R	S	L	EP	R	S	L	EP	R	S	L	R to S	R to L	
C1	Cd only	129.67±12.00^{h}	49.00±9.40^{d}	126.00±12.00^{i}	0.09±0.01^{g}	0.08±0.02^{d}	0.18±0.02^{g}	0.35±0.05^{g}	0.09 mg	0.08 mg	0.18 mg	0.35 mg	25.59	23.45	50.96	0.38±0.04^{a}	0.97±0.01^{a}	1.85±0.22^{h}
T1	Cd+GA_3 10^{-2} M	278.00±9.20^{g}	60.00±12.00^{d}	145.00±9.20^{hi}	0.36±0.02^{f}	0.13±0.03^{cd}	0.33±0.03^{f}	0.82±0.07^{f}	4.07	1.61	1.86	2.37	44.01	15.95	40.04	0.22±0.04^{b}	0.52±0.02^{d}	2.85±0.21^{g}
T2	Cd+GA_3 10^{-4} M	346.33±13.40^{f}	72.00±9.20^{d}	167.00±13.40^{gh}	0.50±0.02^{e}	0.17±0.02^{bc}	0.39±0.04^{ef}	1.06±0.09^{ef}	5.65	2.01	2.21	3.05	47.54	15.55	36.92	0.21±0.02^{b}	0.48±0.02^{e}	3.48±0.22^{fg}
T3	Cd+GA_3 10^{-6} M	432.33±11.20^{e}	80.50±13.40^{cd}	189.00±11.20^{g}	0.71±0.03^{c}	0.19±0.04^{abc}	0.45±0.04^{de}	1.36±0.10^{d}	8.03	2.36	2.57	3.92	52.51	14.15	33.34	0.19±0.03^{b}	0.44±0.01^{f}	4.22±0.23^{f}
T4	Cd+EDTA 40 mg	1267.00±12.60^{a}	143.00±11.20^{ab}	425.00±12.60^{cd}	0.54±0.03^{de}	0.19±0.02^{abc}	0.47±0.02^{de}	1.20±0.07^{de}	6.13	2.30	2.65	3.46	45.36	15.70	38.94	0.11±0.01^{b}	0.34±0.01^{hi}	8.43±0.32^{bcd}
T5	Cd+EDTA 10 mg	1245.00±16.20^{a}	149.00±12.60^{ab}	456.00±16.20^{c}	0.62±0.02^{d}	0.21±0.03^{ab}	0.55±0.04^{d}	1.38±0.09 ^{cd}	7.01	2.60	3.10	3.98	45.05	15.39	39.56	0.12±0.01^{b}	0.37±0.01^{gh}	8.84±0.25^{bc}
T6	Cd+GA_3 10^{-6} M+EDTA 40 mg	1123.00±16.00^{b}	112.00±16.20^{bc}	345.00±12.90^{f}	0.75±0.02^{bc}	0.22±0.04^{ab}	0.67±0.04^{c}	1.65±0.10^{bc}	8.47	2.68	3.81	4.74	45.78	13.31	40.91	0.10±0.01^{b}	0.31±0.01^{i}	7.18±0.28^{e}
T7	Cd+GA_3 10^{-6} M+EDTA 10 mg	1098.00±15.20^{b}	119.00±12.90^{b}	365.00±15.20^{f}	0.83±0.04^{ab}	0.22±0.03^{ab}	0.69±0.04^{bc}	1.75±0.11^{ab}	9.40	2.72	3.91	5.03	47.79	12.71	39.50	0.11±0.01^{b}	0.33±0.01^{i}	7.73±0.34^{de}
T8	Cd+GA_3 10^{-4} M+EDTA 40 mg	992.33±16.00^{c}	120.00±15.20^{b}	379.00±9.60^{ef}	0.83±0.04^{ab}	0.21±0.03^{abc}	0.70±0.03^{bc}	1.74±0.10^{ab}	9.39	2.59	3.95	5.02	47.82	12.15	40.02	0.12±0.01^{b}	0.38±0.03^{g}	7.83±0.30^{de}
T9	Cd+GA_3 10^{-4} M+EDTA 10 mg	968.00±12.00^{c}	129.00±9.60^{ab}	413.00±9.20^{de}	0.84±0.04^{a}	0.22±0.02^{ab}	0.74±0.03^{abc}	1.80±0.09^{ab}	9.48	2.71	4.16	5.18	46.81	12.32	40.87	0.13±0.01^{b}	0.43±0.02^{f}	8.23±0.24^{cd}
T10	Cd+GA_3 10^{-2} M+EDTA 40 mg	908.00±9.00^{d}	147.33±9.20^{ab}	510.00±18.00^{b}	0.86±0.03^{a}	0.24±0.02^{ab}	0.80±0.06^{ab}	1.90±0.11^{ab}	9.71	2.99	4.51	5.48	45.36	12.84	41.80	0.16±0.01^{b}	0.56±0.01^{c}	9.13±0.22^{ab}
T11	Cd+GA_3 10^{-2} M+EDTA 10 mg	878.00±9.00^{d}	166.33±18.00^{a}	570.00±23.45^{a}	0.87±0.04^{a}	0.26±0.03^{a}	0.83±0.04^{a}	1.97±0.11^{a}	9.79	3.21	4.71	5.65	44.27	13.36	42.37	0.19±0.02^{b}	0.65±0.01^{b}	9.75±0.34^{a}

