Scientific J. Flowers & Ornamental Plants
www.ssfop.com/journal
ISSN: 2356-7864
doi: 10.21608/sjfop.2020.114571

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

N.A. El-Shanhorey and A.A. Barakat
Botanical Gardens Research Department, Horticultural Research Institute, ARC, Alexandria, Egypt

Scientific J. Flowers & Ornamental Plants, 7(3):321-335 (2020).

Received:
19/7/2020
Accepted:
20/8/2020

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 lead 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 planted individually in plastic pots (20 cm diameter) filled with 5 kg of sandy soil. Four concentrations of lead 0, 100, 200 and 300 mg/l were applied in the irrigation water. The plants were treated with malic acid at concentrations of 0, 250 and 500 mg/l by monthly spraying in both seasons. The results showed that for vegetative and flowering growth parameters, there was no significant interaction between lead concentrations and foliar spray by malic acid, while a significant reduction was observed in all parameters after irrigation with contaminated water with lead and a significant increase in vegetative and flowering growth parameters was observed after 500 mg/l malic acid application. For chlorophyll and carbohydrate contents, the highest significant values were obtained from plants irrigated with tap water and sprayed with 500 mg/l malic acid while the highest significant lead content in leaves, stem and roots was obtained in the treatment of 300 mg/l without application of malic acid.

Key words: *Salvia splendens***,** lead, 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).

Plants need trace amount of heavy metal but their excessive availability may cause plant toxicity (Sharma *et al*., 2006). Phytotoxic concentration of the heavy metals referred in the literature does not always specify the levels (Wua *et al*., 2010). Lead is a toxic heavy metal that has an environmental concern (Mahler *et al*., 1981). There are many sources of environmental lead pollution, including fuel combustion, industrial sludges, phosphate fertilizers, and mine tailings (Unhalekhana and Kositanont, 2008).

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, they 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 lead on salvia plants and to investigate the response of these plants to malic acid spray treatments to decrease the harmful effect of lead pollution in the irrigation water, determine the potential of salvia in removing lead from the soil and contaminated irrigated water and to investigate on the ability of salvia in removing lead.

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 lead on *Salvia splendens* plants grown in a sandy soil and the possibility of using malic acid spray treatments to overcome the effects of lead pollution. The seedlings used to establish the experiment were brought from a commercial nursery in Alexandria as local produced seedlings of *Salvia splendens*.

On the 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 Tables (1 and 2).

On the 15th of January in both seasons, the contaminated irrigation water treatments were started. Four concentrations of lead acetate [Pb $(CH_3COO)_2$] 0, 100, 200 and 300 mg/l were applied. The plants were irrigated three times per week, one irrigation level was used to keep the soil moisture at the field capacity of the sandy soil at 100%. The reduction in the moisture level was determined by using Moisture Tester Model KS-DI (Gypsum Block) during growing season. At the end of the experiment the total amount of irrigation water for each pot was calculated and presented in Table (3), every plant received about 65.4 liters per pot of contaminated water. The field capacity of the

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. Chemical analyses of the tap water for the season 2018.

Season	pH	EC (dSm^{-1})	Soluble anions (meq/l)		
			HCO_3^{-}	Cl^{-}	SO_2^{--}
2018	7.82	3.45	3.6	21.0	14.8

Table 3. Total amount of the water used for each plant (l/pot) in each treatment during the growing two 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

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 the 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 the 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), branches 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:

- Total 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 leaves was determined according to Bates *et al.* (1973).
- Determination of lead 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 readied a constant weight. The dried plant samples were ground to a powder. The oven dried samples were digested for extraction of lead, using the method described by Piper (1947) and the concentration of heavy metal was assured using an atomic absorption spectrophotometer.
- Available lead 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 plots were the contaminated irrigation water 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

Vegetative growth parameters:

Leaves parameters:

Data presented in Table (4) show that, in both seasons, irrigation water polluted with lead decreased the leaves parameters of *Salvia splendens* plants, compared to plants irrigated with tap water (control). Plants irrigated with tap water had the highest mean values of number of leaves per plant (148.94 and 159.27), leaves dry weight (5.46 and 5.85 g) and leaves area (1220.30 and 1332.85 cm^2) in the first and second season, respectively. Moreover, raising the lead concentration caused steady significant reductions in leaves parameters, with the highest concentration (300 mg/l) giving significantly the smallest plants with mean number of leaves per plant (120.33 and 127.33), leaves dry weight (4.40 and 4.72 g) and leaves area (733.30 and 797.61 cm^2) in the first and second season, respectively, than those received the other lead concentration.

Leaves parameters were also significantly affected by spraying the plants with malic acid. In both seasons, leaves parameters were gradually increased when the malic acid concentration was raised from 0 mg/l (control) to 500 mg/l. Accordingly, it

Table 4. Means of number of leaves per plant, leaves dry weight (g) and leaves area (cm^2) of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb × MA) in the two seasons of 2018 and 2019.

