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Scientific Research

Cadmium (Cd) Removal from Saline Water by *Veronica anagallis* and *Epilobium laxum* Plants in Hydroponic System

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Abstract

Present study was conducted to investigate the Cadmium (Cd) phytoextraction potential of two plants (*Veronica anagallis-aquatic* and *Epilobium laxum Royle*) for Cd removal from induced saline water. In hydroponic system, various concentrations of the Cd (50, 100, and 150 ppm) and NaCl salt (1000, 3000, and 6000 ppm) were used alone and in various combinations to evaluate the effect of salt (NaCl) concentrations on Cd absorption and accumulation in *Veronica anagallis* and *Epilobium* plants. The Cd at higher concentrations (100 and 150 ppm) significantly reduced the growth and biomass of both plants and addition of salt (NaCl) to growth media (Hoagland solution) further reduced the growth. The Cadmium (Cd) translocation factor (TF) of *Epilobium* plant was more than one (1), while the *Veronica* plant showed translocation factor less than 0.5. *Veronica* plant showed higher Bio-concentration factor (BCF) as more than 3.5 and *Epilobium* plant demonstrated Bio-concentration factor less than 1 (BCF 1 is a threshold limit for a plant to be hyper-accumulator of Cd). Conclusively, the *Veronica anagallis* plant is reported as Cd hyper-accumulator, while *Epilobium laxum* plant as non hyper-accumulator on the basis of BCF values in the present findings. Further study on *Veronica* and *Epilobium* plants is recommended.

Keywords

Cadmium, Saline Water, Metal Phytoaccumulation, *Veronica anagallis*, *Epilobium laxum*

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1. Introduction

Water is essential for survival and existence of life. The increasing competition for clean water, due to ever increasing demand for drinking and irrigation water, resulted in a steady and irreversible spread of salinization thus disturbing fresh water reservoirs mostly in arid and semiarid regions of the world [1]. It has been estimated that about 20% of cultivated land and 50% of cropland in the world is saline (containing high concentration of NaCl salt) and affecting agricultural yield [2]. Saline water may also contain substantial amount of toxic heavy metals [3].

Heavy metals can enter the water through urban and municipal runoff, sewage, storm water, industrial effluents, atmospheric deposition, mining operations and agricultural activity [4]-[6]. The water and soil salinity problem and their pollution with heavy metals which may be intensified in future, and severity of toxic effect in plants will be increased [7]. Continuous irrigation of crops with contaminated water not only contaminates soil but also compromise the quality and safety of food and consequently the human health [8]. Cadmium (Cd) is a toxic heavy metal and plants can easily absorb Cd due to its strong bio-accumulative capacity [9] [10]. Cadmium is a potential carcinogenic and can leads to impairment of liver and kidneys in human beings [11] [12]. The removal of toxic heavy metals from contaminated water is of great importance and needs an effective and affordable technological solution. Plants have the natural ability to absorb any thing dissolved in water solution by its roots and this ability of plants can be exploited for the decontamination of heavy metals contaminated water. Many researchers have investigated different aspects of the process of metals removal from water, such as the degree of toxicity of these metals causing harm to plants [13] [14], the use of plants as bio-filters for polluted water [15] and the bio-monitoring of metals [16].

Veronica anagallis-aquatica belongs to family *Plantaginaceae*. It is also known as water speedwell and blue speedwell. It is a perennial herb with root-like subterranean stem, commonly horizontal in position and occurs in moist, wet and semi-aquatic habitat. The *Epilobium laxum Royle*, commonly known as willow herbs, is a dicot angiosperm plant belongs to family *Onagraceae*. It is herbaceous, annual or perennial having stunted stem with simple alternate, ovate to lanceolate leaves. It is mostly present in moist temperate regions, very common in the western Himalaya. These two plants were selected for the hydroponic experiment due to their natural aquatic and semi aquatic habitat, and the objectives were to evaluate the Cd removal (phytoaccumulation) potential of *Veronica* and *Epilobium* plants (from induced saline water).

2. Materials and Methods

2.1. Plant Materials

Uniform size plantlets of *Veronica anagallis* and *Epilobium laxum* (2 cm roots and 3 cm shoot) were collected from river of Miandam, Pakistan, in the month of April 2012. The plantlets were kept under cool condition to avoid evaporation and wilting during transportation to laboratory for experiments. .

2.2. Preparation of Growth Media and Addition of Cadmium and Sodium SALTS

Hoagland's solution was used as plant growth media (GM). The solution was prepared and then poured into flasks (150 ml media per flask). Cadmium was added to the flasks in the form of Cadmium acetate dihydrate [$Cd(CH_3COO)_2 \cdot 2H_2O$] and three different concentrations (50, 100 and 150 ppm) of Cd were used during the experiment. Salt of Sodium (NaCl) was added to flasks in different concentrations (1000, 3000 and 6000 ppm). Calculated amount of Cadmium acetate dihydrate and sodium chloride was added into their respective flask and thoroughly mixed and dissolved. Three replicate flasks were used for each treatment and control. The following treatments and control (**Table 1**) were used during the experiment.

2.3. Seedlings Transplantation in Growth Media

The selected plantlets were transplanted into the flasks (one plant per flask). After transplantation, each flask was covered with aluminum foil to avoid evaporation. To keep the volume of media at constant level (150 ml) fresh media was regularly added to the flasks. The experiment was conducted under natural light/dark conditions (14/10) with temperature 30/25˚C.

Table 1. Different Treatments of Cd and NaCl used during the whole experiment. The Hoagland solution was used as Growth Media (GM). C is compared with all treatments to find out the effect of Cd alone and in combinations with salt (NaCl) on plant growth. While C1, C2, and C3 are compared with all other treatments for NaCl effect on Cd phytoaccumulation.

Treatments	Denoted	Treatments	Denoted
Growth media (GM) only	C	GM + 100 ppm Cd + 1000 ppm NaCl	T4
GM + 50 ppm Cd	C1	GM + 100 ppm Cd + 3000 ppm NaCl	T5
GM + 100 ppm Cd	C2	GM + 100 ppm Cd + 6000 ppm NaCl	T6
GM + 150 ppm Cd	C3	GM + 150 ppm Cd + 1000 ppm NaCl	T7
GM + 50 ppm Cd +1000 ppm NaCl	T1	GM + 150 ppm Cd + 3000 ppm NaCl	T8
GM + 50 ppm Cd + 3000 ppm NaCl	T2	GM + 150 ppm Cd + 6000 ppm NaCl	T9
GM + 50 ppm Cd + 6000 ppm NaCl	T3		

2.4. Harvesting of Plants and Measurement of Different Parameters

The plants were harvested after four weeks treatments. After harvesting, the root, stem and leaf length was measured using centimeter ruler. Then the plants were separated into three parts *i.e*. roots, stem and leaves. The fresh biomass of the different parts was measured for each plant using analytical balance, and each part was packed in labeled paper envelope, dried at 80˚C for 48 hrs in oven. The dried samples were crushed into powdered form using mortar and pestle, and each sample was kept in small polythene bags for further use.