GA_3 was applied in four split doses, and 40 mg EDTA was added in a single dose while four doses of 10 mg EDTA added to a pot. Different letters show significant difference among values of different parameters

SD standard deviation, *DBM* dry biomass, *R* stands for roots, *S* for stem, *L* for leaf, *EP* entire plant, *TF* translocation factor, *BCF* for bioconcentration factor

*For C1 (control with Cd only), actual values of extracted Cd (mg) are given

Table 4 Effect of gibberellic acid and EDTA treatments on free proline, total phenolics and chlorophyll (a/b) contents of *Parthenium hysterophorus* plant grown in Cd-contaminated soil (100 mg Cd/kg soil)

Treatments		Free proline (ppm)±SD			Total phenolics±SD			Chlorophyll±SD		
		Roots	Stem	Leaf	Root	Stem	Leaf	a	b	a+b
C	Control (without Cd)	12.00±2.80^{b}	15.00±3.40^{c}	10.00±1.88^{d}	15.00±4.20^{g}	8.50±3.50^{d}	27.00±3.50^{d}	5.10±0.36^{bc}	4.67±0.15^{a}	9.77±0.31^{b}
C1	Cd only	57.60±3.33^{a}	24.00±3.33^{bc}	28.57±5.40^{cd}	29.23±3.33^{f}	20.00±3.33^{c}	90.80±9.40^{c}	3.65±0.20^{gh}	2.81±0.03^{h}	6.46±0.23^{f}
T1	Cd+GA_3 10^{-2} M	58.00±3.00^{a}	25.00±3.00^{abc}	39.00±5.06^{abc}	50.00±3.00^{cd}	29.00±3.00^{abc}	120.00±6.80^{b}	5.20±0.19^{bc}	4.32±0.03^{b}	9.52±0.22^{b}
T2	Cd+GA_3 10^{-4} M	64.30±5.96^{a}	25.80±5.96^{ab}	45.00±9.00^{abc}	57.00±2.96^{bc}	33.00±2.96^{a}	139.00±9.20^{ab}	5.70±0.19^{ab}	4.54±0.05^{a}	10.24±0.24^{a}
T3	Cd+GA_3 10^{-6} M	62.50±3.60^{a}	35.00±3.60^{a}	48.57±6.75^{abc}	65.00±3.60^{b}	36.00±3.60^{a}	145.00±9.80^{ab}	5.98±0.25^{a}	4.70±0.05^{a}	10.68±0.30^{g}
T4	Cd+EDTA 40 mg	60.00±2.50^{a}	24.00±2.50^{bc}	32.00±8.61^{bcd}	33.00±2.50^{f}	23.00±2.50^{bc}	138.00±5.90^{ab}	3.05±0.32^{hi}	2.35±0.02^{i}	5.40±0.34^{g}
T5	Cd+EDTA 10 mg	59.70±2.70^{a}	27.00±2.70^{ab}	30.00±12.66^{cd}	36.00±2.70^{ef}	22.00±2.70^{bc}	123.00±6.50^{b}	2.95±0.17^{i}	2.27±0.05^{i}	5.22±0.22^{c}
T6	Cd+GA_3 10^{-6} M+EDTA 40 mg	63.00±2.25^{a}	32.00±2.25^{ab}	43.40±9.45^{abc}	79.00±2.25^{a}	29.95±2.25^{ab}	123.00±11.00^{b}	4.76±0.09^{cd}	3.87±0.04^{c}	8.63±0.13^{cd}
T7	Cd+GA_3 10^{-6} M+EDTA 10 mg	59.00±3.94^{a}	26.00±3.94^{ab}	44.29±8.10^{abc}	76.00±3.94^{a}	30.00±3.94^{ab}	143.00±12.90^{ab}	4.67±0.02^{cde}	3.59±0.03^{d}	8.26±0.05^{de}
T8	Cd+GA_3 10^{-4} M+EDTA 40 mg	63.00±2.50^{a}	32.00±2.50^{ab}	46.43±5.06^{abc}	65.00±2.50^{b}	28.00±2.50^{abc}	130.00±10.40^{ab}	4.37±0.10^{def}	3.36±0.03^{e}	7.73±0.13^{de}
T9	Cd+GA_3 10^{-4} M+EDTA 10 mg	60.00±3.58^{a}	31.00±3.58^{ab}	49.29±9.00^{abc}	60.00±3.58^{b}	28.90±3.58^{abc}	145.00±9.60^{ab}	4.30±0.14^{defg}	3.31±0.03^{ef}	7.61±0.17^{e}
T10	Cd+GA_3 10^{-2} M+EDTA 40 mg	63.00±3.38^{a}	29.00±3.38^{ab}	54.29±8.86^{ab}	47.00±3.38^{d}	31.00±3.38^{ab}	130.00±9.20^{ab}	4.10±0.33^{efg}	3.15±0.06^{fg}	7.25±0.39^{ef}
T11	Cd+GA_3 10^{-2} M+EDTA 10 mg	61.67±2.50^{a}	32.00±2.50^{ab}	57.14±6.75^{a}	44.00±2.50^{de}	34.00±2.50^{a}	156.00±13.20^{a}	3.95±0.25^{fg}	3.12±0.03^{g}	7.07±0.28^{ab}

