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THE USE OF *Senecio cineraria* PLANTS SPRAYED WITH CITRIC ACID FOR LEAD POLLUTION PHYTOREMEDIATION

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ABSTRACT

The present study was carried out at Antoniadis Research Branch, Horticulture Research Institute A.R.C. Alexandria, Egypt during 2017 and 2018 seasons. This study aim to investigate the effect of for levels of lead 0,100, 200 and 300 ppm) in water irrigation and three different concentrations of citric acid (0,250 and 500 ppm) as foliar application on the vegetative growth and chemical composition of *Senecio cineraria*. Moreover, this investigation aim to study the ability of using *Senecio cineraria* as lead phytoremediation.

The results exhibited significant reduction in all vegetative parameters as affected by lead concentration treatments. On the other hand, significant increment in vegetative growth parameters was detected by using 500 ppm citric acid. Concerning chlorophyll and carbohydrates contents, the highest significant values were obtained under tap water irrigation (0 and 0 levels of lead and citric acid).

Moreover,the results showed no significant effect of the combinations (interaction) between lead and citric acid concentrations on vegetative growth parameters. Meanwhile, the results showed, the highest amount of lead sentient in leaves and roots was detected under the treatments combination (300 ppm load 0 citric acid).

Finally, the results also showed, it is possible to use *Senecio cineraria* hands as lead phytoremediation by using citric acid (300 ppm) as foliar application.

***Key words**: Senecio cineraria – Lead – Citric acid - Phytoremediation.*

1.INTRODUCTION

Phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soils. Phytoremediation is the use of plants to clean up a contamination from soils, sediments and water. This technology is environment friendly and potentially cost effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants (Choruk *et al.,* 2006). Plants need trace amounts of heavy metals but their excessive availability may cause plant toxicity (Sharma *et al*., 2006). 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 such as nitrogen assimilation, photosynthesis, respiration, water uptake, and transcription (Kurepa *et al.,* 1997 and Boussama *et al.,* 1999). Lead may inactivate 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 the 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 is a six carbon organic acid, having a central role in citric acid cycle in mitochondria that creates cellular energy by phosphorylative oxidation reactions. 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).

Senecio cineraria var. Dusty miller is a sub-shrub. It is a species in the genus *Senecio* which contains approximately 1562 to 2834 species and belongs to the family *Asteraceae*. This family includes members that have lead hyperaccumulators which reflect their potential to survive and sequester high level of lead in tissues (from several thousands of mg/ kg up to 5% of dry biomass) without exhibiting phytotoxicity (Prasad, 2005). The plant height reaches 40 to 60 cm. It is grown primarily for its attractive silver-gray foliage rather than its yellow flowers. In fact, most gardeners prefer to cut off the flowers to encourage leaf growth. The plant is often grown in formal bedding schemes, but looks equally effective in informal or cottage-style designs. Dusty Miller is a nice addition to a colorful container garden, and makes a nice edging (Christoper, 2003).

The aim of this work was to study the growth of *Senecio cineraria* plants irrigated with lead contaminated water and the possible effect of citric acid spray on alleviating lead pollution stress, and to test *Senecio cineraria* as a phytoremediator plant.

2.MATERIALS AND METHODS

The present study was carried-out at Antoniades Research Branch, Horticulture Research Institute, Agricultural Research Center, Alexandria, Egypt during the two successive seasons of 2017 and 2018.

On 1stApril, homogenous seedlungs of *Senecio cineraria var.* Dusty Miller with average leaf number of 25- 27 were planted individually in plastic pots (14 cm diameter) filled with a mixture of sand and clay1:1(v/v). The physical and chemical properties of the used soil are shown in Table (1) as described by Jackson (1958).