2.5. Acid Digestion and Cd Analysis

Dried and powdered sample each (0.25 g) was taken into 50 ml volumetric flask, then 5 ml of Nitric acid (HNO_3), 0.5 ml of perchloric acid ($HClO_4$) and 1 ml sulfuric acid (H_2SO_4) was added to it (Allen, 1974). The flask was then kept on hot plate for 15 minutes at 300˚C until white fumes comes out from the flasks. The samples were cooled, filtered into plastic bottle and the volume of filtrate was raised up to 50 ml by addition of distilled water. This procedure was repeated for all the samples. The digested samples were then analyzed for the Cd concentration using atomic absorption spectrometer.

2.6. Statistical Analysis

The data was subjected to ANOVA and the mean values were compared by using Turkey's Honestly Significant Difference (HSD) test, at $P < 0.05$. The data was analyzed using SPSS-16 and MS-excel (2010).

3. Results and Discussion

3.1. Effect of Different Concentration of Cd on Growth (Root, Stem and Leaf Length) and Biomass (Fresh and Dry) of *Veronica* and *Epilobium* Plants

Cadmium induced the significant reduction in root and stem length of *Veronica* plant when the treatments having Cd (C1 = 50, C2 = 100 and C3 = 150 ppm) were compared with the control without Cd (C) as shown in **Table 2** and **Figure 1(a)**. The reduction in plant growth might be due to the effect of cadmium on nutrient uptake and distribution within the plant cell [17] and also its harmful effect on the permeability of plasma membrane, thus decreasing the cells elongation and growth [18] [19] Similar effects of Cd toxicity on plant growth were reported on *Cucumus sativus* [20], on *Lemna polyrrhiza* [21], and on *Glycyrrhiza uralensis* [22]. The plant biomass (fresh and dry) and total water content (TWC) also demonstrated a decrease in plants treated with Cd, when compared the control C (without Cd) with C1 (50 ppm Cd), C2 (100 ppm Cd), and C3 (150 ppm Cd) as given in (**Table 2**). In stem the decrease in biomass and TWC was significant at all the Cd concentrations (C1 to C3) while in root and leaves, this reduction was significant only at higher Cd concentrations (100 and 150 ppm) as compared to C (without Cd). From the results it is clear that the growth, biomass and water contents of *Veronica* plant showed decrease with increase in Cd concentration in the media.

In *Epilobium* plant, the lower concentration of Cd (C1) has no significant effect on growth, biomass and water content in any part of *Epilobium* plant as compared to control C (**Table 3**, **Figure 1(b)**). Significant reduction in

Table 2. Effect of different concentrations of Cd alone and in combinations with different concentrations of NaCl on growth, biomass and total water content of *Veronica anagallis-aquatica.* ± SD denote standard deviation. Different alphabets represent the significant difference (Turkey's Honestly Significant Difference (HSD) test, at $P < 0.05$). C (control without Cd and NaCl), C1 to C3 (50, 100, 150 ppm Cd), T1 to T3 (50 ppm Cd with 1000, 3000, 6000 ppm NaCl), T4 to T6 (100 ppm Cd with 1000, 3000, 6000 ppm NaCl), T7 to T9 (150 ppm Cd with 1000, 3000, 6000 NaCl). In all treatments and control the Hoagland solution was used as growth medium.

