

Response of St John's wort (*Hypericum empetrifolium*) plants to cadmium (Cd) treatment in relation to substrate acidity/alkalinity

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Abstract

The effect of cadmium (Cd) on growth and Cd accumulation in shoots and roots St John's wort (*Hypericum empetrifolium*) was studied over three months in a greenhouse. Plants were cultivated in pots containing a uniform mixture of either acid or alkaline substrate consisting of peat and perlite (1:1 v/v). The pots were arranged in a completely randomized block design within two groups (acid substrate and alkaline substrate) with four Cd treatments (0-control, 1, 2, and 5 mg Cd L⁻¹) and six replicates per treatment. Cadmium was applied as CdSO₄*8/3H₂O. The total amount of Cd applied per pot was 260 ml, corresponding to 0.26, 0.52, and 1.3 mg Cd per pot for doses 1, 2, and 5 mg L⁻¹, respectively. No visual symptoms of toxicity or nutrient deficiency, as well as no differences in plant height were observed in response to Cd application, irrespective of the growth stage or substrate. There were also no differences in height development rate between the plants grown in an acidic or alkaline substrate. Cd accumulation in shoots and roots increased with increasing concentrations of applied Cd and was higher in the acidic substrate. Thus, St John's wort plant is a Cd accumulator, especially in an acidic environment, and this in combination with its high tolerance to Cd, makes it a suitable species to remove Cd from cadmium-contaminated sites. However, for its use in the preparation of medical products, St John's wort must be grown in a Cd-free soil so as not to pose a risk to human health. Cd extraction by (DTPA-TEA) can be employed to predict Cd accumulation in this plant.

Keywords: Cd accumulation; heavy metals; medicinal plants; shoots; roots

Introduction

The cadmium concentration in soils depends on geogenic and anthropogenic factors. Generally, Cd concentrations are higher in sedimentary rocks (0.01-2.6 mg kg⁻¹) than in igneous and metamorphic rocks (0.11 to 1.0 mg kg⁻¹). Anthropogenic Cd sources include mining, atmospheric deposition of combustion emissions, and the use of Cd-containing fertilizers (Kubier *et al.*, 2019). The cadmium content of P mineral fertilizers

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depends on its concentration in the rock phosphate used for their manufacture. Because of its high mobility in the soil-plant system and its low retention by soil colloids, Cd can easily enter the food chain (Alloway, 1995). Ingested cadmium is excreted slowly and so may accumulate in the human body (biological half-life 10-35 years), particularly the kidney, ultimately resulting in kidney and bone disease (Genghi *et al.*, 2020). Hence, Cd is one of the metals for which the European Food Safety Authority (EFSA, 2012) has set limits - the maximum tolerable monthly intake of Cd being 25 $\mu\text{g kg}^{-1}$ body weight. Reducing the amount of cadmium in the environment would help reduce Cd exposure and concomitant health risks. St John's wort (*Hypericum empetrifolium*) is small evergreen shrub that is native to the Mediterranean region and commonly found at low altitudes in the southern Greek Aegean and western Turkey (Davis, 1988). St John's wort is a traditional medicinal plant with high antioxidant and antibacterial activity. Decoctions of the flowers are drunk for their diuretic activity and are applied externally for the treatment of wounds and herpes (Vokou *et al.*, 1993). Naphthodianthrones and flavonoids have been identified in crude extracts of the flowers (Kitanov, 2001) and the composition of essential oil has been determined (Petraakis *et al.*, 2005). Since Cd may enter the human food chain through medicinal plants, it is important to determine their potential toxicity and health risk. The aim of this study was to examine the effects of Cd on the growth, dry weight and Cd accumulation of St John's wort grown in acid and alkaline substrates.