GA_3 was applied in four split doses, and 40 mg EDTA was added in a single dose while four doses of 10 mg EDTA added to a pot. Different letters show significant difference among values of different parameters

Table 5 Correlations among different parameters measured in roots of *P. hysterophorus* plant

		Length	FBM	DBM	TWC	Cd concentration	Cd accumulation	Proline	Phenolics
Length	Pearson correlation	1	0.945**	0.833**	0.950**	−0.452	0.131	0.415	0.728**
	Sig. (1-tailed)		0.001	0.001	0.001	0.070	0.343	0.090	0.004
FBM	Pearson correlation	0.945**	1	0.921**	0.994**	−0.588*	−0.010	0.397	0.537*
	Sig. (1-tailed)	0.001		0.001	0.001	0.022	0.488	0.101	0.036
DBM	Pearson correlation	0.833**	0.921**	1	0.872**	−0.674**	−0.001	0.371	0.299
	Sig. (1-tailed)	0.001	0.001		0.001	0.008	0.499	0.117	0.173
TWC	Pearson correlation	0.950**	0.994**	0.872**	1	−0.546*	−0.012	0.393	0.590*
	Sig. (1-tailed)	0.001	0.001	0.001		0.033	0.485	0.103	0.022
Cd concentration	Pearson correlation	−0.452	−0.588*	−0.674**	−0.546*	1	0.661**	0.132	0.163
	Sig. (1-tailed)	0.070	0.022	0.008	0.033		0.010	0.341	0.306
Cd accumulation	Pearson correlation	0.131	−0.01	−0.001	−0.012	0.661**	1	0.527*	0.554*
	Sig. (1-tailed)	0.343	0.488	0.499	0.485	0.010		0.039	0.031
Proline	Pearson correlation	0.415	0.397	0.371	0.393	0.132	0.527*	1	0.423
	Sig. (1-tailed)	0.090	0.101	0.117	0.103	0.341	0.039		0.085
Phenolics	Pearson correlation	0.728**	0.537*	0.299	0.590*	0.163	0.554*	0.423	1
	Sig. (1-tailed)	0.004	0.036	0.173	0.022	0.306	0.031	0.085	

**p=0.01, correlation is significant at this level (1-tailed); *p=0.05, correlation is significant at this level (1-tailed)

Discussion

Plant growth and biomass

Plant growth and biomass have been reported to be highly sensitive to heavy metal stress (Arun et al. 2005; Hadi and Bano 2009; John et al. 2009; Hadi et al. 2010). Heavy metal like cadmium in plant tissues reduces plant growth due to toxicity (Khatamipour et al. 2011). In the present investigation, cadmium demonstrated significant reduction in growth and biomass of *P. hysterophorus* plant (comparing C with C1). Decrease in growth of plants in cadmium-contaminated soil is often observed, and this reduction in length (of root and shoot) and biomass could be directly related to the negative effect of heavy metal on the division of meristematic cells and on the cell elongation and expansion (Houshmandfar and Moraghebi 2011). One of the reasons for inhibition of cell elongation might be the effect of metals on cell wall components and their structures (Poschenrieder et al. 1989). Present results showed reduction in fresh biomass and consequently in the

Table 6 Correlations among different parameters measured in stem of *P. hysterophorus* plant

		Length	FBM	DBM	TWC	Cd concentration	Cd accumulation	Proline	Phenolics
Length	Pearson correlation	1	0.944**	0.937**	0.941**	−0.543*	−0.070	0.437	0.745**
	Sig. (1-tailed)		0.001	0.001	0.001	0.034	0.415	0.078	0.003
FBM	Pearson correlation	0.944**	1	0.972**	0.999**	−0.572*	−0.132	0.333	0.771**
	Sig. (1-tailed)	0.001		0.001	0.001	0.026	0.341	0.145	0.002
DBM	Pearson correlation	0.937**	0.972**	1	0.964**	−0.689**	−0.257	0.267	0.651*
	Sig. (1-tailed)	0.001	0.001		0.001	0.007	0.210	0.201	0.011
TWC	Pearson correlation	0.941**	0.999**	0.964**	1	−0.553*	−0.114	0.341	0.785**
	Sig. (1-tailed)	0.001	0.001	0.001		0.031	0.362	0.139	0.001
Cd concentration	Pearson correlation	−0.543*	−0.572*	−0.689**	−0.553*	1	0.867**	0.275	0.040
	Sig. (1-tailed)	0.034	0.026	0.007	0.031		0.001	0.193	0.451
Cd accumulation	Pearson correlation	−0.070	−0.132	−0.257	−0.114	0.867**	1	0.577*	0.481
	Sig. (1-tailed)	0.415	0.341	0.210	0.362	0.001		0.025	0.057
Proline	Pearson correlation	0.437	0.333	0.267	0.341	0.275	0.577*	1	0.640*
	Sig. (1-tailed)	0.078	0.145	0.201	0.139	0.193	0.025		0.013
Phenolics	Pearson correlation	0.745**	0.771**	0.651*	0.785**	0.040	0.481	0.640*	1
	Sig. (1-tailed)	0.003	0.002	0.011	0.001	0.451	0.057	0.013	

