

Cadmium effects on valerian (*Valeriana officinalis* L.) morphology and Cd uptake in relation to substrate acidity/alkalinity

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Abstract

Experiments were conducted on valerian (*Valeriana officinalis* L.) grown under glasshouse conditions to evaluate the effect of Cadmium (Cd) on plant morphological characteristics and Cd uptake. Valerian plants were grown in pots containing a uniform mixture of either moderately acidic or slightly alkaline substrate consisting of peat and perlite (1:1 v/v) over two periods. Pots were arranged in a completely randomized block design within two groups (moderately acid substrate and slightly alkaline substrate) with four Cd treatments (0, 1, 2 and 5 mg Cd L⁻¹) and six replications per treatment. Cadmium was applied as CdSO₄*8/3H₂O. No visual symptoms of toxicity or nutrient deficiency were attributed to Cd application irrespective of the growth stage or substrate in either experimental period. Cadmium did not affect the dry mass of shoots or roots. Cd concentration in both shoots and roots increased with increasing Cd application, indicating valerian to be a Cd accumulator with accumulation occurring mainly in the roots both in moderately acidic or slightly alkaline substrates. The amount of Cd extracted by Diethylene triamine penta acetic acid-triethanol amine (DTPA-TEA) increased with increasing Cd doses and significantly correlates with the Cd concentrations within the shoots and roots indicating that this extractant could be used to predict Cd concentrations within the plant parts.

Keywords: cadmium; DTPA-TEA; roots uptake; shoots uptake; valerian

Introduction

Cadmium is a heavy metal that remains in the soil for a much longer period than other heavy metals of the biosphere, so soil pollution tends to be permanent (Alloway, 1995); for example, the half-life of Cd in soil varies from 15-1100 years (Oliver, 1997). Heavy metals are not biodegradable but become converted to other forms that accumulate in the soil and plants and are washed away to only a small extent. The mobility of heavy metals within plants varies and generally depends on the particular metal, the type of plant, the plant organ and the age of the plant (Oliver, 1997). Cadmium is one of the most toxic heavy metals and moves relatively easily from the roots to the shoots (Tiller, 1989). Cadmium can enter the soil through mining, metallurgical activities,

the plastics industry, the use of fossil fuels, the disposal of urban wastewater and especially through the use of phosphate fertilizers. The growth of industry in combination with the intensification of agriculture has caused a significant increase in cadmium pollution of the soil over the past century (Alloway, 1995). Its concentration in fertilizers ranges from 2-156 mg kg⁻¹ (Tiller, 1989). Due to the many ways it can be found in soil, cadmium is considered one of the most serious toxic heavy metals. Cadmium is taken up by a variety of plants, but is not involved in any important biological function and is toxic (Andriano, 1986; Wagner, 1993). Cd adsorption/uptake depends on soil pH, temperature, organic matter, cation exchange capacity, humidity, Fe and Al concentration, salt concentration and its relationship with competing elements (Jackson and Alloway, 1991). Several medicinal plants have a tendency to absorb higher amounts of Cd than other plants (Moustakas *et al.*, 2011), while Cd competition with zinc (Zn), iron (Fe) and manganese (Mn) in the soil can cause deficiency of these micronutrients during plant growth (Adiloglu, 2002). Cd is highly mobile and toxic even at low concentrations in plant tissues (Andriano, 1986) and high concentrations of Cd in plant tissues can alter RNA synthesis, cause leaf rot, decrease photosynthesis rate, inhibit mouth opening in plants, and reduce the activity of various plant enzymes. Consequently, it can inhibit plant growth and reduce biomass (Kessler and Brand, 1995; Salt *et al.*, 1995; Gallego *et al.*, 1996; Obata and Umebayashi, 1997). Cd enters the human body mainly through the gastrointestinal tract and lungs following exposure to contaminated air, water or food (Hallenbeck, 1984) to accumulate in vital organs, such as the kidneys. Extensive exposure to this element has been linked to pulmonary failure, kidney disorders and bone diseases. Finally, Cd has been implicated in the development of hypertension and various types of cancer (Bernard *et al.*, 1986). However, even small amounts of Cd in the edible parts of the plant can have a significant impact on human health in the long run due to accumulation (Edmunds and Smedley, 1996).