Table (1):The physical and chemical properties of the used mixture soil for the two seasons.

pH	EC ds/m	Cations (meq/l)				Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
8.08	**2.53**	18.20	14.20	23.91	4.49	7.20	21.00	27.10
Soil particles		**Clay**		**Silt**		**Sand**	**Soil texture**	
%		**54.93**		**16.78**		**28.29**	**Clay sandy loam**	

Irrigation treatments started on 1st May in both seasons. Four concentrations of lead (II) acetate ($Pb(CH_3COO)_2$) 0,100,200 and 300 ppm 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 mixture soil at 90%.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 water irrigation for each pot was calculated and presented in Table (2). Every plant received about 36 liters per pot of contaminated water. The field capacity of the mixture soil was determined by the pressure Cooker method at 1/3 atm., as described by Israelsen and Hansen (1962). The plants were also sprayed with citric acid at concentrations of 0, 250 and 500 ppm, monthly from 1st June till the 1st September (Four times per season). Control plants were sprayed with tap water. The plants were collected on 1st November in both seasons.

The plants received NPK requirements using a fertilizer (Milagro Amino leaf 20-20-20) at the rate of 2g/ pot. Fertilization was repeated every 30 days throughout the growing season (from 15thMay till 15thSeptember). In addition, all other agricultural practices were performed as usual.

Data recorded :

(1) The following vegetative growth parameters recorded: number of leaves per plant, leaves area (cm^2) according to Koller (1972), leaf dry weight per plant (g), tillers number per plant, stem height (cm), stem diameter (cm), stem dry weight (g), root length (cm) and root dry weight (g).

(2) Chemical analysis determination:

Total chlorophylls content were determined as a SPAD unites from the fresh leaves of the 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 carbohydrates percentage in the dry leaves was determined according to Dubios *et al.* (1956).

Determination of lead content: Plant samples were divided into leaves and roots. They were then dried at 72°C in an oven until completely dried. The dried plant samples were ground to powder. Element extraction was done according to Piper (1947) method and the concentration of heavy metal

Table (2): Total amount of water used for each plant (L/pot) in each treatment during the growing two seasons of 2017 and 2018.

Field Capacity (%)	Months of the first and second seasons						
	May	**June**	**July**	**August**	**September**	**October**	**Total**
90 %	4.50	5.50	6.50	7.50	6.50	5.50	**36.00**

was determined using an atomic absorption spectrophotometer.

Available lead in soil samples were extracted by DPTA solution according to Lindsay and Norvell (1978) and determined by Inductively Coupled Plasma Spectrometry.

Transfer factor (TF) is calculated by the relation: the concentration of metal in the shoots and 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 experimental design was split plot with three replicates. Each replicate contained three plants. The main plot was lead contaminated water concentration, while the subplot was citric acid treatments. 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).

3.RESULTS

3.1. Leaf characteristics:

Data presented in Table (3) showed that plants irrigated with tap water had the highest number of leaves (46.60 and 65.60 leaves per plant), leaves area (850.83 and 942.23 cm^2) and leaves dry weights (7.63 and 11.41 g per plant) in the first and second seasons, respectively. On the other hand, the lowest number of leaves (41.77 and 61.99 leaves per plant), leaves area (706.65 and 828.88 cm^2) and leaves dry weight (7.10 and 10.39 g per plant) were obtained from plants irrigated with water with lead concentration at 300 ppm, in the first and the second seasons, respectively.

Also, the data presented in Table (3) showed that, the different citric acid treatments had a significant effect on *Senecio cineraria* plants. Foliar application of citric acid at 500 ppm increased the number of leaves significantly (47.33 and 67.28 leaves per plant), the leaves area of (946.53 and 1051.50 cm^2) and leaves dry weight (8.20 and 11.78 g per plant) in the first and second seasons, respectively, compared with the control plants that recorded the lowest leaves number (39.83 and 60.78 leaves per plant), leaves area (603.45 and 739.52 m^2) and leaves dry weight (6.43 and 9.94 g per plant) in the two seasons, respectively.

Regarding the interaction between the effect of irrigation using water contaminated with lead and citric acid concentrations on the leaves characteristics, the data in Table (3) showed that the lowest mean values in the leaves number (36.66 and 59.16 leaves per plant), leaves area (569.92 and 675.04m^2) and leaves dry weight (6.21 and 9.58g per plant) in the first and second seasons, respectively, were obtained in the plants irrigated with 300 ppm contaminated lead water and sprayed with tap water, while the highest mean values in the leaves number (48.33 and 68.16 leaves per plant), leaves area (1039.39 and 1100.27m^2) and leaves dry weight (8.69 and 12.95g per plant) were recorded in plants irrigated with water contaminated with at 0 ppm and sprayed with citric acid at 500 ppm, in the first and second seasons, respectively.