**p=0.01, correlation is significant at this level (1-tailed); *p=0.05, correlation is significant at this level (1-tailed)

Table 7 Correlation among different parameters measured in leaves of *P. hysterophorus* plant

		FBM	DBM	TWC	Cd concentration	Cd accumulation	Proline	Phenolics	Chlorophyll a	Chlorophyll b	Total chlorophyll
FBM	Pearson correlation	1	0.971**	0.998**	−0.614*	−0.223	0.402	0.219	0.986**	0.136	0.146
	Sig. (1-tailed)		0.001	0.001	0.017	0.243	0.098	0.247	0.001	0.337	0.326
DBM	Pearson correlation	0.971**	1	0.952**	−0.608*	−0.166	0.377	0.189	0.984**	0.043	0.052
	Sig. (1-tailed)	0.001		0.001	0.018	0.303	0.114	0.279	0.001	0.447	0.436
TWC	Pearson correlation	0.998**	0.952**	1	−0.609*	−0.238	0.405	0.226	0.976**	0.162	0.171
	Sig. (1-tailed)	0.001	0.001		0.018	0.228	0.096	0.24	0.004	0.308	0.297
Cd concentration	Pearson correlation	−0.614*	−0.608*	−0.609*	1	0.859**	0.399	0.494	−0.576*	−0.398	−0.337
	Sig. (1-tailed)	0.017	0.018	0.018		0.001	0.101	0.051	0.025	0.101	0.142
Cd accumulation	Pearson correlation	−0.223	−0.166	−0.238	0.859**	1	0.723**	0.630*	−0.141	−0.458	−0.39
	Sig. (1-tailed)	0.243	0.303	0.228	0.001		0.004	0.014	0.331	0.067	0.105
Proline	Pearson correlation	0.402	0.377	0.405	0.399	0.723**	1	0.693**	0.448	−0.147	−0.087
	Sig. (1-tailed)	0.098	0.114	0.096	0.101	0.004		0.006	0.072	0.324	0.394
Phenolics	Pearson correlation	0.219	0.189	0.226	0.494	0.630*	0.693**	1	0.252	0.013	0.106
	Sig. (1-tailed)	0.247	0.279	0.240	0.051	0.014	0.006		0.215	0.484	0.371
Chlorophyll a	Pearson correlation	0.986**	0.984**	0.976**	−0.576*	−0.141	0.448	0.252	1	0.137	0.147
	Sig. (1-tailed)	0.001	0.001	0.001	0.025	0.331	0.072	0.215		0.336	0.324
Chlorophyll b	Pearson correlation	0.136	0.043	0.162	−0.398	−0.458	−0.147	0.013	0.137	1	0.993 **
	Sig. (1-tailed)	0.337	0.447	0.308	0.101	0.067	0.324	0.484	0.336		0.001
Total chlorophyll		0.146	0.052	0.171	−0.337	−0.39	0.017	0.106	0.147	0.993**	1
		0.326	0.436	0.297	0.142	0.105	0.394	0.371	0.324	0.001	

**$p=0.01$, correlation is significant at this level (1-tailed); *$p=0.05$, correlation is significant at this level (1-tailed)

water contents within different plant parts under cadmium stress (comparing C with C1). Similar reduction in fresh biomass under cadmium stress was reported by Zheng et al. (2010) and Khatamipour et al. (2011) in *Glycyrrhiza uralensis* plant. Cadmium has also been found to cause physiological drought by altering water content in plant tissues (Barcelo and Poschenriede 1990).

Addition of EDTA into the cadmium-contaminated soil further reduced the plant growth while application of GA_3 foliar spray enhanced the growth and biomass of the *P. hysterophorus* plant. This decreasing effect of EDTA on plant growth and biomass might probably be due to increase in mobility of cadmium by EDTA in soil (Lou et al. 2007; Epelde et al. 2008). The increase in growth and biomass might be due to the role of GA_3 in promotion of cell enlargement (Buchanan et al. 2000) and on the rate of cell division (Moore 1989; Arteca 1996), two main processes for the increase in growth and biomass. GA_3 enhances the synthesis of DNA, RNA and protein (Benjerano and Lips 1970; Broughton and McComb 1971) and ribose and polyribosome multiplication (Evins and Varner 1972) would increase biomass of a plant. GA_3 treatment also increases permeability of cell membrane (Wood and Paleg 1974; Crozier and Turnbull 1984) that would enhance absorption of mineral nutrients and their transport and utilization (Crozier and Turnbull 1984; Aloni et al. 1986; Al-Wakeel et al. 1995; Ansari 1996; Khan et al. 1998), thus, enhancing the capability of the GA_3-treated plants for high biomass production as demonstrated in our experiment (Table 2). Increase in biomass due to GA_3 application has been observed in tomato (Masroor et al. 2006) and in maize plant (Hadi et al. 2010).

Plant cadmium contents

The results showed that *P. hysterophorus* plant is a hyperaccumulator of cadmium at its flowering stage obvious from its high bioconcentration factor (1.85). GA_3 and EDTA in combination greatly increase cadmium concentration within different parts of the plant. The reason might be that EDTA increases the metal bioavailability in soil solution (Elless and Blaylock 2000; Chen and Cutright 2001; Thayalakumaran et al. 2003; Meers et al. 2005; Mamindy-Pajany et al. 2014;) while the GA_3 increases absorption and translocation of cadmium into different parts of the plant (Tassi et al. 2008; Hadi et al. 2014).