Treatments	Length (cm) ± SD			Fresh Biomass (g) ± SD			Dry Biomass (g) ± SD			Total Water Content (g) ± SD		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
C	9.5 ± 0.5 ^{a}	20.5 ± 0.5 ^{a}	5.4 ± 0.4 ^{a}	3.89 ± 0.27 ^{a}	8.4 ± 0.35 ^{a}	2.21 ± 0.13 ^{a}	1.95 ± 0.14 ^{a}	4.2 ± 0.18 ^{a}	1.1 ± 0.06 ^{a}	1.95 ± 0.13 ^{a}	4.2 ± 0.18 ^{a}	1.1 ± 0.06 ^{a}
C1	7.5 ± 0.5 ^{b}	14.75 ± 0.25 ^{b}	4.8 ± 0.2 ^{ab}	2.94 ± 0.60 ^{ab}	5.71 ± 0.71 ^{b}	1.85 ± 0.19 ^{ab}	1.47 ± 0.30 ^{ab}	2.86 ± 0.36 ^{b}	0.93 ± 0.09 ^{ab}	1.47 ± 0.30 ^{ab}	2.86 ± 0.36 ^{b}	0.93 ± 0.09 ^{ab}
C2	5.25 ± 0.15 ^{cd}	13.25 ± 0.75 ^{bc}	4.15 ± 0.15 ^{bc}	1.68 ± 1.24 ^{bcde}	3.98 ± 2.77 ^{bcde}	1.34 ± 0.99 ^{abcde}	0.84 ± 0.62 ^{bcde}	1.99 ± 1.39 ^{bcde}	0.67 ± 0.49 ^{abcde}	0.84 ± 0.62 ^{bcde}	1.99 ± 1.39 ^{bcde}	0.67 ± 0.49 ^{abcde}
C3	4.2 ± 0.2 ^{ef}	11 ± 1 ^{cd}	2.8 ± 0.3 ^{efg}	0.83 ± 0.21 ^{cdef}	2.13 ± 0.24 ^{def}	0.54 ± 0.05 ^{def}	0.42 ± 0.10 ^{cdef}	1.06 ± 0.12 ^{def}	0.27 ± 0.03 ^{def}	0.42 ± 0.10 ^{cdef}	1.06 ± 0.12 ^{def}	0.27 ± 0.03 ^{def}
T1	5.8 ± 0.2 ^{c}	14 ± 1 ^{b}	4.25 ± 0.25 ^{bc}	2.07 ± 0.28 ^{bc}	4.93 ± 0.15 ^{bc}	1.5 ± 0.07 ^{abc}	1.03 ± 0.14 ^{bc}	2.47 ± 0.08 ^{bc}	0.75 ± 0.03 ^{abc}	1.03 ± 0.14 ^{bc}	2.47 ± 0.08 ^{bc}	0.75 ± 0.03 ^{abc}
T2	4.5 ± 0.5 ^{def}	10.5 ± 0.5 ^{cde}	3.8 ± 0.2 ^{cd}	1.3 ± 0.01 ^{cdef}	3.09 ± 0.46 ^{cdef}	1.11 ± 0.05 ^{bcdef}	0.65 ± 0.01 ^{cdef}	1.54 ± 0.23 ^{cdef}	0.55 ± 0.03 ^{cdef}	0.65 ± 0.01 ^{cdef}	1.54 ± 0.23 ^{cdef}	0.55 ± 0.03 ^{cdef}
T3	3.95 ± 0.35 ^{efg}	8.5 ± 0.5 ^{def}	2.5 ± 0.5 ^{fg}	0.53 ± 0.009 ^{def}	1.14 ± 0.04 ^{f}	0.33 ± 0.04 ^{f}	0.26 ± 0.001 ^{def}	0.57 ± 0.02 ^{f}	0.17 ± 0.02 ^{f}	0.26 ± 0.001 ^{def}	0.57 ± 0.02 ^{f}	0.17 ± 0.02 ^{f}
T4	5.25 ± 0.25 ^{cd}	13 ± 1 ^{bc}	4.25 ± 0.25 ^{bc}	1.74 ± 0.005 ^{bcd}	4.34 ± 0.53 ^{bcd}	1.41 ± 0.02 ^{abcd}	0.87 ± 0.01 ^{bcd}	2.17 ± 0.26 ^{bcd}	0.71 ± 0.01 ^{bcd}	0.87 ± 0.01 ^{bcd}	2.17 ± 0.26 ^{bcd}	0.71 ± 0.01 ^{bcd}
T5	4.5 ± 0.5 ^{def}	8 ± 1 ^{ef}	3.3 ± 0.3 ^{def}	0.93 ± 0.67 ^{cdef}	1.4 ± 0.84 ^{ef}	0.67 ± 0.48 ^{cdef}	0.46 ± 0.33 ^{cdef}	0.7 ± 0.42 ^{ef}	0.34 ± 0.24 ^{ef}	0.46 ± 0.34 ^{cdef}	0.7 ± 0.42 ^{ef}	0.34 ± 0.24 ^{ef}
T6	3.15 ± 0.15 ^{gh}	6 ± 1 ^{fg}	2.75 ± 0.25 ^{efg}	0.4 ± 0.14 ^{ef}	0.73 ± 0.18 ^{f}	0.37 ± 0.18 ^{f}	0.2 ± 0.07 ^{ef}	0.36 ± 0.09 ^{f}	0.19 ± 0.09 ^{f}	0.2 ± 0.07 ^{ef}	0.36 ± 0.09 ^{f}	0.19 ± 0.09 ^{f}
T7	4.85 ± 0.15 ^{cde}	12.5 ± 0.5 ^{bc}	3.45 ± 0.25 ^{cde}	1.04 ± 0.05 ^{cdef}	2.71 ± 0.33 ^{cdef}	0.75 ± 0.12 ^{cdef}	0.52 ± 0.02 ^{cdef}	1.35 ± 0.17 ^{cdef}	0.37 ± 0.06 ^{cdef}	0.52 ± 0.02 ^{cdef}	1.35 ± 0.17 ^{cdef}	0.37 ± 0.06 ^{cdef}
T8	3.7 ± 0.2 ^{fgh}	7.5 ± 2.5 ^{f}	2.25 ± 0.25 ^{gh}	0.69 ± 0.03 ^{def}	1.4 ± 0.48 ^{ef}	0.42 ± 0.05 ^{ef}	0.34 ± 0.01 ^{def}	0.7 ± 0.24 ^{ef}	0.21 ± 0.03 ^{ef}	0.34 ± 0.01 ^{def}	0.7 ± 0.24 ^{ef}	0.21 ± 0.03 ^{ef}
T9	2.75 ± 0.25 ^{h}	4.5 ± 0.5 ^{g}	1.45 ± 0.05 ^{h}	0.32 ± 0.003 ^{f}	0.52 ± 0.02 ^{f}	0.17 ± 0.02 ^{f}	0.16 ± 0.07 ^{f}	0.26 ± 0.01 ^{f}	0.08 ± 0.01 ^{f}	0.16 ± 0.01 ^{f}	0.26 ± 0.01 ^{f}	0.08 ± 0.01 ^{f}

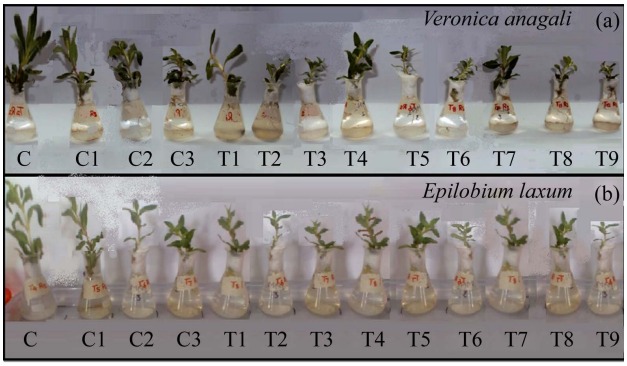

Figure 1. Effect of cadmium alone and in combination with salt (NaCl) on the growth of *Veronica* plant (a) and *Epilobium* Plant (b). C (control without Cd and NaCl), C1 to C3 (100, 200, 300 ppm Cd), T1 to T3 (100 ppm Cd with 1000, 3000, 6000 ppm NaCl), T4 to T6 (200 ppm Cd with 1000, 3000, 6000 ppm NaCl), T7 to T9 (300 ppm Cd with 1000, 3000, 6000 NaCl). In all treatments and control the Hoagland solution was used as growth medium.

root and stem length of *Epilobium* plant occurred at 100 ppm (C2) and 150 ppm (C3) Cd concentrations while the leaf length was reduced only at the highest Cd concentration C3 (150 ppm) when compared with control C (without Cd). With a few exceptions (*i.e.* root and leaves dry biomass) the biomass and water contents of the *Epilobium* plant showed significant reduction only at the highest Cd concentration 150 ppm (C3) as compared to the control C as shown in (**Table 3**).