3.2. Stem characteristics

Data in Table (4) showed that increasing lead concentration in irrigation water caused a significant reduction in stem characteristics. The highest significant reduction was obtained from the concentration of 300 ppm which gave tillers number (3.21 and 6.71 tillers per plant), stem height (4.63 and 6.49 cm), stem diameter (0.69 and 0.92 cm) and stem dry weight (1.31 and 1.50 g per plant) in the first and second seasons, respectively, compared with the control which gave tillers number (3.82 and 7.11tillers per plant), stem height (5.05 and 7.44 cm), stem diameters (0.80 and 0.99 cm) and stem dry weight (1.71 and 1.95 g per plant) in the first and the second seasons, respectively.

In contrast to the effect of lead treatments, citric acid treatments improved growth of *Senecio cineraria* plants. The highest significant number was obtained from plants sprayed with 500 ppm citric acid which gave tillers number per plant (4.32 and 7.49 tillers per plant), stem height (5.55 and 8.14 cm), stem diameters (0.83 and 1.01 cm) and stem dry weight (2.02 and 2.23 g per plant) in the first and the second seasons, respectively, compared with control value gave tillers number per plant (2.95 and 5.99 tillers per plant), stem height (4.22 and 5.85 cm), stem diameters (0.67 and 0.90 cm) and stem dry weight (1.06 and 1.25 g per plant) in the first and the second seasons, respectively.

Regarding the interaction between the effect of

Table (3): Means of leaves characteristics of *Senecio cineraria* plants as influenced by lead concentrations in water irrigation, foliar application of citric acid and their interaction (Lead ×citric acid) in the two seasons of 2017 and 2018.

Treatments		Number of leaves per plant		Leaves area (cm^2)		Leaves dry weight per plant (g)	
Lead (ppm)	**Citric acid (ppm)**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
0 ppm	**0 ppm**	45.33	62.83	697.44	793.84	6.61	10.12
	250 ppm	46.16	65.83	815.66	932.59	7.59	11.18
	500 ppm	48.33	68.16	1039.39	1100.27	8.69	12.95
Mean (Lead)		**46.60**	**65.60**	**850.83**	**942.23**	**7.63**	**11.41**
100 ppm	**0 ppm**	37.50	60.50	574.83	772.57	6.51	10.09
	250 ppm	43.33	64.16	831.49	919.65	7.36	10.66
	500 ppm	47.83	67.83	979.49	1099.56	8.18	11.56
Mean (Lead)		**42.88**	**64.16**	**795.27**	**930.59**	**7.35**	**10.77**
200 ppm	**0 ppm**	39.83	60.66	571.63	716.64	6.42	9.97
	250 ppm	42.16	63.66	638.42	915.34	7.36	10.38
	500 ppm	45.33	67.83	917.20	1003.85	8.07	11.38
Mean (Lead)		**42.44**	**64.05**	**709.08**	**878.61**	**7.28**	**10.57**
300 ppm	**0 ppm**	36.66	59.16	569.92	675.04	6.21	9.58
	250 ppm	40.83	61.50	699.98	809.30	7.23	10.38
	500 ppm	47.83	65.33	850.06	1002.32	7.87	11.23
Mean (Lead)		**41.77**	**61.99**	**706.65**	**828.88**	**7.10**	**10.39**
Mean (Citric acid)	**0 ppm**	39.83	60.78	603.45	739.52	6.43	9.94
	250 ppm	43.12	63.78	746.38	894.22	7.38	10.65
	500 ppm	47.33	67.28	946.53	1051.50	8.20	11.78
L.S.D. at 0.05	**Lead**	**2.37**	**4.71**	**109.26**	**48.19**	**0.68**	**0.34**
	Citric acid	**2.11**	**3.03**	**65.35**	**36.67**	**0.39**	**0.35**
	Lead × Citric acid	**2.80**	**3.48**	**75.11**	**42.15**	**0.45**	**0.40**

Table (4): Means of stem characteristics of *Senecio cineraria* plants as influenced by Lead concentrations in water irrigation, foliar application of citric acid and their interaction (Lead ×Citric acid) in the two seasons of 2017 and 2018.