Proline concentration

Accumulation of proline in plant tissue is often considered as an indicator of environmental stress such as drought, salinity and heavy metal stress. It has been found that free proline chelates cadmium ion in plant tissues and converts them into non-toxic complex of Cd-proline (Sharma et al. 1998). Our results showed a strong correlation between free proline and cadmium accumulation within different tissues of the *P. hysterophorus* plant. This suggests that free proline may play an important role in Cd accumulation and also in the reduction of cadmium toxicity within plants. Several plants have been reported to accumulate high concentration of free proline under heavy metal stress such as sunflower, wheat, tomato, milk thistle, *Solanum nigrum* and *Vigna unguiculata* (Lalk and Dorfling 1985; Bhattacharjee and Mukherjee 1994; Costa and Morel 1994; De and Mukherjee 1998; Zengin and Munzuroglu 2006; Sun et al. 2007; Khatamipour et al. 2011). Higher concentration of free proline was recorded in the roots of *P. hysterophorus* plant as compared to the stem and leaves. Similarly high root proline concentration in *V. unguiculata* plant was found by Bhattacharjee and Mukherjee (1994). GA_3 treatment demonstrated high concentrations of free proline, which suggest an important role of GA3 in the synthesis of proline.

Phenolic concentration within plant tissues

Phenolic compounds play an important role in protection, restoration and degradation processes caused by toxic chemicals (Rice-Evans et al. 1997). High concentration of total phenolics has been found in different plants under various environmental stresses (Grace and Logan 2000; Lavola et al. 2000; Diaz et al. 2001; Sakihama and Yamasaki 2002). Soluble phenolic compounds showed important antioxidant activity and are thus considered to be closely related to stress situations (Wild and Schmitt 1995). Schwitzguébel et al. (2001) reported that Scots pine accumulates high concentration of soluble phenolics subjected to Cd stress. Phenolic compounds act as antioxidant during heavy metal stress (Michalak 2006). High concentration of phenolics was recorded in leaves of the *P. hysterophorus* plant compared to roots and stem. Similarly high concentration of phenolic compounds in leaves of *Crotalaria juncea* was reported (Uraguchi et al. 2006).

Chlorophyll contents

Excess of cadmium in soil decreases content of chlorophyll (Ngayila et al. 2008), its synthesis rate (Vajpayee et al. 2000), efficiency of photosystems (Chugh et al. 1997), photosynthetic enzymes (Mobin and Nafees 2007; Thapar et al. 2008) and plant water balance and consequently reduces plant growth and biomass (Zhou and Qiu 2005). Present results demonstrated that negative correlation existed between cadmium contents of leaf and the chlorophyll contents (Table 7) which are in agreement with earlier reports of Mobin and Nafees (2007), Sun et al. (2008), Ekmekci et al. (2008) and Xue et al. (2014), who found that heavy metal suppressed the photosynthetic activity of plants. Faller et al. (2005) demonstrated that Cd^{2+} has inhibitory effect on the photoactivation of photosystem II

as result of its competitive binding with the Ca^{2+} site. The net photosynthetic rate has been shown to decrease conspicuously with high concentrations of cadmium (Lakshaman and Surinder 1999). Different physiological activities influence the metabolization of chlorophyll in plants. The chlorophyll a was first synthesized and transformed into chlorophyll b (Guo et al. 2006). Present results showed higher content of chlorophyll a in *Parthenium* plant as compared to the chlorophyll b (Table 4) and showed agreement with the findings of Mobin and Nafees (2007).

Conclusions and recommendations

Phytoextraction capabilities of *P. hysterophorus* plant highly increased at flowering stage (reproductive stage), and on bases of its bioconcentration factor, it is suggested as hyperaccumulator of cadmium. GA_3 increased cadmium accumulation in plant. The effect of GA_3 was more pronounced at higher concentration in combination with split doses of EDTA at low concentrations. Free proline and total phenolics significantly increased with the increase in Cd concentration in plant tissues, especially in the GA_3-treated plants. This suggests that GA_3 has some role in the synthesis of these compounds. Proline and phenolics showed positive correlation with the plant dry biomass as well as with the Cd accumulation in different parts of the plant. Further study is recommended to investigate the biochemical and molecular basis of proline and phenolic synthesis and the mechanism through which GA3 enhance their biosynthesis in plant during Cd stress.

Acknowledgments The Directorate of Science and Technology, Khyber Pakhtunkhwa, Pakistan, is highly acknowledged for the full financial support. This manuscript is a part of the Ph.D. thesis of the principal author.

