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RESPONSE OF *LANTANA CAMARA* PLANTS TO FOLIAR APPLIED CITRIC ACID FOR DECREASING THE HARMFUL EFFECT OF HEAVY METALS POLLUTION IN THE IRRIGATION WATER (C) EFFECT OF LEAD

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ABSTRACT: The present study was carried-out at Antoniadis Research Branch, Horticultural Research Institute, Agriculture Research Center (ARC), Alexandria, Egypt during two successive seasons of 2018 and 2019. The aim of this study was to evaluate the effects of irrigation water contaminated with lead on the growth of *Lantana camara* plants and the possibility of using citric acid spray treatments to overcome the effects of lead pollution. Seedlings of *Lantana camara* were planted individually in plastic pots (20 cm diameter) filled with 5 kg of sandy soil. The lead contaminated irrigation water treatments were 0,100, 200 and 300 mg/l. The plants were also monthly sprayed by citric acid at concentrations of 0, 250 and 500 mg/l. The results showed that for vegetative growth parameters there were no significant difference in the interaction between lead concentrations in water of irrigation and foliar spray by citric acid, while significant reductions were observed in all parameters after irrigation with lead contaminated water. However, significant increases in vegetative growth parameters were observed after 500 mg/l citric acid application. For chlorophyll and carbohydrate contents, the highest significant value was obtained in plants irrigated with tap water and sprayed with 250 mg/l citric acid while the highest significant level of lead content in leaves, stem and roots was obtained due treatment by 300 mg/l lead without application of citric acid.

Key words: *Lantana camara*, polluted irrigation water, lead, citric acid.

INTRODUCTION

Phytoremediation has become an effective and affordable technological solution used to extract or remove toxic metals from polluted soil. Phytoremediation is the use of plants to clean polluted soils, sediments and water. This technology is environment friendly and potentially cost effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulators (Choruk *et al.*, 2006). Plants need trace amounts of heavy metals but their excessive availability may cause plant toxicity (Sharma *et al.*, 2006). Phytotoxic concentration of 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).

Lead is one of the main sources of environmental pollution. Many studies have shown that lead inhibits metabolic processes in the plants such as nitrogen assimilation, photosynthesis, respiration, water uptake, and transcription (Kurepa *et al.*, 1997 and Boussama *et al.*, 1999). Lead may inactivates various enzymes by binding to their SH-groups (Rauser, 1995), and can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress (Cuypers *et al.*, 1999; Prasad *et al.*, 1999). In addition, lead can negatively affect mitochondria structure by decreasing number of mitochondrial cristae, which in turn can lower the capability of oxidative phosphorylation (Malecka *et al.*, 2001).

Endogenous organic acids are the source of both carbon skeleton and energy for cells and are used in the respiratory cycle and other biochemical pathways (Da Silva, 2003). Citric acid (CA) is an organic acid mainly served as antioxidant and involved in plant metabolism through its role in Krebs cycle during the respiration in mitochondria which produces cellular energy by oxidative phosphorylation (Taize and Zaiger, 2002). This energy is required for different physiological processes and development of the mechanisms of resistance systems under different stressful factors. It is created by addition of acetyl-CoA to oxaloacetic acid that is converted to succinate and malate in next steps (Wills *et al.*, 1981).

Lantana camara L., which is a new plant owing to its remarkable capacity to extract lead and cadmium from polluted soils in Vietnam (Huynh, 2009) has been suggested as a model species for research on phytoextraction of metals. Moreover, this plant has a rapid growth and developing very fast. It can also grow in extreme conditions and is able to endure long periods of drought or heavy rains. Finally, its multicolored flowers allow integration into a floral arrangement of a landscaping project (Huynh, 2009).

In this study *Lantana camara* was selected due to its characteristics as non-edible plant which can grow in tropical areas and it has many uses in landscaping. Therefore, the objective of this study was to determine the potential of *Lantana camara* in removing lead from soil affected by lead polluted irrigation water and to investigate it is ability for removing heavy metals.

MATERIALS AND METHODS

The present study was carried-out at Antoniades Research Branch, Horticultural Research Institute, Agriculture Research Center (ARC), Alexandria, Egypt during two successive seasons of 2018 and 2019.

On 15th of February, 2018 and 2019 in the first and second seasons, respectively, uniform transplants of *Lantana camara* (15-20 cm height and 10-15 leaves per plant) were planted individually in plastic pots (20 cm diameter) filled with 5 kg sandy soil. The chemical characteristics of the soil were measured as described by Jackson (1958) and presented in Table (1).