Table 3. Effect of different concentrations of Cd alone and in combinations with different concentrations of NaCl on growth, biomass and total water content of *Epilobium laxum* plant. ± SD denote standard deviation and different letter represent the significant difference. C (control without Cd and NaCl), C1 to C3 (50, 100, 150 ppm Cd), T1 to T3 (50 ppm Cd with 1000, 3000, 6000 ppm NaCl), T4 to T6 (100 ppm Cd with 1000, 3000, 6000 ppm NaCl), T7 to T9 (150 ppm Cd with 1000, 3000, 6000 NaCl). In all treatments and control the Hoagland solution was used as growth medium.

Treatments	Length (cm) ± SD			Fresh Biomass (g) ± SD			Dry Biomass (g) ± SD			Total Water Content (g) ± SD		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
C	10.20 ± 2.35 ^{a}	21.00 ± 1.00 ^{a}	3.50 ± 0.50 ^{a}	1.35 ± 0.05 ^{a}	2.43 ± 0.78 ^{a}	0.55 ± 0.05 ^{a}	0.61 ± 0.04 ^{a}	0.97 ± 0.31 ^{a}	0.23 ± 0.02 ^{a}	0.74 ± 0.05 ^{a}	1.46 ± 0.47 ^{a}	0.32 ± 0.03 ^{a}
C1	7.75 ± 0.25 ^{ab}	17.33 ± 2.08 ^{b}	3.15 ± 0.15 ^{ab}	1.19 ± 0.35 ^{ab}	1.90 ± 0.10 ^{ab}	0.45 ± 0.05 ^{ab}	0.56 ± 0.05 ^{ab}	0.74 ± 0.41 ^{ab}	0.2 ± 0.01 ^{a}	0.62 ± 0.3 ^{abc}	1.15 ± 0.31 ^{ab}	0.25 ± 0.05 ^{abc}
C2	6.50 ± 0.50 ^{bc}	16.00 ± 1.00 ^{bc}	2.50 ± 0.50 ^{abc}	1.10 ± 0.10 ^{abc}	1.68 ± 0.16 ^{abc}	0.33 ± 0.04 ^{bcde}	0.46 ± 0.03 ^{bc}	0.67 ± 0.06 ^{ab}	0.16 ± 0.01 ^{b}	0.64 ± 0.11 ^{abcd}	1.01 ± 0.09 ^{abc}	0.17 ± 0.04 ^{abcd}
C3	4.50 ± 0.50 ^{cde}	12.50 ± 0.50 ^{d}	2.17 ± 0.29 ^{bc}	0.80 ± 0.01 ^{cde}	1.47 ± 0.13 ^{bcd}	0.24 ± 0.04 ^{cdef}	0.37 ± 0.02 ^{cd}	0.59 ± 0.05 ^{abc}	0.11 ± 0.01 ^{cde}	0.43 ± 0.02 ^{bcd}	0.88 ± 0.08 ^{bc}	0.14 ± 0.04 ^{bcd}
T1	5.50 ± 0.50 ^{bcd}	13.50 ± 0.50 ^{cd}	2.83 ± 0.29 ^{ab}	0.85 ± 0.05 ^{bcd}	1.6 ± 0.18 ^{bcd}	0.39 ± 0.09 ^{abc}	0.35 ± 0.05 ^{cde}	0.64 ± 0.07 ^{abc}	0.13 ± 0.02 ^{bc}	0.50 ± 0.1 ^{ab}	0.96 ± 0.11 ^{abc}	0.27 ± 0.07 ^{ab}
T2	5.50 ± 0.50 ^{bcd}	11.00 ± 1.00 ^{de}	2.50 ± 0.20 ^{abc}	0.73 ± 0.10 ^{cde}	1.18 ± 0.25 ^{bcde}	0.35 ± 0.13 ^{bcd}	0.32 ± 0.04 ^{def}	0.47 ± 0.10 ^{bcd}	0.12 ± 0.02 ^{c}	0.41 ± 0.12 ^{abcd}	0.71 ± 0.15 ^{bcd}	0.23 ± 0.15 ^{abcd}
T3	4.00 ± 1.00 ^{cde}	7.50 ± 0.50 ^{f}	2.25 ± 0.25 ^{bc}	0.57 ± 0.15 ^{de}	1.14 ± 0.10 ^{bcdef}	0.22 ± 0.02 ^{cdef}	0.24 ± 0.04 ^{efg}	0.46 ± 0.04 ^{bcd}	0.1 ± 0.002 ^{cde}	0.32 ± 0.11 ^{bcd}	0.68 ± 0.06 ^{bcd}	0.12 ± 0.02 ^{bcd}
T4	5.33 ± 0.577 ^{bcd}	13.00 ± 1.00 ^{d}	2.50 ± 0.50 ^{abc}	0.73 ± 0.03 ^{cde}	1.17 ± 0.38 ^{bcde}	0.31 ± 0.01 ^{bcde}	0.25 ± 0.05 ^{efg}	0.47 ± 0.15 ^{bcd}	0.11 ± 0.01 ^{cd}	0.47 ± 0.04 ^{abcd}	0.70 ± 0.23 ^{bcd}	0.20 ± 0.02 ^{abcd}
T5	5.25 ± 1.75 ^{bcd}	11.33 ± 0.58 ^{de}	2.25 ± 0.25 ^{bc}	0.61 ± 0.09 ^{de}	1.07 ± 0.11 ^{cdef}	0.30 ± 0.02 ^{bcde}	0.25 ± 0.05 ^{efg}	0.46 ± 0.04 ^{bcd}	0.1 ± 0.01 ^{cde}	0.36 ± 0.04 ^{abcd}	0.61 ± 0.15 ^{bcd}	0.20 ± 0.04 ^{abcd}
T6	3.25 ± 0.25 ^{de}	7.83 ± 0.76 ^{f}	1.50 ± 0.50 ^{cd}	0.43 ± 0.07 ^{ef}	0.90 ± 0.10 ^{cdef}	0.16 ± 0.02 ^{ef}	0.18 ± 0.04 ^{hi}	0.31 ± 0.01 ^{bcd}	0.07 ± 0.001 ^{ef}	0.25 ± 0.03 ^{cd}	0.59 ± 0.10 ^{cd}	0.09 ± 0.02 ^{cd}
T7	4.00 ± 1.00 ^{cde}	9.00 ± 1.00 ^{ef}	1.75 ± 0.25 ^{cd}	0.73 ± 0.15 ^{cde}	0.85 ± 0.05 ^{def}	0.20 ± 0.1 ^{def}	0.24 ± 0.04 ^{ghi}	0.38 ± 0.05 ^{bcd}	0.08 ± 0.005 ^{de}	0.49 ± 0.17 ^{bcd}	0.47 ± 0.03 ^{cd}	0.13 ± 0.09 ^{bcd}
T8	3.25 ± 0.25 ^{de}	6.67 ± 0.58 ^{fg}	1.15 ± 0.15 ^{d}	0.53 ± 0.06 ^{def}	0.50 ± 0.01 ^{ef}	0.19 ± 0.01 ^{def}	0.23 ± 0.03 ^{ghi}	0.18 ± 0.12 ^{cd}	0.07 ± 0.01 ^{ef}	0.30 ± 0.04 ^{bcd}	0.32 ± 0.12 ^{d}	0.12 ± 0.02 ^{bcd}
T9	2.25 ± 0.25 ^{e}	4.27 ± 0.25 ^{g}	0.75 ± 0.25 ^{d}	0.19 ± 0.01 ^{f}	0.35 ± 0.05 ^{f}	0.10 ± 0.01 ^{f}	0.12 ± 0.02 ^{i}	0.11 ± 0.01 ^{d}	0.04 ± 0.005 ^{f}	0.07 ± 0.01 ^{d}	0.24 ± 0.06 ^{d}	0.07 ± 0.01 ^{d}

