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RESEARCH ARTICLE

Enhanced phytoremediation of cadmium polluted water through two aquatic plants *Veronica anagallis-aquatica* and *Epilobium laxum*

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Abstract Toxic metal-contaminated water is a major threat to sustainable agriculture and environment. Plants have the natural ability to absorb and concentrate essential elements in its tissues from water solution, and this ability of plants can be exploited to remove heavy/toxic metals from the contaminated water. For this purpose, two plants *Veronica anagallis-aquatica* and *Epilobium laxum* were hydroponically studied. The effect of different fertilizers (NPK) and plant growth regulators (GA_3 and IAA) were evaluated on growth, biomass, free proline, phenolics, and chlorophyll contents, and their role in Cd phytoaccumulation was investigated. Results showed that in both plants, fertilizer addition to media (treatment T4) produced the highest significant increase in growth, biomass (fresh and dry), cadmium concentration, proline, phenolics, and chlorophyll concentrations. The significant effect of GA_3 in combination with NPK foliar spray (treatment T12) was observed on most of the growth parameters, Cd concentration, and proline and phenolic contents of the plants. The free proline and total phenolics showed positive correlation with cadmium concentration within plant tissues. Proline showed significantly positive correlation with phenolic contents of root and shoot. *Veronica* plant demonstrated the hyperaccumulator potential for cadmium as bioconcentration factor (BCF >1) which was much higher than 1, while *Epilobium* plant showed non-hyperaccumulator potential. It is recommended for further study to investigate the role of *Veronica* plant for other metals and to study the role of phenolics and proline contents in heavy metal phytoextraction by various plant species.

Keywords Cadmium · Hyperaccumulators · Growth regulators · Proline · Phenolics

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Introduction

Heavy metal-polluted soil and water poses serious threat to the environment and consequently human health. Cadmium (Cd) is a heavy metal of great concern, which mainly enters the soil and water through anthropogenic activities such as mining, industrial effluents, and application of phosphorus fertilizer to the fields (Ali and Hadi 2015). Various methods (both physical and chemical) have been used for the decontamination of metal-polluted water and soil, but these methods are usually expensive and laborious and produce negative effects on the ecosystem. Green plants, which are often thought as a source of food, fibers, and fuel, can also be exploited for the purpose of decontamination of metal-polluted soil and water. The plant-based technologies are collectively known as phytoremediation. One of the phytoremediation techniques, known as phytoextraction, makes use of plants to absorb and accumulate pollutant in its harvestable parts, and the harvested parts are then disposed off (Kumar et al. 1995; Junliang et al. 2013). The phytoextraction potential of more than 400 plant species have been documented till now; among them, species of *Brassica*, *Thlaspi*, *Arabidopsis*, and *Sedum* have been studied mostly (lone et al. 2008). Production of high biomass and tolerance of high concentration metals in the biomass

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are the two basic requirements for a plant to be used for phytoextraction purposes (Ali and Hadi 2015).

Plants grown on heavy metal-polluted water and soil often show reduction in biomass, and for increasing the biomass several strategies have been adopted by scientists. The use of fertilizers and plant growth hormones are commonly used to increase the biomass of plant for better phytoextraction purposes (Li et al. 2012; Hadi et al. 2014). Fertilizer application not only increases the biomass of plants but can also increase the absorption of metals from soil. Increase in metal bioabsorption due to fertilizer application has been reported in plants such as *Pteris vittata* (Mandal et al. 2012). Plant growth regulators are produced naturally inside plants at low concentrations and can promote or inhibit the growth of a plant. One of the plant growth regulators, known as gibberellic acid (GA_3), enhances plant growth by stimulating cells' elongation growth in stem and thus increases dry biomass and yield of the plant (Deotale et al. 1998).

Toxic heavy metals when entering a plant can harm its tissues and disturb the metabolic reaction. Plants have developed certain homeostatic mechanisms to keep an optimal concentration of important metal ions in cellular compartments and also to reduce the harmful effects of heavy metals. Toxic heavy metals often cause oxidative stress in plants by the production of reactive oxygen species (ROS) that cause the oxidation of macromolecules especially lipids, by activating the lipoxygenase enzyme that stimulates lipid peroxidation (Michalak 2006). Like other heavy metals, a high concentration of cadmium in plants causes oxidative stress probably by interacting with the antioxidative defense and by interfering with the respiratory electron transport chain (Yan et al. 2013). In response to the oxidative stress caused by heavy metals, plants increase the synthesis of phenolic compounds; they can directly scavenge molecular species of active oxygen (Michalak 2006). Proline is a vital organic compound accumulated in various plant species due to abiotic stress (Handique and Handique 2009; Ali and Hadi 2015). The exact process by which proline accumulation protects plants against heavy metal stress is unknown, but there are some evidences that proline shields some of the key enzymes from being deactivated by toxic heavy metals and also acts as an osmoregulator (Handique and Handique 2009). *Veronica anagallis-aquatica* is an aquatic plant which belongs to the family Scrophulariaceae (Hutchinson 1975). It is also known as water speedwell and blue speedwell. It is a perennial herb with root-like subterranean stem. It has horizontal stem with smooth leaves and racemose inflorescence. It occurs in moist, wet, and semi-aquatic habitat. *Epilobium laxum* Royle plant is commonly known as willow herbs, a dicot angiosperm plant which belongs to the family Onagraceae. It is herbaceous, annual, or perennial having a stunted nody stem with simple alternate, ovate, to lanceolate leaves. It is mostly present in moist temperate regions, very common in the western Himalaya at middle and upper elevations (Nasir 2005). In the present experiment, two native plants (*Veronica anagallis-aquatica* and *Epilobium laxum*) were tested hydroponically under various treatments of fertilizers and growth regulators for their phytoextraction potentials. The endogenous total phenolics and free proline levels in plants were investigated and correlated with the Cd accumulation in plants.

Materials and methods

Plant materials

Veronica anagallis and *Epilobium laxum* plantlets (2-cm roots and 3-cm shoot for each plantlet) were collected from the river of Miandam valley, Pakistan. To prevent dehydration and flaccidity, the plantlets were kept under cool condition in an insulation box and brought to the laboratory for further experimentations.

Preparation of growth media and cadmium addition

Hoagland's solution (growth media) was prepared, and cadmium was added to it in the form of cadmium acetate dehydrate [Cd (CH3COO)2°2H2O] at the rate of 50 ppm and thoroughly mixed. Then, media were equally distributed into flasks. Three replicate flasks were used for each treatment and control. Growth media without cadmium were used as control.

Seedling transplantation into growth media

The healthy seedlings were selected and transplanted into the flasks containing growth media (one seedling per flask). To keep the volume of media at constant level (150 mL) fresh media were regularly added to the flasks. The experiment was conducted under natural light/dark conditions (14/10) with temperature 30/25 °C.

Treatments applied during experiment

Plants were treated with different fertilizers and plant growth regulators, alone and in different combinations as given in Table 1.

Fertilizer treatments

Three different fertilizers (urea, single super phosphate, and potassium sulfate) were used as a source of macronutrients (nitrogen, phosphorus, and potassium, respectively). These fertilizers were used in two ways: added directly to the media

Table 1 Treatments used during experiment

Treatments	Denoted by	Treatments	Denoted by
Without Cd	C	Cd + P foliar spray	T6
With Cd	C1	Cd + K foliar spray	T7
Cd + N added to growth media	T1	Cd + NPK foliar spray	T8
Cd + P added to growth media	T2	Cd + IAA foliar spray	T9
Cd + K added to growth media	T3	Cd + GA3 foliar spray	T10
Cd + NPK added to growth media	T4	Cd + (IAA + NPK foliar spray)	T11
Cd + N foliar spray	T5	Cd + (GA3 + NPK foliar spray)	T12

Cd concentration 50 ppm and Hoagland solution was used as growth media

in treatments (T1 to T4) and applied as foliar spray on the plant leaves in treatments (T5 to T12). In both the cases, the fertilizers were applied alone (N, P, and K) and also in combination of the three (NPK). The macronutrients (N, P, and K) were used at concentrations of 1000, 500, and 700 ppm, respectively. The same concentration of each nutrient was used in combination treatments.

Hormonal treatments

Two different growth regulators, GA_3 and indole acetic acid (IAA), were used as foliar spray at a concentration of 10^{-5} M.

Combination treatments of hormones and NPK

Plants of T9 to T12 were treated with the NPK foliar spray in combinations with GA_3 and IAA (i.e., GA_3 + NPK and IAA + NPK). The concentrations of NPK, GA_3, and IAA used were the same as discussed earlier. All the treatments were applied in four doses at 1-week interval. The first dose of the treatments was given after 1 week of plant transplantation into the media.

Plant harvesting and data collection

All of the experimental plants were harvested after 30 days of treatment, then the roots were washed thoroughly with EDTA solution to remove surface-bounded metal from the plant surface. The root, stem, and leaf length of each plant was measured using a centimeter ruler. Then, immediately, each plant was cut into root, stem, and leaves and the fresh biomass of each part was measured with analytical balance. Each part of the plants was kept in separate paper bags and was labeled. The samples in paper bags were then dried by placing in an oven at 70 °C for 48 h. The dried samples were then weighed again for the dry biomass using analytical balance. Then, samples were crushed into fine powder with the help of a commercial blender. The powdered samples were then stored in small plastic bags and labeled.