Treatments		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm^2)	
Pb (mg/l)	**MA (mg/l)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
	0	145.50	156.50	5.34	5.77	983.53	1081.60
0	**250**	148.33	158.33	5.44	5.82	1248.36	1378.39
	500	153.00	163.00	5.60	5.97	1429.03	1538.56
Mean (Pb)		148.94	159.27	5.46	5.85	1220.30	1332.85
	0	120.00	139.00	4.44	5.15	745.39	895.90
100	**250**	132.00	143.00	4.80	5.31	898.49	1000.21
	500	140.00	148.00	5.08	5.49	1069.37	1120.45
Mean (Pb)		130.66	143.33	4.77	5.31	904.41	1005.52
	0	122.00	129.00	4.46	4.77	720.83	781.57
200	**250**	129.00	138.00	4.71	5.07	839.07	920.83
	500	135.00	138.00	4.91	5.11	931.79	970.89
Mean (Pb)		128.66	135.00	4.69	4.98	830.56	891.09
	0	111.00	120.00	4.08	4.45	615.15	684.58
300	**250**	123.00	131.00	4.47	4.85	723.83	800.04
	500	127.00	131.00	4.66	4.88	860.93	908.23
Mean (Pb)		120.33	127.33	4.40	4.72	733.30	797.61
	0	124.62	136.12	4.58	5.03	766.22	860.91
Mean (MA)	**250**	133.08	142.58	4.85	5.26	927.43	1024.86
	500	138.75	145.00	5.06	5.36	1072.78	1134.53
	Pb	5.27	4.54	0.18	0.14	48.05	36.72
L.S.D. at 0.05	**MA**	2.64	2.78	0.08	0.09	23.14	17.39
	Pb × MA	2.14	3.19	0.09	0.10	26.59	19.98

can be seen from the data in Table (4) that *Salvia splendens* plants sprayed with 500 mg/l malic acid were significantly highest with mean number of leaves per plant (138.75 and 145.00), leaves dry weight (5.06 and 5.36 g) and leaves area (1072.78 and 1134.53 cm^2) in the first and second seasons, respectively, than plants sprayed with any other malic acid concentration.

Regarding the interaction between the effects of irrigation with contaminated lead water and malic acid treatments on the leaves parameters, the highest values were obtained in the plants irrigated with tap water and sprayed with malic acid at 500 mg/l with mean number of leaves per plant (153 and 163), leaves dry weight (5.60 and 5.97 g) and leaves area (1429.03 and 1538.56 cm^2) in the first and second seasons, respectively). On the other hand, the shortest plants with mean number of leaves per plant (111 and 120), leaves dry weight (4.08 and 4.45 g) and leaves area (615.15 and 684.58 cm^2) in the first and second seasons, respectively, were resulted from the plants irrigated with the highest lead concentration (300 mg/l) without malic acid treatment. It can also be seen from the data presented in Table (4) that in many cases, spraying the plants with malic acid reduced the undesirable effect of contaminated water with lead.

Stem parameters:

Data presented in Table (5) showed that irrigation with lead polluted water decreased stem parameter, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had

Table 5. Means of plant height (cm), branches number per plant and branches dry weight (g) of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb × MA) in the two seasons of 2018 and 2019.

Treatments		Plant height (cm)		Branches number per plant		Branches dry weight (g)	
Pb (mg/l)	**MA (mg/l)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
	0	49.95	54.12	6.00	6.00	6.02	6.59
0	**250**	50.87	54.62	6.16	6.16	5.96	6.61
	500	52.53	56.28	6.33	6.50	6.26	6.74
Mean (Pb)		51.11	55.00	6.16	6.22	6.08	6.64
	0	40.70	48.03	4.83	5.50	4.97	5.38
100	**250**	44.45	49.70	5.00	6.00	5.37	5.72
	500	47.20	51.45	5.33	6.33	5.55	6.08
Mean (Pb)		44.11	49.72	5.05	5.94	5.29	5.72
	0	41.03	44.20	4.83	5.00	4.52	4.97
200	**250**	43.62	47.28	5.00	5.50	5.56	5.90
	500	45.62	47.62	5.16	5.50	5.73	6.08
Mean (Pb)		43.42	46.36	4.99	5.33	5.27	5.65
	0	37.12	40.95	4.16	5.00	4.97	5.42
300	**250**	41.12	44.95	5.16	5.33	5.06	5.59
	500	43.03	45.28	5.00	5.16	5.25	5.69
Mean (Pb)		40.42	43.72	4.77	5.16	5.09	5.56
	0	42.20	46.82	4.95	5.37	5.12	5.59
Mean (MA)	**250**	45.01	49.13	5.33	5.74	5.48	5.95
	500	47.09	50.15	5.45	5.87	5.69	6.14
	Pb	1.93	1.42	0.12	0.31	0.20	0.21
L.S.D. at 0.05	**MA**	0.86	0.91	0.23	0.23	0.07	0.07
	Pb × MA	0.99	1.04	0.27	0.27	0.08	0.08

the thickest stem, with mean plant height (51.11 and 55.00 cm), number of branches per plant (6.16 and 6.22) and branch dry weight (6.08 and 6.64 g) in the first and second seasons, respectively. Increasing the lead concentration in irrigation water caused a steady reduction in stem parameters. This reduction in stem parameters was significant compared to the control, even at the highest lead concentration (300 mg/l), which gave plant height (40.42 and 43.72 cm), number of branches per plant (4.77 and 5.16) and branches dry weight (5.09 and 5.56 g) in the first and second seasons, respectively, compared with the other concentrations.