References

Adewole MB, Sridhar MKC, Adeoye GO (2010) Removal of heavy metals from soil polluted with effluents from a paint industry using *Helianthus annuus* L. and *Tithonia diversifolia* (Hemsl.) as influenced by fertilizer applications. Biorem J 14(4):169–179

Åkesson A, Bjellerup P, Lundh T, Lidfeldt J, Nerbrand C, Samsioe G (2006) Cadmium-induced effects on bone in a population-based study of women. Environ Health Perspect 114:830–834

Alkorta I, Allica JH, Becerril JM, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. RESB 3:71–90

Allen SE (1974) Chemical analysis of ecological materials. Blackwell Scientific Publication, Oxford, London

Aloni B, Daie J, Wyse RE (1986) Enhancement of (14C)-sucrose export from source leaves of *Vicia faba* by gibberellic acid. Plant Physiol 82:962–966

Al-Wakeel SAM, Hamed AA, Dadoura SS (1995) Interactive effects of water stress and gibberellic acid on mineral composition of fenugreek plant. Egypt J Physiol Sci 18:269–282

Ambedkar G, Muniyan M (2013) Bioaccumulation of heavy and essential metals in the selected freshwater fish species from Perumal lake Cuddalore District, Tamilnadu, India. ISRJ 2(12):1–7

Ansari HR (1996) Effect of some phytohormones and NPK on growth and metabolism of mustard, PhD Thesis, Aligarh Muslim University, Aligarh, India

Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenoloxidae in Beta vulgaris. Plant Physiol 24:1–15

Arteca RN (1996) Plant growth substances: principles and applications. Chapman and Hall Inc, New York

Arun KS, Carlos C, Herminia LZ, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution ecology and phytochemistry. Biorecovery 1:811–826

Barcelo J, Poschenriede C (1990) Plant water relations as affected by heavy metal stress: a review. J Plant Nutr 13:1–37

Bates LS, Waldren SP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Benjerano NR, Lips SH (1970) Hormonal regulation of nitrate reductase activity in leaves. New Phytol 69(1):165–169

Bhattacharjee S, Mukherjee AK (1994) Influence of cadmium and lead on physiological and biochemical responses of *Vigna unguiculata* (L). Walp. Seedling germination behaviour, total protein, proline content and protease activity. Pollut Res 13:269–277

Broughton WJ, McComb AJ (1971) Changes in the pattern of enzyme development in gibberellin-treated pea internodes. Annu Bot J 35: 213–228

Buchanan BB, Gruissem W, Jones RL (2000) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, Maryland

Chai MW, Li RL, Shi FC, Liu FC, Pan X, Cao D, Wen X (2012) Effects of cadmium stress on growth, metal accumulation and organic acids of Spartina alterniflora Loisel. Afr J Biotechnol 11(22):6091–6099

Chen H, Cutright T (2001) EDTA and HEDTA effects on Cd, Cr and Ni uptake by *Helianthus annus*. Chemosphere 45:21–28

Chen Y, Shen Z, Li X (2004) The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. Appl Geo-chem 19:1553–1565

Choudhary NL, Sairam RK, Tyagi A (2005) Expression of delta1-pyrroline-5-carboxylate synthetase gene during drought in rice (Oryza sativa L.). Ind J Biochem Biophys 42:366–370

Chugh LK, Sawhney SK, Ghorbal MN, Ferjani EE (1997) Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzymes activities in bean (*Phaseolus vulgaris* L.). Plant Sci 127:139–147

Costa G, Morel J (1994) Water relations, gas exchange and amino acid content in Cd-treated lettuce. Plant Physiol Biochem 32:561–570

Crozier A, Turnbull CGN, (1984) Gibberellins: biochemistry and action in extension growth. What's New in Plant Physiol 15: 9–12

De B, Mukherjee AK (1998) Mercury induced metabolic changes in seedlings and cultured cells of tomato. Geobios 23:83–88

Dhawan SR, Dhawan P (1996) Regeneration in *Parthenium hysterophorus* L. World Weeds 3:181–182

Diaz J, Bernal A, Pomar F, Merino F (2001) Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annum* L.) seedlings in response to copper stress and its relation to lignification. Plant Sci 1:161–179

Ekmekci Y, Tanyolac D, Ayhan B (2008) Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. J Plant Physiol 165:600–611

Elless MP, Blaylock MJ (2000) Amendment optimization to enhance lead extractability from contaminated soils for phytoremediation. Int J Phytoremed 2:75–89

Entry JA, Watrud LS, Reeves M (1999) Accumulation of 137Cs and 90Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi. Int J Environ Pollut 104:449–457

Epelde L, Hernández-Allica J, Becerril JM, Blanco F, Garbisu C (2008) Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. Sci Total Environ 401:21–28

Evins WH, Varner JE (1972) Hormonal control of polyribosome formation in barley aleurone layers. Plant Physiol 49:348–352

Fabro G, Kovács I, Pavet V, Szabados L, Alvarez ME (2004) Proline accumulation and AtP5CS2 gene activation are induced by plant-pathogen incompatible interactions in Arabidopsis. Mol Plant-Microbe Interact 17:343–350

Falkowska M, Pietryczuk A, Piotrowska A, Bajguz A, Grygoruk A, Czerpak R (2011) The effect of gibberellic acid (GA_3) on growth, metal biosorption and metabolism of the green algae Chlorella vulgaris (Chlorophyceae) Beijerinck exposed to cadmium and lead stress. Pol J Environ Stud 20:53–59

Faller P, Kienzler K, Krieger-Liszkay A (2005) Mechanism of Cd2+ toxicity: Cd2+ inhibits photoactivation of photosystem II by competitive binding to the essential Ca2+ site. Biochim Biophys Acta 1706:158–164

Genrich I, Burd D, George D, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbial 46:237–245