Acid digestion and cadmium analysis

Allen method (1974) was used for acid digestion of samples; 0.25 g of powder was weighed from each sample, kept in 50-mL volumetric flasks, and mixed with 6.5 mL of acid solution (nitric acid and sulfuric acid in a ratio of 5:1). After shaking, the flasks were then kept on electric hot plates for complete dissolution. When a white fume started from boiling solution, the volume was made up to 50 mL with distilled water and the samples were allowed gradually to cool, then filtered through filter paper and analyzed by atomic absorption/flame spectrophotometer for Cd contents in roots, stem, and leaves .

Bioconcentration factor

The bioconcentration factor (BCF) is the ration of metal concentration in plants to that of the growth medium: BCF = metal concentration in plant / metal concentration in growth medium. The BCF is considered as index for evaluating the efficiency of plants for their hyperaccumulation potential (Fitz and Wenzel 2002).

Free proline analysis in root and leaves

Bates et al. (1973) method was used for the biochemical analysis and extraction of protein. One hundred milligrams of fresh tissues of roots and leaves were weighed, put in 2-mL tubes, and homogenized in 1.5 mL of 3 % sulfosalicylic acid. The samples were centrifuged for 5 min at 13,000 rpm. Through micropipettes, 300 μL aliquot was taken out from the supernatant in separate tubes. Then, 2 mL glacial acetic acid and acid ninhydrin was added to it and warmed for 60 min in a boiling water bath. The tubes were immediately dipped into ice after the removal of water bath to finalize the reaction. Then, 1 mL of toluene was mixed vigorously with the reaction mixture for 10–30 s. With the help of a pipette, the chromophore layer containing toluene was removed from the aqueous phase and warmed to room temperature. Absorbance of each sample was measured at a 520-nm wavelength using a

spectrophotometer. Toluene was used as a blank (control). The concentration of proline in different samples was determined from a standard curve. The reaction for each sample was performed in triplicate.

Total phenolic measurement in roots and leaves

Air-dried plant samples were ground, and 200 mg of the sample was taken, mixed with 10 mL of 80 % methanol, and shaked in a closed vessel (flask) with a shaker for half an hour. A 2-mL aliquot was taken from the extract and centrifuged at the rate of 13,000 rpm for 3 to 5 min. Folin-Ciocalteu (FC) reagent method (Singleton and Rossi 1965) was used for the analysis of total phenolics in extract. Two hundred fifty microliters of FC reagent and methanolic extract or gallic acid standard solutions at 100 μL were mixed then kept for a short interval up to 3–5 min in the dark at room temperature. Then, the solution was treated with 500 μL (7 %) sodium carbonate (Na_2CO_3) solutions, and the volume was made up to 5 mL with dH_2O and allowed for 120 min in the dark at room temperature prior to absorbance measurement. A spectrophotometer was used for the measurement of absorbance at 760 nm. Triplicates of all samples were measured. A standard solution with different concentrations of gallic acid (10, 30, 50, 100, 150 mg/L) was prepared in methanol (80 %), and phenolic concentration in samples was calculated as milligrams of gallic acid equivalent per gram of dry mass. Methanol (80 %) was used as blank solution (control).

Analysis of chlorophyll (a/b) contents

Chlorophylls a, b and total chlorophyll were determined according to the method of Arnon (1949). Fresh leaves were collected from the controls and treated plants. From each sample, 200 mg of leaves was mixed with 2 mL (80 %) acetone and then ground properly. The ground samples were transferred to Eppendorf tubes and centrifuged at 10,000 rpm for 5 min. After centrifugation, the supernatant was poured into a clean test tube. Then, 6 mL acetone was added to the test tube. Absorbance of the samples was measured at 645 and 663 nm using a spectrophotometer. Chlorophylls a, b and total chlorophyll were calculated by using the following formulas:

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

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

Statistical analysis

The data was subjected to ANOVA, and the mean values were compared by using Tukey's honest significant difference (HSD) test, at $P < 0.05$. The correlations among different parameters were analyzed. The data was analyzed using SPSS-16 and MS excel (2010).

Results

Effects of fertilizers and growth regulators on plant growth

The present results showed that Cd in hydroponic condition significantly decreased the growth, biomass, and total water content of both the *Veronica* and *Epilobium* plants (except root and leaf length) when control without Cd (C) was compared with C1 (Cd treated) (Tables 2 and 3 and Fig. 1). The treatments showed a significant increase in root, stem, and leaf length of the *Veronica* plant, except treatments T9 (IAA foliar spray), T10 (GA_3 foliar spray), and T11 (IAA + NPK foliar spray), as compared to control C1 (Table 2). In case of plant biomass (fresh and dry) and water content, all the treatments (except T2) demonstrated a significant increase as compared to control C1. The highest significant increase was recorded for treatment T4 (Cd + NPK in media) as shown in Table 2. Most of the treatments have no significant effect on the leaf dry biomass of the *Veronica* plant as compared to C1 (Table 2).

The *Epilobium* plant showed the highest significant increase in length, biomass, and total water content in plants treated with fertilizers in combination with T4 (NPK added to growth media) as shown in Table 3. Fresh biomass and total water content of root and leaves and dry biomass of stem were highly significant in treatment T12 (NPK + GA3 foliar spray) (Table 3). The effects of all other treatments (except treatment T2 and T9) were found significant on most of the growth parameters as compared to C1 (Table 3).

Effects of fertilizers and plant growth regulators on cadmium concentration and accumulation in plants

Tables 4 and 5 showed the effect of different fertilizers and growth regulators separately and in combination on Cd concentration (ppm), accumulation (mg/DBM), Cd percentage, Cd translocation, and bioconcentration in different parts of the *Veronica* and *Epilobium* plant, respectively. Both the plants showed a significant increase in Cd concentration of root, stem, and leaf for all the treatments as compared to control C1. In *Veronica* plant, the highest root (1666.30 ± 25.00 ppm), stem (739.10 ± 16.00 ppm), and leaf (785.40 ± 23.40 ppm) cadmium concentration was found in the treatment T4 (NPK addition into media) as shown in Table 4. Accumulation of cadmium (mg Cd/plant DBM) within plant tissues was significantly increased by all the treatments (compared to the control plants) except treatments T2 (in all parts of the plant), T6 and T9 (only in root and leaves), and T7 (in

Table 2 Different fertilizers and growth regulator effect on length, fresh biomass, dry biomass, and water content of *Veronica* plant in Cd-contaminated growth media

Treatments		length (cm) ± SD			Fresh biomass (g) ± SD			
		Root	Stem	Leaves	Root	Stem	Leaves	Entire plant
C	Without Cd	7.667 ± 0.58^{bcd}	17.33 ± 1.15^{bcd}	8.33 ± 0.57^{de}	8.77 ± 0.77^{a}	16.34 ± 1.51^{abc}	8.54 ± 1.10^{bcde}	33.70 ± 2.89^{abc}
C1	Only Cd	5.667 ± 0.58^{d}	13.00 ± 1.00^{f}	7.33 ± 1.52^{e}	2.70 ± 0.95^{d}	4.64 ± 1.15^{f}	2.39 ± 0.64^{f}	9.75 ± 2.75^{e}
T1	Cd + N added to media	9.50 ± 0.50^{b}	18.00 ± 1.00^{abc}	11.00 ± 1.00^{abcd}	8.07 ± 0.60^{ab}	15.68 ± 1.49^{abcd}	8.67 ± 1.64^{bcde}	32.40 ± 1.21^{bc}
T2	Cd + P added to media	9.00 ± 1.00^{b}	18.83 ± 1.60^{abc}	11.00 ± 2.00^{abcd}	4.62 ± 0.68^{cd}	9.22 ± 2.41^{ef}	6.07 ± 1.52^{e}	19.90 ± 4.62^{de}
T3	Cd + K added to media	8.50 ± 0.50^{bc}	17.67 ± 0.57^{bcd}	9.50 ± 0.50^{bcde}	7.48 ± 0.50^{abc}	16.63 ± 1.58^{abc}	8.98 ± 1.97^{bcde}	33.10 ± 3.13^{bc}
T4	Cd + NPK added to media	12.67 ± 0.58^{a}	20.50 ± 0.50^{ab}	12.50 ± 0.50^{a}	10.30 ± 1.49^{a}	20.80 ± 0.96^{a}	12.73 ± 0.64^{a}	43.90 ± 1.71^{a}
T5	Cd + N foliar spray	8.50 ± 0.50^{bc}	16.50 ± 1.50^{cde}	11.50 ± 0.50^{abc}	8.07 ± 0.57^{ab}	15.98 ± 3.48^{abcd}	10.95 ± 0.94^{abc}	35.00 ± 5.00^{abc}
T6	Cd + P foliar spray	6.50 ± 0.50^{cd}	17.17 ± 1.25^{cd}	11.50 ± 0.50^{abc}	5.67 ± 0.84^{bcd}	14.38 ± 3.47^{cbcde}	8.69 ± 1.87^{bcde}	28.70 ± 6.13^{cd}
T7	Cd + K foliar spray	8.50 ± 0.50^{bc}	16.50 ± 0.50^{cde}	10.00 ± 1.00^{abcd}	7.87 ± 0.71^{ab}	17.73 ± 1.66^{abc}	10.66 ± 0.25^{abcd}	36.30 ± 1.78^{abc}
T8	Cd + NPK foliar spray	9.33 ± 1.53^{b}	19.00 ± 1.00^{abc}	12.00 ± 1.00^{ab}	9.17 ± 1.94^{a}	19.00 ± 1.00^{ab}	11.87 ± 0.51^{ab}	40.00 ± 2.49^{ab}
T9	Cd + IAA foliar spray	6.50 ± 0.50^{cd}	13.67 ± 1.52^{ef}	8.00 ± 1.00^{e}	4.50 ± 0.50^{cd}	9.83 ± 1.26^{def}	7.16 ± 0.76^{de}	21.50 ± 1.32^{d}
T10	Cd + GA3 foliar spray	7.50 ± 0.50^{bcd}	14.50 ± 0.50^{def}	8.50 ± 0.50^{de}	7.40 ± 1.84^{abc}	12.54 ± 4.29^{cde}	7.94 ± 1.22^{cde}	27.90 ± 6.29^{cd}
T11	Cd + (IAA + NPK foliar spray)	6.50 ± 0.50^{cd}	16.00 ± 1.00^{cdef}	9.00 ± 1.00^{cde}	8.30 ± 0.26^{ab}	15.69 ± 0.60^{abcd}	10.33 ± 1.52^{abcd}	34.30 ± 2.06^{abc}
T12	Cd + (GA3 + NPK foliar spray)	8.00 ± 1.00^{bc}	21.00 ± 1.00^{a}	12.80 ± 1.04^{a}	10.10 ± 0.86^{a}	18.00 ± 1.00^{abc}	13.00 ± 1.00^{a}	41.10 ± 0.87^{ab}