In contrast to the effect of lead treatments, malic acid treatments improved stem parameters of *Salvia splendens* plants, compared to the control. Moreover, plants sprayed with 500 mg/l malic acid had significantly mean plant height (47.09 and 50.15 cm), number of branches per plant (5.41 and 5.87) and branch dry weight (5.63 and 6.11 g) in the first and second seasons, respectively, compared with the other concentrations.

Regarding the interaction between the irrigation with lead polluted water and spraying with malic acid on stem parameters, the recorded results for the two seasons are presented in Table (5) showed that significant differences were detected between the values obtained from plants receiving the different treatment combinations. The highest values of plant height (52.53 and 56.28 cm), number of branches per plant (6.33 and 6.50) and branches dry weight (6.26 and 6.74 g) in the first and second seasons, respectively, were obtained from plants irrigated with tap water and sprayed with malic acid at 500 mg/l. On the other hand, the least values of plant height (37.12 and 40.95 cm), number of branches per plant (4.16 and 5.00) and branches dry weight (4.97 and 5.42 g) in the first and second seasons, respectively, were obtained for plants irrigated by the highest lead concentration (300 mg/l) and sprayed with malic acid at 0 mg/l treatment. In many cases, spraying the plants with malic acid reduced the adverse effect of lead polluted water.

Root parameters:

Data presented in Table (6) showed that irrigation with lead polluted water decreased root parameters, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the highest values of root length (45.55 and 49.99 cm) and root dry weight (4.64 and 5.07 g) in the first and second seasons, respectively. Increasing lead concentration in irrigation water caused a steady reduction in root parameter. These reductions were significant as compared to the control even at the highest lead concentration (300 mg/l), which gave root length (33.54 and 40.48 cm) and root dry weight (3.79 and 4.13 g) in the first and second seasons, respectively.

In contrast to the effect of lead treatments, malic acid treatments improved root parameters, compared to the control. Moreover, plants sprayed with 500 mg/l malic acid had significantly longer root (41.06 and 46.15 cm) and heavier root dry weight (4.24 and 4.59 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 lead polluted water and malic acid treatments on root parameters of *Salvia splendens* plants, the results in Table (6) showed significant differences between the values obtained for plants receiving the different treatment combinations. The highest values of root length (47.23 and 51.11 cm) and root dry weight (4.79 and 5.16 g) in the first and second seasons, respectively, were obtained for the plants irrigated with tap water and sprayed with malic acid at 500 mg/l. On the other hand, the lowest values of root length (29.86 and 39.66 cm) and root dry weight (3.65 and 3.96 g) in the first and second seasons, respectively, were obtained for the plants irrigated with the highest lead concentration

Table 6. Means of root length (cm) and root dry weight (g) of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb × MA) in the two seasons of 2018 and 2019.

Treatments		Root length (cm)		Root dry weight (g)	
Pb (mg/l)	**MA (mg/l)**	**2018**	**2019**	**2018**	**2019**
	0	44.18	49.14	4.57	5.01
0	**250**	45.26	49.73	4.57	5.04
	500	47.23	51.11	4.79	5.16
Mean (Pb)		45.55	49.99	4.64	5.07
	0	33.91	38.87	3.85	4.23
100	**250**	38.05	42.91	4.04	4.47
	500	41.21	45.19	4.15	4.54
Mean (Pb)		37.72	42.32	4.01	4.41
	0	34.25	35.32	3.62	3.97
200	**250**	37.07	42.42	4.13	4.42
	500	39.34	46.37	4.14	4.43
Mean (Pb)		36.88	41.37	3.96	4.27
	0	29.86	39.66	3.65	3.96
300	**250**	34.30	39.86	3.85	4.21
	500	36.48	41.93	3.89	4.24
Mean (Pb)		33.54	40.48	3.79	4.13
	0	35.55	40.74	3.92	4.29
Mean (MA)	**250**	38.67	43.73	4.14	4.53
	500	41.06	46.15	4.24	4.59
	Pb	2.11	1.61	0.15	0.15
L.S.D. at 0.05	**MA**	0.97	0.77	0.03	0.05
	Pb × MA	1.11	0.88	0.03	0.05

(300 mg/l) and sprayed with malic acid at 0 mg/l treatment. It is shown from Table (6) that in many cases, spraying the plants with malic acid reduced the harmful effect of lead polluted water.

Flowering growth parameters:

Data presented in Table (7) show the effect of contaminated water with lead on flowering of *Salvia splendens* plants. In both seasons, plants irrigated with tap water had the highest number of florets per spike (31.86 and 35.03), spike length (14.98 and 16.53 cm) and florets dry weight (11.34 and 12.54 g) in the first and second seasons, respectively.