Valeriana officinalis L. (common name valerian) is a perennial herb of the Fabaceae family and is native to Europe, North America and parts of Asia. Morphologically it is characterized by its numerous slender roots and rhizomes, light green foliage with linear to lanceolate, entire or dentate leaves and flowering stems 30-120 cm. The white to pink flowers appears in July, in compound inflorescences, up to 10 cm in diameter. The plant blooms from May of the second year of cultivation (Strid and Kit, 1991). Valerian is mainly cultivated for its roots, which are harvested in autumn (September-October) from two-year-old plants. Valerian is a constituent of most medicines sold in pharmacies or health food stores as mild sedatives and sleep aids. Dried valerian roots are used for the preparation of herbal remedies to reduce stress, facilitate sleep, and relieve gastrointestinal disturbances (Foss and Houghton, 1997). Valerian was well known by ancient Greeks as a medicinal plant for the treatment of digestive problems, epilepsy and urinary tract infections (Foss and Houghton, 1997). It thrives in most soils, especially medium-textured or sandy soils with humus content (Bernath, 1997). Since Cd may enter the human food chain through medicinal plants, it is important to determine its potential toxicity and health risk in this plant category. Therefore, the aim of this study was to investigate the effect of Cd on valerian morphology and Cd uptake.

Materials and Methods

Experimental conduction and design

Pot experiments were conducted under unheated greenhouse conditions at the Agricultural University of Athens over two periods of approximately three to four months each. Valerian plants were grown in pots filled with a uniform mixture of peat and perlite (1:1 v/v). Two types of peat were used: one moderately acidic and one slightly alkaline. For moderately acidic substrates, the “Baltica peat moss” of Klasmann-Deilmann with a pH of 4.5–5.6 was used. It is a light to moderately decomposed (0-25 mm) blonde peat with no addition of fertilizer or lime. The alkaline substrate comprised “Base substrate peat moss” of Klasmann-Deilmann with a balanced pH of 5.5-6.5. It is a natural blond peat with moderate structure (0-25 mm) and calcium oxide (CaO) was added to adjust the pH to slightly alkaline. The organic matter and moisture content were 90% and 50-

65% by weight, respectively. The perlite particles were 1 to 5 mm in diameter (Perloflor; ISOCON S.A., Athens, Greece). The expanded perlite can hold up to 3 times its weight in water while allowing for good root aeration; it has neutral pH and provides root protection against sudden temperature changes. The depth of substrate in the pots was 10 cm. The pH of the moderately acidic substrate was 5.6 and that of the slightly alkaline substrate was 7.4. Pots were arranged in a completely randomized block design in two groups (moderately acid substrate and slightly alkaline substrate) with four Cd treatments (0, 1, 2 and 5 mg Cd L⁻¹) and six replications per treatment. Cadmium was applied as CdSO₄*8/3H₂O. The treatments, replications per treatment and the cultivation techniques were the same in both experimental periods.

1st experiment

The 1st experiment started on February 21st and ended on May 29th, 2018. Two-month-old seedlings of valerian (kindly sponsored by the “Vioma” estate, Greece) were transplanted on 21st of February 2018 in plastic pots (12x12 cm). On March 16th began the addition of 20 ml of each treatment per pot in both substrates and ended on May 29th 2018. Fertilization was performed three times, i.e. on April 3rd and on May 5th and 19th, using a commercial fertilizer (Nutrileaf-60) with 2 mg N, 2 mg P₂O₅, and 2 mg K₂O per pot (the content of Cd in the fertilizer was negligible). The total amount of Cd applied per pot throughout the cultivation period for both substrates was 200 ml i.e. 10 applications, corresponding to 0.2 mg Cd for level 1 mg L⁻¹, 0.4 mg Cd for the level 2 mg L⁻¹, and 1 mg Cd for the level 5 mg L⁻¹.

2nd experiment

The 2nd experiment began on 20th of November 2018 and ended on 17th of April 2019. Five-month-old seedlings of valerian (kindly sponsored by the “Vioma” estate, Greece) were transplanted on November 20th. On December 16th, began the addition of 20 ml of each treatment to each pot at concentrations indicated above and ended on March 9th. Fertilization was performed once in both substrates on 16th February using a 20-20-20 commercial fertilizer. The total amount of Cd applied per pot was the same as that for the 1st experiment. Throughout the experiments, the frequency of irrigation time varied according to the prevailing weather conditions. Pots were irrigated in such a way as to maintain the moisture at a soil matrix potential of -100 cm. This matrix potential ensured the maintenance of moisture in the substrate without the occurrence of leaching.