Grace SC, Logan BA (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Phil Trans R Soc Lond B 355:1499–1510

Guo CN, Liu F, Xu XM (2006) Chlorophyll-b deficiency and photosynthesis in plants. Plant Physiol Commun 42:967–973

Hadi F, Bano A (2009) Utilization of *Parthenium hysterophorus* for the remediation of lead-contaminated soil. Weed Biol Manag 9(4):307–314

Hadi F, Ali N, Ahmad A (2014) Enhanced phytoremediation of cadmium-contaminated soil by Parthenium hysterophorus plant: effect of gibberellic acid (GA3) and synthetic chelator, alone and in combinations. Bioremediat J 18(1):46–55

Hadi F, Bano A, Fuller MP (2010) The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA_3 and IAA) and EDTA alone and in combinations. Chemosphere 80:475–462

Haudecoeur E, Tannieres M, Cirou A, Raffoux A, Dessaux Y, Faure D (2009) Proline antagonizes GABA-induced quenching of quorum-sensing in *Agrobacterium tumefaciens*. Proc Natl Acad Sci U S A 106:14587–14592

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: a review. Plant Signal Behav 7(11):1456–1466

Houshmandfar A, Moraghebi F (2011) Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower. Afr J Agric Res 6(5):1182–1187

John R, Ahmad P, Gadgil K, Sharma S (2009) Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. Int J Plant Prod 3:65–76

John R, Ahmad P, Gadgil K, Sharma S (2008) Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ 54(6):262–270

Kafel A, Rozpędek K, Szulińska E, Zawisza-Raszka A, Migula P (2014) The effects of cadmium or zinc multigenerational exposure on metal tolerance of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environ Sci Pollut Res 21:4705–4715. doi:10.1007/s11356-013-2409-z

Kamnev AA, Van Der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20:239–258

Khan NA, Ansari HR, Samiullah (1998) Effect of gibberellic acid spray during ontogeny of mustard on growth, nutrient uptake and yield characteristics. J Agron Crop Sci 181:61–73

Khatamipour M, Piri E, Esmaeilian Y, Tavassoli A (2011) Toxic effect of cadmium on germination, seedling growth and proline content of milk thistle (*Silybum marianum*). Ann Biol Res 2(5):527–532

Kidd PS, Domínguez-Rodríguez MJ, Díez J, Monterroso C (2007) Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere 66:1458–1467

Lakshaman KC, Surinder KS (1999) Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. Plant Physiol Biochem 37:297–303

Lalk I, Dorfling K (1985) Hardening, ABA, proline and freezing resistance in the winter wheat varieties. Physiol Plant 63:287–29

Lavola A, Julkunen-Titto R, Delarosa TM, Lehto T, Aphalo PJ (2000) Allocation of carbon to growth and secondary metabolites in birch seedlings under UV-B radiation and CO2 exposure. Physiol Plant 109:260–267

Li YM, Chaney R, Brewer E, Roseberg R, Angle JS, Baker AJM, Reeves R, Nelkin J (2003) Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. Plant Soil 249:107–115

Liu J, Zhu Q, Zhang Z, Xu J, Yang J, Wong MH (2005) Variations in cadmium accumulation among rice cultivars and types and the selection of cultivars for reducing cadmium in the diet. J Sci Food Agric 85:147–153

Liu Z, He X, Chen W, Yuan F, Yan K, Tao D (2009) Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator—*Lonicera japonica* Thunb. Water Air Soil Pollut 196:29–40

Lone MI, He Z, Stoffella PJ, Yang X (2008) Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. J Zhejiang Univ Sci B 9:210–220

Lou LQ, Ye ZH, Wong MH (2007) Solubility and accumulation of metals in Chinese brake fern, vetiver and *rostrate sesbania* using chelating agents. Int J Phytoremediat 9:325–343

Mamindy-Pajany Y, Sayen S, Guillon E (2014) Impact of lime-stabilized biosolid application on Cu, Ni, Pb and Zn mobility in an acidic soil. Environ Sci Pollut Res 21:4473–4481

Masroor M, Khan A, Gautam C, Mohammad F, Siddiqui MH, Naeem M, Khan MN (2006) Effect of gibberellic acid spray on performance of tomato. Turk J Biol 30:11–16

Mazharia M, Homaeeb M (2012) Annual halophyte *Chenopodium botrys* can phytoextract cadmium from contaminated soils. J Basic Appl Sci Res 2(2):1415–1422

Meers E, Lesage E, Lamsal S, Hopgood M, Vervaekle P, Tack FMG, Verloo MG (2005) Enhanced phytoextraction: I. Effect of EDTA and citric acid on heavy metal uptake by *Heliantus annuus* from a calcareous soil. Int J Phytoremed 7:129–142

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol J Environ Stud 15(4): 523–530

Mobin M, Nafees AK (2007) Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. J Plant Physiol 164:601–610

Moore TC (1989) Biochemistry and physiology of plant hormones. Springer-Verlag Inc, New York

Mubeen H, Naeem I, Taskeen A (2010) Phytoremediation of Cu(II) by *Calotropis procera* roots. N Y Sci J 3(3):1–5

Ngayila N, Botineau M, Baudu M, Basly JP (2008) *Myriophyllum alterniflorum* DC. Effect of low concentrations of copper and cadmium on somatic and photosynthetic endpoints: a chemometric approach. Ecol Indic 9:379–384

