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EFFECT OF LEAD AND CADMIUM IN IRRIGATION WATER AND FOLIAR APPLIED MALIC ACID ON VEGETATIVE GROWTH, FLOWERING AND CHEMICAL COMPOSITION OF *SALVIA SPLENDENS* PLANTS (B) EFFECT OF CADMIUM

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ABSTRACT: The present study was carried-out at Antoniadis Botanical Garden Research Branch, Horticultural Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons of 2018 and 2019. The aim of this study was to evaluate the effects of irrigation water contaminated with cadmium on *Salvia splendens* plants grown in a sandy soil, for possibilities of using malic acid spray treatments to overcome the effects of lead pollution. Seedlings of *Salvia splendens* were individually planted in plastic pots (20 cm diameter) filled with 5 kg of sandy soil. Four concentrations of cadmium 0,100, 200 and 300 mg/l were applied in the irrigation water. The plants were sprayed with malic acid at concentrations of 0, 250 and 500 mg/l at monthly internals in both seasons. The results showed that for vegetative and flowering growth parameters, there was no significant interaction between cadmium concentrations and foliar spray by malic acid, while significant reduction were observed in all parameters after irrigation with contaminated water with cadmium and significant increase in vegetative and flowering growth parameters were observed after 250 mg/l malic acid application. For chlorophyll and carbohydrate content the significantly highest value was obtained from plants irrigated with tap water and sprayed with 500 mg/l malic acid while the significantly highest cadmium content in leaves, stem and roots was obtained due to the treatment of 300 mg/l without application of malic acid.

Key words: *Salvia splendens***,** cadmium, malic acid, polluted irrigation water, phytoremediation.

INTRODUCTION

The genus *Salvia* (Fam. Lamiaceae), consists of approximately 900 species including shrubs, herbaceous perennials and annuals (Karousou *et al*., 2000). *Salvia splendens* plants were originated in Brazil. Many studies have focused only on sage's secondary metabolites which has aromatic and medicinal properties (Topcu, 2006). *Salvia splendens* is considered as one of the most commonly observed ornamental plants in the landscape. Wu *et al.* (2001) mentioned that landscape and floricultural plants, are damaged due to low or moderate salinity in the irrigation water (Clebsch and Barner, 2003).

Phytoremediation has become an effective and affordable technological solution used to extract or remove toxic metals from contaminated soil. Phytoremediation is the use of plants to clean up a contamination from soils, sediments and water. This technology is friendly environment and potentially cost effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants (Choruk *et al.,* 2006). Plants need trace amount of heavy metal but their excessive availability may cause plant toxicity (Sharma *et al.*, 2006). Phytotoxic concentration of heavy metals referred to in the literature does not always specify the levels (Wua *et al.*, 2010). Cadmium is a toxic metal that has an environmental concern (Mahler *et al.*, 1981). There are many sources of environmental pollution by cadmium, including fuel combustion, industrial sludges, phosphate fertilizers, and mine tailings (Unhalekhana and Kositanont, 2008). Cadmium can be absorbed by human body through respiration and consumption, and cadmium then accumulates in the liver and kidney, causing acute and chronic symptoms such as nausea, abdominal pain, diarrhea, kidney dysfunction, and osteomalacia (Simmons *et al.*, 2005).

Malic acid is an organic dicarboxylic acid formed in the metabolic cycles in the cells of plants, and plays a key role in the energy-producing Krebs cycle. Therefore, it can influence the cut flower's vase life (da Silva, 2003). Malic acid is the organic acid which could be metabolized by reaction of malic enzyme in plant mitochondria by reaction of malic enzyme and this is considered as ability limited to plant (Day and Hanson, 1977).

In this study *Salvia splendens* was selected due to its characteristics as non-edible plant, therefore the objective of this study is to evaluate the effects of irrigation water contaminated with cadmium on Salvia plants and to investigate the response of these plants to malic acid spray treatments to decrease the harmful effect of cadmium pollution in the irrigation water, determine the potential of Salvia in removing cadmium from the soil and contaminated water irrigated and to investigate on the ability of Salvia in removing cadmium.

MATERIALS AND METHODS

The present study was carried-out at Antoniadis Research Branch, Horticultural Research Institute, A.R.C., Alexandria, Egypt during the two successive seasons of 2018 and 2019. The aim of this study was to evaluate the effects of irrigation water contaminated with cadmium on *Salvia splendens* plants grown in a sandy soil and the possibility of using malic acid spray treatments to overcome the effects of cadmium pollution. The seedlings used to establish the experiment were brought from a commercial nursery in Alexandria as local produced seedlings of *Salvia splendens*.

On 3rd of January, 2018 and 2019 in the first and second seasons, respectively, homogeneous seedlings of *Salvia splendens* (14-16 cm height and 9-11 leaves per plant) were individually planted in plastic pots (20 cm diameter) filled with 5 kg of a sandy soil. The chemical constituents of the soil were determined as described by Page *et al.* (1982) in Table (1).

On 15th of January in both seasons, the contaminated water irrigation treatments were started. Four concentrations (0, 100, 200 and 300 mg/l) of cadmium acetate [$Cd(CH_3COO)_2H_2O$] were applied. The plants were irrigated three times per week, one irrigation level was used to keep the soil moisture at the field capacity at 100% of the sandy soil. The reduction in the moisture level was determined by using Moisture Tester Model KS-DI (Gypsum Block) during the growing season. At the end of the experiment the total amount of irrigation water for each pot was calculated and presented in Tables (2), every plant received

Table 1. Chemical and physical analyses of the used sandy soil for the two successive seasons 2018 and 2019.