Accordingly, the lowest number of florets per spike (26.38 and 29.02), spike length (11.90 and 13.19 cm) and florets dry weight (9.26 and 10.26 g) in the first and second seasons, respectively, were obtained from the plants irrigated with the highest lead concentration (300 mg/l).

Concerning the effect of malic acid treatments on the flowering growth, the data recorded in Table (7) show that the treatment of malic acid 500 mg/l caused a significant increase in the number of florets per spike (29.75 and 32.25), spike length (13.95 and 15.17 cm) and florets dry weight (10.54 and 11.48 g) in the first and second seasons, respectively, compared to that of the control plants in the number of florets per spike (26.53 and 29.15), spike length (12.39 and 13.65 cm) and florets dry weight (9.31 and 10.31 g) in the two seasons, respectively.

Data presented in Table (7) showed significant interaction in both seasons

Table 7. Means of florets number per spike, spike length (cm) and florets dry weight (g) of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb × MA) in the two seasons of 2018 and 2019.

Treatments		Florets number per spike		Spike length (cm)		Florets dry weight (g)	
Pb (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	31.56	34.72	14.83	16.37	11.22	12.42
0	250	31.17	34.84	14.65	16.43	11.08	12.46
	500	32.87	35.55	15.48	16.79	11.72	12.74
Mean (Pb)		31.86	35.03	14.98	16.53	11.34	12.54
	0	25.73	27.96	11.99	13.08	9.01	9.86
100	250	27.88	29.86	13.05	14.01	9.83	10.58
	500	28.96	31.88	13.57	14.99	10.24	11.35
Mean (Pb)		27.52	29.90	12.87	14.02	9.69	10.59
	0	23.15	25.72	12.00	13.20	8.04	9.013
200	250	28.96	30.88	13.57	14.51	10.24	10.97
	500	29.95	31.87	14.05	14.99	10.61	11.34
Mean (Pb)		27.35	29.49	13.20	14.23	9.63	10.44
	0	25.70	28.20	10.75	11.98	9.00	9.95
300	250	26.20	29.16	12.22	13.67	9.19	10.32
	500	27.25	29.70	12.73	13.93	9.59	10.52
Mean (Pb)		26.38	29.02	11.90	13.19	9.26	10.26
	0	26.53	29.15	12.39	13.65	9.31	10.31
Mean (MA)	250	28.55	31.18	13.37	14.65	10.08	11.08
	500	29.75	32.25	13.95	15.17	10.54	11.48
	Pb	1.12	1.24	0.58	0.60	0.42	0.47
L.S.D. at 0.05	MA	0.43	0.45	0.18	0.21	0.16	0.17
	Pb × MA	0.49	0.51	0.19	0.24	0.18	0.19

between the effects of irrigation with contaminated lead water and malic acid treatments on flowering parameters formed by *Salvia splendens* plants.

Combination between irrigation using tap water and spraying the plants with malic acid at 500 mg/l gave the highest number of florets per spike (32.87 and 35.55), spike length (15.48 and 16.79 cm) and florets dry weight (11.72 and 12.74 g) in the first and second seasons, respectively. On the other hand, the lowest number of florets per spike (25.70 and 28.20), spike length (10.75 and 11.98 cm) and florets dry weight (9.00 and 9.95 g) in the first and second seasons, respectively, were obtained from plants irrigated with the highest lead concentration (300 mg/l) and sprayed without malic acid.

Chemical composition:

Leaf chemical analysis:

The results presented in Table (8) show that the highest content of total chlorophyll and carbohydrates (%) were obtained in the plant irrigated with tap water had the highest total chlorophyll content (30.74 and 30.85 SPAD) and carbohydrate (%) (14.24 and 14.23 %) in the first and second seasons, respectively. However, the maximum proline content (2.61 and 2.58 mg/g) was obtained in the plants irrigated with lead polluted water at (300 mg/l). Raising the lead concentration in irrigation water resulted in steady significant reductions in the total chlorophyll content and carbohydrate (%), which reached its lowest total chlorophyll content (28.18 and 28.39 SPAD) and carbohydrate (%)

Table 8. Means of chlorophyll content (SPAD), carbohydrate content (%) and proline content (mg/g d.w.) of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb × MA) in the two seasons of 2018 and 2019.