3.2. Effect of Different Concentrations of Cd and NaCl Salt in Various Combinations on Growth and Biomass of *Veronica* and *Epilobium* Plants

Addition of different concentration of salt (NaCl) to media induced reduction in all of the growth parameters when the treatments T1 to T9 (Cd + NaCl) were compared with C1 to C3 as shown in **Table 2** and **Table 3** respectively for *Veronica* and *Epilobium* plants. Increasing the salt concentration in media gradually reduced the growth and biomass of both the plant (*Veronica* and *Epilobium*) and the reduction was found more significant at the highest concentration (6000 ppm) of salt (NaCl). Salt stress is considered to have main effects *i.e.* it reduces water potential, causes ion imbalance or disturbance in ion homeostasis, and is toxic. Thus higher concentration of salt alters the status of the water significantly, thus leading to a reduction in the initial growth of the plant and consequently limits its productivity. Since salt stress entails both osmotic as well as ionic stress [23] [24]. The suppression of growth is directly related to the total concentration of soluble salts [25] [26]. The highest significant reduction in growth and biomass was recorded for the plants grown in highest concentrations of Cd and salt *i.e.* treatment T9 (150 ppm Cd + 6000 ppm salt). Both plant showed almost identical reduction in growth and biomass.

3.3. Cadmium Concentration and Accumulation in Different Parts of Both Plants

The effect of different concentrations of salt (NaCl) on Cd concentration and accumulation in different parts of *Veronica* and Epilobium plant is presented respectively in **Table 4** and **Table 5**. *Veronica* plant accumulated more than 100 ppm Cd (a threshold limit for a plant to be hyper-accumulator of Cd) in plants treated with Cd only (C1 (50 ppm Cd), C2 (100 ppm Cd) and C3 (150 ppm Cd)) as shown in **Table 4**. The plant showed a gradual increase in Cd concentration in plant tissues with the increase in Cd concentration in growth media, by

Table 4. Effect of different concentration of Cd alone and in combination with different concentrations of salt (NaCl) on Cd uptake in different parts of *Veronica anagallis-aquatic* plant. Different letters indicate the significant difference. C (control without Cd and NaCl), C1 to C3 (50, 100, 150 ppm Cd), T1 to T3 (50 ppm Cd with 1000, 3000, 6000 ppm NaCl), T4 to T6 (100 ppm Cd with 1000, 3000, 6000 ppm NaCl), T7 to T9 (150 ppm Cd with 1000, 3000, 6000 NaCl). In all treatments and control the Hoagland solution was used as growth medium.

Treatment	Cd concentration (ppm)			Cd accumulation (mg/DBM)				Cd accumulation %			Cd translocation factor (TF)		Cd bio-concentration factor (BCF)
	Root	Stem	Leaves	Root	Stem	Leaves	Entire plant Cd (mg/DBM)	Root	Stem	Leaf	Root to stem	Root to leaves	
C1	928.6 ± 2.86 ^{d}	329 ± 1.58 ^{de}	231.4 ± 3.54 ^{g}	1.347 ± 0.0032 ^{a}	0.94 ± 0.01 ^{a}	0.214 ± 0.0011 ^{a}	2.5 ± 0.02 ^{a}	53.66	37.72	8.62	0.36	0.25	9.55
C2	985.4 ± 1.36 ^{c}	385 ± 0.9 ^{bcd}	244 ± 1.28 ^{f}	0.827 ± 0.0019 ^{ab}	0.77 ± 0.003 ^{abc}	0.163 ± 0.0007 ^{ab}	1.76 ± 0.01 ^{abc}	46.27	44.66	9.07	0.39	0.25	5.00
C3	1102.6 ± 1.34 ^{c}	418 ± 1.8 ^{bc}	388 ± 0.6 ^{d}	0.46 ± 0.0009 ^{b}	0.44 ± 0.002 ^{abcde}	0.105 ± 0.0004 ^{cdef}	1.01 ± 0.01 ^{bcd}	45.2	44.34	10.5	0.38	0.35	3.83
T1	890 ± 1.02 ^{e}	380 ± 1.54 ^{cd}	107.4 ± 1.6 ^{j}	0.92 ± 0.0021 ^{cd}	0.94 ± 0.003 ^{a}	0.07 ± 0.0016 ^{bcd}	1.92 ± 0.01 ^{ab}	47.82	49.03	3.15	0.43	0.09	9.01
T2	945 ± 0.9 ^{d}	466 ± 1.58 ^{ab}	128.4 ± 1.56 ^{i}	0.616 ± 0.0019 ^{c}	0.71 ± 0.005 ^{abcd}	0.071 ± 0.0018 ^{bc}	1.4 ± 0.01 ^{abcd}	44.2	50.71	5.09	0.49	0.14	10.23
T3	1155.2 ± 0.58 ^{d}	468 ± 0.86 ^{ab}	451 ± 1.8 ^{c}	0.305 ± 0.0023 ^{cde}	0.27 ± 0.003 ^{cde}	0.075 ± 0.0003 ^{bcd}	0.65 ± 0.001 ^{cd}	47.21	41.25	11.5	0.41	0.39	12.93
T4	860 ± 0.86 ^{f}	408 ± 1.02 ^{bcd}	217.2 ± 3 ^{h}	0.75 ± 0.0016 ^{e}	0.89 ± 0.007 ^{ab}	0.153 ± 0.0012 ^{bcde}	1.79 ± 0.008 ^{abc}	41.99	49.42	8.59	0.48	0.25	4.78
T5	1040 ± 2.86 ^{b}	468 ± 1.04 ^{ab}	381.8 ± 1.34 ^{d}	0.479 ± 0.0021 ^{e}	0.32 ± 0.002 ^{cde}	0.128 ± 0.0015 ^{bcde}	0.93 ± 0.004 ^{bcd}	49.3	37.48	13.2	0.45	0.37	6.17
T6	1198 ± 1.58 ^{e}	521.6 ± 1.18 ^{a}	732.6 ± 14.28 ^{a}	0.24 ± 0.0017 ^{e}	0.19 ± 0.001 ^{e}	0.135 ± 0.0011 ^{def}	0.57 ± 0.0034 ^{cd}	42.39	34.42	23.2	0.44	0.61	7.52
T7	900 ± 1.84 ^{c}	278 ± 0.92 ^{e}	232 ± 1.2 ^{g}	0.469 ± 0.0021 ^{cd}	0.38 ± 0.0025 ^{bcde}	0.087 ± 0.0005 ^{ef}	0.93 ± 0.002 ^{bcd}	50.46	40.26	9.28	0.31	0.26	2.77
T8	1040 ± 1.3 ^{b}	351.6 ± 1.4 ^{cde}	369.6 ± 1.22 ^{e}	0.357 ± 0.002 ^{c}	0.25 ± 0.005 ^{de}	0.077 ± 0.0007 ^{fg}	0.68 ± 0.002 ^{bcd}	53.14	35.51	11.4	0.34	0.36	3.67
T9	1480 ± 2.32 ^{a}	372.2 ± 0.88 ^{cd}	680.2 ± 1.38 ^{b}	0.234 ± 0.0015 ^{de}	0.1 ± 0.0007 ^{e}	0.057 ± 0.0004 ^{g}	0.39 ± 0.0006 ^{d}	60.4	24.82	14.8	0.25	0.46	5.16