On 1st of March (in both seasons), the polluted of irrigation water were initiated. Four concentrations of lead (II) acetate (Pb $(CH_3COO)_2$): 0, 100, 200 and 300 mg/l were prepared and dissolved in the irrigation water. The plants were irrigated three times per week. At the end of the experiment every pot had received about 127 liters of lead polluted water (Table, 2). In both seasons, the plants were monthly sprayed by citric acid from 15th May till 15th August in both seasons at concentrations of 0, 250 and 500 mg/l. The control plants were sprayed with tap water. On 30th of September in the both seasons, the plants were harvested.

In the two seasons, the plants received N, P and K chemical fertilizers in the form of soluble fertilizer (Kristalon 19-19-19) at rate of 1.5 g/pot. Fertilization was repeated every 30 days throughout the growing season (from 1st of March till 30th of September). In addition, weeds were removed manually upon emergence.

Data recorded:

1. Vegetative growth parameters:

Plant height (cm), number of leaves per plant, leaves dry weight per plant (g), leaves area (cm^2) according to Koller (1972), branch number per plant, stem diameter

Table 1. The chemical properties of the used sandy soil for the two seasons 2018 and 2019.

Season	pH	EC dS/m	Water soluble cations (meq/l)				Water soluble anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
2018	7.93	1.55	3.4	3.4	6.5	1.3	3.6	6.7	2.4
2019	7.91	1.52	3.2	3.0	6.3	1.2	3.3	6.5	2.2

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

Field Capacity (%)	Months of first and second seasons							
	March	April	May	June	July	August	September	Total
100 %	14.00	15.00	16.00	17.00	20.00	23.00	22.00	127.00

(cm), stem dry weight (g), root length (cm), root dry weight (g), flower number per plant and flower dry weight (g) were also measured.

2. Chemical analysis:

- Total chlorophyll index was measured as a SPAD for the fresh middle leaves of plants, for the different treatments under the experiment at the end of the season using Minolta (chlorophyll meter) SPAD 502 according to Yadava (1986).
- Total carbohydrate 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. 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 the soil sample was extracted by DPTA-Solution according to Lindsay and Norvell (1978) and measured by Inductively Coupled Plasma Spectrometry.
- Transfer factor (TF) is calculated the ratio between the concentration of metal in the shoots to the concentration of metal in the soil (Chen *et al.*, 2004) as indicates to the efficiency of any plant to transfer any metal from soil to the aerial parts.

The pot experimental design was split plot with three replicates. Each replicate contained three plants. The main plot was lead concentration, while the subplot was citric acid treatments. The obtained data were subjected to analysis of variance (ANOVA) using the SAS program, SAS Institute (SAS Institute, 2002). 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

1. Vegetative growth:

a. Leaves parameters:

Data presented in Table (3) showed that, in both seasons, irrigation water polluted with lead decreased the tested leaves parameters of *Lantana camara* plants. While plants irrigated with tap water (control) had the highest mean values of number of leaves per plant (100.50 and 101.66), leaves dry weight (2.05 and 2.41 g) and leaves area

Table 3. Means of number of leaves per plant, leaves dry weight (g) and leaves area (cm^2) of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) 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)	CA (mg/l)	2018	2019	2018	2019	2018	2019
	0	98.50	100.33	2.01	2.36	370.44	261.95
0	**250**	100.50	101.66	2.05	2.40	496.36	282.53
	500	102.50	103.00	2.11	2.47	401.09	287.42
Mean (Pb)		100.50	101.66	2.05	2.41	422.63	277.30
	0	95.50	97.33	1.96	2.31	342.22	216.70
100	**250**	96.50	98.33	1.98	2.35	360.02	246.52
	500	100.50	101.66	2.06	2.40	360.33	266.42
Mean (Pb)		97.50	99.10	2.00	2.35	354.19	243.21
	0	91.00	92.83	1.87	2.25	311.81	236.11
200	**250**	93.83	94.83	1.91	2.26	317.24	241.95
	500	94.83	96.66	1.94	2.29	319.23	246.56
Mean (Pb)		93.22	94.77	1.90	2.26	316.09	241.54
	0	88.50	90.33	1.82	2.16	219.55	191.02
300	**250**	90.00	91.83	1.84	2.20	233.67	199.43
	500	91.16	92.66	1.87	2.24	274.81	215.54
Mean (Pb)		89.88	91.60	1.84	2.20	242.67	201.99
	0	93.37	95.20	1.91	2.27	311.00	226.44
Mean (CA)	**250**	95.20	96.66	1.94	2.30	351.82	242.60
	500	97.24	98.49	1.99	2.35	338.86	253.98
	Pb	1.68	1.56	0.03	0.03	49.37	26.34
L.S.D. at 0.05	**CA**	1.95	1.81	0.04	0.04	26.80	15.79
	Pb × CA	2.25	2.09	0.04	0.04	30.80	18.15

(422.63 and 277.30 cm^2) in the first and second seasons, respectively. Increasing the lead concentration significantly reduced in the tested leaves parameters. The highest lead concentration (300 mg/l) significantly produced the lowest mean values of plant number of leaves per plant (89.88 and 91.60), leaves dry weight (1.84 and 2.20 g) and leaves area (242.67 and 201.99 cm^2) in the first and second seasons, respectively, compared with the other concentrations.

