

Cadmium Effects on Hyssop (*Hyssopus officinalis* L.) Morphology and Cd Uptake in Relation to Substrate Acidity/Alkalinity

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Abstract

Hyssop (*Hyssopus officinalis*), is a herb with a wide range of use in food preparation and herbal medicine. It is a perennial shrub through which pollutants such as Cd may enter the human food chain. Therefore, the aim of this research was to examine the extent to which Cd added to the growth substrate is accumulated by hyssop plants and whether Cd affects the plant's morphology. Hyssop 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) to which Cd ($\text{CdSO}_4 \cdot 8/3\text{H}_2\text{O}$) was added (0-control, 1, 2 and 5 mg Cd L⁻¹) during the course of growth. No symptoms of toxicity or nutrient deficiency as well as no differences in plant height were attributed to Cd application irrespective of the growth stage or substrate. Cadmium uptake by aerial organs (shoots) and underground organs (roots) of hyssop increased with Cd application and was higher in the moderately acidic than in the slightly alkaline soil environment. Hyssop is a Cd accumulator and accumulation occurred mainly in the roots in the acidic substrate. Measurement of extractable Cd by diethylene triamine penta acetic acid – triethanol amine (DTPA-TEA) could be used to predict Cd uptake by hyssop plants.

Keywords: extractable Cd; cadmium accumulation; hyssop; medicinal plants; uptake

Introduction

Cadmium (Cd) is a widespread pollutant and Cd soil pollution has increased during the 20th century due to mining, atmospheric deposition during zinc smelting, combustion of fossil fuels and disposal of wastes such as cadmium-containing plastic containers and batteries (Alloway and Steinnes, 1999). Phosphate fertilizers are considered to be the most common cause of cadmium contamination in agricultural soils and Cd concentrations in fertilizers range from 2 to 156 mg kg⁻¹ (Tiller, 1989). Cadmium accumulates in the surface soil creating a long-term problem due to its long half-life, which ranges from 15 to 1100 years (Oliver, 1997). Due to its high mobility in the plant-soil system and low degree of attraction to soil colloids, Cd can easily enter the food chain, with adverse effects on human health (Alloway, 1995). For example, cadmium has been linked to bone diseases, lung edema, liver

damage, anemia and hypertension (Staessen *et al.*, 1999; WHO, 1996). The World Health Organization (WHO) has established a provisional tolerable weekly intake (PTWI) for cadmium of 7 µg/kg of body weight, which corresponds to a daily tolerable intake level of 70 µg of cadmium for an average 70-kg man and 60 µg of cadmium per day for an average 60-kg woman. The uptake and accumulation of cadmium in plants varies widely among species, varieties, and tissues (Oliver, 1997) and is influenced by soil factors such as soil cadmium content, pH, salinity, organic matter and the concentration of iron (Fe), manganese (Mn), calcium (Ca), zinc (Zn) and chlorine (Cl) (McBride, 2002). Hyssop (*Hyssopus officinalis*), a perennial, aromatic shrub of the Lamiaceae family, native to southern Europe, the Middle East and temperate regions of Asia, is widely used in pharmacy and cooking. Hyssop has a woody basal stem, from which upright shoots arise and grow to a height of 40-70 cm. The leaves are narrow, serrated, shiny, dark green and aromatic, up to 2.5 cm long. The flowers, which are aromatic, tubular purple-blue, red or white with

protruding stamens, form in dense oblong inflorescences from mid- to late- summer (late May to September) and the seeds ripen from August to October. Hyssop leaves and shoots are used as a fresh, aromatic condiment to flavor soups, salads etc. while its flowers may be added to salads. Extracted essence is used to flavor foods and beverages (Bown, 1995; Grieve, 1984). In herbal or traditional medicine hyssop is recommended for the treatment of coughs, colds, and asthma. In particular, the leaves and inflorescence tops are antiseptic, expectorant, and vasodilatory, while leaf extracts possess antimicrobial properties and may be used for the treatment of bronchitis and respiratory infections (Chevallier, 1996). Nowadays, hyssop is considered an undervalued herb often used as a home remedy, especially as a stomach expectorant and tonic. Since Cd may enter the human food chain through medicinal plants, it is important to determine the potential toxicity and health risk. Therefore, the aim of this research was to examine the morphological characteristics and Cd content of hyssop plants grown in a moderately acidic or slightly alkaline substrate to which Cd was added.