comparing C1, C2, and C3 (**Table 4**). Salt (NaCl) addition to growth media showed an increasing effect on the Cd concentration in different parts of the plant. The increase in Cd phytoaccumulation by salt might be due to two reasons *i.e.* exchange of metals ions and formation of stable metal complexes with the chloride anion [27]. Addition of NaCl increased Cd concentration in the soil solution and accumulation in the leaf of Swiss chard [28]. The bioavailability of Cd is enhanced under saline conditions [29] [30]. Higher salt concentrations (3000 and 6000 ppm) significantly increased the concentration of Cd within plant parts as compared to C1, C2 and C3 (only Cd added, without NaCl) (**Table 4**). Lower concentration of salt (1000 ppm) showed no significant effect. Increasing salinity increased the Cd accumulation in *Salix* shoots [31]. The increase in salinity elevated the Cd translocation due to the formation of chloro-complexes with Cd, resulted in enhanced Cd solubility, increased uptake of Cd in the form of chloro-complexes and increased the transport of Cd within the root apoplasts [31]. Similar increase in Cd concentration in potato and sunflower in relation with the increase in the Cl concentration in soil has been reported [29].

The highest total Cd accumulation in different parts of *Veronica* plant (root = 1.347 ± 0.0032, stem = 0.94 ± 0.01 and leaves = 0.214 ± 0.0011 mg/DBM) was shown by plants treated with lowest concentration of Cd (C1) in growth media, this increase might be due to high biomass production of plants in C1 as already described in **Table 2**. Roots accumulated the highest percentage of Cd as compared to stem and leave at all the treatments, except T1, T2, and T4 where Cd % accumulation was higher in stem (**Table 4**). The treatments of Cd in combination with NaCl (T7, T8 and T9) showed more than 50% of total plant Cd accumulation in roots (**Table 4**).

All the treatments showed translocation factor (TF) values less than 0.5 but the Cd bio-concentration factor (BCF) for all the treatments was found higher than 2.5 and the highest BCF value (12.93) was recorded for the treatment T3 (Cd 50 ppm + NaCl 6000 ppm). It shows that treatment T3 has efficiently concentrated Cd from the growth media into the plant tissues. The plants treated with Cd (C1 - C3) showed the BCF greater than 1 and

Table 5. Effect of different concentration of Cd alone and in combination with different concentrations of salt (NaCl) on Cd uptake in different parts of *Epilobium laxum* plant. Different letters indicate the significant difference. C (control without Cd and NaCl), C (control without Cd and NaCl), C1 to C3 (50, 100, 150 ppm Cd), T1 to T3 (50 ppm Cd with 1000, 3000, 6000 ppm NaCl), T4 to T6 (100 ppm Cd with 1000, 3000, 6000 ppm NaCl), T7 to T9 (150 ppm Cd with 1000, 3000, 6000 NaCl). In all treatments and control the Hoagland solution was used as growth medium.