Leaves parameters were also significantly affected by spraying the plants with citric acid. In both seasons, the tested leaves parameters were gradually increased when the citric acid concentration was increased from 0 mg/l (control) to 500 mg/l. Accordingly, data presented in Table (3) showed that *Lantana camara* plants sprayed with 500 mg/l citric acid gave significantly higher mean values of number of leaves per plant (97.24 and 98.49), leaves dry weight (1.99 and 2.35 g) and leaves area (338.86 and 253.98 cm^2) in the first and second seasons, respectively, compared with the other concentrations.

Regarding the interaction between irrigation with lead polluted water and citric acid treatments on the tested leaves parameter of *Lantana camara* plants, the recorded results in the two seasons showed that, the highest values were obtained for plants irrigated with tap water (control) and sprayed with citric acid at 500 mg/l with mean values of number of leaves per plant (102.50 and 103.00) and leaves dry weight (2.11 and 2.47 g) in the first and second seasons, while irrigated plants with tap water (control) and sprayed with citric acid at 250 mg/l gave mean values of leaves area (496.36 cm^2) in the first season while these irrigated with tap water (control) and sprayed with citric acid at 500 mg/l recorded mean values of leaves area (287.42 cm^2) in the second season, respectively, compared with the other concentrations. On the other hand, the lowest values of number of leaves per plant (88.50 and 90.33), leaves dry weight (1.82 and 2.16 g) and leaves area (219.55 and 191.02 cm^2) in the first and second seasons, respectively, were resulted when the plants were irrigated using the highest lead concentration (300 mg/l) and sprayed with citric acid at 0 mg/l. It is also shown that in many cases, spraying plants with citric acid reduced the adverse effect of polluted water with lead (Table, 3).

b. Stem parameters:

Data presented in Table (4) 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 the thickest stem, with mean plant height (34.69 and 40.19 cm), number of branch per plant (7.33 and 12.33), stem diameter (0.54 and 0.71 cm) and stem dry weight (3.13 and 4.75 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 parameter was significant compared to the control, even at the highest lead concentration (300 mg/l), which gave plant height (30.60 and 36.66 cm), number of branch per plant (6.10 and 9.49), stem diameter (0.40 and 0.60 cm), and stem dry weight (2.45 and 3.94 g) in the first and second seasons, respectively, compared with the other concentrations.

In contrast to the effect of lead treatments, citric acid treatments improved stem parameters of *Lantana camara* plants, compared to the control. Moreover, plants sprayed with 500 mg/l citric acid had significantly mean plant height (33.31 and 39.16 cm), number of branch per plant (7.04 and 11.24), stem diameter (0.51 and 0.68 cm) and stem dry weight (2.90 and 4.55 g)

Table 4. Means of plant height (cm), branch number per plant, stem diameter (cm) and stem dry weight (g) of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) in the two seasons of 2018 and 2019.

Treatments		Plant height (cm)		Branch number per plant		Stem diameter (cm)		Stem dry weight (g)	
Pb (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019	2018	2019
	0	33.58	39.41	7.00	11.83	0.53	0.68	2.84	4.41
0	250	34.91	40.08	7.16	12.33	0.54	0.71	2.97	4.87
	500	35.58	41.08	7.83	12.83	0.56	0.75	3.58	4.98
Mean (Pb)		34.69	40.19	7.33	12.33	0.54	0.71	3.13	4.75
	0	32.58	38.41	6.66	10.66	0.49	0.66	2.54	4.06
100	250	33.58	38.75	6.83	10.83	0.51	0.67	2.58	4.14
	500	34.25	40.08	7.00	11.66	0.52	0.71	2.72	4.51
Mean (Pb)		33.47	39.08	6.83	11.05	0.50	0.68	2.61	4.23
	0	31.08	36.91	6.33	9.83	0.47	0.65	2.36	3.73
200	250	31.25	37.41	6.66	10.16	0.49	0.66	2.49	3.90
	500	32.25	38.08	7.00	10.50	0.50	0.67	2.66	4.37
Mean (Pb)		31.52	37.46	6.66	10.16	0.48	0.66	2.50	4.00
	0	30.25	36.08	5.83	8.83	0.42	0.59	2.17	3.69
300	250	30.41	36.50	6.16	9.66	0.44	0.60	2.53	3.79
	500	31.16	37.41	6.33	10.00	0.46	0.61	2.65	4.36
Mean (Pb)		30.60	36.66	6.10	9.49	0.44	0.60	2.45	3.94
	0	31.87	37.70	6.45	10.28	0.47	0.64	2.47	3.97
Mean (CA)	250	32.53	38.18	6.70	10.74	0.49	0.66	2.64	4.17
	500	33.31	39.16	7.04	11.24	0.51	0.68	2.90	4.55
	Pb	0.60	0.52	0.41	1.81	0.04	0.03	0.22	0.32
L.S.D. at 0.05	CA	0.69	0.58	0.27	0.74	0.02	0.03	0.12	0.28
	Pb × CA	0.80	0.67	0.31	0.85	0.02	0.03	0.13	0.32