Materials and Methods

Experimental conduction and design

The experiments were conducted in a greenhouse of the Agricultural University of Athens Greece, over a period of approximately five months. St John's wort plants were grown in pots (15 cm in diameter) filled 2.0 kg of a dry, uniform mixture of peat and perlite (1:1 v/v). Two types of peat were employed: one acidic and one alkaline. The organic matter and moisture contents were 90% and 50-65% by weight, respectively and the electrical conductivity of both forms of peat was 10 mS cm^{-1} . The perlite particles were 1-5 mm in diameter (Perloflor™; ISOCON S.A., Athens, Greece). The pH of the acidic substrate was 5.6 (moderately acid) and that of the alkaline substrate was 7.4 (slightly alkaline). Pots were arranged in a completely randomized block design within two groups: acid substrate and alkaline substrate with four Cd treatments (0-control, 1, 2, and 5 mg Cd L^{-1}) and six replications per treatment. All pots were lined with clear polyethylene bags. Cadmium was applied as $\text{CdSO}_4 \cdot 8/3\text{H}_2\text{O}$. The experiment for both substrates started on 1st March and ended on May 29th, 2019. Two months' seedlings of St John's wort were transplanted to each pot on March 1st, 2019. Cd application began with the addition of 20 mL of each treatment to each pot at the concentrations indicated above on the following dates: March 10th, March 16th, March 21st, March 24th, March 28th, March 31st, April 4th, April 7th, April 11th, April 14th, April 20th, April 24th. The total amount applied per pot throughout the cultivation was 260 mL, corresponding to 0.26 mg Cd for dose 1 mg L^{-1} , 0.52 mg Cd for the dose 2 mg L^{-1} , and 1.3 mg Cd for the dose 5 mg L^{-1} . The experiment was terminated on 29 May 2019. The cultivation techniques were the same in both substrates. 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. Nutrileaf-60 fertilizer (2 mg each of N, P_2O_5 , and K_2O) was applied to each pot on April 3rd and on May 5th; the Cd content of the fertilizer was negligible.

Plant analysis

Plant height was measured on 3 and 18 of April and 3 and 18 May in six plant per treatment. On 29 May, 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

were 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). Cadmium uptake by shoots and roots was calculated by multiplying the dry weight of shoots (SDW) and roots (RDW) by the Cd concentration in shoots (Cd-shoots) and Cd concentration in roots (Cd-roots), respectively, and expressed as $\mu\text{g Cd}$ per plant. The sum of the shoot and root accumulation corresponded to the total plant Cd uptake.

Substrate analysis

Samples of the 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). A certified reference material (ERM-CC141 European Reference Material) was used to check the analytical procedures.

Statistical analysis

The influence of Cd treatment on plant growth (dry weight and height), Cd accumulation, and the Cd concentration extracted by DTPA-TEA 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. The Cd content of shoots was examined by regression analysis to establish relationships between Cd concentration in the plant parts and applied Cd. The Cd extracted by DTPA-TEA was examined by regression analysis to assess the suitability of this extraction method for predicting tissue Cd concentrations.

Results and Discussion

Cadmium treatments up to 5 mg L^{-1} for six weeks did not affect plants height irrespective of the growth stage or substrate (Table 1). Similar results were reported for purple coneflower (Salta *et al.*, 2019) using the same Cd application rates (0-5 mg Cd L^{-1}). The shoot and root length of St John's wort and chamomile six weeks after planting was not affected significantly by Cd treatments up to 250 $\mu\text{mol L}^{-1}$ (Masarovicova *et al.*, 2004).

Table 1. Height of St. John's wort plants grown in acid and alkaline substrates

Cd added	1 st measurement (3 rd April)	2 nd measurement (18 st April)	3 rd measurement (3 rd May)	4 th measurement (18 th May)
mg L^{-1}	cm			
	Acid substrate			
0	29.9 (1.8)	37.13 (3.2)	41.77 (3.1)	43.03 (3.1)
1	28.0 (4.4)	35.3 (3.2)	42.6 (3.1)	43.77 (4.5)
2	28.48 (1.9)	34.12 (3.6)	38.07 (2.1)	38.77 (1.8)
5	25.95 (3.3)	31.87 (3.8)	38.15 (3.7)	39.63 (3.7)
	Alkaline substrate			
0	23.2 (4.6)	25.53 (4.4)	29.02 (3.8)	31.85 (3.8)
1	26.43 (2.5)	30.42 (3.8)	35.02 (4.9)	37.55 (5.3)
2	25.38 (1.4)	28.52 (2.5)	32.48 (3.4)	34.85 (3.6)
5	23.97 (4.0)	26.87 (4.6)	30.1 (3.6)	31.25 (3.8)