Treatment	Cd concentration (ppm) ± SD			Cd accumulation (mg/DBM) ± SD			Entire plant Cd (mg/DBM) ± SD	Cd accumulation %			Cd translocation factor (TF)		Cd bio-concentration factor (BCF)
	Root	Stem	Leaves	Root	stem	Leaves		Root	Stem	Leaf	Root to stem	Root to leaves	
C1	57.4 ± 2.86 ^{d}	48.8 ± 1.58 ^{ef}	70 ± 3.54 ^{g}	0.032 ± 0.0032 ^{a}	0.036 ± 0.0191 ^{ab}	0.014 ± 0.0011 ^{bc}	0.082 ± 0.0213 ^{ab}	40.34	41.48	18.18	0.85	1.22	0.31
C2	64.4 ± 1.36 ^{c}	54.2 ± 0.9 ^{d}	114 ± 1.28 ^{c}	0.03 ± 0.0019 ^{ab}	0.036 ± 0.0037 ^{a}	0.018 ± 0.0007 ^{a}	0.084 ± 0.0032 ^{a}	35.14	43.21	21.65	0.84	1.77	0.41
C3	66 ± 1.34 ^{c}	59 ± 1.8 ^{c}	128 ± 0.6 ^{b}	0.025 ± 0.0009 ^{b}	0.035 ± 0.0026 ^{ab}	0.013 ± 0.0004 ^{bc}	0.073 ± 0.0028 ^{abc}	33.86	47.68	18.46	0.89	1.94	0.28
T1	45 ± 1.02 ^{e}	50.8 ± 1.54 ^{de}	86 ± 1.6 ^{f}	0.016 ± 0.0021 ^{cd}	0.032 ± 0.0033 ^{ab}	0.011 ± 0.0016 ^{cd}	0.059 ± 0.0071 ^{cd}	26.86	55.07	18.07	1.13	1.91	0.28
T2	54.8 ± 0.9 ^{d}	62.2 ± 1.58 ^{bc}	118 ± 1.56 ^{bc}	0.018 ± 0.0019 ^{c}	0.029 ± 0.0057 ^{ab}	0.014 ± 0.0018 ^{bc}	0.061 ± 0.0056 ^{bcd}	29.04	48.02	22.94	1.13	2.15	0.44
T3	55 ± 0.58 ^{d}	72.8 ± 0.86 ^{a}	148 ± 1.8 ^{a}	0.013 ± 0.0023 ^{cde}	0.033 ± 0.0031 ^{ab}	0.015 ± 0.0003 ^{b}	0.061 ± 0.0012 ^{bcd}	21.99	54.29	23.72	1.32	2.69	1.22
T4	32 ± 0.86 ^{f}	50.4 ± 1.02 ^{e}	86 ± 3 ^{f}	0.008 ± 0.0016 ^{e}	0.023 ± 0.0073 ^{abc}	0.009 ± 0.0012 ^{de}	0.041 ± 0.0081 ^{de}	19.65	56.48	23.87	1.58	2.69	0.11
T5	34.2 ± 2.86 ^{f}	52 ± 1.04 ^{de}	96 ± 1.34 ^{ef}	0.009 ± 0.0021 ^{e}	0.024 ± 0.0023 ^{abc}	0.009 ± 0.0015 ^{de}	0.042 ± 0.0041 ^{de}	20.37	57.26	22.37	1.53	2.82	0.44
T6	42.4 ± 1.58 ^{e}	64.4 ± 1.18 ^{b}	110 ± 14.3 ^{cd}	0.008 ± 0.0017 ^{e}	0.02 ± 0.001 ^{abc}	0.008 ± 0.0011 ^{de}	0.035 ± 0.0034 ^{e}	21.81	56.66	21.53	1.52	2.59	0.52
T7	65.2 ± 1.84 ^{c}	43.6 ± 0.92 ^{g}	42 ± 1.2 ^{h}	0.015 ± 0.0021 ^{cd}	0.016 ± 0.0025 ^{bc}	0.003 ± 0.0005 ^{f}	0.035 ± 0.0021 ^{e}	43.9	47.05	9.041	0.67	0.64	0.10
T8	73 ± 1.3 ^{b}	45.8 ± 1.4 ^{fg}	90 ± 1.22 ^{ef}	0.017 ± 0.002 ^{c}	0.008 ± 0.0053 ^{c}	0.006 ± 0.0007 ^{ef}	0.031 ± 0.0026 ^{e}	54.01	25.72	20.28	0.63	1.23	0.17
T9	86.2 ± 1.4 ^{a}	61 ± 0.88 ^{bc}	100 ± 1.38 ^{de}	0.01 ± 0.0015 ^{de}	0.007 ± 0.0007 ^{c}	0.004 ± 0.0004 ^{f}	0.021± 0.0006 $_{e}$	50.11	32.78	17.11	0.71	1.16	0.27

on the basis of its BCF the *Veronica* plant can be reported as hyper-accumulator for Cd. In C1 (50 ppm Cd) the BCF is highest as compared to C2 (100 ppm Cd) and C3 (150 ppm Cd) (**Table 4**). This increase of BCF in C1 could be due to higher biomass than C2 and C3 as shown in (**Table 2**).

In comparison to *Veronica*, the *Epilobium* plant showed much lower Cd concentration in its different parts under the same treatments used for *Veronica* plant (by comparing **Table 4** and **Table 5**). The highest Cd concentration in *Epilobium* roots (86.2 ± 1.4 ppm) was recorded for the treatment T9 (Cd 150 ppm + 6000 ppm NaCl) while in stem (72.8 ± 0.86 ppm) and leaves (148 ± 1.8 ppm) treatment T3 (Cd 50 ppm + 6000 ppm NaCl) in (**Table 5**). Like in *Veronica*, the combination treatments of Cd and salt (NaCl) showed a negative effect on the biomass of *Epilobium* plant. Although combination treatments (Cd + NaCl) increased the Cd concentration within plant tissues, while biomass was reduced, as a result the total Cd accumulation in different parts of the plant was very low (**Table 5**). The highest total Cd accumulation in *Epilobium* roots (0.032 ± 0.0032 mg/DBM) was shown by C1 (Cd 50 ppm) while in stem (0.036 ± 0.019 mg Cd/DBM) and leaves (0.014 ± 0.0011 mg Cd/DBM) it was shown by C2 in (**Table 5**). In all the treatments, stem of the plant accumulated the highest percentage of Cd as compared to roots and leaves (except the treatments T8 and T9 which showed more than 50% of Cd accumulation in plant roots). The results showed that addition of salt (1000 and 3000 ppm concentrations) into media had increased the stem Cd accumulation about 50% of the total plant. The Cd translocation factor TF values for *Epilobium* were much higher than that of *Veronica* but its Cd bio-concentration BCF values were very low as compared to the BCF values of *Veronica* (comparing values of **Table 4** and **Table 5**), which shows that *Epilobium* is efficient in translocation of Cd from roots to aerial parts but its efficiency in extraction of total Cd from polluted water was much less as compared to the *Veronica* plant. The highest Cd translocation (root to stem) in *Veronica* was recorded for the treatment T4 (100 ppm Cd + 1000 ppm NaCl) while the treatment T5 (100 ppm Cd + 3000 ppm NaCl) showed the highest Cd translocation from root to leaves (**Table 5**). The treatment T3 (50 ppm Cd + 6000 ppm NaCl) possessed the highest Cd bio-concentration value (1.22) compared to

all treatments. These results demonstrate *Veronica* as the Cd hyper-accumulator, while *Epilobium* plant as non hyper-accumulator.