Treatments		Tillers number per plant		Stem height (cm)		Stem diameter (cm)		Stem dry weight (g)	
Lead (ppm)	**Citric acid (ppm)**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
0 ppm	**0 ppm**	3.16	5.83	4.41	6.25	0.77	0.92	1.22	1.40
	250 ppm	3.66	7.00	5.08	7.58	0.79	0.95	1.64	1.90
	500 ppm	4.66	8.50	5.66	8.50	0.86	1.10	2.27	2.56
Mean (Lead)		**3.82**	**7.11**	**5.05**	**7.44**	**0.80**	**0.99**	**1.71**	**1.95**
100 ppm	**0 ppm**	3.00	5.83	4.16	5.83	0.66	0.92	1.11	1.32
	250 ppm	3.50	7.00	4.75	7.08	0.75	0.95	1.53	1.78
	500 ppm	4.66	7.16	5.75	8.25	0.85	1.05	2.18	2.34
Mean (Lead)		**3.72**	**6.66**	**4.88**	**7.05**	**0.75**	**0.97**	**1.60**	**1.81**
200 ppm	**0 ppm**	3.00	6.16	4.25	5.75	0.64	0.90	1.03	1.27
	250 ppm	3.16	6.83	4.66	6.50	0.70	0.94	1.42	1.64
	500 ppm	4.33	7.16	5.66	8.16	0.84	0.96	1.86	2.08
Mean (Lead)		**3.49**	**6.71**	**4.85**	**6.80**	**0.72**	**0.93**	**1.43**	**1.66**
300 ppm	**0 ppm**	2.66	6.16	4.08	5.58	0.61	0.87	0.91	1.03
	250 ppm	3.33	6.83	4.66	6.25	0.68	0.93	1.26	1.52
	500 ppm	3.66	7.16	5.16	7.66	0.80	0.96	1.77	1.97
Mean (Lead)		**3.21**	**6.71**	**4.63**	**6.49**	**0.69**	**0.92**	**1.31**	**1.50**
Mean (Citric acid)	**0 ppm**	2.95	5.99	4.22	5.85	0.67	0.90	1.06	1.25
	250 ppm	3.41	6.91	4.78	6.85	0.73	0.94	1.46	1.71
	500 ppm	4.32	7.49	5.55	8.14	0.83	1.01	2.02	2.23
L.S.D. at 0.05	**Lead**	**0.38**	**0.26**	**0.65**	**0.39**	**0.03**	**0.02**	**0.17**	**0.13**
	Citric acid	**0.39**	**0.27**	**0.34**	**0.27**	**0.03**	**0.06**	**0.13**	**0.20**
	Lead × Citric acid	**0.45**	**0.32**	**0.39**	**0.31**	**0.03**	**0.07**	**0.15**	**0.23**

irrigation using water contaminated with lead and citric acid concentrations on the stem characteristics, the data in Table (4) showed that the lowest mean values in the tillers number per plant (2.66 and 6.16 tiller per plant), stem height (4.08 and 5.58 cm), stem diameter (0.61 and 0.87 cm) and stem dry weight (0.91 and 1.03 g per plant) in the first and the second seasons, respectively, were obtained in plants irrigated with 300 ppm contaminated lead water and sprayed with tap water, while the highest mean values in the tillers number per plant (4.66 and 8.50 tiller per plant), stem height (5.66 and 8.50 cm) stem diameter (0.86 and 1.10 cm) and stem dry weight (2.27 and 2.56 g per plant) in the first and the second season, respectively, were recorded in the plants irrigated with lead contaminated water at 0 ppm and sprayed with citric acid at 500 ppm.

3.3. Root characteristics

Data presented in Table (5) showed that the tested lead concentrations in water irrigation significantly decreased the root characteristics of *Senecio cineraria*, compared with the plants irrigated with tap water (control). Plants irrigated with tap water had the highest mean root length of (12.58 and 18.24 cm) and root dry weight of (2.02 and 3.19 g per plant) in the first and the second seasons, respectively, while the lowest root length of 11.72 cm in the first season and 17.08 cm in the second season were obtained from plants irrigated by the highest lead concentration 200 and 300 ppm and root dry weight of (1.87 and 2.84 g per plant) in the first and the second seasons, respectively, were obtained from plamts treated with lead at 300 ppm.