Treatments	Dry biomass (g) ± SD				Total water content (g) ± SD			
	Root	Stem	Leaves	Entire plant	Root	Stem	Leaves	Entire plant
C	4.24 ± 0.69^{a}	8.14 ± 0.03^{abc}	4.28 ± 0.62^{a}	16.66 ± 2.30^{ab}	4.53 ± 0.51^{abc}	8.20 ± 1.87^{abc}	4.25 ± 0.61^{de}	16.99 ± 2.64^{cde}
C1	1.41 ± 0.31^{d}	2.57 ± 0.07^{f}	1.62 ± 0.38^{c}	5.61 ± 0.54^{g}	1.28 ± 1.11^{c}	2.07 ± 1.24^{d}	0.77 ± 0.93^{f}	4.13 ± 3.17^{f}
T1	3.33 ± 0.57^{abc}	6.20 ± 0.03^{bcde}	3.46 ± 0.66^{abc}	13.01 ± 0.70^{abcde}	4.73 ± 0.87^{ab}	9.47 ± 1.34^{abc}	5.20 ± 0.98^{cde}	19.46 ± 1.91^{abcde}
T2	2.20 ± 0.24^{cd}	4.58 ± 0.02^{ef}	2.56 ± 0.61^{abc}	9.35 ± 1.26^{efg}	2.41 ± 0.69^{bc}	4.63 ± 2.17^{cd}	3.51 ± 0.98^{ef}	10.56 ± 3.85^{ef}
T3	3.48 ± 0.51^{abc}	5.65 ± 0.06^{de}	3.06 ± 1.44^{abc}	12.13 ± 2.21^{cdef}	4.00 ± 0.86^{abc}	10.9 ± 1.76^{ab}	5.92 ± 1.16^{bcde}	20.90 ± 2.59^{abcd}
T4	4.10 ± 0.62^{a}	8.66 ± 0.08^{a}	4.37 ± 0.54^{a}	17.15 ± 0.79^{a}	6.20 ± 1.10^{a}	12.6 ± 0.68^{a}	8.35 ± 0.58^{ab}	26.70 ± 1.20^{a}
T5	3.23 ± 0.23^{abc}	7.05 ± 0.04^{abcd}	3.79 ± 0.71^{ab}	14.08 ± 0.14^{abcd}	4.84 ± 0.34^{ab}	8.92 ± 3.14^{abc}	7.15 ± 1.53^{abcd}	20.91 ± 4.87^{abcd}
T6	2.26 ± 0.57^{cd}	5.94 ± 0.03^{de}	3.34 ± 0.75^{abc}	11.55 ± 2.36^{def}	3.40 ± 0.57^{abc}	8.43 ± 2.36^{abc}	5.35 ± 1.17^{bcde}	17.19 ± 3.76^{bcde}
T7	3.33 ± 0.57^{abc}	6.01 ± 0.14^{cde}	3.50 ± 0.50^{abc}	12.84 ± 0.36^{bcde}	4.53 ± 1.26^{abc}	11.70 ± 1.90^{ab}	7.15 ± 0.24^{abcd}	23.42 ± 1.62^{abc}
T8	3.50 ± 0.50^{abc}	6.40 ± 0.01^{bcde}	4.13 ± 0.34^{ab}	14.03 ± 1.01^{abcd}	5.67 ± 2.22^{ab}	12.1 ± 1.24^{a}	7.73 ± 0.49^{abc}	26.00 ± 3.22^{ab}
T9	1.18 ± 0.02^{d}	4.85 ± 0.01^{e}	2.20 ± 0.29^{bc}	8.24 ± 0.25^{fg}	3.31 ± 0.48^{abc}	4.97 ± 1.55^{cd}	4.96 ± 0.84^{cde}	13.25 ± 1.45^{de}
T10	2.55 ± 0.68^{bcd}	6.35 ± 0.01^{bcde}	2.82 ± 0.82^{abc}	11.73 ± 2.49^{def}	4.83 ± 2.03^{ab}	6.19 ± 3.48^{bcd}	5.12 ± 0.41^{cde}	16.15 ± 4.59^{cde}
T11	3.40 ± 0.52^{abc}	7.25 ± 0.02^{abcd}	3.33 ± 0.58^{abc}	13.98 ± 0.95^{abcd}	4.90 ± 0.79^{ab}	8.43 ± 1.27^{abc}	7.00 ± 2.00^{abcd}	20.33 ± 2.88^{abcd}
T12	3.83 ± 0.28^{ab}	8.28 ± 0.01^{ab}	3.97 ± 0.31^{ab}	16.09 ± 0.57^{abc}	6.27 ± 0.88^{a}	9.71 ± 0.39^{abc}	9.02 ± 0.68^{a}	25.01 ± 0.72^{abc}

Hoagland solution was used as growth media

The different superscript letters show significant difference among treatments

N nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole 3-acetic acid, GA_3 gibberellic acid

Table 3 Effect of fertilizers and plant growth regulators on length, fresh biomass, dry biomass, and water content of *Epilobium* plant in Cd-contaminated aqueous media

Treatments		Length (cm) ± SD			Fresh biomass (g) ± SD			
		Root	Stem	Leaves	Root	Stem	Leaves	Entire plant
C	Without Cd	7.67 ± 0.53^{bcd}	18.33 ± 0.57^{bcd}	8.33 ± 0.57^{cd}	9.65 ± 0.84^{ab}	17.98 ± 1.65^{abc}	9.39 ± 1.21^{bcd}	37.03 ± 3.17^{abc}
C1	Only Cd	5.67 ± 0.57^{d}	12.16 ± 0.76^{e}	7.33 ± 1.52^{d}	2.97 ± 1.04^{e}	5.11 ± 0.26^{f}	2.63 ± 0.71e	10.72 ± 2.02e
T1	Cd + N added to media	9.50 ± 0.50^{b}	18.00 ± 1.00^{bcd}	11.00 ± 1.00^{abc}	8.43 ± 0.65^{abc}	17.27 ± 0.59^{abc}	9.51 ± 1.80^{bcd}	35.24 ± 2.41^{bc}
T2	Cd + P added to media	9.00 ± 1.00^{b}	19.16 ± 1.04^{bc}	11.00 ± 2.00^{abc}	5.37 ± 0.66^{de}	10.14 ± 2.65^{def}	6.68 ± 1.67^{d}	22.28 ± 4.65^{d}
T3	Cd + K added to media	8.50 ± 0.50^{bc}	18.00 ± 0.01^{bcd}	9.50 ± 0.50^{bcd}	8.72 ± 0.59^{abc}	16.81 ± 0.80^{abcd}	9.88 ± 2.16^{bcd}	35.4 ± 83.54^{bc}
T4	Cd + NPK added to media	12.66 ± 0.57^{a}	22.50 ± 0.50^{a}	12.50 ± 0.50^{a}	10.80 ± 0.29^{ab}	22.88 ± 1.05^{a}	12.79 ± 0.97^{ab}	46.42 ± 0.30^{a}
T5	Cd + N foliar spray	8.50 ± 0.50^{bc}	18.00 ± 1.00^{bcd}	11.50 ± 0.50^{ab}	8.88 ± 0.63^{ab}	17.57 ± 3.82^{abc}	11.04 ± 0.04^{abc}	37.49 ± 4.49^{abc}
T6	Cd + P foliar spray	6.50 ± 0.50^{cd}	17.10 ± 0.45^{cd}	12.00 ± 1.00^{ab}	5.86 ± 1.44^{cde}	15.82 ± 3.81^{bcde}	9.56 ± 2.05^{bcd}	31.25 ± 7.28^{cd}
T7	Cd + K Foliar spray	8.50 ± 0.50^{bc}	16.50 ± 0.50^{d}	10.00 ± 1.00^{abcd}	9.28 ± 1.42^{ab}	19.51 ± 1.82^{abc}	11.42 ± 0.31^{ab}	40.21 ± 2.50^{abc}
T8	Cd + NPK foliar spray	9.33 ± 1.52^{b}	19.93 ± 0.40^{b}	12.00 ± 1.00^{ab}	11.01 ± 0.99^{ab}	21.53 ± 0.56^{ab}	12.76 ± 0.58^{ab}	45.38 ± 0.94^{ab}
T9	Cd + IAA foliar spray	6.50 ± 0.50^{cd}	16.00 ± 1.00^{d}	10.50 ± 0.50^{abc}	5.24 ± 0.05^{de}	9.32 ± 1.13^{ef}	7.58 ± 0.63^{cd}	22.15 ± 1.52^{d}
T10	Cd + GA3 foliar spray	7.50 ± 0.50^{bcd}	16.46 ± 0.50^{d}	8.50 ± 0.50^{cd}	8.137 ± 2.02^{bcd}	13.80 ± 4.72^{cde}	9.42 ± 1.17^{bcd}	31.36 ± 6.70^{cd}
T11	Cd + (IAA + NPK foliar spray)	6.50 ± 0.50^{cd}	16.00 ± 1.00^{d}	7.50 ± 0.50^{d}	8.77 ± 0.62^{abc}	17.26 ± 0.65^{abc}	11.70 ± 1.30^{ab}	37.73 ± 1.79^{abc}
T12	Cd + (GA3 + NPK foliar spray)	8.00 ± 1.00^{bc}	19.66 ± 1.52^{b}	10.50 ± 0.50^{abc}	11.32 ± 0.98^{a}	19.80 ± 1.10^{abc}	13.75 ± 0.55^{a}	44.88 ± 1.17^{ab}