Ogawa T, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Nogawa K (2004) Relationship among prevalence of patients with itai-itai disease, prevalence of abnormal urinary findings, and cadmium

concentrations in rice of individual hamlets in the Jinzu river basin, Toyama prefecture of Japan. Int J Environ Health Res 14:243–252
Okedeyi OO, Dube S, Awofolu OR, Nindi MN (2014) Assessing the enrichment of heavy metals in surface soil and plant (*Digitaria eriantha*) around coal-fired power plants in South Africa. Environ Sci Pollut Res 21:4686–4696. doi:10.1007/s11356-013-2432-0
Parsons WT, Cuthbertson EG (1992) Noxious weeds of Australia. Inkata Press, Melbourne
Persans M, Salt DE (2000) Possible molecular mechanisms involved in nickel, zinc and selenium hyperaccumulation in plants. Biotechnol Genet Eng Rev 17:385–409
Poschenrieder C, Gunse G, Barcelo J (1989) Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. Plant Physiol 90:1365–1371
Ramadevi S, Prasad MNV (1998) Copper toxicity in *Ceratophyllum demersum* l. (coontail), a free floating macrophyte: response of antioxidant enzymes and antioxidants. Plant Sci. 138–157
Reeves RD, Brooks RR (1983) Hyperaccumulation of lead and zinc by two metallophytes from a mining area of Central Europe. Environ Pollut A 31:277–287
Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152–159
Sakihama Y, Yamasaki H (2002) Lipid peroxidation induces by phenolics in conjunction with aluminium ions. Biol Plantarum 45:249–254
Schwitzguébel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL, Polle A (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. Plant Physiol 127:887–898
Schwitzguebel JP, Kumpiene J, Comino E, Vanek T (2009) From green to clean: a promising and sustainable approach towards environmental remediation and human health for the 21 st century. Agrochimica 53:209–237
Sharma SS, Schat H, Vooijs R (1998) In vitro alleviation of heavy metal-induced enzyme inhibition by proline. Phytochemistry 46:1531–1535
Simmons RW, Pongsakul P, Saiyasitpanich D, Klinphoklap S (2005) Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: implications for public health. Environ Geochem Health 27:501–511
Singh OV, Labana S, Pandey G, Budhiraja R, Jain RK (2003) Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61:405–412
Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic—phosphotungstic acid reagents. Am J Enol Vitic 16:144–158
Sun Q, Ye ZH, Wang XR, Wong MH (2007) Cadmium hyperaccumulation leads to an increase of glutathione rather than phytochelatins in the cadmium hyperaccumulator *Sedum alfredii*. J Plant Physiol 164:1489–1498
Sun YB, Zhou QX, Diao CY (2008) Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator *Solanum nigrum* L. Bioresour Technol 99:1103–1110
Tassi E, Pouget J, Petruzzelli G, Barbafieri M (2008) The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. Chemosphere 71:66–73
Thapar R, Srivastava AK, Bhargava P, Mishra Y, Rai LC (2008) Impact of different abiotic stress on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated *Anabaena doliolum*. J Plant Physiol 165:306–316
Thayalakumaran T, Robinson BH, Vogeler I, Scotter DR, Clothier BE, Percival HJ (2003) Plant uptake and leaching of copper during EDTA-enhanced phytoremediation of repacked and undisturbed soil. Plant Soil 254:415–423
Uraguchi S, Watanabe I, Yoshitomi A, Kiyono M, Kuno K (2006) Characteristics of cadmium accumulation and tolerance in novel Cd-accumulating crops, *Avena strigosa* and *Crotalaria juncea*. J Exp Bot 57:2955–2965
Vajpayee P, Tripathi RD, Rai UN, AliSingh SN (2000) Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. Chemosphere 41:1075–1082
Wild A, Schmitt V (1995) Diagnosis of damage to Norway spruce (*Picea abies*) through biochemical criteria. Physiol Plant 93:357–382
Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol 5:218–223
Wood A, Paleg LG (1974) Alteration of liposomal membrane fluidity by gibberellic acid. Aust J Plant Physiol 1:31–40
Xu J, Yin X, Li X (2009) Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, *Solanum nigrum* L. Plant Cell Rep 28:325–333
Xue Z, Gao H, Zhao S (2014) Effects of cadmium on the photosynthetic activity in mature and young leaves of soybean plants. Environ Sci Pollut Res 21:4656–4664
Yang SL, Lan SS, Gong M (2009) Hydrogen peroxide-induced proline and metabolic pathway of its accumulation in maize seedlings. J Plant Physiol 166:1694–1699
Zengin FK, Munzuroglu O (2006) Toxic effects of cadmium (Cd++) on metabolism of sunflower (*Helianthus annuus* L.) seedlings. Acta Agric Scand Sect B Plant Soil Sci 56:224–229
Zheng HP, Gao LS, Wang SR (2010) Effects of cadmium on growth and antioxidant responses in *Glycyrrhiza uralensis* seedlings. Plant Soil Environ 56(11):508–515
Zhou WB, Qiu BS (2005) Effects of cadmium hyperaccumulation on physiological characteristics of *Sedum alfredii* Hance (Crassulaceae). Plant Sci 169:737–745