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 citric acid on stem parameters of *Lantana camara* plants, the recorded results for the two seasons are presented in presented in Table (4) showed that significant differences were detected between the values obtained from plants receiving the different treatment combinations. The highest values of plant height (35.58 and 41.08 cm), number of branch per plant (7.83 and 12.83), stem diameter (0.56 and 0.75 cm) and stem dry weight (3.58 and 4.98 g) in the first and second seasons, respectively, were obtained for plants irrigated with tap water and sprayed with citric acid at 500 mg/l. On the other hand, the least values of plant height (30.25 and 36.08 cm), number of branch per plant (5.83 and 8.83), stem diameter (0.42 and 0.59 cm) and stem dry weight (2.17 and 3.59 g) in the first and second seasons, respectively, were obtained for plants irrigated by the highest lead concentration (300 mg/l) and sprayed with citric acid at 0 mg/l treatment. In many cases, spraying the plants with citric acid reduced the adverse effect of lead polluted water.

c. Root parameters:

Data presented in Table (5) 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 (16.82 and 20.14 cm) and root dry weight (2.25 and 3.62 g) in the first and second seasons, respectively. Increasing lead concentration in irrigation water caused a steady reduction in root parameter. These reductions in root parameters were significant as compared to the control even at the highest lead concentration (300 mg/l), which gave root length (15.00 and 18.29 cm) and root dry

Table 5. Means of root length (cm) and root dry weight (g) of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) in the two seasons of 2018 and 2019.

Treatments		Root length (cm)		Root dry weight (g)	
Pb (mg/l)	CA (mg/l)	2018	2019	2018	2019
	0	16.47	19.80	2.07	3.44
0	**250**	16.83	20.14	2.13	3.51
	500	17.16	20.49	2.56	3.91
Mean (Pb)		16.82	20.14	2.25	3.62
	0	15.96	19.27	1.85	2.89
100	**250**	16.13	19.44	1.89	2.94
	500	16.82	20.14	1.98	3.19
Mean (Pb)		16.30	19.61	1.90	3.00
	0	15.19	18.48	1.75	2.67
200	**250**	15.66	18.97	1.84	2.78
	500	15.84	19.14	1.93	3.09
Mean (Pb)		15.56	18.86	1.84	2.84
	0	14.76	18.06	1.60	2.63
300	**250**	15.01	18.30	1.84	2.71
	500	15.23	18.52	1.93	3.09
Mean (Pb)		15.00	18.29	1.79	2.81
	0	15.59	18.90	1.81	2.90
Mean (CA)	**250**	15.90	19.21	1.92	2.98
	500	16.26	19.57	2.10	3.32
	Pb	0.27	0.28	0.14	0.52
L.S.D. at 0.05	**CA**	0.33	0.33	0.08	0.29
	Pb × CA	0.38	0.38	0.09	0.33

weight (1.79 and 2.81 g) in the first and second seasons, respectively.

In contrast to the effect of lead treatments, citric acid treatments improved root parameters of *Lantana camara* plants, compared to the control. Moreover, plants sprayed with 500 mg/l citric acid had significantly longer root (16.26 and 19.57 cm) and heavier root dry weight (2.10 and 3.32 g) in the first and second seasons, respectively, compared to those of control plants, or plants sprayed with any other citric acid concentration.

Regarding the interaction between the irrigation with lead polluted water and citric acid treatments on root parameters of *Lantana camara* plants, 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 (17.16 and 20.49 cm) and root dry weight (2.56 and 3.91 g) in the first and second seasons, respectively, were obtained for the plants irrigated with tap water and sprayed with citric acid at 500 mg/l. On the other hand, the lowest values of root length (14.76 and 18.06 cm) and root dry weight (1.60 and 2.63 g) in the first and second seasons, respectively, were obtained for the plants irrigated with the highest lead concentration (300 mg/l) and sprayed with citric acid at 0 mg/l treatment. It is shown from Table (5) that in many cases, spraying the plants with citric acid reduced the harmful effect of lead polluted water with lead.

d. Flowering parameters:

Data presented in Table (6) showed that, in both seasons, irrigation with lead polluted water decreased the flowering parameters of *Lantana camara* plants. Plants irrigated with tap water (control) had the highest mean values of number of flowers per plant (22.94 and 29.94) and flower dry weight (1.91 and

Table 6. Means of flower number per plant and flower dry weight (g) of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) in the two seasons of 2018 and 2019.