Data in Table (5) indicated that citric acid treatments had a significant effect on the root characteristics. Plants sprayed with citric acid at 500 ppm gave the tallest root length of (13.37 and 19.56 cm) and root dry weight of (2.16 and 3.39 g per plant) in the first and the second seasons, respectively, compared with the control (10.93 and 15.95 cm) and root dry weight of (1.66 and 2.66 g per plant) in the first and the second seasons, respectively.

Regarding the interaction between the effect of irrigation using water contaminated with lead and citric acid concentrations on the root characteristics, the data in Table (5) showed that the lowest mean values in the root length of (10.83 and 15.25 cm), and root dry weight (1.61 and 2.40 g per plant) in the first and second seasons, respectively, were obtained for plants irrigated with 300 ppm contaminated lead water and sprayed with tap water, while the highest mean values in the root length of (13.83 and 20.00 cm) and root dry weight (2.19 and 3.53 g per plant) in the first and the

Table (5): Means of root characteristics of *Senecio cineraria* plants as influenced by Lead concentrations in water irrigation, foliar application of citric acid and their interaction (Lead ×citric acid) in the two seasons of 2017 and 2018.

Treatments		Root length (cm)		Root dry weight (g)	
Lead (ppm)	**Citric acid (ppm)**	**2017**	**2018**	**2017**	**2018**
0 ppm	**0 ppm**	11.08	16.41	1.78	2.93
	250 ppm	12.83	18.33	2.07	3.13
	500 ppm	13.83	20.00	2.19	3.53
Mean (Lead)		**12.58**	**18.24**	**2.02**	**3.19**
100 ppm	**0 ppm**	11.00	16.16	1.67	2.78
	250 ppm	12.66	17.66	1.99	3.06
	500 ppm	13.66	19.83	2.18	3.47
Mean (Lead)		**12.44**	**17.88**	**1.95**	**3.10**
200 ppm	**0 ppm**	10.83	16.00	1.61	2.54
	250 ppm	11.50	17.33	1.90	3.06
	500 ppm	12.83	19.75	2.15	3.37
Mean (Lead)		**11.72**	**17.69**	**1.88**	**2.99**
300 ppm	**0 ppm**	10.83	15.25	1.61	2.40
	250 ppm	11.58	17.33	1.87	2.94
	500 ppm	13.16	18.66	2.14	3.20
Mean (Lead)		**11.85**	**17.08**	**1.87**	**2.84**
Mean (Citric acid)	**0 ppm**	10.93	15.95	1.66	2.66
	250 ppm	12.14	17.66	1.95	3.04
	500 ppm	13.37	19.56	2.16	3.39
L.S.D. at 0.05	**Lead**	**0.50**	**1.58**	**0.09**	**0.17**
	Citric acid	**0.60**	**1.30**	**0.11**	**0.09**
	Lead × Citric acid	**0.68**	**1.49**	**0.12**	**0.10**

Table (6): Means of the chemical characteristics of *Senecio cineraria* plants as influenced by Lead concentrations in water irrigation, foliar application of citric acid and their interaction (Lead ×citric acid) in the two seasons of 2017 and 2018.