Season	pH	EC (dSm^{-1})	Soluble cations (meq/l)				Soluble anions (meq/l)			Soil particles		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_2^{--}	Sand (%)	Silt (%)	Clay (%)
2018	7.94	1.57	3.4	3.4	6.5	1.2	3.6	6.7	2.4	94.0	4.0	2.0
2019	7.91	1.52	3.2	3.0	6.3	1.1	3.3	6.5	2.2	92.0	5.0	3.0

Table 2. Total amount of the water used for each plant (l/pot) in each treatment during the two growing seasons 2018 and 2019.

Field capacity (%)	Irrigation water (l) at months of first and second seasons						
	January	February	March	April	May	June	Total
100	4.80	9.75	11.25	12.00	13.20	14.40	65.4

about 65.4 liters per pot of contaminated water. The field capacity of the sandy soil was determined by the pressure Cooker method at 1/3 atm., as described by Israelsen and Hansen (1962). In both seasons, the plants were received monthly spraying from 1st March till 1st May in both seasons. The plants were also sprayed with malic acid at concentrations of 0, 250 and 500 mg/l. Control plants were sprayed with tap water. On 30th of June in both seasons, the plants were harvested.

In the two seasons, all plants received NPK chemical fertilization using soluble fertilizer (Agrico 20-20-20) at the rate of 1 g/pot. Fertilization was repeated every 15 days throughout the growing season (from the 15th of January till 15th of June). In addition, weeds were manually removed upon emergence.

Data recorded:

Vegetative growth parameters:

Plant height (cm), number of leaves per plant, leaves dry weight per plant (g), middle leaves area (cm^2) according to Koller (1972), branch number per plant, branches dry weight (g), root length (cm) and root dry weight (g).

Flowering growth parameters:

Number of florets per spike, spike length (cm) and florets dry weight (g).

Chemical analysis determination:

- Chlorophyll content was determined as a SPAD unites from the fresh leaves of plants for the different treatments under the experiment at the middle of the season using Minolta (chlorophyll meter) SPAD 502 according to Yadava (1986).
- Carbohydrates percentage in the leaves was determined according to Dubios *et al.* (1956).
- Proline content (mg/g) in the dried leaves was determined according to Bates *et al.* (1973).
- Determination of cadmium content in plant samples was determined as follows: Plant samples were divided into leaves, stem and roots, oven dried at 70 °C for 72 hrs in an oven until reached a constant weight. The dried plant samples were ground to a powder. The oven dried samples were digested for extraction of cadmium, using the method described by Piper (1947) and the concentration of heavy metal was assured using an atomic absorption spectrophotometer.
- Available cadmium in soil samples was extracted by DTPA solution according to Lindsay and Norvell (1978) and determined by Inductively Coupled Plasma Spectrometry.
- Transfer factor (TF) is given by the relation: the ratio of the concentration of metal in the shoots to the concentration of metal in the soil (Chen *et al.*, 2004). The transfer factor is a value used in evaluation studies on the impact of routine or accidental releases of pollutant into the environment.

The layout of the experimental design was split plot design with three replicates. Each replicate contained three plants. The main plot was the contaminated water irrigation levels, while the sub plots were the concentrations of malic acid. Data were subjected to analysis of variance (ANOVA) using the SAS program, SAS Institute (SAS Institute, 2002). The means of the individual factors and their interactions were compared by L.S.D test at 5% level of probability according to Snedecor and Cochran (1989).

RESULTS

Leaves parameters:

Data presented in Table (3) show that, in both seasons, irrigation with polluted water with cadmium decreased the leaves parameters, compared to plants irrigated with tap water (control). Plants irrigated with tap water had the highest mean values of number of leaves per plant (143.22 and 153.22), leaves dry weight (4.90 and 5.30 g) and leaves area (1170.22 and 1289.60 cm^2) in the first and second season, respectively. Moreover, raising the cadmium concentration caused steady significant reductions in leaf parameters, with the highest concentration (300 mg/l) giving significantly the smallest plants with mean number of leaves per plant (117.33 and 124.33), leaves dry weight (3.96 and 4.29 g) and leaves area (743.44 and 807.75 cm^2) in the first and second season, respectively, than those which received the other cadmium concentration.

Leaf parameters were also significantly affected by spraying the plants with malic acid. In both seasons, leaf parameters were gradually increased when the malic acid concentration was raised from 0 mg/l (control) to 500 mg/l. Accordingly, it can be seen from data presented in Table (3) that plants sprayed with 250 mg/l malic acid were significantly highest with mean number of leaves per plant (135.58 and 142.45), leaves dry weight (4.69 and 4.97 g) and leaves area (1082.92 and 1144.67 cm^2) in the first and second seasons, respectively, more than plants sprayed with any other malic acid concentration.