Treatments		Chlorophyll content (SPAD)		Carbohydrate (%)		Proline content (mg/g d.w.)	
Pb (mg/l)	**MA (mg/l)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
	0	29.91	30.12	13.96	13.89	1.51	1.54
0	**250**	30.74	30.79	14.18	14.21	1.48	1.51
	500	31.59	31.65	14.59	14.61	1.37	1.42
Mean (Pb)		30.74	30.85	14.24	14.23	1.45	1.49
	0	26.60	26.99	12.25	12.43	1.96	2.02
100	**250**	31.38	31.43	14.18	14.34	1.94	1.95
	500	30.49	30.70	13.88	14.15	1.92	1.94
Mean (Pb)		29.49	29.70	13.43	13.64	1.94	1.97
	0	27.96	28.01	12.72	12.91	2.39	2.45
200	**250**	30.16	30.21	13.59	13.72	2.37	2.40
	500	29.69	29.74	13.21	13.28	2.33	2.40
Mean (Pb)		29.27	29.32	13.17	13.30	2.36	2.41
	0	27.14	27.51	12.49	12.67	2.62	2.60
300	**250**	29.22	29.44	13.14	13.58	2.61	2.58
	500	28.18	28.24	12.99	13.01	2.60	2.57
Mean (Pb)		28.18	28.39	12.87	13.08	2.61	2.58
	0	27.90	28.15	12.85	12.97	2.12	2.15
Mean (MA)	**250**	30.37	30.46	13.77	13.96	2.10	2.11
	500	29.98	30.08	13.66	13.76	2.05	2.08
	Pb	0.36	0.42	0.51	0.18	0.05	0.04
L.S.D. at 0.05	**MA**	0.43	0.41	0.27	0.22	0.02	0.02
	Pb × MA	0.48	0.46	0.31	0.24	0.02	0.02

(12.87 and 13.08 %) in the first and second seasons, respectively, in plants which received the highest lead concentration (300 mg/l), while, the least proline content (1.45 and 1.49 mg/g) were obtained for the plants irrigated with tap water.

The results of leaf chemical analysis presented in Table (8) show also that the tested malic acid treatments had clear effect on the total chlorophyll content and carbohydrate (%). The recorded mean values were ranged from total chlorophyll content (30.37 and 30.46 SPAD) and carbohydrate (%) (13.77 and 13.96 %) in the first and second seasons, respectively, in plants sprayed with 250 mg/l malic acid, while, the higher proline contents (2.12 and 2.15 mg/g) were obtained in plants sprayed without malic acid (tap water). total chlorophyll content (27.90 and 28.15 SPAD) and carbohydrate (%) (12.85 and 12.97 %) in the first and second seasons, respectively, from plants sprayed with 0 mg/l malic acid, while, the lowest proline contents (2.05 and 2.08 mg/g) were obtained from plants sprayed with 500 mg/l malic acid.

Regarding the interaction between the effects of irrigation contaminated water with lead and malic acid treatments, data presented in Table (8) showed that the highest total chlorophyll contents of (31.59 and 31.65 SPAD) and carbohydrate (%) (14.59 and 14.61 %) 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 higher proline contents (2.62 and 2.60 mg/g) were resulted for the plants irrigated by the highest lead concentration (300 mg/l) without malic acid treatment.

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

Data resulting from plant parts chemical analysis presented in Table (9) showed that, the lead content (mg/l) in the plant part of *Salvia splendens* plants was steadily raised with raising the lead concentration in the irrigation water. The lowest mean lead content in leaves (0.178 and 0.191 mg/l), lead content in stem (0.067 and 0.075 mg/l) and lead content in root (0.017 and 0.016 mg/l) in the first and second seasons, respectively, were found in the control plants, whereas the highest contents in leaves (0.446 and 0.476 mg/l), lead content in stem (0.252 and 0.276 mg/l) and lead content in root (0.103 and 0.119 mg/l) in the first and second seasons, respectively, was found in plants irrigated with water containing the highest lead concentration (300 mg/l).

Concerning the effect of malic acid treatments on lead content in plant parts, data recorded in the two seasons (Table 9) show that malic acid treatment 500 mg/l caused a significant decrease in the lead content in leaves giving mean values (0.247 and 0.263 mg/l), lead content in stem (0.117 and 0.127 mg/l) and lead content in root (0.041 and 0.043 mg/l) in the first and second seasons, respectively, compared to that of control plants that had the highest lead content in leaves (0.343and 0.359 mg/l), lead content in stem (0.188 and 0.198 mg/l) and lead content in root (0.080 and 0.085 mg/l) in the first and second seasons, respectively.

Concerning the interaction between the

Table 9. Means of chemical constituents characteristics of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb × MA) in the two seasons of 2018 and 2019.

Treatments		Lead content in leaves (mg/kg)		Lead content in stem (mg/kg)		Lead content in roots (mg/kg)	
Pb (mg/l)	**MA (mg/l)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
	0	0.204	0.235	0.084	0.102	0.019	0.023
000	**250**	0.186	0.190	0.071	0.073	0.018	0.015
	500	0.144	0.150	0.046	0.050	0.015	0.012
Mean (Pb)		0.178	0.191	0.067	0.075	0.017	0.016
	0	0.376	0.377	0.226	0.226	0.117	0.121
100	**250**	0.349	0.340	0.184	0.182	0.067	0.072
	500	0.209	0.229	0.094	0.106	0.032	0.035
Mean (Pb)		0.311	0.315	0.168	0.171	0.072	0.076
	0	0.531	0.552	0.312	0.327	0.134	0.143
200	**250**	0.402	0.415	0.223	0.234	0.090	0.098
	500	0.292	0.315	0.140	0.154	0.037	0.042
Mean (Pb)		0.408	0.427	0.225	0.238	0.087	0.094
	0	0.639	0.693	0.377	0.426	0.153	0.193
300	**250**	0.438	0.463	0.249	0.264	0.103	0.109
	500	0.261	0.272	0.131	0.138	0.053	0.055
Mean (Pb)		0.446	0.476	0.252	0.276	0.103	0.119
	0	0.343	0.359	0.188	0.198	0.080	0.085
Mean (MA)	**250**	0.345	0.355	0.182	0.191	0.069	0.076
	500	0.247	0.263	0.117	0.127	0.041	0.043
	Pb	0.006	0.009	0.004	0.005	0.003	0.003
L.S.D. at 0.05	**MA**	0.003	0.005	0.002	0.003	0.002	0.001
	Pb × MA	0.003	0.006	0.003	0.004	0.003	0.002