Numbers in brackets represent standard deviation of six measurements

Due to heterogeneity in plant height at the beginning of the experiment and in order to study the differences in plant height between the two substrates at different Cd applications, we calculated the height development rate using the following formula: Height Development Rate (HDR) = difference between the height of two consecutive measurements divided by the number of intervening days. No differences in HDR were detected between Cd treatments on the same substrate (Table 2) or between the two substrates at the same Cd concentration (except for one measurement in the control). Moreover, no symptoms of toxicity or nutrient deficiency were observed in any of the plants, indicating good tolerance to Cd irrespective of substrate acidity or alkalinity. No toxicity or nutrient deficiency symptoms were reported in lettuce, cucumber, radish (Moustakas *et al.*, 2001), endive and rocket (Akoumianakis *et al.*, 2008) at Cd application rates of up to 20 mg Cd kg⁻¹ or in purple coneflower (Salta *et al.*, 2019) at Cd application rates of up to 5 mg L⁻¹.

Table 2. The height development rate of St John's wort plants in acid and alkaline substrates

Cd (mg L ⁻¹)	Acid substrate			Alkaline substrate		
	HDR-1	HDR-2	HDR-3	HDR-1	HDR-2	HDR-3
	cm					
0	0.482	0.397	0.083	0.156	0.232	0.189
1	0.433	0.596	0.079	0.266	0.307	0.169
2	0.339	0.388	0.050	0.209	0.264	0.158
5	0.394	0.419	0.099	0.193	0.216	0.077

HDR: Height development rate, HDR-1: Height development rate between 1st and 2nd measurement, HDR-2: Height development rate between 2nd and 3rd measurement, HDR-3: Height development rate between 3rd and 4th measurement.

Acidic substrate

A significant difference in shoot dry weight (SDW) was observed between the control and the Cd treatments, but not between the different Cd concentrations (1, 2, and 5 mg Cd L⁻¹). Root dry weight (RDW) increased at a Cd concentration of 5 mg L⁻¹. The cadmium content of shoots (Cd-shoots) and roots (Cd-roots) increased with increasing Cd concentration, as did shoot (SU_{pt}), root (RU_{pt}) and total (TU_{pt}) Cd uptake (Table 3). A significant linear correlation existed between Cd applied to the medium and Cd extracted by DTPA-TEA(Cd-soil), expressed by the equation: Cd-soil = 1.91x(Cd-added) - 0.19 (r= 0.98, p<0.001).

Table 3. Effect of Cd on shoot dry weight (SDW), root dry weight (RDW), Cd concentration in shoots (Cd-shoots), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SU_{pt}), Cd uptake by roots (RU_{pt}), and total Cd uptake (TU_{pt}) by plants of St. John's wort cultivated in the acidic substrate

Cd added	SDW	RDW	Cd-shoots	Cd-roots	SU _{pt}	RU _{pt}	TU _{pt}
mg L ⁻¹	mg plant ⁻¹ (DW)		µg Cd g ⁻¹ (DW)		µg plant ⁻¹ (DW)		
0	1.89 a	0.28 a	6.33 a	15.54 a	15.54 a	11.23 a	4.35 a
1	2.35 b	0.28 a	8.33 b	19.04 b	15.54 a	11.23 a	4.35 a
2	2.53 b	0.32 a	10 c	23.37 c	20.05 ab	19.55 b	5.33 ab
5	2.88 b	0.34 b	11.50 c	30.02 d	23.37 bc	25.43 b	6.66 b
F=	6.28	4.81	15.5	23.41	27.39 c	33.12 c	10.86 c

Column means followed by a different letter are significantly different according to Duncan's multiple range test at p≤0.05

Alkaline substrate

No significant differences were detected in SDW with increasing Cd, while RDW increased in the 5 mg L⁻¹ treatment. Cadmium concentrations in shoots and roots increased with increasing Cd application doses. Consequently, shoots, roots, and total Cd uptake by St John's wort plants increased (Table 4). A significant linear correlation existed between Cd applied in the medium and Cd extracted by DTPA-TEA (Cd-soil), expressed by the equation: Cd-soil = 1.66x(Cd-added) - 0.22 (r= 0.99, p<0.001).