Regarding the interaction between the effects of irrigation with contaminated cadmium water and malic acid treatments on

Table 3. Means of number of leaves per plant, leaves dry weight (g) and leaves area (cm^2) of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm^2)	
Cd (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	140.00	150.66	4.77	5.20	993.67	1091.74
0	250	149.33	159.50	5.25	5.51	1439.17	1548.71
	500	144.83	154.50	4.97	5.35	1258.50	1388.53
Mean (Cd)		143.22	153.22	4.90	5.30	1170.22	1289.60
	0	116.33	135.50	3.97	4.68	755.53	906.04
100	250	136.33	144.50	4.71	5.13	1079.51	1130.59
	500	128.33	139.33	4.33	4.85	908.63	1010.35
Mean (Cd)		126.99	139.77	4.33	4.88	914.55	1015.66
	0	118.33	125.33	4.00	4.30	730.97	791.71
200	250	131.33	136.33	4.54	4.74	941.93	981.03
	500	125.33	134.00	4.24	4.60	849.21	930.97
Mean (Cd)		124.99	131.88	4.26	4.54	840.70	901.23
	0	107.33	116.50	3.61	3.99	625.29	694.72
300	250	125.33	129.50	4.29	4.51	871.07	918.37
	500	119.33	127.00	4.00	4.38	733.97	810.18
Mean (Cd)		117.33	124.33	3.96	4.29	743.44	807.75
	0	120.49	131.99	4.08	4.54	776.36	871.05
Mean (MA)	250	135.58	142.45	4.69	4.97	1082.92	1144.67
	500	129.45	138.70	4.38	4.79	937.57	1035.00
	Cd	5.10	4.47	0.17	014	48.05	36.72
L.S.D. at 0.05	MA	2.64	2.78	0.08	0.09	23.14	17.39
	Cd × MA	3.03	3.19	0.09	0.10	26.59	19.98

the leaves parameters, the highest values were obtained for plants irrigated with tap water and sprayed with malic acid at 250 mg/l with mean number of leaves per plant (149.33 and 159.50), leaves dry weight (5.25 and 5.51 g) and leaves area (1439.17 and 1548.71 cm^2) in the first and second seasons, respectively. On the other hand, the shortest plants with mean number of leaves per plant (107.33 and 116.50), leaves dry weight (3.61 and 3.99 g) and leaves area (625.29 and 694.72 cm^2) in the first and second seasons, respectively, were resulted from the plants irrigated with the highest cadmium concentration (300 mg/l) without malic acid treatment. It can also be seen from data presented in Table (3) that in many cases, spraying plants with malic acid reduced the undesirable effects of polluted water with cadmium.

Stem parameters:

Data presented in Table (4) showed that irrigation with cadmium polluted water decreased stem parameter, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the thickest stem, with mean plant height (49.32 and 53.20 cm), number of branches per plant (5.27 and 5.27) and branch dry weight (6.47 and 7.04 g) in the first and second seasons, respectively. Increasing the cadmium concentration in water irrigation caused a steady reduction in stem parameters. These reduction in stem parameters were significant compared to the control, even at the highest cadmium concentration (300 mg/l), which gave plant height (39.96 and 43.25 cm), number of branches per plant (3.77 and 4.16) and branches dry weight (5.48 and 5.95 g) in the first and second seasons, respectively,

Table 4. Means of plant height (cm), branches number per plant and branches dry weight (g) of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Plant height (cm)		Branches number per plant		Branches dry weight (g)	
Cd (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	46.82	50.98	5.00	5.00	6.35	7.01
0	250	51.40	55.15	5.66	5.66	6.65	7.13
	500	49.74	53.48	5.16	5.16	6.41	6.99
Mean (Cd)		49.32	53.20	5.27	5.27	6.47	7.04
	0	39.57	46.90	3.83	4.50	5.36	5.77
100	250	48.07	52.32	4.33	5.33	5.95	6.47
	500	43.32	48.56	4.00	5.00	5.76	6.11
Mean (Cd)		43.65	49.26	4.05	4.94	5.69	6.11
	0	39.90	43.06	3.83	4.00	4.91	5.36
200	250	46.49	48.49	4.33	4.50	6.12	6.47
	500	42.49	46.15	4.00	4.50	5.95	6.29
Mean (Cd)		43.65	49.26	4.05	4.94	5.69	6.11
	0	35.99	39.81	3.16	4.00	5.36	5.81
300	250	43.90	46.15	4.16	4.33	5.64	6.08
	500	39.99	43.81	4.00	4.16	5.46	5.98
Mean (Cd)		39.96	43.25	3.77	4.16	5.48	5.95
	0	40.57	45.18	3.95	4.37	5.49	5.98
Mean (MA)	250	47.46	50.52	4.62	4.95	6.09	6.53
	500	43.88	48.00	4.29	4.70	5.89	6.34
	Cd	1.93	1.42	0.20	0.24	0.20	0.21
L.S.D. at 0.05	MA	0.86	0.91	0.22	0.22	0.07	0.07
	Cd × MA	0.99	1.04	0.26	0.25	0.08	0.08

compared with the other concentrations.

In contrast to the effect of cadmium treatments, malic acid treatments improved stem parameters of plants, compared to the control. Moreover, plants sprayed with 250 mg/l malic acid had significantly mean plant height (47.46 and 50.52 cm), number of branches per plant (4.62 and 4.95) and branch dry weight (6.09 and 6.53 g) in the first and second seasons, respectively, compared with the other concentrations.

Regarding the interaction between the irrigation with cadmium polluted water and spraying with malic acid on stem parameters, the recorded results for the two seasons are presented in Table (4). They showed that significant differences were detected between the values obtained from plants receiving the different treatment combinations. The highest values of plant height (51.40 and 55.15 cm), number of branches per plant (5.66 and 5.66) and branches dry weight (6.65 and 7.13 g) in the first and second seasons, respectively, were obtained from plants irrigated with tap water and sprayed with malic acid at 250 mg/l. On the other hand, the least values of plant height (35.99 and 39.81 cm), number of branches per plant (3.16 and 4.00) and branches dry weight (5.36 and 5.81 g) in the first and second seasons, respectively, were obtained from plants irrigated with the highest cadmium concentration (300 mg/l) and sprayed with malic acid at 0 mg/l. In many cases, spraying plants with malic acid reduced the adverse effects of cadmium polluted water.