Materials and Methods

Experimental conduction and design

Pot experiments were conducted under greenhouse conditions at the Agricultural University of Athens over two periods of approximately three months each. The plants were grown in pots filled with a uniform mixture of peat and perlite (1:1 v/v). Two types of peat were employed: one moderately acidic and one slightly alkaline. The electrical conductivity of both forms of peat was 10 mS cm^{-1} , and 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 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 within two groups (moderately acid substrate and slightly alkaline substrate) with four Cd treatments (0-control, 1, 2 and 5 mg Cd L^{-1}) and six replications per treatment. Cadmium was applied as $\text{CdSO}_4 \cdot 8/3\text{H}_2\text{O}$. The 1st experiment for both substrates started on February 22nd and ended on May 29th 2018; the second experiment began on 20th of November 2018 and ended on 17th of April 2019. The treatments, replications per treatment and the cultivation techniques were the same in both study periods. 1st experiment: On March 16th (two months old seedlings), Cd application began with the addition of 20 ml of each treatment to each pot at the concentrations indicated above and ended on April 20th. The total amount applied per pot throughout the cultivation was 260 ml, corresponding to 0.26 mg Cd for level 1 mg L^{-1} , 0.52 mg Cd for the level 2 mg L^{-1} , and 1.3 mg Cd for the level 5 mg L^{-1} . 2nd experiment: On December 18th (five months old seedlings), Cd application began with the addition of 20 ml of each treatment to each pot at the concentrations indicated above and ended on March 9th. The total amount applied per pot was the same as that for the 1st experiment. Throughout the experiments, the frequency and duration of irrigation 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. Fertilization was performed three times in both experiments; using a commercial fertilizer (Nutrileaf-60) with 2 mg N , 2 $\text{mg P}_2\text{O}_5$, and 2 $\text{mg K}_2\text{O}$ for each pot (the content of Cd in the fertilizer was negligible).

Plant analysis

The plants were carefully removed from the pots and separated into the aerial organs (shoots) and underground organs (roots). The dry weight of both plant parts from each pot was measured after drying to a constant weight in an oven at 60 °C. Dried samples were ground in a stainless steel Wiley mill and passed through a 150 μm plastic sieve to ensure uniformity. 0.5 g of dried samples smaller than 150 μm in diameter was 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 (Baker and Amacher, 1982). Cd uptake by shoots and roots of hyssop 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 taken 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 (Lindsay and Norvell, 1978).

Statistical analysis

The influence of Cd treatments on Cd uptake by hyssop and the concentrations of Cd extracted by DTPA- TEA from the substrate were evaluated by analysis of variance (ANOVA), using STATISTICA (StatSoft, 2008). Where a significant difference was found, Duncan's Multiple Range Test at the 5% level of probability was used to compare individual treatment means.

Results and Discussion

No symptoms of toxicity or nutrient deficiency over the application range of 0 - 5 mg Cd kg^{-1} were detected 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 substrate, Cd concentration in shoots and roots of hyssop, increased at Cd doses higher than 2 and 1 mg L^{-1} , respectively. Root dry weight increased at Cd doses greater than 2 mg L^{-1} . Shoot dry weight also tended to increase with Cd dose. Consequently, total Cd uptake by hyssop plants increased with Cd dose (Table 1). The relationships between Cd applied and Cd uptake by

the different plant parts are illustrated in Fig. 1. In slightly alkaline substrate, Cd concentration in shoots and roots, as well as shoot dry weight, increased with Cd doses greater than 1, 2 and 1 mg L⁻¹, respectively. Consequently, total Cd uptake by hyssop plants here too increased with increasing Cd dose (Table 1). The relationships between Cd applied and Cd uptake by the different plant parts are illustrated in Fig. 2. Shoot, root and total Cd uptake by hyssop plants grown in the moderately acidic substrate was higher than that of plants grown in the slightly alkaline substrate (Table 3), possibly due to the higher bioavailability of Cd in an acid soil environment. Soil pH is the most important factor affecting Cd bioavailability (hence its uptake by plants), which increases linearly with decreasing pH (Kirkham, 2006; Kim *et al.*, 2009).