Treatments		Flower number per plant		Flower dry weight (g)	
Pb (mg/l)	**CA (mg/l)**	**2018**	**2019**	**2018**	**2019**
	0	22.50	29.33	1.72	3.10
0	**250**	23.00	30.00	1.79	3.17
	500	23.33	30.50	2.23	3.57
Mean (Pb)		22.94	29.94	1.91	3.28
	0	22.00	28.83	1.51	2.55
100	**250**	22.16	29.00	1.56	2.60
	500	23.00	30.00	1.64	2.85
Mean (Pb)		22.38	29.27	1.57	2.66
	0	21.00	27.66	1.40	2.33
200	**250**	21.66	28.33	1.51	2.46
	500	21.83	28.50	1.59	2.75
Mean (Pb)		21.49	28.16	1.50	2.51
	0	20.33	27.16	1.27	2.30
300	**250**	20.83	27.50	1.51	2.36
	500	21.16	27.66	1.58	2.75
Mean (Pb)		20.77	27.44	1.45	2.47
	0	21.45	28.24	1.47	2.57
Mean (CA)	**250**	21.91	28.70	1.59	2.64
	500	22.33	29.16	1.76	2.98
	Pb	0.24	0.37	0.15	0.53
L.S.D. at 0.05	**CA**	0.47	0.47	0.08	0.29
	Pb × CA	0.54	0.54	0.09	0.33

3.28 g) in the first and second seasons, respectively. Increasing lead concentration caused significant reductions in flowering parameters, with the highest lead concentration (300 mg/l) giving significantly the least values of number of flowers per plant (20.77 and 27.44) and flower dry weight (1.45 and 2.47 g) in the first and second seasons, respectively, compared with the other concentrations.

Flowering parameters were also significantly affected by spraying the plants with citric acid. In both seasons, gradual increasing of citric acid concentration was increased from 0 mg/l (control) to 500 mg/l. Accordingly, Table (6) showed that *Lantana camara* plants sprayed with 500 mg/l citric acid were significantly increased the values of number of flowers per plant (22.33 and 29.16) and flower dry weight (1.76 and 2.98 g) in the first and second seasons, respectively, compared with the other concentrations.

Regarding the interaction between the irrigation with lead polluted water and citric acid treatments, the recorded results in the two seasons showed that, the highest values were obtained for the plants irrigated with tap water and sprayed with citric acid at 500 mg/l with values of number of flowers per plant (23.33 and 30.50) and flower dry weight (2.23 and 3.57 g) in the first and second seasons, respectively. On the other hand, the plants with mean values of number of flowers per plant (20.33 and 27.16) and flower dry weight (1.27 and 2.30 g) in the first and second seasons, respectively, were recorded for the plants which were irrigated using the highest lead concentration of (300 mg/l) and sprayed with citric acid at 0 mg/l. It is shown that in many cases, spraying the plants with citric acid reduced the harmful effect of polluted water with lead.

2. Chemical composition:

a. Leaf chemical analysis:

Data presented in Table (7) showed that the highest values of total chlorophyll were obtained for plants irrigated with tap water (57.21 and 57.69 SPAD) as well as total carbohydrates (17.72 and 17.88%) in the first and second seasons, respectively, while the maximum proline content (2.52 and 2.49 mg/g) was obtained in the plants irrigated with lead polluted water at 300 mg/l. Increasing the lead concentration in irrigation water resulted in steady significant reductions in the chlorophyll and carbohydrates content, which reached lowest mean value for chlorophyll (51.81 and 52.51 SPAD) and total carbohydrates (15.87 and 16.12%) in the first and second seasons, respectively, for plants receiving the highest lead concentration (300 mg/l), while, the least proline content (1.36 and 1.40 mg/g) were obtained for the plants irrigated with tap water.

Data in Table (7) showed also that citric acid treatments had possible effect on leaf chlorophyll (55.29 and 55.75 SPAD) and total carbohydrates (17.66 and 17.23 %) in the first and second seasons, respectively, in plants sprayed with citric acid at 500 mg/l, while, the higher proline contents (2.03 and 2.06 mg/g) were obtained in plants sprayed without citric acid (tap water).