Root parameters:

Data presented in Table (5) showed that irrigation with cadmium polluted water decreased root parameters, compared to that of plants irrigated with tap water (control). In

Table 5. Means of root length (cm) and root dry weight (g) of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Root length (cm)		Root dry weight (g)	
Cd (mg/l)	MA (mg/l)	2018	2019	2018	2019
	0	44.64	49.60	4.70	5.24
0	250	47.70	51.57	5.02	5.39
	500	45.72	50.19	4.80	5.34
Mean (Cd)		46.02	50.45	4.84	5.32
	0	34.37	39.33	4.09	4.46
100	250	41.67	46.65	4.48	4.86
	500	38.51	43.38	4.27	4.70
Mean (Cd)		38.18	43.12	4.28	4.67
	0	34.71	35.78	3.85	4.20
200	250	39.80	46.83	4.47	4.76
	500	37.53	42.88	4.37	4.65
Mean (Cd)		37.34	41.83	4.23	4.53
	0	30.32	40.12	3.88	4.19
300	250	37.94	43.39	4.23	4.58
	500	34.76	40.32	4.09	4.44
Mean (Cd)		34.34	41.27	4.06	4.40
	0	36.01	41.20	4.13	4.52
Mean (MA)	250	41.77	47.11	4.55	4.89
	500	39.13	44.19	4.38	4.78
	Cd	2.11	1.61	0.15	0.15
L.S.D. at 0.05	MA	0.97	0.77	0.03	0.04
	Cd × MA	1.11	0.62	0.04	0.05

both seasons, plants irrigated with tap water had the highest values of root length (46.02 and 50.45 cm) and root dry weight (4.84 and 5.32 g) in the first and second seasons, respectively. Increasing cadmium concentration in irrigation water caused a steady reduction in root parameters. These reductions were significant as compared to the control even at the highest cadmium concentration (300 mg/l), which gave root length (34.34 and 41.27 cm) and root dry weight (4.06 and 4.40 g) in the first and second seasons, respectively.

In contrast to the effect of cadmium treatments, malic acid treatments improved root parameters, compared to the control. Moreover, plants sprayed with 250 mg/l malic acid had significantly longer root (41.77 and 47.11 cm) and heavier root dry weight (4.55 and 4.89 g) in the first and second seasons, respectively, compared to those of control plants, or plants sprayed with any other malic acid concentration.

Regarding the interaction between the irrigation with cadmium polluted water and malic acid treatments on root parameters, the results in Table (5) showed significant differences between the values obtained for plants receiving the different treatment combinations. The highest values of root length (47.70 and 51.57 cm) and root dry weight (5.02 and 5.39 g) in the first and second seasons, respectively, were obtained from the plants irrigated with tap water and sprayed with malic acid at 250 mg/l. On the other hand, the lowest values of root length (30.32 and 40.12 cm) and root dry weight (3.88 and 4.19 g) in the first and second seasons, respectively, were obtained from the plants irrigated with the highest cadmium concentration (300 mg/l) and sprayed with malic acid at 0 mg/l treatment. It is shown from Table (5) that in many cases, spraying the plants with malic acid reduced the harmful effects of cadmium polluted water.

Flowering parameters:

Data presented in Table (6) show the effect of polluted water with cadmium on flowering of *Salvia splendens* plants. In both seasons, plants irrigated with tap water had the highest number of florets per spike (33.21 and 36.38), spike length (15.21 and 16.76 cm) and florets dry weight (10.88 and 12.08 g) in the first and second seasons, respectively. Accordingly, the lowest number of florets per spike (28.39 and 31.02), spike length (12.79 and 14.09 cm) and florets dry weight (9.13 and 10.13 g) in the first and second seasons, respectively, were obtained from the plants irrigated with the highest cadmium concentration (300 mg/l).

Concerning the effect of malic acid treatments on the flowering, data recorded in Table (6) show that the treatment of malic acid 250 mg/l caused a significant increases in number of florets per spike (32.27 and 34.91), spike length (16.18 and 17.40 cm) and florets dry weight (10.95 and 11.96 g) in the first and second seasons, respectively, compared to that of the control plants in number of florets per spike (27.70 and 30.20), spike length (12.07 and 13.38 cm) and florets dry weight (8.73 and 9.68 g) in the two seasons, respectively.

Data presented in Table (6) showed significant interaction in both seasons between irrigation with polluted cadmium water and malic acid treatments on flowering parameters. Combination between irrigation using tap water and spraying the plants with malic acid at 250 mg/l gave the highest number of florets per spike (34.90 and 38.18), spike length (17.71 and 19.02 cm) and florets dry weight (11.76 and 13.01 g) in the first and second seasons, respectively. On the other hand, the lowest number of florets per spike (27.04 and 29.54), spike length (10.98 and 12.21 cm) and florets dry weight (8.54 and 9.49 g) in the first and second seasons, respectively, were obtained from plants irrigated with the highest cadmium concentration (300 mg/l) and non-sprayed with malic acid.