Treatments	Dry biomass (g) ±SD				Total water content (g) ±SD			
	Root	Stem	Leaves	Entire plant	Root	Stem	Leaves	Entire plant
C	4.66 ± 0.76^{a}	9.32 ± 0.73^{a}	4.71 ± 0.68^{a}	18.33 ± 2.53a	4.98 ± 0.56^{abc}	9.03 ± 2.05^{abc}	4.68 ± 0.67^{cd}	18.69 ± 2.91^{cde}
C1	1.55 ± 0.34e	2.04 ± 0.05^{e}	1.79 ± 0.41^{c}	5.39 ± 0.11^{g}	1.41 ± 1.22^{d}	3.06 ± 0.24^{d}	0.85 ± 1.02^{e}	5.33 ± 1.91^{f}
T1	3.66 ± 0.63^{abcd}	5.84 ± 0.01^{bcd}	3.82 ± 0.72^{abc}	13.36 ± 0.60^{abcde}	4.76 ± 1.13^{abcd}	11.46 ± 0.75^{abc}	5.72 ± 1.08^{bcd}	21.91 ± 1.81^{abcde}
T2	2.42 ± 0.27^{de}	3.91 ± 0.02^{cde}	2.82 ± 0.67^{abc}	9.16 ± 1.77^{efg}	2.94 ± 0.91^{cd}	6.23 ± 1.54^{cd}	3.86 ± 1.08^{de}	13.04 ± 2.99^{ef}
T3	3.82 ± 0.56^{abcd}	6.02 ± 0.08^{bc}	3.37 ± 1.58^{abc}	13.21 ± 2.02^{bcde}	4.89 ± 0.08^{abcd}	10.79 ± 1.01^{abc}	6.51 ± 1.28^{abcd}	22.21 ± 2.25^{abcd}
T4	4.51 ± 0.68^{a}	8.42 ± 0.02^{ab}	4.82 ± 0.59^{a}	17.75 ± 0.91^{ab}	6.28 ± 0.50^{abc}	14.46 ± 0.67^{a}	7.97 ± 1.45^{ab}	28.72 ± 0.86^{a}
T5	3.55 ± 0.25^{abcd}	6.36 ± 0.045^{abc}	4.17 ± 0.78^{ab}	14.08 ± 1.15^{abcde}	5.32 ± 0.37^{abc}	11.21 ± 3.34^{abc}	6.86 ± 0.81^{abcd}	23.41 ± 4.41^{abcd}
T6	2.49 ± 0.63^{cde}	5.99 ± 0.02^{bc}	3.68 ± 0.82^{abc}	12.17 ± 2.43^{cdef}	3.37 ± 0.89^{bcd}	9.82 ± 2.81^{abc}	5.88 ± 1.29^{bcd}	19.08 ± 4.87^{cde}
T7	3.66 ± 0.63^{abcd}	6.80 ± 0.07^{ab}	3.85 ± 0.55^{abc}	14.32 ± 1.51^{abcd}	5.61 ± 2.01^{abc}	12.70 ± 2.73^{ab}	7.56 ± 0.77^{abc}	25.89 ± 3.55^{abc}
T8	4.18 ± 0.79^{abc}	8.12 ± 0.06^{ab}	4.66 ± 0.56^{a}	16.96 ± 1.43^{abc}	6.90 ± 1.70^{ab}	13.41 ± 1.42^{ab}	8.10 ± 0.54^{ab}	28.42 ± 1.24^{ab}
T9	2.29 ± 0.02^{de}	3.19 ± 0.02^{de}	2.10 ± 0.02^{bc}	7.59 ± 0.01^{fg}	2.94 ± 0.03^{cd}	6.12 ± 1.14^{cd}	5.48 ± 0.61^{bcd}	14.55 ± 1.52^{de}
T10	2.81 ± 0.75^{bcde}	5.81 ± 0.02^{bcd}	3.11 ± 0.89^{abc}	11.73 ± 3.36^{def}	5.32 ± 2.23^{abc}	7.98 ± 3.10^{bcd}	6.32 ± 1.16^{abcd}	19.63 ± 4.76^{bcde}
T11	3.74 ± 0.58^{abcd}	6.54 ± 0.02^{abc}	4.00 ± 0.60^{abc}	14.28 ± 1.31^{abcd}	5.03 ± 1.18^{abc}	10.71 ± 0.17^{abc}	7.70 ± 1.90^{abc}	23.45 ± 2.82^{abcd}
T12	4.21 ± 0.31^{ab}	7.08 ± 0.02^{ab}	4.38 ± 0.34^{a}	15.68 ± 1.02^{abcd}	7.11 ± 1.108^{a}	12.71 ± 1.98^{ab}	9.37 ± 0.45^{a}	29.20 ± 2.19^{a}

Hoagland solution was used as growth media

The different superscript letters show significant difference among treatments

N nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole 3-acetic acid, GA_3 gibberellic acid

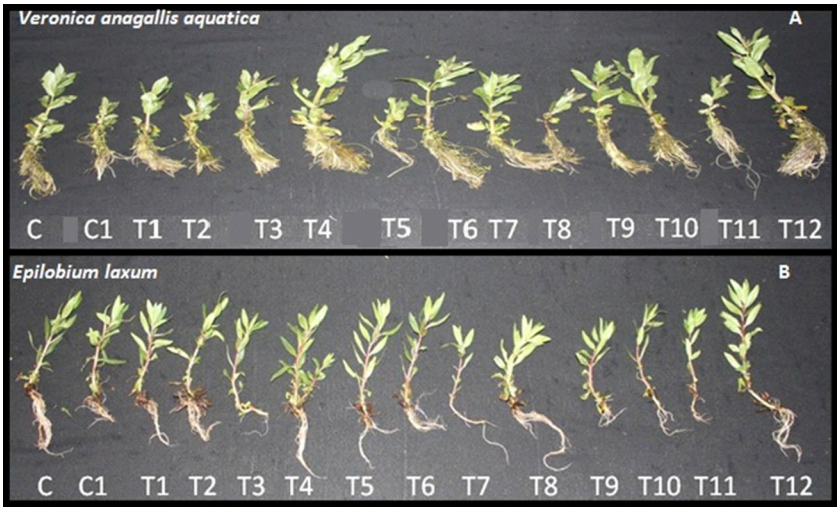

Fig. 1 Effect of NPK fertilizers and plant growth regulators on the growth of **a** *Veronica* plant and **b** *Epilobium* plant under cadmium stress. *C* without Cd, *C1* Cd in media, *T1* Cd + (N added to media), *T2* Cd + (P added to media), *T3* Cd + (K added to media), *T4* Cd + (NPK added to media), *T5* Cd + (N Foliar spray), *T6* Cd + (P Foliar spray), *T7* Cd + (K Foliar spray), *T8* Cd + (NPK foliar spray), *T9* Cd + (IAA foliar spray), *T10* Cd + (GA3 foliar spray), *T11* Cd + (IAA + NPK foliar spray), *T12* Cd + (GA3 + NPK foliar spray). *N* nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole acetic acid, GA_3 gibberellic acid. Hoagland solution was used as growth media

leaves only) as shown in Table 4. Due to the high biomass and high cadmium concentration in treatment T4, it accumulated the highest significant amount of cadmium in its roots (6.83 ± 0.93 mg Cd/DBM), stem (6.41 ± 0.62 mg Cd/DBM), and leaves (3.44 ± 0.44 mg Cd/DBM) and consequently within the entire plant (16. 68 ± 0.51 mg/DBM) as given in Table 4. Cadmium translocation from root to stem or leaves was recorded less than 1 for all the treatments which showed that cadmium concentration of roots is much higher than the stem and leaf cadmium concentration. Control C1 (Cd only) showed a BCF of 8.79 in *Veronica* plant (hyperaccumulator) as shown in Table 4. All the treatments demonstrated significantly high Cd bioconcentration values as compared to control C1, and the highest significant Cd bioconcentration values (19.46 ± 0.59) among all the other treatments were recorded for treatment T4 as shown in Table 4.