2nd experimental period

In moderately acid substrate, Cd concentration in hyssop shoots and roots increased with Cd doses higher than 2 and 1 mg L⁻¹, respectively. Shoot and root dry weight also increased with Cd dose. Therefore, total Cd uptake by hyssop plants increased with increasing Cd doses (Table 2). The relationships between Cd applied and Cd uptake by the different plant parts are illustrated in Fig. 3. In slightly alkaline substrate, Cd concentration in shoots and roots, and root dry weight increased with Cd doses greater than 1, 2 and 1 mg L⁻¹, respectively. Shoot dry weight also tended to increase with Cd doses. Hence total Cd uptake by hyssop plants increased with increasing Cd doses (Table 2). The relationships between Cd applied and Cd uptake by the different plant parts are illustrated in Fig. 4. In both experimental periods shoot, root and total Cd uptake by hyssop plants increased with increasing Cd application. These results are in accordance with the effects of Cd concentration on the leaves of lettuce, radish, cucumber (Moustakas *et al.*, 2001), endive and rocket (Akoumianakis *et al.*, 2008).

In control treatments during the 1st experimental period, Cd concentration in shoots and roots was 11.5 and 14.33 µg g⁻¹, respectively for plants grown in moderately acid substrate (Table 1), and 10.83 and 16.32 µg g⁻¹, respectively for plants grown in slightly alkaline substrate (Table 2). Also in control treatments during the 2nd experimental period, the Cd concentration in shoots and roots was 4.0 and 4.0 µg g⁻¹, respectively for plants grown in moderately acid substrate (Table 4), and 2.8 and 2.0 µg g⁻¹, respectively for plants grown in slightly alkaline substrate (Table 5). These values are much higher than those for Cd extracted by DTPA – TEA (Tables 1, 2, 4, 5). These findings in both experimental periods are particularly noteworthy since they indicate significant accumulation of Cd by hyssop independent of pH. Akoumianakis *et al.* (2008), Bingham (1983) and Davis (1984) classified rocket, endive, lettuce, spinach, turnip grass, celery and cabbage as Cd accumulator plants.

No differences in Cd uptake by shoots of hyssop grown either in moderately acid or slightly alkaline substrate were detected in either experimental period (Table 2, 4). However, differences were observed in Cd uptake by roots of hyssop grown in moderately acidic substrate compared to those in a slightly alkaline substrate. These findings in both experimental periods indicate that Cd in the acid soil environment accumulated mainly in the roots.

The amount of Cd in the substrate that was extractable by DTPA-TEA correlated with that in the shoots and roots, and therefore total Cd, independently of the experimental period, indicating that this extractant could be used effectively to predict Cd uptake in hyssop grown in this substrate (Table 5). The relationship between Cd applied and extractant Cd by DTPA-TEA is shown in Figs. 1, 2. Moustakas *et al.* (2001) and Akoumianakis *et al.* (2008) reported that DTPA-TEA could also be used to predict Cd uptake by lettuce, radish, cucumber, endive and rocket plants grown in a slightly alkaline substrate.

Table 1. 1st experiment period - moderately acid substrate - effect of Cd on shoot dry weight (SDW), Cd concentration in shoots (Cd-SDW), root dry weight (RDW), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by plants of hyssop cultivated in a moderately acid substrate, during the 1st experimental period

Cd added ml L ⁻¹	SDW	RDW	Cd-SDW	Cd-RDW	SUpt	RUpt	TUpt	Cd soil (µg g ⁻¹ soil)
	(g plant ⁻¹)		(µg g ⁻¹ DW)					
0	1.86 a	2.26 a	11.5	14.33 a	21.03 a	31.14 a	52.16 a	0.03 a
1	2.26 ab	1.98 a	14.17	25.08 b	31.21 b	48.15 ab	79.35 b	0.1 b
2	2.52 ab	2.71 ab	14.33	25.28 b	34.39 b	68.32 b	102.71 c	0.22 c
5	2.88 b	3.36 c	14.67	34.67 c	41.64 c	116.1 c	157.74 d	0.58 d
F=	3.19	4.78		27.42	14.04	25.19	33.28	950.8

Column means followed by the same letter are not significantly different according to Duncan's multiple range test at p≤0.05; column means without letters indicate no significance by Duncan's test at p≤0.05.