Regarding to the interaction between the irrigation with lead polluted water and citric acid treatments, the highest total chlorophyll contents (58.87 and 59.25 SPAD) and total carbohydrates (18.29 and 18.42 %) in the first and second seasons, respectively, were found in leaves of plants irrigated with tap water and sprayed with citric acid at 250 mg/l, while, the higher proline contents (2.53 and 2.51 mg/g) were resulted for the plants irrigated by the highest lead concentration (300 mg/l) without citric acid treatment.

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

Data presented in Table (8) showed that, the lead content (mg/l) in the dried plant parts of *Lantana camara* plants was increased with increasing the lead concentration in the irrigation water. Generally, the lowest mean lead content of leaves (0.182 and 0.343 mg/l), lead content

Table 7. Means of some chlorophyll content (SPAD), carbohydrates content (%) and proline content (mg/g D.W) of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) in the two seasons of 2018 and 2019.

Treatments		Chlorophyll content (SPAD)		Carbohydrate content (%)		Proline content (mg/g d.w.)	
Pb (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019
	0	55.55	56.26	17.16	17.39	1.42	1.45
0	**250**	58.87	59.25	18.29	18.42	1.39	1.42
	500	57.22	57.58	17.72	17.85	1.28	1.33
Mean (Pb)		57.21	57.69	17.72	17.88	1.36	1.40
	0	51.71	52.09	15.85	15.97	1.87	1.93
100	**250**	58.45	58.83	18.14	18.27	1.84	1.86
	500	56.67	57.42	17.54	17.80	1.83	1.85
Mean (Pb)		55.61	56.11	17.17	17.34	1.84	1.88
	0	50.11	51.13	15.30	15.65	2.30	2.35
200	**250**	56.04	56.43	17.32	17.46	2.27	2.31
	500	55.12	55.49	17.01	17.13	2.24	2.30
Mean (Pb)		53.75	54.35	16.54	16.74	2.27	2.32
	0	49.07	50.11	14.95	15.30	2.53	2.51
300	**250**	54.20	54.91	16.69	16.94	2.52	2.49
	500	52.17	52.53	15.99	16.14	2.51	2.48
Mean (Pb)		51.81	52.51	15.87	16.12	2.52	2.49
	0	51.61	52.39	15.81	16.07	2.03	2.06
Mean (CA)	**250**	56.89	57.35	17.61	17.77	2.00	2.02
	500	55.29	55.75	17.06	17.23	1.96	1.99
	Pb	0.52	0.48	0.17	0.16	0.05	0.04
L.S.D. at 0.05	**CA**	0.38	0.38	0.13	0.13	0.01	0.01
	Pb × CA	0.44	0.43	0.15	0.15	0.02	0.01

of stem (0.222 and 0.240 mg/l) and lead content of root (0.136 and 0.123 mg/l) in the first and second seasons, respectively, were recorded in leaves of plants irrigated with tap water, whereas the highest mean values of lead content of the leaves (0.995 and 1.034 mg/l), stem (0.627 and 0.697 mg/l) and root (0.502 and 0.541 mg/l) in the first and second seasons, respectively, were registered for plants irrigated with water containing the highest lead concentration (300 mg/l).

Concerning the effect of citric acid treatments on the lead content in plant parts, citric acid treatment of 500 mg/l caused a significant decrease in the mean values of lead content in the leaves (0.621 and 0.712 mg/l), stem (0.448 and 0.488 mg/l) and root (0.349 and 0.374 mg/l) in the first and second seasons, respectively, compared to those of control plants had the highest lead content in the leaves (0.676 and 0.769 mg/l), stem (0.468 and 0.511 mg/l) and root (0.365 and 0.386 mg/l) in the first and second seasons, respectively.

Concerning the interaction between irrigation using lead polluted water with lead and citric acid treatments on the lead content in plant parts, the lowest mean values of lead content in the leaves (0.141 and 0.306 mg/l), stem (0.219 and 0.226 mg/l) and root (0.131 and 0.119 mg/l) in the first and second seasons, respectively, were recorded for plants irrigated with tap water and sprayed with citric acid at 500 mg/l. On the other hand, the highest lead content was obtained in the plant parts irrigated with lead polluted water at 300 mg/l and void of citric acid giving lead content in the leaves (1.003 and 1.049 mg/l), stem (0.641 and 0.710 mg/l) and root (0.513 and 0.542 mg/l).