effects of irrigation contaminated lead water and malic acid treatments on lead content in plant parts (leaves stem and root), the presented results in Table (9) show that the lowest lead content in leaves (0.144 and 0.150 mg/l), lead content in stem (0.046 and 0.050 mg/l) and lead content in root (0.015 and 0.012 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 lead content were obtained in the plant parts that treated with lead at (300 mg/l) and receiving no malic acid treatment lead content in leaves (0.639 and 0.693 mg/l), lead content in stem (0.377 and 0.426 mg/l) and lead content in root (0.153 and 0.193 mg/l) in the first and second seasons, respectively.

Transfer factor (TF) of heavy metals:

1. Lead content in soil samples (mg/l):

Data presented in Table (10) showed that the lowest average of lead content was observed in soil cultured by untreated plants, while the highest content was observed in soil after the treatment with (300 mg/l) lead.

2. Transfer factor to plant parts:

From data presented in Table (11), it can be stated that the transfer factor in the parts of *Salvia splendens* plants was increased steadily with raising the lead concentration in the irrigation water. Accordingly, the lowest transfer factor in leaves (0.253 and 0.269 mg/l), transfer factor in stem (0.095 and 0.105 mg/l) and transfer factor in root (0.024 and 0.023 mg/l) in the first and second seasons, respectively, was found in plants irrigated with water containing 0 mg/l lead (control), whereas the highest transfer factor in leaves (0.512 and 0.539 mg/l), transfer factor in stem (0.290 and 0.313 mg/l) and transfer factor in root (0.118 and 0.135 mg/l) in the first and second seasons, respectively, was found in plants irrigated with water contained (300 mg/l) lead.

The results in Table (11) 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 (0.562 and 0.588 mg/l), transfer factor in stem (0.318 and 0.340 mg/l) and transfer factor in root (0.134 and 0.149 mg/l) in the first and second

Table 10. Average values of lead content in soil samples as influenced by lead concentrations in irrigation water and foliar application of malic acid on *Salvia splendens* in the two seasons of 2018 and 2019.

Treatments		Lead content in soil (mg/kg)			
Lead (mg/l)	Malic acid (mg/l)	2018		2019	
		Before	After	Before	After
	0	0.229	0.658	0.234	0.669
0	250	0.258	0.717	0.264	0.728
	500	0.276	0.752	0.281	0.763
	0	0.368	0.737	0.374	0.748
100	250	0.389	0.779	0.395	0.790
	500	0.408	0.816	0.413	0.827
	0	0.397	0.794	0.402	0.805
200	250	0.413	0.826	0.418	0.837
	500	0.436	0.873	0.442	0.884
	0	0.419	0.839	0.426	0.852
300	250	0.445	0.891	0.451	0.902
	500	0.459	0.918	0.464	0.929

Table 11. Means of transfer factor to leaves, stem and root of *Salvia splendens* plants as influenced by lead (Pb), malic acid (MA) and their combinations (Pb ×MA) in the two seasons of 2018 and 2019.

Treatments		Transfer factor to leaves (TFL)		Transfer factor to stem (TFS)		Transfer factor to root (TFR)	
Pb (mg/l)	MA (mg/l)	2018	2019	2018	2019	2018	2019
	0	0.310	0.351	0.127	0.152	0.028	0.034
0	250	0.259	0.260	0.099	0.100	0.025	0.020
	500	0.191	0.196	0.061	0.065	0.019	0.015
Mean (Pb)		0.253	0.269	0.095	0.105	0.024	0.023
	0	0.510	0.504	0.306	0.302	0.158	0.161
100	250	0.448	0.430	0.236	0.230	0.086	0.091
	500	0.256	0.276	0.115	0.128	0.039	0.042
Mean (Pb)		0.404	0.403	0.219	0.220	0.094	0.098
	0	0.668	0.685	0.392	0.406	0.168	0.177
200	250	0.486	0.495	0.269	0.279	0.108	0.117
	500	0.334	0.356	0.160	0.174	0.042	0.047
Mean (Pb)		0.496	0.512	0.273	0.286	0.106	0.113
	0	0.761	0.813	0.449	0.500	0.182	0.226
300	250	0.491	0.513	0.279	0.292	0.115	0.120
	500	0.284	0.292	0.142	0.148	0.057	0.059
Mean (Pb)		0.512	0.539	0.290	0.313	0.118	0.135
	0	0.562	0.588	0.318	0.340	0.134	0.149
Mean (MA)	250	0.421	0.424	0.220	0.225	0.083	0.087
	500	0.266	0.280	0.119	0.128	0.039	0.040

seasons, respectively, were recorded in the parts of control plants, whereas plants sprayed with the malic acid concentration at (500 mg/l) had the lowest transfer factor in leaves (0.266 and 0.280 mg/l), transfer factor in stem (0.119 and 0.128 mg/l) and transfer factor in root (0.0395 and 0.040 mg/l) in the first and second seasons, respectively.