Plant analysis

The plants were cut in the neck area, carefully removed from the pots and divided into aerial (shoots) and underground (roots) plant parts, the fresh weight of which was measured immediately. The dry weight of both plant parts was measured after drying to a constant weight in an oven at 60 °C. Dry samples were ground in a stainless-steel Wiley mill and passed through a 150 μm plastic sieve to ensure uniformity. Porcelain beakers were loaded with 0.5 g dry ground tissue smaller than 150 μm in diameter from each pot and ashed at 550 °C for 4 hours. Upon completion of combustion the residue was dissolved in 5 ml of 6N HCL. The suspension was filtered into 100 ml volumetric flasks and made up to volume with deionized water. Cd was determined by flame atomic absorption spectrophotometry at 213.9 nm using an air-acetylene flame. Cd uptake by shoots and roots of valerian was calculated by multiplying the dry weight of shoots and roots by the corresponding Cd concentration and expressed as mg Cd per plant and pot. The sum of shoot and root uptake corresponded to the plant Cd uptake.

Substrate analysis

Samples of air-dried substrate from each pot at the end of each experiment were passed through a 500 μm plastic sieve and analysed for extractable Cd using the diethylene triamine penta acetic acid–triethanol amine (DTPA-TEA), method of Lindsay and Norvell (1978).

Statistical analysis

The influence of Cd application on valerian morphology, plant Cd uptake and the concentration of Cd extracted from the soil by DTPA-TEA were evaluated by analysis of variance (ANOVA), using STATISTICA (StatSoft, 2008). All data were subjected to Duncan's Multiple Range Test to determine statistical significance of the effects due to treatments with Cd.

Results and Discussion

Macroscopic examination of treated plants did not reveal visible symptoms of toxicity or nutrient deficiency over the application range of 0-5 mg Cd kg⁻¹ in plants grown either in moderately acidic or slightly alkaline substrate in either experimental period. Similarly, no differences in plant height were attributed to Cd application irrespective of the growth stage or substrate in either experimental period.

1st experimental period

In the moderately acidic and the slightly alkaline substrate, shoot and root dry matter was not affected by Cd doses up to 5 mg L⁻¹. In the moderately acidic substrate Cd concentration in shoots and roots increased with Cd doses greater than 2 and 1 mg L⁻¹, respectively (Figure 1). In the slightly alkaline substrate Cd concentration in shoots and roots increased with Cd doses greater than 1 mg L⁻¹ (Figure 1). Consequently, Cd uptake by valerian increased with Cd doses, independent of substrate acidity (Figures 3, 4). Cd concentration in the roots of plants grown in either substrate was higher than that of the shoots at each level of application (Figure 1).

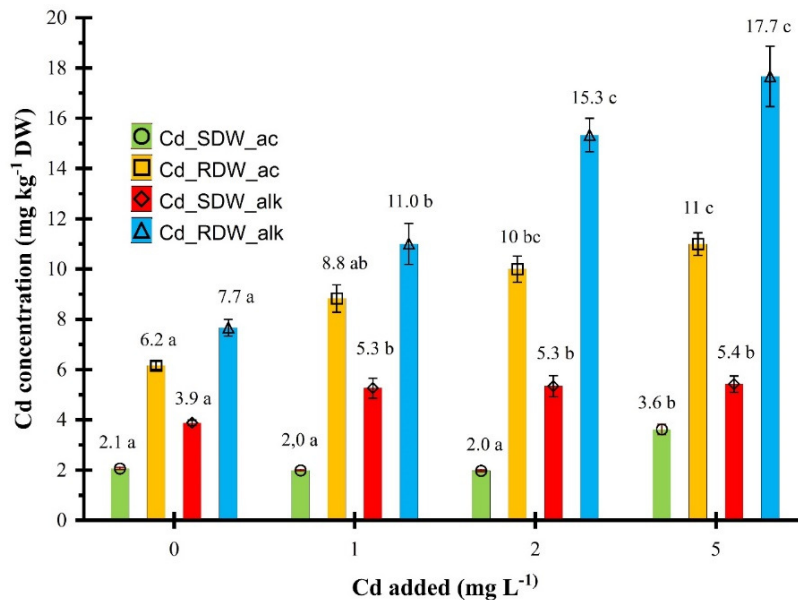


Figure 1. Effect of Cd application on the Cd concentration in shoots (Cd_SDW_ac) and roots (Cd_RDW_ac) of valerian plants grown in a moderately acidic substrate and in shoots (Cd_SDW_alk) and roots (Cd_RDW_alk) of valerian plants grown in slightly alkaline substrate during the 1st experimental period