Table 8. Means of lead content of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) 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)	**CA (mg/l)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
	0	0.234	0.391	0.225	0.248	0.141	0.128
0	**250**	0.172	0.332	0.224	0.246	0.137	0.124
	500	0.141	0.306	0.219	0.226	0.131	0.119
Mean (Pb)		0.182	0.343	0.222	0.240	0.136	0.123
	0	0.556	0.713	0.427	0.496	0.347	0.401
100	**250**	0.538	0.695	0.418	0.488	0.341	0.395
	500	0.494	0.652	0.398	0.468	0.326	0.380
Mean (Pb)		0.529	0.686	0.414	0.484	0.338	0.392
	0	0.912	0.925	0.579	0.591	0.461	0.474
200	**250**	0.879	0.893	0.564	0.576	0.451	0.463
	500	0.859	0.883	0.555	0.572	0.444	0.459
Mean (Pb)		0.883	0.900	0.566	0.579	0.452	0.465
	0	1.003	1.049	0.641	0.710	0.513	0.542
300	**250**	0.993	1.047	0.620	0.693	0.499	0.541
	500	0.990	1.008	0.620	0.688	0.495	0.540
Mean (Pb)		0.995	1.034	0.627	0.697	0.502	0.541
	0	0.676	0.769	0.468	0.511	0.365	0.386
Mean (CA)	**250**	0.645	0.741	0.456	0.500	0.357	0.380
	500	0.621	0.712	0.448	0.488	0.349	0.374
	Pb	0.062	0.056	0.029	0.030	0.029	0.023
L.S.D. at 0.05	**CA**	0.057	0.042	0.028	0.019	0.022	0.019
	Pb × CA	0.065	0.048	0.031	0.022	0.024	0.022

c. Transfer factor (TF) of heavy metals:

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

Data presented in Table (9) showed that the lowest average value of lead content was in untreated soil, while the highest average was observed in soil after the treatment by 300 mg/l lead and 0 mg/l citric acid.

2. Transfer factor to leaves, stem and root:

Data presented in Table (10), showed that the transfer factor (TF) in the dried plant parts of *Lantana camara* plants was steadily increased with increasing lead concentration in irrigation water. Accordingly, the highest lead values in leaves (1.509 and 1.396 mg/l), stem (0.950 and 0.940 mg/l) and root (0.761 and 0.730 mg/l) were recorded plants irrigated with water containing lead concentration of 300 mg/l, whereas the lowest values were for leaves (0.812 and 1.346 mg/l), stem (0.974 and 0.935 mg/l) and root (0.597 and 0.481 mg/l) for plants irrigated with tap water (control).

Data showed also that the transfer factor (TF) in the dried plant parts was steadily reduced with increasing citric acid concentration. Accordingly, the highest lead values in the leaves (1.559 and 1.448 mg/l), stem (0.996 and 0.980 mg/l) and root (0.797 and 0.748 mg/l) were obtained in the leaves of control plants, whereas plants sprayed with the highest citric acid concentration (500 mg/l) had the lowest lead values in the leaves (1.468 and 1.329 mg/l), stem (0.919 and 0.907 mg/l) and root (0.734 and 0.712 mg/l) of the two seasons, respectively.

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 metals. The results

Table 9. Average values of lead content in soil samples as influenced by lead concentrations in irrigation water and foliar application of citric acid on *Lantana camara* in the two seasons of 2018 and 2019.

Treatments		Lead content in soil (mg/kg)			
Lead (mg/l)	Citric acid (mg/l)	2018		2019	
		Before	After	Before	After
	0	0.085	0.204	0.096	0.230
0	**250**	0.098	0.235	0.110	0.264
	500	0.105	0.252	0.118	0.283
	0	0.169	0.405	0.190	0.456
100	**250**	0.178	0.427	0.201	0.482
	500	0.186	0.446	0.209	0.501
	0	0.248	0.595	0.279	0.669
200	**250**	0.254	0.609	0.286	0.686
	500	0.265	0.636	0.298	0.715
	0	0.268	0.643	0.302	0.724
300	**250**	0.275	0.661	0.309	0.741
	500	0.281	0.674	0.316	0.758

Table 10. Means of transfer factor to leaves, stem and roots of *Lantana camara* plants as influenced by lead (Pb), citric acid (CA) and their combinations (Pb × CA) in the two seasons of 2018 and 2019.

Treatments		Transfer factor to leaves (TFL)		Transfer factor to stem (TFS)		Transfer factor to roots (TFR)	
Pb (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019
	0	1.147	1.700	1.102	1.078	0.691	0.556
0	**250**	0.731	1.257	0.953	0.931	0.582	0.469
	500	0.559	1.081	0.869	0.798	0.519	0.420
Mean (Pb)		0.812	1.346	0.974	0.935	0.597	0.481
	0	1.372	1.563	1.054	1.087	0.856	0.879
100	**250**	1.259	1.441	0.978	1.012	0.798	0.819
	500	1.107	1.301	0.892	0.934	0.730	0.758
Mean (Pb)		1.246	1.435	0.974	1.011	0.794	0.818
	0	1.532	1.382	0.973	0.883	0.774	0.708
200	**250**	1.443	1.301	0.926	0.839	0.740	0.674
	500	1.350	1.234	0.872	0.800	0.698	0.641
Mean (Pb)		1.441	1.305	0.923	0.840	0.737	0.674
	0	1.559	1.448	0.996	0.980	0.797	0.748
300	**250**	1.502	1.412	0.937	0.935	0.754	0.730
	500	1.468	1.329	0.919	0.907	0.734	0.712
Mean (Pb)		1.509	1.396	0.950	0.940	0.761	0.730
	0	1.402	1.523	1.031	1.007	0.779	0.722
Mean (CA)	**250**	1.233	1.352	0.948	0.929	0.718	0.673
	500	1.121	1.236	0.888	0.859	0.670	0.632