Regarding the interaction between effect of irrigation with contaminated water and malic acid concentrations on the transfer factor in the plant parts, presented data in Table (10) show that the highest transfer factor in leaves (0.761 and 0.813 mg/l), transfer factor in stem (0.449 and 0.500 mg/l) and transfer factor in root (0.182 and 0.226 mg/l) in the first and second seasons, respectively, were obtained in plants irrigated with lead water at (300 mg/l) and sprayed with tap water, while the lowest transfer factor in leaves (0.191 and 0.196 mg/l), transfer factor in stem (0.061 and 0.065 mg/l) and transfer factor in root (0.019 and 0.015 mg/l) in the first and second seasons, respectively, were recorded in plants applied with 0 mg/l lead and sprayed with (500 mg/l) malic acid.

DISCUSSION

This study revealed that at high heavy metal concentrations, the biomass was significantly reduced. The leaves growth was more sensitive than other parts, as leaves rapidly absorbed water and had higher accumulations 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).

Plants can tolerate lead be either by external exclusion or internal tolerance. By the external exclusion, lead ions are excluded from entering the plant cells and thus lead cannot accumulate in the organelles and excess lead ions are removed out of the plant cell (Sharma and Dubey 2005). The internal tolerance of lead is mainly due to the synthesis of organic lead compounds (cysteine, glutathione, phytochelatin, etc.) and eventually the lead ions are transformed in the cell into chemically bound structures with lower toxicity, alleviating the Pb toxic effect on the plants tissues (Pourrut *et al.,* 2011).

Lead can damage the ultra-structures of the organs, tissues, chloroplast, mitochondria, nucleus, cell wall, and cell membrane in the plants. This damage can cause a loss of organelle function, and can eventually affect the normal physiological functions that include photosynthesis, respiration, protein synthesis, cell division within the plant species (Salazar and Pignata, 2014).

Concerning treatments and the control sample, at a preliminary stage, one should note that the transfer factor of most treatments is lower than one for lead; which means that the physiological need of the plant for these elements is rather limited.

Trace elements 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 trace metals have been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream (Blaylock and Huang, 2000).

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 increased chlorophyll content significantly. 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. The *Salvia splendens* have 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.

REFERENCES

Bah, A.M.; Dai, H.; Zhao, J.; Sun, H.; Cao, F.; Zhang, G. and Wu, F. (2011). Effects of lead, chromium and lead on growth, metal uptake and antioxidative capacity in *Typha angustifolia*. Biol. Trace Elem. Res., 142:77-92.

Bates, L.S.; Waldern, R.P. and Teare, L.D. (1973). Rapid determination of free proline under water stress studies. Plant and Soil, 39:205-207.

Blaylock, M.J. and Huang, J.W. (2000). Phytoextraction of Metals. IN: Raskin, I. and Ensley, B.D. (eds.), Phytoremediation of Toxic Metals: Using Plants to Clean up the environment. John Wiley and Sons, Inc, Toronto, 304 p.

Chen, Y.; Shen, Z. and Li, X. (2004). The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. Applied Geochemistry, 19:1553-1565.

Clebsch, B. and Barner, C.D. (2003). The New Book of Salvias. Timber Press, California, USA, pp. 278-280.

Da Silva, J.A. (2003). The cut flower: postharvest considerations. Journal of Biological Sciences, 3(4)406-442.

Darandeh, N. and Hadavi, E. (2012). Effect of pre-harvest foliar application of citric acid and malic acid on chlorophyll content and post-harvest vase life of *Lilium* cv. Brunello. Frontiers in Plant Science, 2(106):1-3. https://doi.org/ 10.3389/fpls.2011.00106

Day, D.A. and Hanson, J.B. (1977). Pyruvate and malate transport and oxidation in corn mitochondria. Plant Physiology, 59(4):630-635.

Dubios, M.; Gilles, K.; Hamlton, J.; Rebers, P. and Smith, F. (1956). Colourimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3):350-356.

Israelsen, O. and Hansen, V. (1962). Irrigation, Principles and Practices. John Wiley and Sons, Inc. New York, 447 p.

Karousou, R.; Hanlidou, E. and Kokkini, S. (2000). The sage plants in Greece: Di ribution and infraspeci c variation, In: Kintzios, S.E. (ed.), Sage: The genus Salvia, Harwood Academic Publishers, Amsterdam, The Netherlands, pp. 27-46.

Koller, H.R. (1972). Leaf area, leaf weight relationship in the soybean canopy. Crop Sci., 12:180-183.