Note: Numbers shown are means ±SE (n=6). Different letters following the mean values above bars with the same colour indicate significant differences between treatments, according to Duncan's multiple range test at p ≤ 0.05

2nd experimental period

In both the moderately acidic and the slightly alkaline substrate, shoot and root dry matter was not affected by Cd doses up to 5 mg L⁻¹. In the moderately acidic substrate Cd concentration in shoots and roots increased with Cd doses greater than 2 and 1 mg L⁻¹, respectively (Figure 2). In the slightly alkaline substrate Cd concentration in shoots and roots increased with Cd doses greater than 1 and 2 mg L⁻¹, respectively (Figure 2). Hence, Cd uptake by valerian increased with Cd doses, independent of substrate acidity (Figures 5, 6). Cd concentration in the roots of valerian grown in either substrate was higher than that in the shoots at Cd doses greater than 2 mg L⁻¹ (Figure 2).

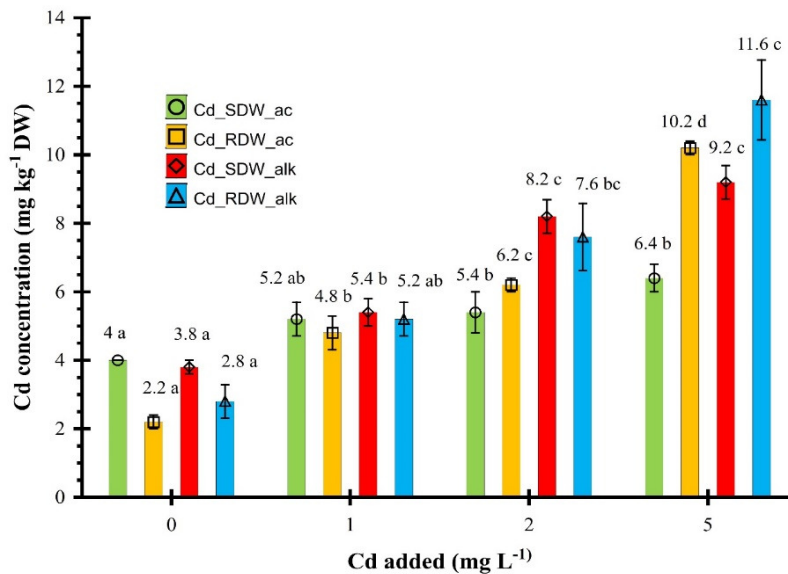


Figure 2. Effect of Cd application on the Cd concentration in shoots (Cd_SDW_ac) and roots (Cd_RDW_ac) of valerian plants grown in a moderately acidic substrate and in shoots (Cd_SDW_alk) and roots (Cd_RDW_alk) of valerian plants grown in slightly alkaline substrate during the 2nd experimental period.

Note: see Figure 1

In both experimental periods shoot, root and total Cd uptake by valerian plants increased with increasing Cd application (Figures 3, 4, 5, 6). These results are in accordance with those reported by Akoumianaki-Ioannidou *et al.* (2019) for hyssop. Moustakas *et al.* (2001) and Akoumianakis *et al.* (2008) reported that Cd application increased Cd concentrations in the leaves of lettuce, radish and cucumber, and in endive and rocket, respectively.

In control treatments during the 1st experimental period, the Cd concentration in shoots and roots was 2.1 and 6.2 mg kg⁻¹, respectively for plants grown in moderately acidic substrate, and 3.9 and 7.7 mg kg⁻¹, respectively for plants grown in slightly alkaline substrate (Figure 1). In control treatments during the 2nd experimental period, the Cd concentration in shoots and roots was 4.0 and 2.2 mg kg⁻¹, respectively for plants grown in moderately acidic substrate, and 3.8 and 2.8 mg kg⁻¹, respectively for plants grown in slightly alkaline substrate (Figure 2). These values are much higher than those for Cd extracted by DTPA-TEA (Table 1). These findings in both experimental periods are particularly noteworthy since they indicate significant accumulation of Cd by valerian independent of substrate acidity. In addition, no growth inhibition was detected even though considerable Cd concentrations were found within the plants. Overall, therefore, valerian can be considered a Cd accumulating plant, like hyssop, rocket, endive, lettuce, spinach, turnip grass, celery and cabbage (Bingham *et al.*, 1983; Davis 1984; Akoumianakis *et al.*, 2008; Akoumianaki-Ioannidou *et al.*, 2019).