Table 4. Effect of Cd on shoot dry weight (SDW), root dry weight (RDW), Cd concentration in shoots (Cd-shoots), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), and total Cd uptake (TUpt) by plants of St. John's wort cultivated in the alkaline substrate

Alkaline substrate							
Cd added	SDW	RDW	Cd-shoots	Cd-roots	SUpt	RUpt	TUpt
mg L ⁻¹	mg plant ⁻¹ (DW)		µg Cd g ⁻¹ (DW)		µg plant ⁻¹ (DW)		
0	1.12	0.27 a	2.00 a	7.49 a	2.29 a	2.16 a	4.45 a
1	1.06	0.26 a	3.83 b	14.50 b	3.99 b	3.88 a	7.86 b
2	1.11	0.3 ab	5.18 c	12.63 b	5.76 c	4.66 a	10.42 b
5	1.15	0.35 b	6.67 d	21.36 c	7.66 d	8.25 b	15.91 c
F=		4.76	56.50	53.96	3.52	7.514	23.01

Column means followed by a different letter are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Statistically significant differences were observed between SDW and RDW in all Cd treatments and substrates (Table 5) and could possibly be explained by the higher sensitivity of young roots to Cd stress (Macarovicova *et al.*, 2004). Statistically significant differences between acidic and alkaline substrates existed in all the data examined, except for RDW and Cd-soil (Table 5). The higher Cd concentration in plants grown in acidic substrate may be due to the higher bioavailability of Cd in an acid environment. Soil pH is the most important factor affecting Cd bioavailability, which increases linearly with decreasing pH (Kirkham, 2006; Kim *et al.*, 2009). Rieuwerts *et al.* (1998) reported that Cd absorption by plants became significant at pH 5-6.5. These results agree with those reported by Moustakas *et al.* (2001) for lettuce, radish and cucumber, and by Akoumianakis *et al.* (2008) for endive and rocket.

Table 5. Comparison of the effects of Cd on shoot dry weight (SDW), root dry weight (RDW), Cd concentration in shoots (Cd-shoots), Cd concentration in roots (Cd-roots), Cd uptake by shoots (SUpt), Cd uptake by roots (RUpt), total Cd uptake (TUpt) by plants of St John's wort plants grown in the acidic and alkaline substrate and on Cd-soil

Cd added	Substrate	SDW	RDW	Cd-shoots	Cd-roots	SUpt	RUpt	TUpt	Cd-soil
mg L ⁻¹		g		µg g ⁻¹ (DW)		µg plant ⁻¹			µg g ⁻¹ l
0	acidic	1.9 a (a)	0.3 (b)	6.3 a (a)	15.5 a (b)	11.9 a (a)	4.3 a (b)	16.3 a	0.02
	alkaline	1.1 b (a)	0.3 (b)	2.0 b (a)	7.5 b (b)	2.2 b (a)	2.0 b (a)	4.3 b	0.02
1	acidic	2.4 a (a)	0.3 (b)	8.3 a (a)	19.0 a (b)	19.6 a (a)	5.3 a (b)	24.9 a	1.5
	alkaline	1.1 b (a)	0.3 (b)	3.8 b (a)	14.5 b (b)	4.1 b (a)	3.9 b (a)	8.0 b	1.3
2	acidic	2.5 a (a)	0.3 (b)	10.0 a (a)	23.4 a (b)	25.4 a (a)	6.7 a (b)	32.1 a	3.2
	alkaline	1.1 b (a)	0.3 (b)	5.2 b (a)	12.6 b (b)	5.8 b (a)	4.0 b (a)	9.8 b	2.9
5	acidic	2.9 a (a)	0.4 (b)	11.5 a (a)	30.0 a (b)	33.1 a (a)	10.9 a (b)	44.0 a	9.5
	alkaline	1.1 b (a)	0.3 (b)	6.7 b (a)	21.4 b (b)	7.7 b (a)	7.3 b (a)	15.0 b	8.2