In *Epilobium* plant, the highest significant cadmium concentration in roots (482.60 ± 6.75 ppm) was found in treatment T4 (Cd + NPK in media) while in stem (312.40 ± 5.26 ppm) and leaves (504.50 ± 6.50 ppm) it was recorded for T11 (Cd + (IAA + NPK foliar spray) as shown in Table 5. All the treatments showed a significant increase in cadmium accumulation in *Epilobium* with respect to control C1 (Table 5). The highest significant cadmium accumulation in roots (2.18 ± 0.30 mg/DBM), stem (2.59 ± 0.16 mg/DBM), leaves (2.36 ± 0.31 mg/DBM) and the entire plant (7.12 ± 0.36 mg/DBM) was observed in plants grown in NPK fertilizers containing media (T4). Different parts (root, stem, and leaves) of the plants showed variation in cadmium accumulation percentage by the application of different treatments. The results showed high cadmium accumulation percentage occurring in the aerial parts (stem and leaves) as compared to the underground part (roots) (Table 5). The cadmium translocation (from roots to stem) factor values were less than 1 (Table 5). In contrast, the Cd translocation (root to leaves) factor value was significantly higher (1.34 ± 0.17) in treatment T11 (Cd + (IAA + NPK foliar spray) and the lowest (0.76 ± 0.03) in treatment T5 (Cd + N foliar spray). Treatments T1, T3, T4, T6, T8, and T11 increased the Cd translocation from roots into leaves and showed translocation values more than 1 while the other treatments possess Cd translocation value less than 1 (Table 5). Treatments T4 and T8 were found significantly efficient in concentrating Cd within plant tissues and as a result showed the highest significant Cd bioconcentration values (4. 15 ± 0.14 and 4.06 ± 0.28, respectively) as compared to the other treatments. In control C1 (Cd only), the BCF was less than 1 which demonstrated the *Epilobium* plant non-hyperaccumulator for cadmium.

Effects of fertilizers and plant growth regulator on proline, phenolics, and chlorophyll production in plants

Cadmium increased the free proline concentration in roots and leaves when C1 (Cd treated) was compared with control C (without Cd), but this increase was non-significant (Table 6). While in case of phenolics and chlorophyll production, cadmium significantly increased the phenolic concentration in roots and leaves of *Veronica* plant on comparison of C1 (Cd treated) with C (without Cd) (Table 6). All other treatments except T6 (P foliar spray), T7 (K foliar spray), and T9 (IAA foliar spray) showed significant increase in root proline concentrations as compared to control C1, and the highest significant concentration of proline in roots was recorded in the

Table 4 Effect of fertilizers and growth regulator on cadmium concentration and accumulation in various parts of the *Veronica* plant

Treatments		Cd concentration (ppm) ± SD			Cd accumulation (mg/kg) ± SD		
		Root	Stem	Leaves	Root	Stem	Leaves
C1	Only Cd	896.00 ± 26.00^{i}	313.80 ± 7.00^{i}	236.62 ± 5.60^{k}	1.26 ± 0.26^{g}	0.81 ± 0.13^{g}	0.39 ± 0.10^{e}
T1	Cd + N added to media	1477.00 ± 34.00^{d}	627.87 ± 8.00^{c}	684.03 ± 2.00^{c}	4.91 ± 0.75^{bc}	3.90 ± 0.41^{bc}	2.37 ± 0.45^{abc}
T2	Cd + P added to media	1023.00 ± 15.00^{h}	363.77 ± 12.00^{fg}	450.26 ± 5.90^{h}	2.26 ± 0.22^{efg}	1.66 ± 0.14^{fg}	1.15 ± 0.27^{de}
T3	Cd + K added to media	1188.67 ± 27.00^{f}	351.90 ± 9.00^{gh}	489.07 ± 4.30^{g}	4.13 ± 0.57^{bcd}	1.99 ± 0.24^{ef}	1.50 ± 0.71^{cd}
T4	Cd + NPK added to media	1666.30 ± 25.00^{a}	739.10 ± 6.00^{a}	785.40 ± 3.40^{a}	6.83 ± 0.93^{a}	6.41 ± 0.62^{a}	3.44 ± 0.44^{a}
T5	Cd + N foliar spray	1091.10 ± 17.00^{g}	565.30 ± 15.00^{d}	618.60 ± 5.00^{e}	3.53 ± 0.28^{cdef}	3.99 ± 0.36^{bc}	2.35 ± 0.45^{bc}
T6	Cd + P foliar spray	1009.67 ± 22.00^{h}	335.60 ± 10.00^{hi}	328.83 ± 9.00^{j}	2.28 ± 0.55^{defg}	1.99 ± 0.32^{ef}	1.10 ± 0.22^{de}
T7	Cd + K foliar spray	990.80 ± 13.00^{h}	383.90 ± 3.00^{f}	388.43 ± 6.00^{i}	3.31 ± 0.61^{cdef}	2.31 ± 0.14^{def}	1.36 ± 0.20^{cde}
T8	Cd + NPK foliar spray	1386.00 ± 19.00^{e}	637.76 ± 11.00^{c}	736.37 ± 2.00^{b}	4.85 ± 0.72^{bc}	4.08 ± 0.33^{bc}	3.04 ± 0.26^{ab}
T9	Cd + IAA foliar spray	1550.33 ± 12.00^{bc}	616.70 ± 9.00^{c}	660.00 ± 6.50^{d}	1.83 ± 0.03^{fg}	3.00 ± 0.36^{cde}	1.46 ± 0.18^{cde}
T10	Cd + GA3 foliar spray	1500.33 ± 10.00^{cd}	493.30 ± 8.00^{e}	530.90 ± 3.50^{f}	3.83 ± 1.01^{bcde}	3.13 ± 0.45^{cd}	1.50 ± 0.42^{cd}
T11	Cd + (IAA + NPK foliar spray)	1603.10 ± 12.00^{b}	635.77 ± 5.00^{c}	682.37 ± 6.50^{c}	5.45 ± 0.86^{ab}	4.61 ± 0.49^{b}	2.28 ± 0.41^{bc}
T12	Cd + (GA3 + NPK foliar spray)	1481.33 ± 22.00^{d}	694.00 ± 7.00^{b}	731.20 ± 4.64^{b}	5.68 ± 0.50^{ab}	5.75 ± 0.51^{a}	2.91 ± 0.21^{ab}

Treatments	Cd accumulation (mg/kg) ± SD	Cd accumulation %			Translocation factor		Cd Bioconcentration factor
	Entire plant	Root	Stem	leaves	Root-stem	Root-leaves	
C1	2.46 ± 0.21^{g}	51.34	33.01	15.65	0.35 ± 0.01^{g}	0.26 ± 0.02^{k}	8.79 ± 0.64^{f}
T1	11.18 ± 0.74^{c}	43.77	34.79	21.43	0.43 ± 0.01^{d}	0.46 ± 0.01^{d}	17.18 ± 0.25^{b}
T2	5.07 ± 0.55^{fg}	44.59	32.87	22.54	0.36 ± 0.01^{g}	0.44 ± 0.03^{e}	10.87 ± 0.33^{e}
T3	7.62 ± 1.31^{def}	54.60	26.37	19.02	0.30 ± 0.01^{i}	0.41 ± 0.01^{g}	12.53 ± 0.40^{d}
T4	16.68 ± 0.51^{a}	40.97	38.43	20.60	0.44 ± 0.01^{c}	0.47 ± 0.01^{d}	19.46 ± 0.59^{a}
T5	9.86 ± 0.29^{cd}	35.72	40.44	23.84	0.52 ± 0.01^{a}	0.57 ± 0.03^{a}	14.00 ± 0.36^{c}
T6	5.37 ± 1.01^{f}	42.26	37.31	20.44	0.33 ± 0.02^{h}	0.33 ± 0.02^{j}	9.30 ± 0.36^{f}
T7	6.97 ± 0.53^{ef}	47.18	33.30	19.52	0.39 ± 0.03^{f}	0.39 ± 0.03^{h}	10.85 ± 0.60^{e}
T8	11.98 ± 1.10^{bc}	40.40	34.13	25.46	0.46 ± 0.01^{b}	0.53 ± 0.01^{b}	17.06 ± 0.42^{b}
T9	6.28 ± 0.21^{ef}	29.14	47.64	23.22	0.40 ± 0.02^{e}	0.43 ± 0.04^{f}	15.24 ± 0.05^{c}
T10	8.46 ± 1.87^{de}	45.03	37.39	17.57	0.33 ± 0.04^{h}	0.35 ± 0.03^{i}	14.40 ± 0.13^{c}
T11	12.34 ± 0.76^{bc}	44.14	37.46	18.39	0.40 ± 0.03^{ef}	0.43 ± 0.02^{f}	17.66 ± 0.89^{b}
T12	14.34 ± 0.75^{ab}	39.58	40.06	20.36	0.47 ± 0.02^{b}	0.49 ± 0.02^{c}	17.81 ± 0.32^{b}

Hoagland solution was used as growth media

The different superscript letters show significant difference among treatments

N nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole 3-acetic acid, GA_3 gibberellic acid

Table 5 Effect of fertilizers and growth regulators on cadmium concentration and accumulation in various parts of the *Epilobium* plant