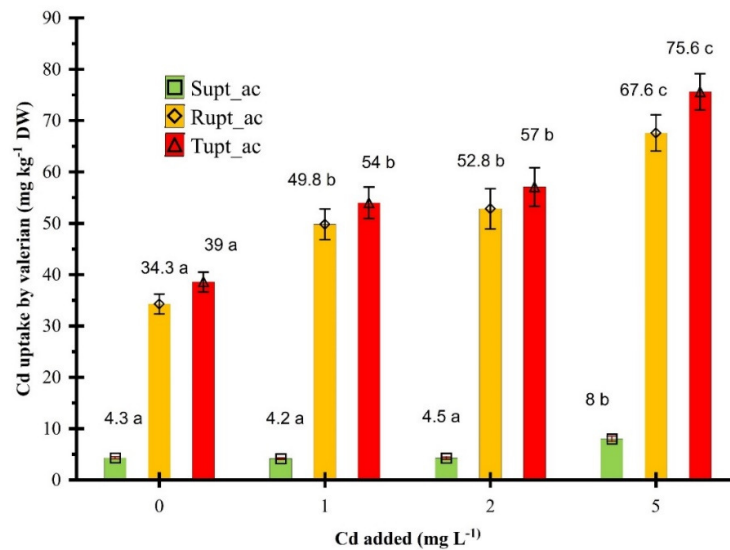


Figure 3. Effect of Cd application on Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt) and total Cd uptake (TUpt) by valerian plants cultivated in moderately acid substrate during the 1st experimental period.

Note: see Figure 1

Table 1. Effect of applied Cd on Cd extracted by DTPA-TEA during two experimental periods

Cd added mg L ⁻¹	1 st experimental period		2 nd experiment period	
	Moderately acidic substrate	Slightly alkaline substrate	Moderately acidic substrate	Slightly alkaline substrate
0	0.03 a	0.02 a	0.14 a	0.12 a
1	0.14 b	0.12 b	0.29 b	0.38 b
2	0.27 c	0.19 c	0.32 b	0.56 c
5	0.68 d	0.53 d	0.66 c	0.89 d

Column means followed by the same letter are not significantly different, according to Duncan's multiple range test, at $p \leq 0.05$

The amount of Cd extractable by DTPA-TEA in the moderately acidic substrate ranged from 0.1 to 0.7 mg kg⁻¹ and from 0.3 to 0.9 mg kg⁻¹ for the 1st and 2nd experimental period, respectively (Table 1). In the slightly alkaline substrate the amount of Cd extractable by DTPA-TEA, ranged from 0.1 to 0.5 and from 0.4 to 0.9 mg kg⁻¹ for the 1st and the 2nd experimental period, respectively (Table 1). The concentrations of Cd extractable by DTPA-TEA in both periods were lower than the maximum tolerable levels (1.6 to 6 mg kg⁻¹) for Cd in agricultural soils in various countries proposed by Kabata-Pendias and Pendias (1992). Cd addition to the substrate led to a significant increase in extractable Cd by DTPA-TEA in both substrates and both periods, as indicated in Figures 7 and 8.

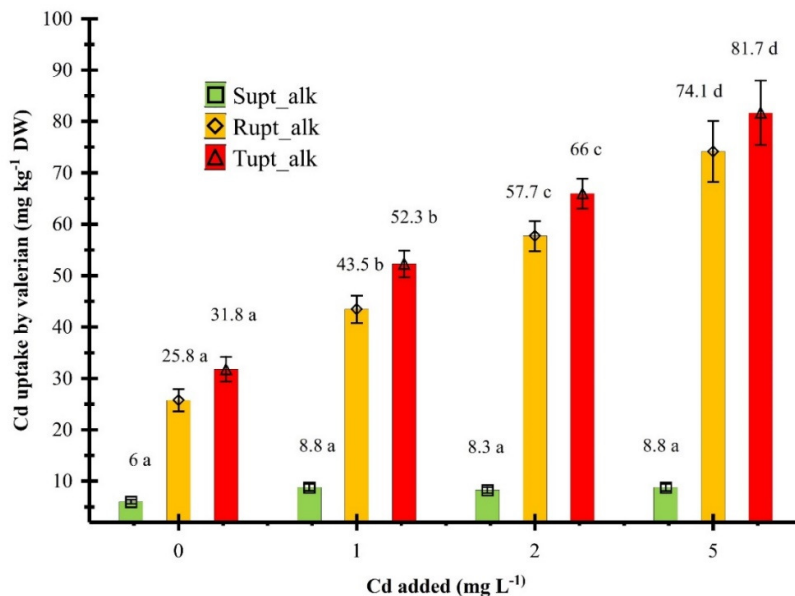
Regression analysis between the amount of Cd in the substrate that was extractable by DTPA-TEA and total Cd uptake by valerian independently of the experimental period, indicated that this extractant could be used effectively to predict Cd uptake in valerian grown in this substrate (Table 2, 3). Moustakas *et al.* (2001), Akoumianakis *et al.* (2008) and Akoumianaki-Ioannidou *et al.* (2019) reported that DTPA-TEA could also be used to predict Cd uptake by lettuce, radish, cucumber, endive, rocket and hyssop.