Column means for the same treatment in different substrates followed by a different letter without parenthesis are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Means for the SDW and RDW, Cd-shoots and Cd-roots as well as SUpt and RUpt followed by different letter in parenthesis in the same row are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Shoot, root, and total Cd uptake by St John's wort plants grown in acidic and alkaline substrate increased with increasing Cd application (Tables 3, 4). More than 70% of the total Cd taken up by the plants grown in the acid substrate was accumulated in the shoots. In alkaline substrate Cd taken up by the plants was equally distributed in shoots and roots (Figure 1). This finding in combination with the plants' high Cd tolerance (plant height and dry weight unaffected) indicates that St John's wort can be used not only in conventional phytotherapy but also in the rehabilitation and recovery of Cd contaminated sites, as suggested by Macarovicova *et al.* (2004). In the controls, Cd-shoots and Cd-roots were 6.3 and 15.5 µg g⁻¹ (DW), respectively

for plants grown in the acidic substrate were 2.0 and 7.5 $\mu\text{g g}^{-1}$ (DW), respectively for plants grown in the alkaline substrate (Table 5). These values, which were much higher (especially in the acid substrate) than those for Cd extracted by DTPA-TEA, are particularly noteworthy since they indicate a significant accumulation of Cd by the plant parts independent of the substrate pH. Akoumianakis *et al.* (2008), Bingham (1983), and Davis (1984) classified rocket, endive, lettuce, spinach, turnip grass, celery, and cabbage as Cd accumulator. Using linear regression analysis, we found a significant relationship between the amount of Cd that was extracted by DTPA-TEA and Cd accumulation in the shoots and roots of St John's wort, independent of the pH of substrate (Table 6, 7). Hence, this extractant could be effectively used to predict Cd content in St John's wort plant parts. Moustakas *et al.* (2001), Akoumianakis *et al.* (2008) reported that Cd extraction by DTPA-TEA could be effectively used to predict Cd uptake by lettuce, radish, cucumber, endive and rocket plants.

Table 6. Regression summary for dependent variable Cd accumulation in shoots and roots of St. John's wort plants grown in acidic substrate and Cd extracted by DTPA-TEA

Acidic substrate (shoots)						
N=24	Regression Summary for Dependent Variable: SU _{pt} , R= 0.749, R ² = 0.56, Adjusted R ² = 0.54, F (1,22) = 28.11, p<.0001, Std. Error of estimate: 6.48					
	b*	Std.Err.	b	Std.Err.	t (22)	p-value
Intercept			15.52	1.867	8.31	0.000
Cd extracted by (DTPA-TEA)	0.749	0.141	1.92	0.362	5.30	0.000
Acidic substrate (roots)						
N=24	Regression Summary for Dependent Variable: RU _{pt} , R= 0.863, R ² = 0.745, Adjusted R ² = 0.734, F (1,22) = 64.42, p<.0000, Std. Error of estimate: 1.49					
	b*	Std.Err.	b	Std.Err.	t (22)	p-value
Intercept			4.357	0.431	10.10	0.000
Cd extracted by (DTPA-TEA)	0.863	0.108	0.671	0.0836	8.024	0.000

Table 7. Regression summary for dependent variable Cd accumulation in shoots and roots of St. John's wort plants grown in alkaline substrate and Cd extracted by DTPA-TEA

Alkaline substrate (shoots)						
N=24	Regression Summary for Dependent Variable: SU _{pt} , R= 0.89, R ² = 0.792, Adjusted R ² = 0.783, F (1,22) = 83.83, p<.0000, Std. Error of estimate: 0.993					
	b*	Std.Err.	b	Std.Err.	t (22)	p-value
Intercept			2.49	0.284	8.755	0.000
Cd extracted by (DTPA-TEA)	0.89	0.097	0.587	0.064	9.156	0.000
Alkaline substrate (roots)						
N=24	Regression Summary for Dependent Variable: RU _{pt} , R= 0.861, R ² = 0.741, Adjusted R ² = 0.729, F (1,22) = 62.92, p<.0000, Std. Error of estimate: 1.152					
	b*	Std.Err.	b	Std.Err.	t (22)	p-value
Intercept			3.10	0.330	9.39	0.000
Cd extracted by (DTPA-TEA)	0.861	1.109	0.59	0.074	7.93	0.000