Table 2. 1st experiment period - Slightly alkaline substrate - Effect of Cd on shoot dry weight (SDW), Cd concentration in shoots (Cd-SDW), root dry weight (RDW), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by plants of hyssop cultivated in a slightly alkaline substrate, during the 1st experimental period

Cd added ml L ⁻¹	SDW	RDW	Cd-SDW	Cd-RDW	SUpt	RUpt	TUpt	Cd soil (µg g ⁻¹ soil)
	(g plant ⁻¹)		(µg g ⁻¹ DW)					
0	1.65 a	1.56	10.83 a	16.22 a	17.76 a	25.07 a	42.83 a	0.02 a
1	1.77 ab	1.59	14.67 b	25.06 b	25.85 b	39.4 ab	65.25 b	0.09 b
2	2.02 b	1.88	17.33 c	28.47 c	34.84 c	50.6 b	85.44 c	0.17 c
5	1.93 ab	2.23	18 c	33.33 c	34.43 c	74.59 c	109.02 d	0.38 d
F=	2.89		13.02	13.95	17.69	15.14	34.55	756.1

Column means followed by the same letter are not significantly different according to Duncan's multiple range test at p≤0.05; column means without letters indicate no significance by Duncan's test at p≤0.05.

Table 3. Comparison effects of Cd on shoot dry weight (SDW), Cd concentration in shoots (Cd-SDW), root dry weight (RDW), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) in relation to the acidity or alkalinity substrate, during the 1st experiment period

Cd added mg L ⁻¹	Acidity	SDW	RDW	Cd-SDW	Cd-RDW	SUpt	RUpt	TUpt	Cd soil ($\mu\text{g g}^{-1}$ soil)
		(g plant ⁻¹)				($\mu\text{g g}^{-1}$ DW)			
0	acid	1.86 a	2.26 a	11.50 a	14.33 a	21.03 a	31.14 a	52.16 a	0.03 a
	alkaline	1.65 a	1.56 b	10.83 a	16.22 a	17.76 a	25.07 b	42.83 b	0.02 a
1	acid	2.26 a	1.98 a	14.17 a	25.08 a	31.21 a	48.15 a	79.35 a	0.10 a
	alkaline	1.77 a	1.59 a	14.67 a	25.06 a	25.85 a	39.40 a	65.25 a	0.09 a
2	acid	2.52 a	2.71 a	14.33 a	25.28 a	34.39 a	68.32 a	102.71 a	0.22 a
	alkaline	2.02 a	1.88 a	17.33 a	28.47 a	34.84 a	50.60 a	85.44 a	0.17 b
5	acid	2.88 a	3.36 a	14.67 a	34.67 a	41.64 a	116.10 a	157.74 a	0.58 a
	alkaline	1.93 b	2.23 b	18.00 b	33.33 a	34.43 a	74.59 b	109.02 b	0.38 b

Means in acid and alkaline acidity in each Cd treatment followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Table 4. 2nd experiment period - Moderately acid substrate - effect of Cd on shoot dry weight (SDW), Cd concentration in shoots (Cd-SDW), root dry weight (RDW), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) of hyssop plants cultivated in moderately acidic substrate, during the 2nd experiment period

Cd added ml L ⁻¹	SDW	RDW	Cd-SDW	Cd-RDW	SUpt	RUpt	TUpt	Cd soil ($\mu\text{g g}^{-1}$ soil)
	(g plant ⁻¹)				($\mu\text{g g}^{-1}$ DW)			
0	7.00	7.22	4.00 a	4.00 b	27.99 a	28.88 b	56.87 b	0.12 a
1	7.13	7.88	4.40 ab	10.80 a	31.12 ab	84.85 a	115.97 a	0.30 b
2	6.58	7.28	5.60 bc	12.00 a	36.86 b	87.87 a	124.73 a	0.50 c
5	6.87	7.94	6.80 c	14.40 c	46.20 c	115.18 c	161.38 c	0.98 d
F=			10.03	53.42	13.75	23.04	30 b.1	185.4

Column means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$; column means without letters indicate no significance by Duncan's test at $p \leq 0.05$.