Table 2. Regression summary for dependent variable Total Cd uptake (TUpt) by valerian plants grown either in moderately acidic or slightly alkaline substrate in the 1st experimental period and Cd extracted by DTPA-TEA

1 st experimental period											
Moderately acid substrate						Slightly alkaline substrate					
N=24	Regression summary for dependent Var: TUpt_ac					N=24	Regression summary for dependent Var: TUpt_alk				
	R= 0.826 R ² = 0.682 Adjusted R ² = 0.668						R= 0.782 R ² =0.613 Adjusted R ² = 0.595				
	F(1.22)= 47.37 p<0.000 Std.Err of estimate: 8.78						F(1.22)=34.85 p<0.000 Std.Err of estimate: 13.18				
	Std.Err	b	Std.Err	t(22)	P-value		Std.Err	b	Std.Err	t(22)	P-value
Intercept		42.528	2.688	15.824	0.00	Intercept		40.318	4.016	10.040	0.00
Cd_ac_soil	0.120	49.301	7.163	6.883	0.00	Cd_alk_soil	0.133	82.184	13.922	5.903	0.00

Table 3. Regression summary for dependent variable Total Cd uptake (TUpt) by valerian plants grown either in moderately acidic or slightly alkaline substrate in the 2nd experimental period and Cd extracted by DTPA-TEA

2 nd Experimental period											
Moderately acid substrate						Slightly alkaline substrate					
N=24	Regression summary for dependent Var: TUpt_ac					N=24	Regression summary for dependent Var: TUpt_alk				
	R= 0.881 R ² =0.776 Adjusted R ² = 0.763						R=0.948 R ² =0.899 Adjusted R ² = 0.893				
	F(1.22)=62.37 p<0.000 Std.Error of estimate: 16.58						F(1.18)=160.47 p<0.000 Std.Error of estimate: 13.97				
	Std.Err	b	Std.Err	t(22)	P-value		Std.Err	b	Std.Err	t(22)	P-value
Intercept		36.651	7.494	4.891	0.00	Intercept		40.975	6.184	6.626	0.00
Cd_ac_soil	0.112	147.313	18.653	7.898	0.00	Cd_alk_soil	0.075	138.527	10.936	12.668	0.00

**Figure 4.** Effect of Cd application on Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by valerian plants cultivated in slightly alkaline substrate during the 1st experimental period.