Table 6. Means of florets number per spike, spike length (cm) and florets dry weight (g) of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Florets number per spike		Spike length (cm)		Florets dry weight (g)	
Cd (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	32.21	34.90	12.88	14.60	10.26	11.28
0	250	34.90	38.18	17.71	19.02	11.76	13.01
	500	32.52	36.07	15.06	16.66	10.62	11.96
Mean (Cd)		33.21	36.38	15.21	16.76	10.88	12.08
	0	27.07	29.30	12.22	13.31	8.56	9.40
100	250	30.30	33.23	15.80	17.22	10.78	11.89
	500	29.22	31.20	13.28	14.24	9.37	10.12
Mean (Cd)		28.86	31.24	13.76	14.92	9.57	10.47
	0	24.49	27.06	12.23	13.43	7.58	8.55
200	250	33.29	35.22	16.28	17.22	11.16	11.88
	500	30.31	32.22	13.80	14.74	9.78	10.51
Mean (Cd)		29.36	31.50	14.10	15.13	9.50	10.31
	0	27.04	29.54	10.98	12.21	8.54	9.49
300	250	30.59	33.04	14.96	16.16	10.13	11.06
	500	27.54	30.50	12.45	13.90	8.73	9.86
Mean (Cd)		28.39	31.02	12.79	14.09	9.13	10.13
	0	27.70	30.20	12.07	13.38	8.73	9.68
Mean (MA)	250	32.27	34.91	16.18	17.40	10.95	11.96
	500	29.89	32.49	13.64	14.88	9.62	10.61
	Cd	1.12	1.24	0.58	0.61	0.42	0.47
L.S.D. at 0.05	MA	0.43	0.45	0.18	0.21	0.16	0.17
	Cd × MA	0.49	0.51	0.19	0.24	0.17	0.17

Chemical composition:

Leaf chemical analysis:

The results presented in Table (7) show that the highest content of chlorophyll and carbohydrates (%) were obtained from plant irrigated with tap water as had the highest chlorophyll content (29.57 and 29.67 SPAD) and carbohydrate (%) (12.78 and 12.77 %) in the first and second seasons, respectively. However, the maximum proline content (2.49 and 2.47 mg/g) was obtained from plants irrigated with cadmium polluted water at (300 mg/l). Raising the cadmium concentration in irrigation water resulted in steady significant reductions in the chlorophyll content and carbohydrate (%), which reached lowest values of chlorophyll content (27.00 and 27.21 SPAD) and carbohydrate (%) (11.75 and 111.95%) in the first and second seasons, respectively, in plants which received the highest cadmium concentration (300 mg/l), while, the least proline content (1.33 and 1.37 mg/g) was obtained from plants irrigated with tap water.

The results of leaf chemical analysis presented in Table (7) show also that the tested malic acid treatments had clear effects on the chlorophyll content and carbohydrate (%). The recorded mean values were ranged for chlorophyll content (29.43 and 29.50 SPAD) and carbohydrate (%) (12.74 and 12.93 %) in the first and second seasons, respectively, in plants sprayed with 500 mg/l malic acid, while, the highest proline contents (2.00 and 2.03 mg/g) were obtained from plants non sprayed with malic acid (tap water). Chlorophyll content (26.97 and 27.21 SPAD) and carbohydrate (%) (11.53 and 11.67 %) in the first and second seasons, respectively, were obtained from plants sprayed with 0 mg/l malic acid, while, the lowest proline contents (1.95 and 1.97 mg/g) were obtained from plants sprayed with 500 mg/l malic acid.

Table 7. Means of chlorophyll content (SPAD), carbohydrate content (%) and proline content (mg/g d.w.) of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Chlorophyll content (SPAD)		Carbohydrate content (%)		Proline content (mg/g d.w.)	
Cd (mg/l)	**MA (mg/l)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
	0	28.73	28.94	12.06	12.08	1.39	1.42
0	**250**	29.57	29.61	12.83	12.76	1.36	1.39
	500	30.41	30.47	13.46	13.48	1.25	1.30
Mean (Cd)		29.57	29.67	12.78	12.77	1.33	1.37
	0	26.42	26.81	11.12	11.30	1.84	1.90
100	**250**	29.31	29.52	12.76	13.02	1.82	1.84
	500	30.20	30.25	13.05	13.21	1.80	1.81
Mean (Cd)		28.64	28.86	12.31	12.51	1.82	1.85
	0	26.78	26.84	11.59	11.78	2.28	2.34
200	**250**	28.51	28.56	12.08	12.15	2.21	2.28
	500	29.08	29.04	12.46	12.59	2.25	2.30
Mean (Cd)		28.12	28.14	12.04	12.17	2.24	2.30
	0	25.96	26.33	11.38	11.54	2.51	2.49
300	**250**	27.00	27.06	11.86	11.88	2.48	2.45
	500	28.04	28.26	12.01	12.45	2.50	2.47
Mean (Cd)		27.00	27.21	11.75	11.95	2.49	2.47
	0	26.97	27.23	11.53	11.67	2.00	2.03
Mean (MA)	**250**	28.59	28.68	12.38	12.45	1.96	1.99
	500	29.43	29.50	12.74	12.93	1.95	1.97
	Cd	0.36	0.41	0.51	0.17	0.05	0.05
L.S.D. at 0.05	**MA**	0.43	0.41	0.27	0.22	0.01	0.02
	Cd × MA	0.50	0.47	0.31	0.26	0.02	0.02

Regarding the interaction between the irrigation with cadmium polluted water and malic acid treatments, data presented in Table (7) showed that the highest chlorophyll contents of (30.41 and 30.47 SPAD) and carbohydrate (%) (13.46 and 13.48 %) in the first and second seasons, respectively, were found in leaves of plants irrigated with tap water and sprayed with malic acid at 500 mg/l, while, the highest proline contents (2.51 and 2.49 mg/g) were resulted for plants irrigated with the highest cadmium concentration (300 mg/l) without malic acid treatment.