Table 5. 2nd experiment period - Slightly alkaline substrate - Effect of Cd on shoot dry weight (SDW), Cd concentration in shoots (Cd-SDW), root dry weight (RDW), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) of hyssop plants cultivated in slightly alkaline substrate, during the 2nd experiment period

Cd added ml L ⁻¹	SDW	RDW	Cd-SDW	Cd-RDW	SUpt	RUpt	TUpt	Cd soil ($\mu\text{g g}^{-1}$ soil)
	(g plant ⁻¹)				($\mu\text{g g}^{-1}$ DW)			
0	7.38	7.05 b	2.80 b	2.00 a	20.61 b	14.10 a	34.70 a	0.11 a
1	6.99	9.05 a	5.20 a	2.00 a	35.61 a	18.11 a	53.72 a	0.22 b
2	7.27	9.12 a	5.60 a	6.00 b	40.98 a	55.75 b	96.72 b	0.32 c
5	7.28	8.67 a	6.40 a	8.60 c	46.75 a	75.24 b	122.00 b	0.33 c
F=		4.88	8.0	23.84	8.24	15.32	21.33	45.7

Column means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$; column means without letters indicate no significance by Duncan's test at $p \leq 0.05$.

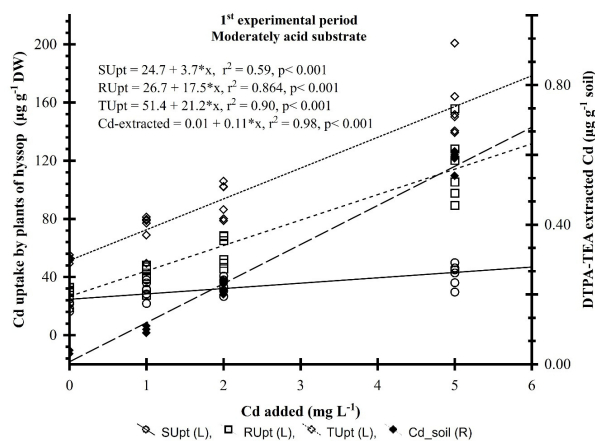
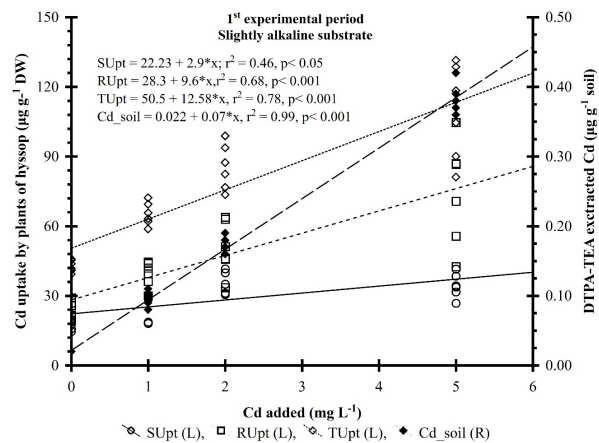
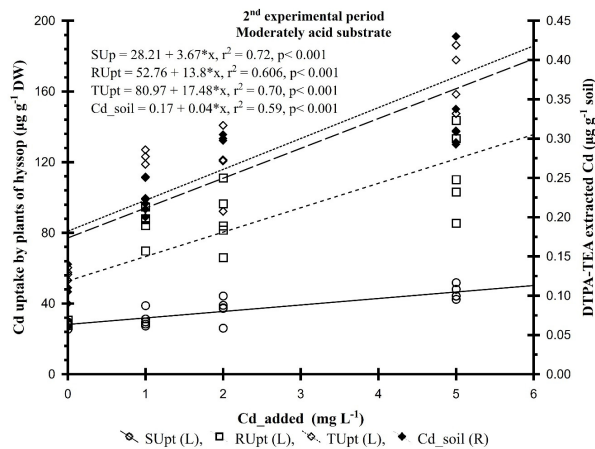
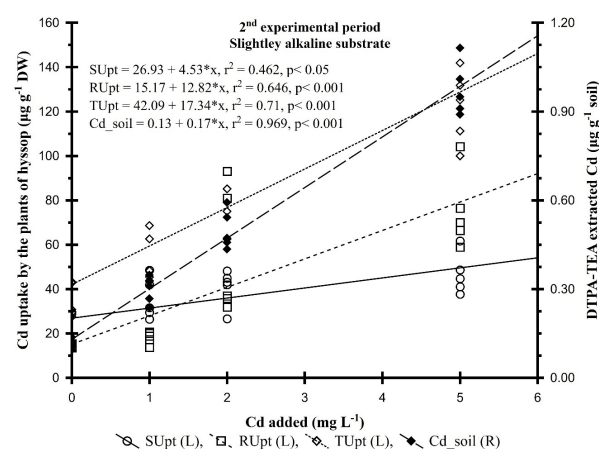
Table 6. Comparison effects of Cd on shoot dry weight (SDW), Cd concentration in shoots (Cd-SDW), root dry weight (RDW), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) of hyssop plants grown in an acidic and in an alkaline substrate, during the 2nd experiment period