3.4. Correlation of Plants Dry Biomass with Cd Concentration in Different Parts of Plant

The correlation of *Veronica* dry biomass (of root, stem and leaves) with the Cd concentration in roots, stem and leaves is presented respectively in **Figures 2(a)-(c)**. The correlation was found negative and it was significant ($R^2 = 0.608$) in case of plant leaves (**Figure 2(c)**). On the other hand *Epilobium* leaves Cd concentration showed positive correlation with dry biomass of leaves while the roots and stem Cd concentration showed negative correlation with their dry biomasses as shown in **Figures 3(a)-(c)**.

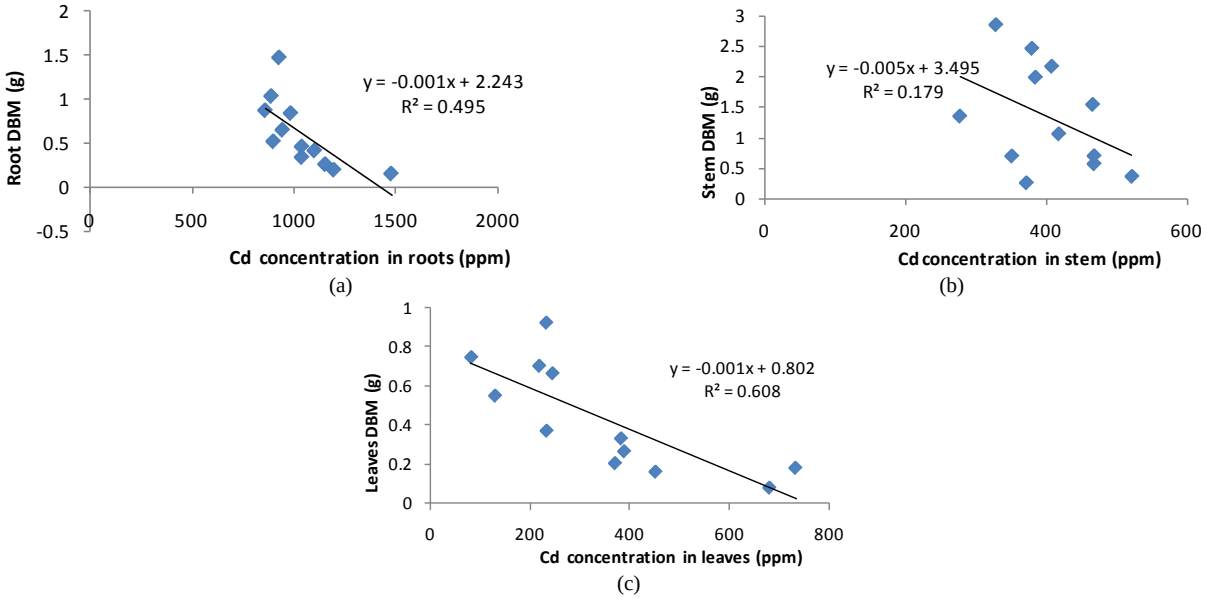

Figure 2. Correlation of root dry biomass (DBM) vs Cd concentration in root (a), stem DBM vs Cd concentration of stem (b), and leave DBM vs Cd concentration in leaves (c) of Veronica plant.

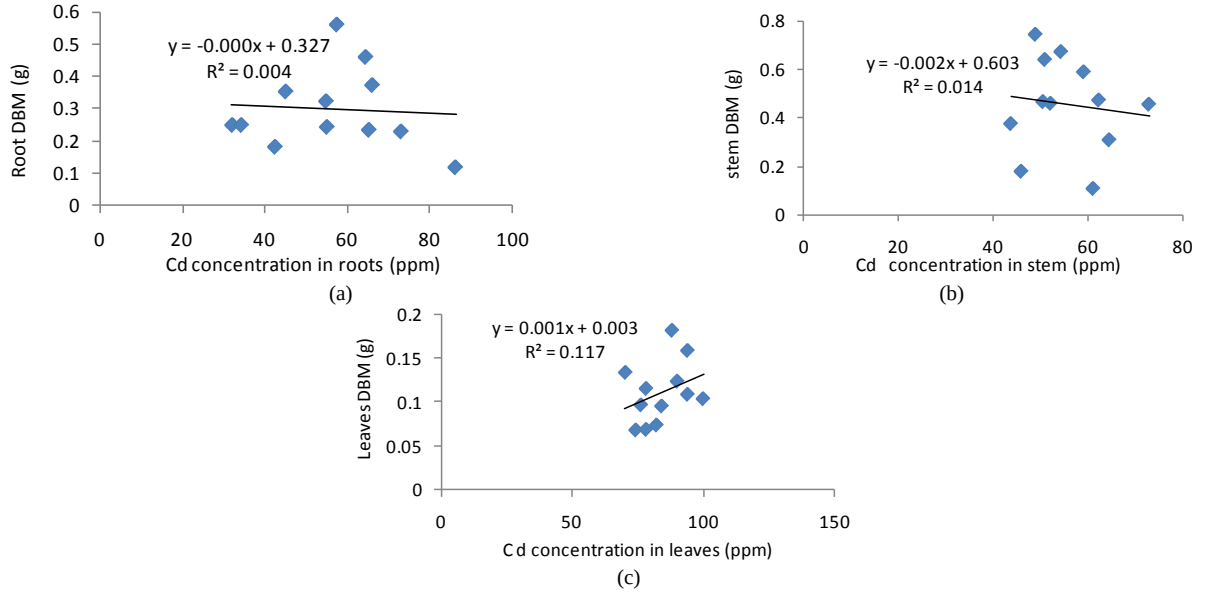

Figure 3. Correlation of root DBM vs root Cd concentration (a), Stem DBM vs stem Cd concentration (b), and leaves DBM vs leaves Cd concentration (c) of *Epilobium* Plant.

4. Conclusion

The result of this study showed that the *Veronica anagallis* plant is hyper-accumulator of Cd, while *Epilobium laxum* plant is non hyper-accumulator, as the *Veronica* plant showed Bio-concentration factor (BCF) more than one (01) and *Epilobium* plant demonstrated Bio-concentration factor less than one (01) (BCF 1 is a threshold limit for a plant to be hyper-accumulator of Cd). The *Veronica* plant demonstrated encouraging results for the removal of Cd from saline water, so it is further recommended to investigate the phytoextraction potential of *Veronica* plant for other metals from the saline water.

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