presented in 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). Other studies with woody plants reported a higher inhibition of root elongation (Dominguez *et al.*, 2009). In particular, Jatropha plants could bioaccumulate and bioconcentrate toxic heavy metals from an aqueous solution (Mohammad *et al.*, 2010) and could be used as phytoremediation candidates in some countries (Juwarkar *et al.*, 2008; Kumar *et al.*, 2008 and Jamil *et al*., 2009).

Plants can tolerate lead either by external exclusion or internal tolerance. By the external exclusion, lead ions are excluded from entering the plant cells and thus lead cannot accumulates 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 plant tissues (Pourrut *et al.,* 2011). Lead can damage the ultrastructures 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).

For the effect of citric acid it is observed that there is a significant increase in all vegetative parameters, chlorophyll content, carbohydrate percentage, significant decrease in lead content in the leaves and roots and decrease in lead content. This may be due to that application of citric acid with any of the concentrations of lead led to a statistically decrease in the uptake of lead. This decrease in uptake of lead in the presence of citric acid resulted in the formation of citric acid–lead complexes that inhibited the uptake (Chen *et al.,* 2003). The decrease in lead uptake helped to overcome the negative effects of lead 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. and (El-Shanhorey *et al*., 2019) on *Senecio cineraria*.

CONCLUSION

Phytoremediation is a new cleanup concept that involves the use of non-edible plants to clean or stabilize contaminated environments. Phytoremediation of metals is the most effective plant-based method to remove pollutants from contaminated areas. This green technology can be applied to remediate the polluted soils without creating any destructive effect of soil structure. Some specific plants, such as woody species, have been proven to have noticeable potential to absorb toxic heavy metals.

We conclude that we can use *Lantana camara* plants as lead phytoremediation plant without spraying with citric acid and if we want to use *Lantana camara* as an ornamental plant and the water irrigation is contaminated with lead, we can spray the plants with citric acid to overcome the negative effect of lead.

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إستجابة نباتات اللانتانا للرش بحمض الستريك لتقليل الأثر الضار للتلوث بالعناصر الثقيلة فى ماء الرى (ج) تأثير الرصاص

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أجريت هذه الدراسة في فرع البحوث بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر خلال الموسمين المتتاليين ٢٠١٨ و ٢٠١٩. وتهدف الدراسة إلى تقييم آثار الري بمياة ملوثة بالرصاص على نمو نباتات اللانتانا المزروعة في تربة رملية، كذلك إمكانية استخدام الرش بحمض الستريك للتغلب على الآثار الضارة للرصاص. ولتحقيق ذلك زرعت شتلات اللانتانا بشكل فردي في أوعية بلاستيكية (قطرها ٢٠ سم) مملوءة ٥ كجم من التربة الرملية. وكانت معاملات مياه الري الملوثة بأربعة تراكيزات من الرصاص وهي صفر، ١٠٠، ٢٠٠، ٣٠٠ مليجرام/لتر. تم رش النباتات شهريا أيضا بحامض الستريك بإستخدام ثلاث تركيزات هي صفر، ٢٥٠، ٥٠٠ مليجرام/لتر في كلا الموسمين. أظهرت نتائج الدراسة أن هناك اختلاف كبير في التفاعل بين تركيزات الرصاص ورش النباتات بحامض الستريك. وقد وجد انخفاض كبير في كافة معاملات الري بالماء الملوث بالرصاص وكذلك لوحظ زيادة كبيرة في معدلات النموالخضري بعد الرش بـ٥٠٠ مليجرام/لتر حمض الستريك. تم الحصول على أعلى قيمة من محتوى الكلوروفيل والكربوهيدرات من النباتات المروية بماء الصنبور والرش بتركيز ٢٥٠ مليجرام/لتر حامض الستريك في حين أن أعلى تركيز من الرصاص في الأوراق و الساق و الجذور من خلال الري بماء ملوث بتركيز ٣٠٠ مليجرام/لتر من الرصاص بدون الرش بحمض الستريك.