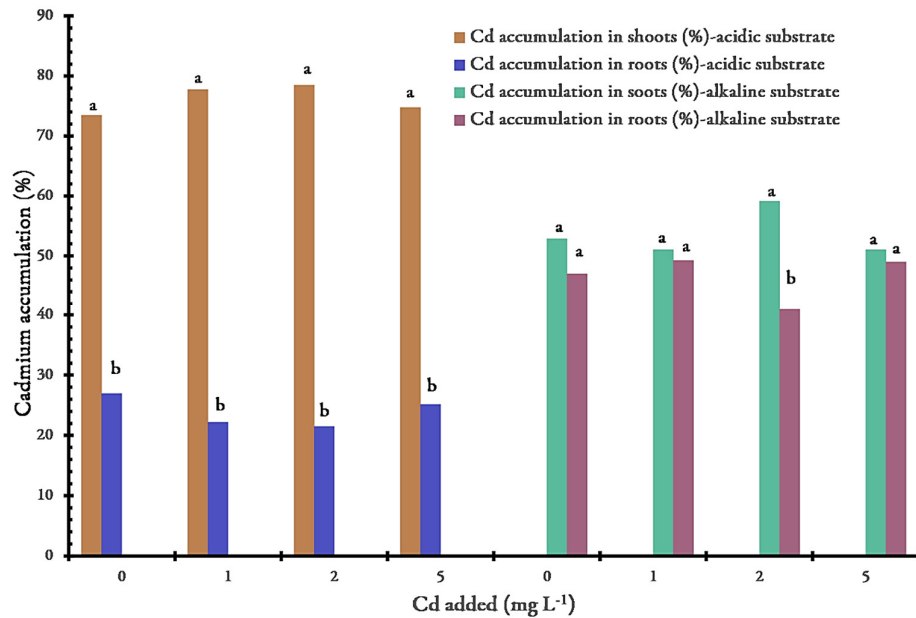


Figure 1. Effects of Cd on Cd content accumulated in shoots and roots of St John's wort plants in the acidic and alkaline substrate
(Different letters above the bars with the same color indicate a significant difference between treatments, according to Duncan's multiple range test, at $p \leq 0.05$, in the acid and alkaline substrate)

The Cd content in aerial (SU_{pt}) and underground (RU_{pt}) plant parts increased with increasing Cd application (Tables 3, 4). The average ratio of SDW/RDW and Cd-roots/Cd-shoots was 7 and 2.4, respectively for plants grown in an acidic environment, and 3.8 and 3.3 respectively for plants grown in an alkaline environment. The Cd accumulation capacity was greater in roots than in shoots in both substrates. In the acidic substrate Cd accumulation by dry weight was greater in shoots than in roots, resulting in a higher Cd concentration in the shoots. In the alkaline substrate, however, the equal dry matter and Cd accumulation capacities of the shoots and roots resulted an approximately equal Cd concentrations in these plant parts. Thus, Cd accumulation in St John's wort depended on both the Cd accumulation capacity and the biomass of the plant.

Finally, because the Cd concentrations in the shoots and roots were invariably higher than the maximum permissible Cd concentration of 0.3 mg kg^{-1} (WHO, 1999), it is clear that to be used safely in the preparation of medicinal products, St John's wort should not be grown in Cd contaminated (especially acidic) soils.

Conclusions

Plant growth (dry weight and height) of St John's wort was not affected by Cd application up to 5 mg L^{-1} no symptoms of toxicity or nutrient deficiency were observed. Our results confirmed that this plant species is a Cd accumulator and supported previous findings that plants of the *Hypericaceae* family are Cd accumulators. Cd accumulation in St John's-wort plants is much higher in an acidic than in an alkaline environment and occurs mainly in the aerial plant parts (shoots). St John's wort may therefore be used to remove Cd from contaminated soils. However, caution is required when this plant is to be used in the preparation of medicinal products, since it must be grown in a Cd-free soil. Cd extraction by DTPA-TEA could be used effectively to predict Cd concentration in shoots and roots of St John's-wort plants.

Authors' Contributions

Conceptualization, supervision, validation, writing original draft, writing review and editing: AAI, NM, PB; Methodology, data curation analysis: PB and AS, review and editing: NM, PB, AAI. All authors read and approved the final manuscript.

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Conflict of Interests

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

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