Cadmium content in the leaves, stem and root (mg/l):

Data resulted from plant parts chemical analysis presented in Table (8) showed that, the cadmium content in the plant parts was steadily raised with raising the cadmium concentration in the water irrigation. The lowest mean cadmium content in leaves (0.118 and 0.126 mg/l), cadmium content in stem (0.044 and 0.049 mg/l) and cadmium content in root (0.011 and 0.011 mg/l) in the first and second seasons, respectively, were found in the control plants, whereas the highest contents in leaves (0.297 and 0.307 mg/l), cadmium content in stem (0.168 and 0.184 mg/l) and cadmium content in root (0.069 and 0.079 mg/l) in the first and second seasons, respectively, was found in plants irrigated with water containing the highest cadmium concentration (300 mg/l).

Concerning the effect of malic acid treatments on cadmium content in plant parts, data recorded in the two seasons Table (8) show that malic acid treatment at 500 mg/l caused significant decreases in the cadmium content in leaves giving mean values (0.150 and 0.160 mg/l), cadmium content in stem (0.068 and 0.074 mg/l) and cadmium content in root (0.022 and 0.023 mg/l) in the first and second seasons,

Table 8. Means of cadmium content of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Cadmium content in leaves (mg/l)		Cadmium content in stem (mg/l)		Cadmium content in roots (mg/l)	
Cd (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	0.136	0.155	0.056	0.068	0.013	0.015
0	250	0.124	0.126	0.047	0.048	0.012	0.011
	500	0.095	0.099	0.030	0.032	0.009	0.007
Mean (Cd)		0.118	0.126	0.044	0.049	0.011	0.011
	0	0.250	0.251	0.151	0.150	0.078	0.080
100	250	0.232	0.227	0.122	0.121	0.044	0.048
	500	0.139	0.152	0.062	0.070	0.021	0.023
Mean (Cd)		0.207	0.210	0.111	0.113	0.047	0.050
	0	0.352	0.367	0.207	0.217	0.089	0.095
200	250	0.268	0.276	0.149	0.156	0.059	0.064
	500	0.194	0.210	0.093	0.102	0.024	0.027
Mean (Cd)		0.271	0.284	0.149	0.158	0.057	0.062
	0	0.427	0.433	0.252	0.285	0.104	0.129
300	250	0.292	0.309	0.165	0.175	0.068	0.073
	500	0.174	0.181	0.087	0.092	0.035	0.036
Mean (Cd)		0.297	0.307	0.168	0.184	0.069	0.079
	0	0.291	0.301	0.166	0.180	0.071	0.079
Mean (MA)	250	0.229	0.234	0.120	0.125	0.045	0.049
	500	0.150	0.160	0.068	0.074	0.022	0.023
	Cd	0.004	0.006	0.002	0.003	0.002	0.003
L.S.D. at 0.05	MA	0.002	0.003	0.001	0.002	0.001	0.001
	Cd × MA	0.0008	0.0044	0.0006	0.0009	0.0005	0.0004

respectively, compared to those of control plants that had the highest cadmium content in leaves (0.291and 0.301 mg/l), cadmium content in stem (0.166 and 0.180 mg/l) and cadmium content in root (0.071 and 0.079 mg/l) in the first and second seasons, respectively.

Concerning the interaction between irrigation with cadmium polluted water and malic acid treatments on cadmium content in plant parts (leaves stem and root), the presented results in Table (8) show that the lowest cadmium content in leaves (0.095 and 0.099 mg/l), cadmium content in stem (0.030 and 0.032 mg/l) and cadmium content in root (0.009 and 0.007 mg/l) in the first and second seasons, respectively, were obtained plant parts irrigated with tap water and sprayed with malic acid at 500 mg/l. On the other hand, the highest cadmium contents were obtained from plant parts that treated with cadmium at (300 mg/l) and non-receiving malic acid treatment as cadmium content in leaves (0.427 and 0.433 mg/l), cadmium content in stem (0.252 and 0.285 mg/l) and cadmium content in root (0.104 and 0.129 mg/l) in the first and second seasons, respectively.

Transfer factor (TF) of cadmium:

Transfer factor (TF) indicates the efficiency of plants to transfer metals from its root to the aerial parts.

Cadmium content in soil samples (mg/l):

Data presented in Table (9) showed that the lowest average of cadmium content was observed in soil cultured by untreated plants, while the highest content was observed in soil after the treatment with (300 mg/l) cadmium.

Transfer factor to plant parts:

From data presented in Table (10), it can be stated that the transfer factor in the parts of *Salvia splendens* plants was increased steadily with raising the cadmium

Table 9. Average values of cadmium content in soil samples as influenced by cadmium concentrations in water irrigation and foliar application of malic acid on *Salvia splendens* in the two seasons of 2018 and 2019.

Treatments		Lead content in soil (mg/kg)			
Cd	MA	2018		2019	
(mg/l)	(mg/l)	Before	After	Before	After
	0	0.063	0.342	0.064	0.348
0	250	0.076	0.391	0.077	0.397
	500	0.071	0.373	0.072	0.379
	0	0.101	0.383	0.103	0.389
100	250	0.112	0.425	0.114	0.430
	500	0.107	0.405	0.108	0.411
	0	0.109	0.413	0.111	0.419
200	250	0.120	0.454	0.122	0.460
	500	0.114	0.430	0.115	0.435
	0	0.116	0.436	0.117	0.443
300	250	0.127	0.478	0.128	0.483
	500	0.123	0.464	0.124	0.469

Table 10. Means of transfer factor to leaves, stem and roots of *Salvia splendens* plants as influenced by cadmium (Cd), malic acid (MA) and their combinations (Cd × MA) in the two seasons of 2018 and 2019.