Lindsay, W.L. and Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J., 42:421-428.

Mahler, R.J.; Bingham, F.T. and Chang, A.C. (1981). Effect of heavy metal pollution on plants. Applied Science, 1:72-109.

Page, A.L.; Miller, R.H. and Keency, D.R. (1982) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Madison, Wisconsin U.S.A., 1159 p.

Piotrowska, A.; Bajguz, A.; Godlewska-Zylkiewicz, B. and Zambrzycka, E. (2010). Changes in growth, biochemical components, and antioxidant activity in aquatic plant *Wolffiaarrhiza* (Lemnaceae) exposed to cadmium and lead. Arch. Environ. Contam. Toxicol., 58:594-604.

Piper, O.S. (1947). Soil and Plant Analysis. Adelaite Univ., Adelaide, Australia, pp. 258-275.

Pourrut, B.; Shahid, M.; Dumat, C.; Winterton, P. and Pinelli, E. (2011). Lead uptake, toxicity, and detoxification in plants. Rev. Environ. Contam., 213:113-136.

Salazar, M.J. and Pignata, M.L. (2014). Lead accumulation in plants grown in polluted soils. Screening of native species for phytoremediation. J. Geochem. Explor., 137:29-36.

SAS Institute (2002). SAS User Guide and Program 20 Version 9.038. cary, North Carolina,USA.

Sharma, B.D.; Mukhopadhyay, S.S. and Katyal, J.C. (2006). Distribution of total and DTPA- extractable zinc, copper, manganese and iron in vertisols of India. Commun Soil Sci Plant Anal., 37:653-672.

Sharma, P. and Dubey, R.S. (2005). Lead toxicity in plants. B. J. Plant Physiol., 17:35-52.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State University Press, Ames, 503 p.

Talebi, M.; Hadavi, E. and Jaafari, N. (2014). Foliar sprays of citric acid and malic acid modify growth, flowering, and root to shoot ratio of gazania (*Gazania rigens* L.). Adv. Agric. doi.org/10.1155/2014/147278

Tiryakioglu, M.; Eker, S.; Ozkutlu, F.; Husted, S. and Cakmak, I. (2006). Antioxidant defense system and lead uptake in barley genotypes differing in lead tolerance. J. Trace Elem. Med. Biol., 20:181-189.

Topcu, G. (2006). Bioactive triterpenoids from Salvia species. J. Nat. Prod., 69:482-487.

Unhalekhana, U.; and Kositanont, C. (2008). Distribution of lead in soil around zinc mining area. Thai Journal of Toxicology, 23:170-174.

Wu, L.; Guo, X. and Harivandi A. (2001). Salt tolerance and salt accumulation of landscape plants irrigated by sprinkler and drip irrigation systems. J. Plant Nutr., 24:1473-1490.

Wua, G.; Kanga, H., Zhangc, X.; Shaob, H.; Chuc, L. and Ruand, C. (2010). A critical review on the bio-removal of hazardous heavy metals from contaminated soils: Issues, progress, eco-environmental concerns and opportunities. Journal of Hazardous Materials, 174(1-3):1-8.

Yadava, U. (1986). A rapid and nondestructive method to determine chlorophyll in intact leaves. Hort. Sci., 21(6):1449-1450.

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

نادر أحمد الشنهورى، أحمد عبد المنعم بركات
قسم بحوث الحدائق النباتية، معهد بحوث البساتين، مركز البحوث الزراعية، الأسكندرية، مصر

أجريت هذه الدراسة في فرع بحوث الحدائق النباتية بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر خلال الموسمين المتتاليين ٢٠١٨ و ٢٠١٩. وكان الهدف من هذه الدراسة تقييم آثار مياه الري الملوثة بالرصاص على نباتات السلفيا المزروعة في تربة رملية، كذلك استخدام الرش بحمض الماليك للتغلب على الآثار الضارة للرصاص. زرعت شتلات السلفيا بشكل فردي في أوعية بلاستيكية (قطرها ٢٠ سم) مملوءة ٥ كجم من التربة الرملية. وكانت معاملات مياه الري الملوثة بأربعة تراكيزات من الرصاص وهى صفر، ١٠٠، ٢٠٠ و ٣٠٠ جزء في المليون. تمت معاملة النباتات أيضا بحامض الماليك في ثلاث تركيزات هى صفر، ٢٥٠ و ٥٠٠ جزء في المليون عن طريق الرش شهريا في كلا الموسمين. أظهرت النتائج أنة بتقييم معايير النمو الخضري وجد أن هناك اختلافا كبيرا في التفاعل بين تركيزات الرصاص ورش النباتات بحامض الماليك. لوحظ انخفاض كبير في كافة معاملات الري بالماء الملوث بالرصاص وكذلك لوحظ زيادة كبيرة في معدلات النموالخضرى بعد الرش بتركيز ٥٠٠ 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 للرصاص كان في الأوراق و الساق و الجذور كنتيجة للري بماء ملوث بتركيز ٣٠٠ جزء في المليون دون الرش بحمض الماليك.