Treatments		Cd concentration (ppm) ± SD			Cd accumulation (mg/kg) ± SD		
		Root	Stem	Leaves	Root	Stem	Leaves
C1	Only Cd	63.00 ± 2.00^{i}	53.50 ± 4.00^{h}	75.40 ± 5.60^{h}	0.10 ± 0.02^{e}	0.11 ± 0.02^{g}	0.14 ± 0.04^{e}
T1	Cd + N added to media	389.30 ± 6.50^{d}	210.70 ± 8.00^{d}	429.30 ± 2.00^{bc}	1.43 ± 0.22^{bcd}	1.23 ± 0.10^{cde}	1.6 ± 0.31^{abc}
T2	Cd + P added to media	384.97 ± 8.50^{d}	178.00 ± 6.00^{e}	365.30 ± 5.90^{e}	0.93 ± 0.08^{cd}	0.70 ± 0.20^{f}	1.03 ± 0.24^{cd}
T3	Cd + K added to media	248.40 ± 3.75^{h}	132.00 ± 9.00^{f}	314.52 ± 4.30^{f}	0.95 ± 0.13^{cd}	0.80 ± 0.12^{ef}	1.06 ± 0.50^{cd}
T4	Cd + NPK added to media	482.60 ± 6.75^{a}	307.20 ± 6.00^{a}	489.00 ± 3.40^{a}	2.18 ± 0.30^{a}	2.59 ± 0.16^{a}	2.36 ± 0.31^{a}
T5	Cd + N foliar spray	413.60 ± 6.25^{c}	167.00 ± 3.00^{e}	314.90 ± 5.00^{f}	1.47 ± 0.12^{bc}	1.06 ± 0.15^{cdef}	1.31 ± 0.25^{bcd}
T6	Cd + P foliar spray	363.40 ± 4.25^{ef}	130.80 ± 5.00^{f}	399.80 ± 9.00^{d}	0.90 ± 0.22^{cd}	0.78 ± 0.11^{ef}	1.47 ± 0.30^{bcd}
T7	Cd + K foliar spray	352.90 ± 5.50^{f}	107.60 ± 3.00^{g}	283.30 ± 6.00^{g}	1.30 ± 0.24^{bcd}	0.73 ± 0.13^{ef}	1.09 ± 0.17^{cd}
T8	Cd + NPK foliar spray	408.37 ± 3.25^{c}	251.20 ± 7.00^{b}	418.80 ± 2.00^{c}	1.71 ± 0.34^{ab}	2.04 ± 0.22^{b}	1.95 ± 0.24^{ab}
T9	Cd + IAA foliar spray	377.80 ± 4.75^{de}	304.40 ± 9.00^{a}	328.60 ± 6.50^{f}	0.87 ± 0.01^{d}	0.97 ± 0.03^{def}	0.69 ± 0.02^{de}
T10	Cd + GA3 foliar spray	330.00 ± 3.00^{g}	235.00 ± 8.00^{bc}	269.40 ± 3.50^{g}	0.93 ± 0.24^{cd}	1.36 ± 0.35^{cd}	0.83 ± 0.23^{de}
T11	Cd + (IAA + NPK foliar spray)	381.43 ± 2.50^{d}	312.40 ± 5.00^{a}	504.50 ± 6.50^{a}	1.43 ± 0.22^{bcd}	2.04 ± 0.17^{b}	2.02 ± 0.33^{ab}
T12	Cd + (GA3 + NPK foliar spray)	446.94 ± 3.00^{b}	216.82 ± 7.00^{cd}	438.25 ± 4.64^{b}	1.89 ± 0.15^{ab}	1.53 ± 0.15^{c}	1.92 ± 0.13^{ab}

Treatments	Cd accumulation (mg/kg) ± SD	Cd accumulation %			Translocation factor		Cd Bioconcentration factor
	Entire plant	Root	Stem	leaves	Root-stem	Root-leaves	
C1	0.34 ± 0.03^{f}	28.78	31.88	39.35	0.85 ± 0.05^{a}	1.20 ± 0.05^{c}	0.61 ± 0.02^{f}
T1	4.30 ± 0.22^{cd}	33.36	28.67	37.98	0.54 ± 0.01^{d}	1.10 ± 0.01^{d}	3.31 ± 0.30^{bc}
T2	2.66 ± 0.43^{e}	35.63	25.99	38.38	0.46 ± 0.01^{f}	0.95 ± 0.01^{f}	2.84 ± 0.23^{cde}
T3	2.81 ± 0.63^{e}	34.65	29.06	36.29	0.53 ± 0.03^{de}	1.27 ± 0.02^{b}	2.29 ± 0.12^{e}
T4	7.12 ± 0.36^{a}	30.61	36.33	33.06	0.64 ± 0.01^{c}	1.01 ± 0.01^{e}	4.15 ± 0.14^{a}
T5	3.85 ± 0.33^{de}	38.32	27.60	34.08	0.40 ± 0.02^{g}	0.76 ± 0.03^{i}	2.73 ± 0.25^{cde}
T6	3.15 ± 0.60^{de}	28.50	24.96	46.54	0.36 ± 0.01^{g}	1.10 ± 0.01^{d}	2.73 ± 0.10^{cde}
T7	3.12 ± 0.39^{de}	41.34	23.50	35.15	0.30 ± 0.03^{h}	0.80 ± 0.02^{hi}	2.43 ± 0.25^{de}
T8	5.70 ± 0.62^{b}	29.88	35.93	34.18	0.62 ± 0.03^{c}	1.03 ± 0.03^{e}	4.06 ± 0.28^{a}
T9	2.53 ± 0.05^{e}	34.26	38.46	27.28	0.81 ± 0.01^{a}	0.87 ± 0.01^{g}	3.07 ± 0.04^{cd}
T10	3.12 ± 0.82^{de}	29.73	43.55	26.73	0.71 ± 0.02^{b}	0.82 ± 0.01^{h}	2.64 ± 0.14^{cde}
T11	5.49 ± 0.56^{bc}	25.91	37.36	36.73	0.82 ± 0.01^{a}	1.32 ± 0.01^{a}	3.94 ± 0.50^{ab}
T12	5.33 ± 0.19^{bc}	35.39	28.70	35.91	0.49 ± 0.01^{ef}	0.98 ± 0.03^{ef}	3.32 ± 0.21^{bc}

Hoagland solution was used as growth media

The different superscript letters show significant difference among treatments

N nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole 3-acetic acid, GA_3 gibberellic acid

Table 6 Effect of different treatments of fertilizers and plant growth regulators on proline, phenolics and chlorophyll (a and b) concentration in roots and leaves of *Veronica* plant grown in cadmium contaminated media

Treatments		Proline (μg/g) ± SD		Phenolics (μg/g) ± SD		Chlorophyll (μg/g) ± SD	
		Root	Leaf	Root	Leaf	A	B
C	Without Cd	29.33 ± 3.51^{e}	42.53 ± 5.09^{b}	32.97 ± 5.46^{e}	44.37 ± 5.22^{e}	1.85 ± 0.14^{c}	1.48 ± 0.11^{d}
C1	Only Cd	41.67 ± 2.08^{de}	63.42 ± 3.42^{b}	60.42 ± 3.02^{d}	128.79 ± 0.81^{bc}	1.10 ± 0.08^{d}	0.88 ± 0.07^{e}
T1	Cd + N added to media	68.67 ± 5.03^{ab}	97.57 ± 7.30^{a}	99.57 ± 7.30^{ab}	148.83 ± 4.58^{a}	2.59 ± 0.16^{b}	2.55 ± 0.18^{abc}
T2	Cd + P added to media	65.73 ± 2.97^{ab}	61.95 ± 2.80^{b}	95.31 ± 4.31^{ab}	82.01 ± 3.44^{d}	3.13 ± 0.12^{a}	2.82 ± 0.10^{a}
T3	Cd + K added to media	66.00 ± 4.00^{ab}	62.21 ± 3.77^{b}	95.70 ± 5.80^{ab}	86.90 ± 7.37^{d}	2.83 ± 0.38^{ab}	2.27 ± 0.30^{bc}
T4	Cd + NPK added to media	75.33 ± 5.03^{a}	111.23 ± 8.30^{a}	109.23 ± 7.30^{a}	153.18 ± 7.98^{a}	3.28 ± 0.17^{a}	2.85 ± 0.33^{a}
T5	Cd + N foliar spray	65.33 ± 5.03^{ab}	98.73 ± 8.35^{a}	94.73 ± 7.30^{ab}	116.82 ± 3.91^{c}	2.54 ± 0.03^{b}	2.12 ± 0.17^{c}
T6	Cd + P foliar spray	48.67 ± 3.06^{cd}	52.93 ± 3.32^{b}	70.57 ± 4.43^{cd}	85.61 ± 5.39^{d}	2.88 ± 0.08^{ab}	2.59 ± 0.07^{abc}
T7	Cd + K foliar spray	56.38 ± 3.44^{bcd}	61.32 ± 3.74^{b}	81.76 ± 4.99^{bcd}	80.80 ± 2.63^{d}	2.65 ± 0.01^{b}	2.12 ± 0.01^{c}
T8	Cd + NPK foliar spray	64.60 ± 13.43^{ab}	97.67 ± 14.37^{a}	93.67 ± 19.47^{ab}	117.60 ± 5.50^{c}	2.93 ± 0.09^{ab}	2.61 ± 0.08^{ab}
T9	Cd + IAA foliar spray	58.08 ± 3.92^{bc}	50.53 ± 3.41^{b}	84.22 ± 5.68^{bc}	114.21 ± 1.35^{c}	1.76 ± 0.19^{c}	1.41 ± 0.15^{d}
T10	Cd + GA3 foliar spray	75.65 ± 4.82^{a}	108.69 ± 6.89^{a}	109.69 ± 6.99^{a}	145.20 ± 9.06^{ab}	1.86 ± 0.11^{c}	1.49 ± 0.09^{d}
T11	Cd + (IAA + NPK foliar spray)	64.10 ± 4.27^{abc}	98.95 ± 5.19^{a}	92.95 ± 6.19^{abc}	121.32 ± 14.79^{c}	1.52 ± 0.16^{cd}	1.21 ± 0.13^{de}
T12	Cd + (GA3 + NPK foliar spray)	75.30 ± 4.30^{a}	110.19 ± 8.21^{a}	109.19 ± 6.23^{a}	153.60 ± 5.50^{a}	2.88 ± 0.10^{ab}	2.74 ± 0.10^{ab}

Hoagland solution was used as growth media. Cd concentration 100 mg kg^{-1}

The different superscript letters show significant difference among treatments

N nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole 3-acetic acid, GA_3 gibberellic acid

treatments T4, T10, and T12 (Table 6). In leaves of the plant, proline concentration was significantly high in all treatments, except T2, T3, T6, T7, and T9, when compared to control C1 (Table 6).