Note: see Figure 1

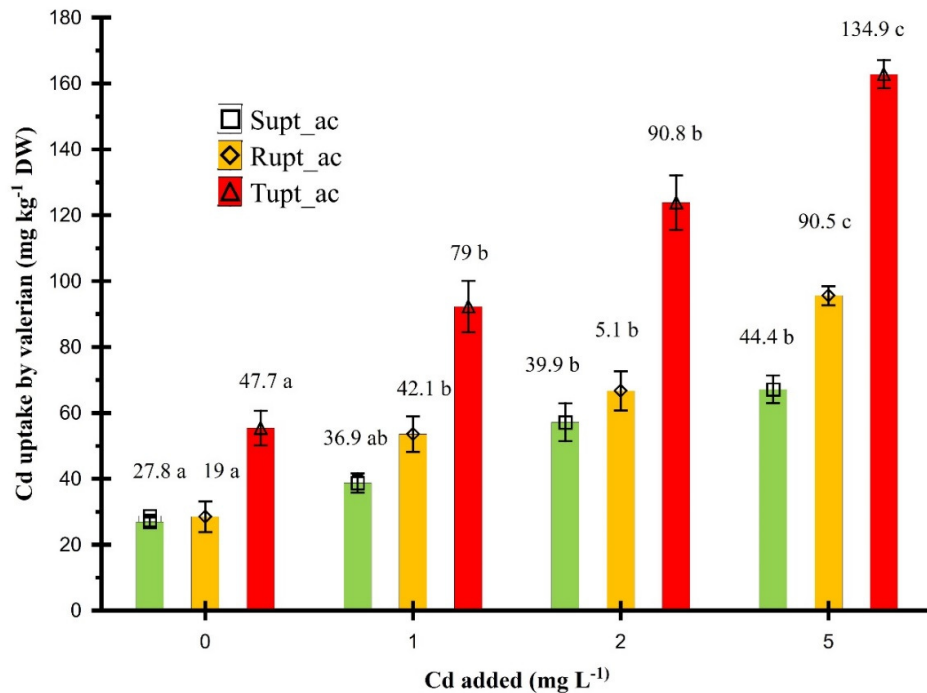


Figure 5. Effect of Cd on Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by valerian plants cultivated in moderately acid substrate during the 2nd experimental period.

Note: see Figure 1

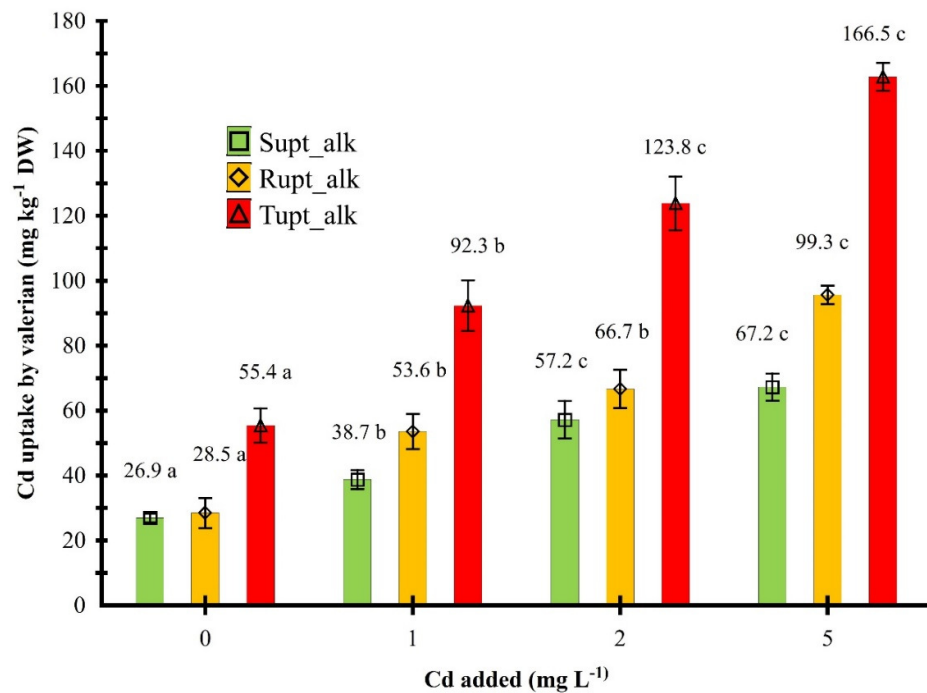


Figure 6. Effect of Cd on Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by valerian plants cultivated in slightly alkaline substrate during the 2nd experimental period.