Treatments		Transfer factor to leaves (TFL)		Transfer factor to stem (TFS)		Transfer factor to roots (TFR)	
Cd (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	0.397	0.445	0.163	0.195	0.038	0.043
0	250	0.317	0.317	0.120	0.120	0.030	0.027
	500	0.254	0.261	0.080	0.084	0.024	0.018
Mean (Cd)		0.322	0.341	0.121	0.133	0.030	0.029
	0	0.652	0.645	0.394	0.385	0.203	0.205
100	250	0.545	0.527	0.287	0.281	0.103	0.111
	500	0.343	0.369	0.153	0.170	0.051	0.055
Mean (Cd)		0.513	0.513	0.278	0.278	0.119	0.123
	0	0.852	0.875	0.501	0.517	0.215	0.226
200	250	0.590	0.600	0.328	0.339	0.129	0.139
	500	0.451	0.482	0.216	0.234	0.055	0.062
Mean (Cd)		0.631	0.652	0.348	0.363	0.133	0.142
	0	0.979	0.977	0.577	0.643	0.238	0.291
300	250	0.610	0.639	0.345	0.362	0.142	0.151
	500	0.375	0.385	0.187	0.196	0.075	0.076
Mean (Cd)		0.654	0.667	0.369	0.400	0.151	0.172
	0	0.720	0.735	0.408	0.435	0.173	0.191
Mean (MA)	250	0.515	0.520	0.270	0.275	0.101	0.107
	500	0.355	0.374	0.159	0.171	0.051	0.052

concentration in the water irrigation. Accordingly, the lowest transfer factor in the leaves (0.322 and 0.341 mg/l), transfer factor in stem (0.121 and 0.133 mg/l) and transfer factor in root (0.030 and 0.029 mg/l) in the first and second seasons, respectively, was found in plants irrigated with water containing 0 mg/l cadmium (control), whereas the highest transfer factor in leaves (0.654 and 0.667 mg/l), transfer factor in stem (0.369 and 0.400 mg/l) and transfer factor in root (0.151 and 0.172 mg/l) in the first and second seasons, respectively, was found in plants irrigated with water which contained (300 mg/l) cadmium.

The results presented in Table (10) show also that the transfer factor in the plant parts was reduced steadily with raising malic acid concentration. Accordingly, the highest transfer factor in leaves (0.720 and 0.735 mg/l), transfer factor in stem (0.408 and 0.435 mg/l) and transfer factor in root (0.173 and 0.191 mg/l) in the first and second seasons, respectively, were recorded in the parts of control plants, whereas plants sprayed with malic acid concentration at 500 mg/l had the lowest transfer factor in leaves (0.355 and 0.374 mg/l), transfer factor in stem (0.159 and 0.171 mg/l) and transfer factor in root (0.051 and 0.052 mg/l) in the first and second seasons, respectively.

Regarding the interaction between irrigation with polluted water and malic acid concentrations on transfer factor in the plant parts, presented data in Table (10) show that the highest transfer factor in leaves (0.979 and 0.977 mg/l), transfer factor in stem (0.577 and 0.643 mg/l) and transfer factor in root (0.238 and 0.291 mg/l) in the first and second seasons, respectively, were obtained from plants irrigated with cadmium water at 300 mg/l and sprayed with tap water, while the lowest transfer factor in leaves (0.254 and 0.261 mg/l), transfer factor in stem (0.080 and 0.084 mg/l) and transfer factor in root (0.024 and 0.018 mg/l) in the first and second seasons, respectively, were recorded in plants treated with 0 mg/l cadmium and sprayed with (500 mg/l) malic acid.

DISCUSSION

This study revealed that at high heavy-metal concentrations, the plant height was significantly reduced, and the biomass was decreased. The root growth was more sensitive than other parameters, as roots rapidly absorbed water and had higher accumulation of heavy metal elements. The results presented by this study were in agreement with earlier reports on other plants, such as aquatic plant *Wolffia arrhiza* (Piotrowska *et al.*, 2010), barley *Hordeum vulgare* (Tiryakioglu *et al*., 2006) and *Typha angustifolia* (Bah *et al.*, 2011) and *Jatropha curcas* (El-Shanhorey and Emam, 2016). Cadmium has been shown to cause many morphological, physiological and biochemical changes in plants, such as growth inhibition, and water imbalance (Benavides *et al*., 2005).

The physiological responses, photosynthetic function, can be ascribed to the different effects of physico-chemical properties of heavy metals on the integrity and function of the photochemical apparatus of plant seedling fronds, as well as the impact on the chlorophyll concentrations in the leaves. The photosynthesis rate, CO_2 assimilation rate, and stomatal conductance in response to cadmium heavy metal have been well documented (Chen *et al*., 2012). The maintenance of an intercellular CO_2 concentration is concomitant with the leaf CO_2 assimilation rate and reflected photosynthesis function of seedling in the different heavy metal-spiked soils. The chlorophyll and carotenoid contents played a central role in the energy manifestation of green plant. Any significant alteration of their contents possibly resulted in a marked effect on the entire metabolism of the plant (Piotrowska *et al*., 2010). In this study, cadmium resulted in a significant reduction in the chlorophyll contents, possibly due to the inhibition of chlorophyll biosynthesis or a breakdown of pigments and their precursors (Agrawal and Mishra, 2009). As cadmium might replace the central Mg from chlorophyll molecules and thereby reduce the photosynthetic light-harvesting ability of plant (Agrawal and Mishra, 2009).