Table 6 showed that all the treatments (except T6 and T7) significantly increased the phenolic concentration in roots, while in leaves of the plant, phenolic concentration was significantly high in treatments T1, T4, and T12 and most of

Table 7 Effect of different treatments of fertilizers and plant growth regulators on the proline, phenolics, and chlorophyll (a and b) concentration in roots and leaves of *Epilobium* plant grown in cadmium contaminated media

Treatments		Proline (μg/g) ± SD		Phenolics (μg/g) ± SD		Chlorophyll (μg/g) ± SD	
		roots	leaf	Root	Leaf	a	B
C	Without Cd	36.30 ± 5.55^{cdef}	50.82 ± 7.77^{efg}	41.33 ± 12.60^{c}	57.87 ± 17.64^{e}	2.50 ± 0.15^{fg}	1.56 ± 0.10^{fg}
C1	Only Cd	41.70 ± 2.70^{bcde}	58.38 ± 3.78^{defg}	51.62 ± 7.89^{c}	72.26 ± 11.04^{e}	1.96 ± 0.02^{g}	1.23 ± 0.01^{g}
T1	Cd + N added to media	50.33 ± 2.93^{abc}	80.52 ± 4.68^{abc}	100.95 ± 6.75^{a}	161.52 ± 10.80^{ab}	4.67 ± 0.13^{ab}	3.12 ± 0.09^{ab}
T2	Cd + P added to media	43.48 ± 9.24^{abcd}	63.04 ± 13.40^{cdef}	84.51 ± 7.18^{ab}	122.54 ± 10.41^{cd}	4.28 ± 0.19^{abc}	3.05 ± 0.14^{ab}
T3	Cd + K added to media	51.00 ± 1.50^{ab}	71.40 ± 2.10^{abcde}	91.03 ± 15.95^{a}	127.45 ± 22.33^{bc}	3.89 ± 0.03^{abcde}	2.78 ± 0.02^{abc}
T4	Cd + NPK added to media	55.28 ± 2.47^{a}	85.24 ± 13.55^{ab}	106.12 ± 6.58^{a}	169.79 ± 10.52^{a}	4.84 ± 0.29^{a}	3.23 ± 0.19^{a}
T5	Cd + N foliar spray	41.83 ± 2.96^{bcde}	66.92 ± 4.73^{bcde}	89.27 ± 14.55^{a}	124.97 ± 20.36^{bc}	3.04 ± 0.19^{def}	2.17 ± 0.14^{cdef}
T6	Cd + P foliar spray	44.13 ± 1.21^{abcd}	67.60 ± 4.26^{bcde}	60.32 ± 13.69^{bc}	84.44 ± 19.16^{de}	3.78 ± 0.02^{bcde}	2.52 ± 0.01^{bcde}
T7	Cd + K foliar spray	34.13 ± 1.88^{def}	51.19 ± 2.81^{efg}	83.50 ± 1.40^{ab}	116.90 ± 1.96^{cd}	2.97 ± 0.09^{ef}	1.91 ± 0.05^{ef}
T8	Cd + NPK foliar spray	27.83 ± 1.43^{ef}	44.52 ± 2.28^{fg}	92.00 ± 4.85^{a}	147.20 ± 7.77^{abc}	3.38 ± 0.15^{cdef}	2.60 ± 0.12^{abcd}
T9	Cd + IAA foliar spray	27.15 ± 3.60^{f}	38.01 ± 5.04^{g}	51.93 ± 8.88^{c}	72.71 ± 12.44^{e}	3.34 ± 0.10^{cdef}	2.01 ± 0.06^{def}
T10	Cd + GA3 foliar spray	49.28 ± 4.28^{abc}	78.84 ± 6.84^{abcd}	81.40 ± 3.40^{ab}	113.96 ± 4.76^{cd}	3.90 ± 0.09^{abcde}	2.60 ± 0.06^{abcd}
T11	Cd + (IAA + NPK foliar spray)	43.88 ± 7.13^{abcd}	70.20 ± 11.40^{abcde}	90.75 ± 1.95^{a}	127.05 ± 2.73^{bc}	3.95 ± 0.93^{abcd}	2.63 ± 0.62^{abcd}
T12	Cd + (GA3 + NPK foliar spray)	57.30 ± 3.08^{a}	91.68 ± 4.92^{a}	108.07 ± 3.98^{a}	172.91 ± 6.37^{a}	4.08 ± 0.61^{abc}	2.92 ± 0.43^{ab}

Hoagland solution was used as growth media. Cd concentration 100 mg kg^{-1}

The different superscript letters show significant difference among treatments

N nitrogen fertilizer, *P* phosphate fertilizer, *K* potassium fertilizer, *IAA* indole 3-acetic acid, GA_3 gibberellic acid

Table 8 Correlations among different parameters measured in roots of *Veronica* plant

Correlations between parameters measured in root of Veronica plant								
	Length	FBM	DBM	TWC	Cd conc.	Cd accumulation	Proline	Phenolics
Length	1.00							
FBM	0.64*	1.00						
DBM	0.68*	0.94***	1.00					
TWC	0.58	0.98***	0.85**	1.00				
Cd conc.	0.28	0.64*	0.44	0.73**	1.00			
Cd accumulation	0.55	0.91***	0.86**	0.90***	0.80**	1.00		
Proline	0.63*	0.75**	0.66*	0.76**	0.72**	0.78**	1.00	
Phenolics	0.63*	0.75**	0.66*	0.76**	0.72**	0.78**	0.98***	1.00

Asterisks show the significant correlations

other treatments showed non-significant increase in leaf phenolic concentration as compared to control C1 as shown in Table 6. Results clearly demonstrated that Cd has a significantly negative effect on the concentration of chlorophyll "a" and "b" in leaves of *Veronica* plant when the control C was compared to C1(Table 6). A highly significant increase in both chlorophylls a and b was recorded in the plant grown in media containing phosphate fertilizer (P) in T2 and also in combination of NPK (T4) as shown in Table 6. In contrast to the *Veronica* plant, the effect of Cd on the proline, phenolic, and chlorophyll content in *Epilobium* plant was found statistically non-significant when cadmium-treated plant (C1) was compared with the control without Cd (C) as given in Table 7. The treatments T4 and T12 showed a significant increase in both root and leaf proline concentrations as compared to C1. All treatments significantly increased the phenolic concentration in root and leaf of the *Epilobium* plant (except phenolics in roots of treatments T6 and T9) when compared with C1 (only Cd treated). The highest significant increase in leaf phenolic concentration was found in treatments T4 and T12 while in roots the increase in phenolic concentration was not significant. The chlorophyll (a, b) contents were significantly increased with all treatments when compared to C1 (Cd only), and the highest increase in chlorophyll contents was recorded in treatment T4 (Cd + NPK added to media) as shown in Table 7.

Correlations among various parameters measured in *Veronica* and *Epilobium* plants

Correlation between different parameters measured for root, stem, and leaves of *Veronica* plant respectively are presented (Tables 8, 9, and 10). Most of the parameters measured in root showed significantly positive correlation except the correlation between root cadmium concentration with length and DBM (Table 8). The cadmium concentration in root showed a strong positive correlation with proline and phenolic content, and the correlation between the proline and phenolic concentration was highly significant (Table 8). The *Veronica* plant stem dry biomass showed highly positive correlation with cadmium accumulation and Cd concentration as shown in Table 9. A strong positive correlation existed between Cd concentration in leaves with proline and phenolic contents while the correlation of Cd concentration was found non-significant with chlorophyll a and b contents (Table 10).