Note: see Figure 1

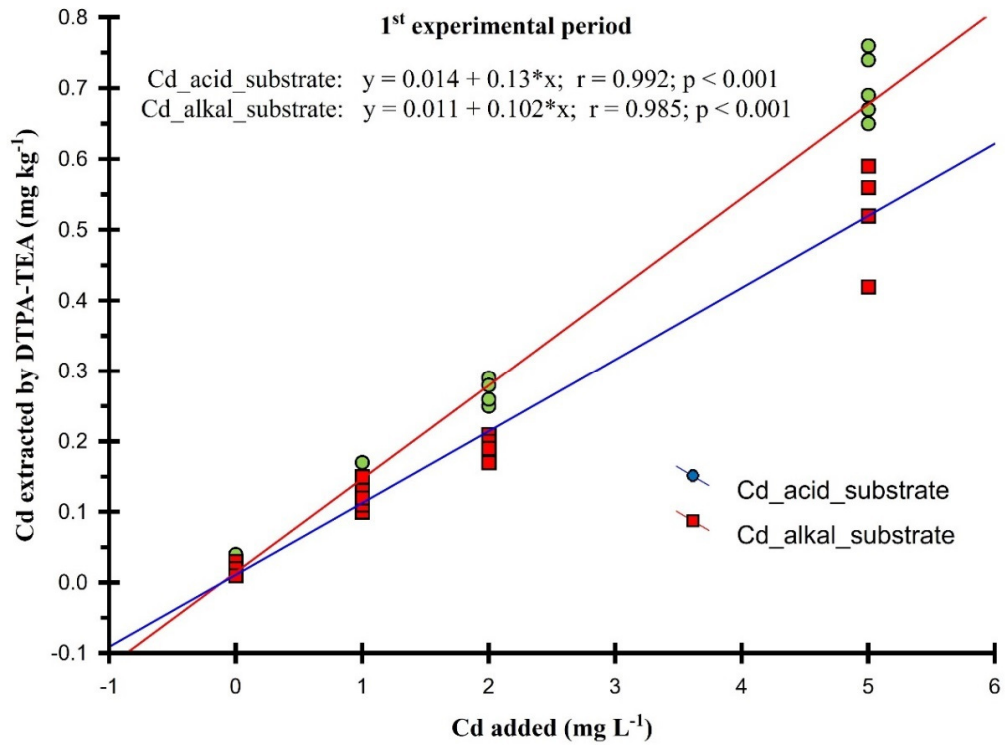


Figure 7. Extractable Cd by DTPA-TEA as affected by Cd additions (1st experimental period)

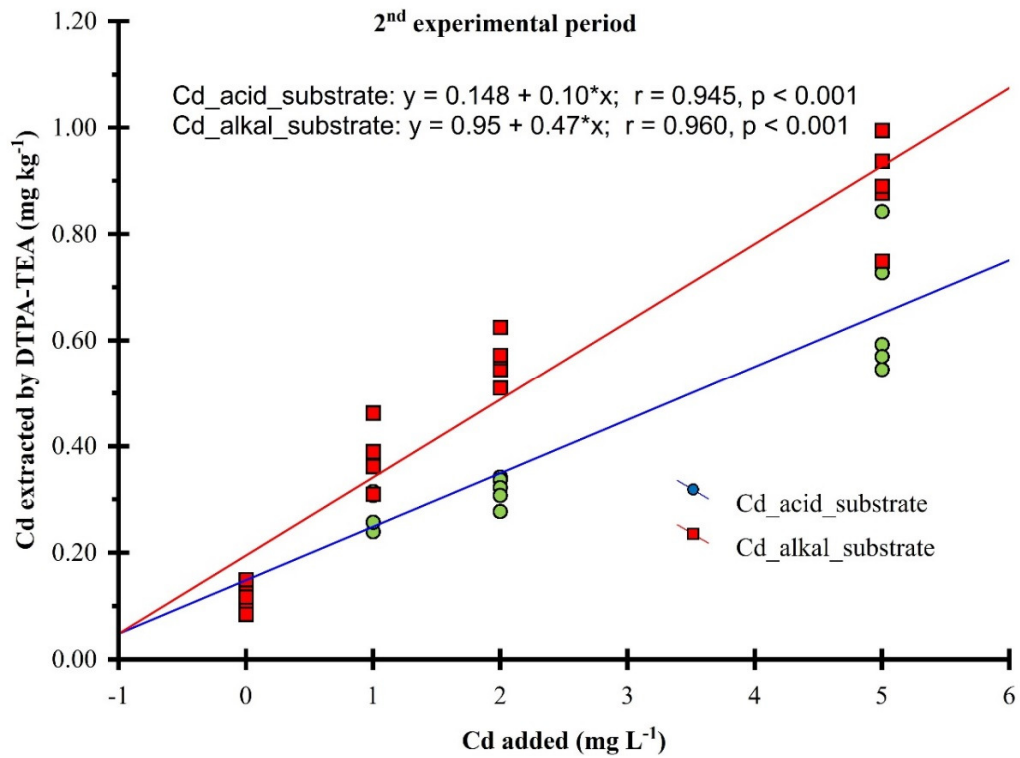


Figure 8. Extractable Cd by DTPA-TEA as affected by Cd additions (2nd experimental period)

Conclusions

Valerian plants did not exhibit visual symptoms of toxicity or deficiency by the application of up to 5 mg Cd L⁻¹ substrate. Valerian plant height as well as shoot and root dry weight were not affected by Cd application. Cadmium uptake by shoots and roots of valerian increased with increasing Cd application irrespective of substrate acidity. Cd extraction by DTPA-TEA could be used to predict Cd uptake by valerian.

Conflict of Interests

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

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