Cd added mg L ⁻¹	Acidity	SDW	RDW	Cd-SDW	Cd-RDW	SUpt	RUpt	TUpt	Cd soil ($\mu\text{g g}^{-1}$ soil)
		(g plant ⁻¹)				($\mu\text{g g}^{-1}$ DW)			
0	acid	7.00 a	7.22 a	4.00 a	4.00 a	27.99 a	28.88 a	56.87 a	0.12 a
	alkaline	7.38 a	7.05 a	2.80 a	2.00 b	20.61 a	14.10 b	34.70 b	0.11 a
1	acid	7.13 a	7.88 a	4.40 a	10.80 a	31.12 a	84.85 a	115.97 a	0.30 a
	alkaline	6.99 a	9.05 a	5.20 a	2.00 b	35.61 a	18.11 b	53.72 b	0.22 b
2	acid	6.58 a	7.28 a	5.60 a	12.00 a	36.86 a	87.87 a	124.73 a	0.50 a
	alkaline	7.27 a	9.12 b	5.60 a	6.00 b	40.98 a	55.75 a	96.72 a	0.32 b
5	acid	6.87 a	7.94 a	6.80 a	14.40 a	46.20 a	115.18 a	161.38 a	0.98 a
	alkaline	7.28 a	8.67 a	6.40 a	8.60 b	46.75 a	75.24 b	122.00 b	0.33 b

Means in acid and alkaline acidity in each Cd treatment followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Table 7. Correlations between DTPA –TEA extractable Cd with Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by plants of hyssop cultivated in moderately acidic substrate and in slightly alkaline substrate in both experimental periods

1 st experimental period			
Moderately acid substrate			
Cd-soil	SUpt	Rupt	Tupt
	0.734*	0.937	0.948
Slightly acid substrate			
	SUpt	RUpt	TUpt
Cd-soil	0.683	0.822	0.879
2 nd experimental period			
Moderately acid substrate			
	SUpt	Rupt	Tupt
Cd-soil	0.657	0.804	0.819
Slightly acid substrate			
	SUpt	Rupt	Tupt
Cd-soil	0.746	0.800	0.861

*All correlations are significant at $p < 0.050$ Fig. 1. The relationship between the Cd applied to the moderately acid substrate and the Cd uptake by shoots (SUpt) and roots (RUpt) and the total plant uptake (TUpt) during the 1st experimental period as well as the relationship between extractable Cd by DTPA-TEA and the amount of Cd added to substrateFig. 2. The relationship between the Cd applied to the slightly alkaline substrates and the Cd uptake by shoots (SUpt) and roots (RUpt) and the total plant uptake (TUpt) during the 1st experimental period as well as the relationship between extractable Cd by DTPA-TEA and the amount of Cd added to substrateFig. 3. The relationship between the Cd applied to the moderately acid substrate and the Cd uptake by shoots (SUpt) and roots (RUpt) and the total plant uptake (TUpt) during the 2nd experimental period as well as the relationship between extractable Cd by DTPA-TEA and the amount of Cd added to substrateFig. 4. The relationship between the Cd applied to the slightly alkaline substrates and the Cd uptake by shoots (SUpt) and roots (RUpt) and the total plant uptake (TUpt) during the 2nd experimental period as well as the relationship between extractable Cd by DTPA-TEA and the amount of Cd added to substrate

Conclusions

Hyssop plants did not exhibit symptoms of toxicity by Cd application up to 5 mg Cd L⁻¹. Hyssop plant height was not affected by Cd. Cadmium uptake by aerial organs (shoots) and underground organs (roots) of hyssop increased with Cd application. Cd uptake by hyssop is higher in moderately acid than in slightly alkaline substrate.

In moderately acid substrate Cd accumulates mainly in the roots. Cd extraction by DTPA-TEA could be used to predict Cd uptake by hyssop.

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Conflict of Interest

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

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