In *Epilobium* plant, Cd concentration and accumulation in roots showed significantly positive correlation with the root phenolic contents as given in Table 11. The fresh biomass

Table 9 Correlations between different parameters measured in stem of *Veronica* plant

Correlations between parameters measured in stem of *Veronica* plant						
	Length	*FBM*	*DBM*	*TWC*	*Cd conc.*	*Cd accumulation*
Length	1.00					
FBM	0.71*	1.00				
DBM	0.67*	0.87**	1.00			
TWC	0.67*	0.97***	0.73*	1.00		
Cd conc.	0.48	0.64*	0.78**	0.51*	1.00	
Cd accumulation	0.60*	0.73*	0.90***	0.58*	0.94***	1.00

Asterisks show the significant correlations

Table 10 Correlations among different parameters measured in leaf of *Veronica* plant

Correlations between parameters measured in leaf of *Veronica* plant

	Length	FBM	DBM	TWC	Cd conc.	Cd accumulation	Proline	Phenolics	Chlorophyll a	Chlorophyll b
Length	1.00									
FBM	0.74**	1.00								
DBM	0.85**	0.95***	1.00							
TWC	0.69*	0.99***	0.91***	1.00						
Cd conc.	0.51	0.75**	0.68*	0.76**	1.00					
Cd accumulation	0.71**	0.87**	0.88**	0.85**	0.92***	1.00				
Proline	0.37	0.58*	0.57*	0.57*	0.78**	0.79**	1.00			
Phenolics	0.14	0.23	0.25	0.22	0.52*	0.54*	0.86**	1.00		
Chlorophyll a	0.86**	0.59*	0.69*	0.54	0.35	0.50	0.08	0.01	1.00	
Chlorophyll b	0.91***	0.56*	0.68*	0.51	0.38	0.52	0.15	0.07	0.98***	1.00

Asterisks show the significant correlations

(FBM), dry biomass (DBM), and total water contents (TWC) of the root showed a highly significant correlation with phenolic concentration in root (Table 11). On the other hand, the correlation of FBM, DBM, and TWC of the stem was found non-significant with the Cd concentration of the stem as shown in Table 12. The leaf of plant demonstrated a significant correlation of chlorophylls a and b with proline and phenolic concentration (Table 13). The leaf phenolic concentration showed a significantly positive correlation with Cd concentration in leaves and FBM, DBM, and TWC of leaves (Table 13).

Discussion

The reduction in plant growth might be due to the effect of cadmium on nutrient uptake and distribution within the plant cell and its harmful effect on permeability of plasma membrane (Khatamipour et al. 2011). Similar effects of Cd toxicity have been reported on *Cucumis sativus* (Abu-Muriefah 2008) and *Lemna polyrhiza* (John et al. 2008). Cadmium accumulation in plants may negatively affect the growth of plant by a decrease in the enzymatic activities (Van Assche and Clijsters 1990). Present results showed that the growth and biomass of the plants were decreased due to Cd stress. Possibly the low nutrient and water uptake might be one of the main causes for root and shoot growth inhibition by cadmium. The application of fertilizers (NPK) and growth regulator (GA3) increased the growth and biomass of plants under Cd stress. This increase might be due to the availability of more nutrients to plants. Two major factors determine the total amount of metal extracted by plants: concentration of the metals in biomass and total biomass of the plant. Both factors are equally important. The present experiment showed that biomass and cadmium concentration and accumulation in different parts of both plants were influenced markedly by application of fertilizers. These findings support the previous results of Li et al. (2012), who reported many fold increase in Cd concentration in *Amaranthus* plant.

Table 11 Correlations among different parameters measured in roots of *Epilobium* plant

Correlations between parameters measured in roots of *Epilobium* plant

	Length	FBM	DBM	TWC	Cd conc.	Cd accumulation	Proline	Phenolics
Length	1.00							
FBM	0.62*	1.00						
DBM	0.69**	0.97**	1.00					
TWC	0.57*	0.99***	0.93***	1.00				
Cd conc.	0.59*	0.67**	0.65**	0.66**	1.00			
Cd accumulation	0.38	0.37	0.34	0.38	0.76**	1.00		
Proline	0.29	0.26	0.30	0.24	0.09	0.011	1.00	
Phenolics	0.73**	0.86***	0.89***	0.83***	0.61*	0.67*	0.55*	1.00

Asterisks show the significant correlations

Table 12 Correlations among different parameters measured in stem of *Epilobium* plant

Correlations between parameters measured in stem of *Epilobium* plant						
	Length	FBM	DBM	TWC	Cd conc.	Cd accumulation
Length	1.00					
FBM	0.74**	1.00				
DBM	0.74**	0.99**	1.00			
TWC	0.74**	1.00***	0.96***	1.00		
Cd conc.	0.42	0.29	0.32	0.27	1.00	
Cd accumulation	0.24	0.09	0.10	0.09	0.77**	1.00

Asterisks show the significant correlations

Accumulation of free proline in plant during metal stress is a defensive mechanism in some plants (Mehta and Gaur 1999). Previous investigators have suggested that proline accumulation in plants might reduce the toxic effect of heavy metal in plants (Mehta and Gaur 1999). The stimulation of proline synthesis and accumulation under abiotic stress might be the result of a degradation or increase in its de novo synthesis (Kasai et al. 1998). Influence of proline on permeability of membrane has also been reported by Pesci and Reggiani (1992). Proline takes part in chlorophyll synthesis and in regulating acidity within the cytoplasm (Gajewska and Sklodowska 2008). Proline plays role in tolerance to stress by stabilization of protein, macromolecules, and organelles and by protecting enzymes from denaturation (John et al. 2008; Gajewska and Sklodowska 2008). The accumulation of proline in plants could be considered as an indicator of tolerance to heavy metal stress. Siripornadulsil et al. (2002) reported that proline decreases cadmium stress by sequestering cadmium ions and also by reducing free radical damage induced by cadmium, thus keeping a more reducing condition within the cell. Sun et al. (2007) reported a high proline level under cadmium stress in hyperaccumulator *Solanum nigrum* plant as compared to the non-hyperaccumulator *S. melongena,* and they suggested that proline might play a role in cadmium accumulation and tolerance in *S. nigrum*. The current results showed a positive correlation between heavy metal (Cd) concentration and proline accumulation. The similar increase in Cd and proline has been previously reported in *Parthenium hysterophorus* (Ali and Hadi 2015) and *Raphanus sativus* plants (Teklic et al. 2008).

An increase in the concentration of total phenolic compounds under heavy metal (Cd) stress might be due to their protective role by metal chelation and ROS scavenging and their act as antioxidant agents (Lavid et al. 2001). In previous literature, it has been shown that phenolic compounds except ascorbate can protect cell against oxidative stress by phenol-coupled APX reaction (Rastgoo and Alemzadeh 2011). The anti-oxidative properties of phenolic compounds are due to their ability to chelate transition metal ion and inhibit superoxide-driven Fenton reaction (Arora et al. 2008). Thus, they provide stability to membranes by decreasing membrane fluidity. The current results showed a significant increase in concentration of total phenolic compound under cadmium stress. The results also showed that chemical fertilizers (NPK)

Table 13 Correlations among different parameters measured in leaf of *Epilobium* plant

Correlations between parameters measured in leaf of *Epilobium* plant										
	Length	FBM	DBM	TWC	Cd conc.	Cd accumulation	Proline	Phenolics	Chlorophyll a	Chlorophyll b
Length	1.00									
FBM	0.44	1.00								
DBM	0.54	0.92**	1.00							
TWC	0.38	0.99**	0.85**	1.00						
Cd conc.	0.47	0.73**	0.73**	0.70**	1.00					
Cd accumulation	0.58*	0.56*	0.52*	0.55*	0.69**	1.00				
Proline	0.02	0.35	0.42	0.30	0.37	0.04	1.00			
Phenolics	0.39	0.74**	0.81**	0.67**	0.66**	0.26	0.63*	1.00		
Chlorophyll a	0.43	0.49	0.49	0.47	0.49	0.27	0.61*	0.67**	1.00	
Chlorophyll b	0.48	0.56*	0.59*	0.52	0.47	0 .32	0.61*	0.76**	0.96**	1.00

Asterisks show the significant correlations

under Cd stress demonstrated the highest significant increase in root and leaf phenolic contents of the plant. The results showed a negative effect of cadmium on the chlorophyll (a and b) contents of the plant. The reduction in chlorophyll content might be due to the decrease in synthesis of chlorophyll as a consequence of inhibition of enzyme activity such as δ-aminolevulinic acid dehydratase and protochlorophyllide reductase, replacement of Mg with heavy metals in chlorophyll structure, and decrease in essential metals (such as Fe^{2+} and Zn^{2+}) that are involved in chlorophyll synthesis (Padmaja et al. 1990; Van Assche and Clijsters 1990). The chloroplast contains different parts that respond to heavy metal stress; therefore, any changes in chlorophyll synthesis and activity used as the index of direct toxic effects of heavy metals and decrease in chlorophyll ratio (a/b) in response to heavy metals demonstrates that chlorophyll a is more sensitive to metals (Rastgoo and Alemzadeh 2011). "Lavid et al. 2001" is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.Lavid N, Schwartz A, Yarden O, Tel-Or E (2001) The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of the waterlily (Nymphaeaceae). Planta 212, 323-331.

Conclusion and recommendations

Veronica plant was found to be a hyperaccumulator of cadmium, based on BCF >1, while *Epilobium* plant showed BCF <1. The Cd concentration severely affects the plant growth and biomass while addition of NPK fertilizers into the growth media showed the most significant increase in biomass and cadmium concentration of both the plants. GA_3 application in combination with NPK foliar spray showed a significant effect on biomass and cadmium accumulation. Total phenolics and free proline production in plant showed a positive correlation with Cd phytoaccumulation and plant biomass. Overall proline concentration was higher in roots while phenolics were higher in leaves. Further investigation is recommended to study the role of endogenous phenolics and proline in heavy metal phytoextraction by various plant species.

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