In the present study, cadmium exposure significantly decreased photosynthetic pigments concentration in comparison with control plants. Reduction in photosynthetic pigments by excess cadmium has been reported by some authors i.e., Abdel-Latif (2008). Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. In addition, cadmium-induced decrement in chlorophyll content was attributed to impairment in the supply of magnesium and iron to the leaves (Greger and Ogren, 1991). Alternatively, cadmium may substitute magnesium in chlorophyll molecules (Kupper *et al*., 1998).

Proline, an amino acid is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Saradhi *et al*., 1993). Plants have been shown proline accumulation under environmental stress (Ahmad and Jhon, 2005; Ahmad *et al*., 2006; Ahmad *et al*., 2008). It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles. Increase in proline content may be either due to *de novo* synthesis or decreased degredation or both (Kasai *et al*., 1998). Proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to Cd^{2+} toxicity has been demonstrated by Dhir *et al*. (2004). Similar results of increasing proline content by Cd^{2+} was also reported by Zengin and Munzuroglu, (2006) in sunflower.

Concerning treatments, at a preliminary stage, one should note that the transfer factor (TF) of most treatments is lower than one for cadmium; which means that the physiological need of the plant for this element is rather limited. Translocation from roots to shoots via a number of physiological processes, including metal unloading into root xylem cells, long-distance carrying from the xylem to the shoots and metal reabsorption, by leaf mesophyll cells, from the xylem stream. Once the cadmium has been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream (Blaylock and Huang, 2000).

The decrease in cadmium uptake helped to overcome the negative effects of cadmium on the previous studied parameters. These results are in agreement with those mentioned by Talebi *et al*., (2014) on Gazania plants and (Jaafari and Hadavi, 2012) on *Ocimum basilicum* L. In addition, we can use *Senecio cineraria* plants as cadmium phytoremediation and if we want to use *Senecio cineraria* as an ornamental plant and the irrigation water is contaminated with cadmium we can spray the plants to overcome the negative effect of cadmium (El-Shanhorey and EL-Sayed, 2017), (El-Shanhorey and Emam, 2020) on *Lantana camara* and (El-Shanhorey and Ahmed, 2020) on *Polianthes tuberosa.*

In malic acid, while there were no significant differences between applied concentrations, but in the root, fresh weight, and root dry weight, we see that only the higher concentration is considered significantly different from the control. When comparing with earlier reports, here we observe more similarity between responses of selected traits to organic acids. In some traits, the lower concentration gave good results that give us the idea of possible distinct patterns of response to concentration of applied organic acids. Therefore, we suggest testing both lower and higher concentrations of these organic acids to reach a better understanding in this regard (Talebi *et al.,* 2014). Malic acid spray significantly increased chlorophyll content. Chlorophyll content was highest in plants treated with malic acid alone with SPAD reading compared with control. All factor-levels containing malic acid had significantly higher chlorophyll content compared with control (Darandeh and Hadavi, 2012)

CONCLUSION

Phytoremediation is a new cleanup concept that involves the use of plants to clean or stabilize contaminated environments. Phytoremediation of heavy metals is the most effective plant-based method to remove pollutants from contaminated lands as a result of irrigation with water contaminated with heavy metals. This green technology can be applied to remediate the polluted soils without creating any destructive effect of soil structure. Phytoremediation of contaminated water and soil using non-edible plant like *Salvia splendens* offers an environmental friendly and cost-effective method for remediating the polluted soil with heavy metals. *Salvia splendens* has noticeable potential to absorb toxic heavy metals. This method has been able to use wastewater contaminated with heavy metals in the irrigation of ornamental plants while maintaining soil fertility.

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تأثير الرصاص والكادميوم فى ماء الرى والرش بحمض الماليك على النمو الخضرى والإزهار والتركيب الكيماوى لنباتات السلفيا المستديمة (ب) تأثير الكادميوم

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قسم بحوث الحدائق النباتية، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر

أجريت هذه الدراسة في فرع بحوث الحدائق النباتية بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر خلال الموسمين المتتاليين ٢٠١٨ و٢٠١٩. وكان الهدف من هذه الدراسة تقييم آثار مياه الري الملوثة بالكادميوم على نباتات السلفيا المزروعة في تربة رملية، كذلك استخدام الرش بحمض الماليك للتغلب على الآثار الضارة للكادميوم. زرعت شتلات السلفيا بشكل فردي في أوعية بلاستيكية (قطرها ٢٠ سم) مملوءة ٥ كجم من التربة الرملية. وكانت معاملات مياه الري الملوثة بأربعة تركيزات من الكادميوم وهي صفر، ١٠٠، ٢٠٠ , ٣٠٠ جزء في المليون. تمت معاملة النباتات أيضا بحامض الماليك بثلاث تركيزات هي صفر، ٢٥٠ و٥٠٠ جزء في المليون عن طريق الرش شهريا

في كلا الموسمين. أظهرت النتائج أنه بتقييم معايير النمو الخضري وجد أن هناك اختلافاً كبيراً في التفاعل بين تركيزات الكادميوم ورش النباتات بحامض الماليك. لوحظ إنخفاض كبير في كافة معاملات الري بالماء الملوث بالكادميوم وكذلك لوحظت زيادات كبيرة فى معدلات النموالخضري بعد الرش بتركيز ٢٥٠ جزء في المليون حمض الماليك. تم الحصول على أعلى قيمه من محتوى الكلوروفيل والكربوهيدرات فى النباتات المروية بماء الصنبور والرش بتركيز ٥٠٠ جزء في المليون حامض الماليك في حين أن أعلى تركيز للكادميوم كان في الأوراق والساق والجذور كنتيجة للرى بماء ملوث بتركيز ٣٠٠ جزء في المليون دون الرش بحمض الماليك.