Treatments		Total chlorophyll content (SPAD)		Total carbohydrates percentage (%)		Lead content in leaves (ppm)		Lead content in stem (ppm)		Lead content in roots (ppm)	
Lead (ppm)	**Citric acid (ppm)**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
0 ppm	**0 ppm**	73.70	78.10	9.57	9.55	0.141	0.171	0.109	0.132	0.019	0.024
	250 ppm	71.90	72.82	9.60	9.67	0.123	0.127	0.092	0.095	0.013	0.012
	500 ppm	70.25	71.63	9.07	9.02	0.082	0.088	0.060	0.066	0.004	0.008
Mean (Lead)		**71.95**	**74.18**	**9.41**	**9.41**	**0.115**	**0.128**	**0.087**	**0.097**	**0.012**	**0.014**
100 ppm	**0 ppm**	63.58	64.48	8.52	8.50	0.309	0.311	0.286	0.289	0.116	0.120
	250 ppm	67.70	69.03	8.84	8.80	0.283	0.274	0.236	0.233	0.067	0.072
	500 ppm	66.58	67.15	8.67	8.66	0.146	0.165	0.122	0.137	0.033	0.036
Mean (Lead)		**65.95**	**66.88**	**8.67**	**8.65**	**0.246**	**0.250**	**0.214**	**0.219**	**0.072**	**0.076**
200 ppm	**0 ppm**	57.85	58.27	7.55	7.53	0.461	0.482	0.399	0.419	0.133	0.142
	250 ppm	61.32	62.13	7.95	7.92	0.335	0.348	0.285	0.305	0.089	0.098
	500 ppm	60.38	60.97	7.78	7.76	0.227	0.251	0.179	0.198	0.038	0.043
Mean (Lead)		**59.85**	**60.45**	**7.76**	**7.73**	**0.341**	**0.360**	**0.287**	**0.307**	**0.086**	**0.094**
300 ppm	**0 ppm**	46.38	47.23	6.78	6.72	0.567	0.620	0.481	0.543	0.151	0.191
	250 ppm	52.65	53.85	7.13	7.09	0.370	0.395	0.317	0.337	0.102	0.107
	500 ppm	53.83	56.72	7.03	6.91	0.197	0.206	0.169	0.177	0.053	0.055
Mean (Lead)		**50.95**	**52.60**	**6.98**	**6.90**	**0.378**	**0.407**	**0.322**	**0.352**	**0.102**	**0.117**
Mean (Citric acid)	**0 ppm**	60.37	62.02	8.10	8.07	0.369	0.396	0.318	0.345	0.104	0.119
	250 ppm	63.39	64.45	8.38	8.37	0.277	0.286	0.232	0.242	0.067	0.072
	500 ppm	62.76	64.11	8.13	8.08	0.163	0.177	0.132	0.144	0.032	0.035
L.S.D. at 0.05	**Lead**	**1.18**	**0.68**	**0.07**	**0.03**	**0.006**	**0.009**	**0.005**	**0.006**	**0.003**	**0.002**
	Citric acid	**0.91**	**0.89**	**0.03**	**0.06**	**0.003**	**0.005**	**0.003**	**0.004**	**0.002**	**0.002**
	Lead×Citric acid	**1.04**	**1.02**	**0.03**	**0.06**	**0.003**	**0.006**	**0.003**	**0.005**	**0.0008**	**0.0007**

second season, respectively, were recorded in the plants irrigated with lead water at 0 ppm and sprayed with citric acid at 500 ppm.

3.4. Chemical analysis

3.4.1. Total chlorophylls content (SPAD) units

The results presented in Table (6) showed that the highest content of total chlorophylls was obtained in plants irrigated with tap water (71.95 and 74.18 SPAD) in the first and the second seasons, respectively. Increasing lead concentration in irrigation water resulted in steady significant reductions in the total chlorophylls content, which reached its lowest values after treatment with 300 ppm (50.95 and 52.60 SPAD) in the first and the second seasons, respectively.

Moreover, Table (6) illustrated that citric acid treatments had a clear positive effect on the total chlorophylls content. Mean values ranged from (60.37 and 62.02 SPAD) in the first and second seasons, respectively, in plants sprayed with 0 ppm citric acid to (63.39 and 64.45 SPAD) in plants sprayed with 250 ppm citric acid in the first and the second seasons, respectively.

Data in Table (6) clearly showed that a significant interaction was detected between the effects of plants irrigated with lead contaminated water and citric acid treatments. The highest total chlorophylls contents (73.70 and 78.10 SPAD) in the first and the second seasons, respectively, were formed by plants irrigated with tap water and sprayed with citric acid at 0 ppm. On the other hand, the lowest chlorophyll content (46.38 and 47.23 SPAD) were recorded in the first and the second seasons, respectively, for plants irrigated by 300 ppm contaminated water combined with 0ppm citric acid treatment.

3.4.2. Total carbohydrates percentage (%)

Table (6) showed that, the percentage of total carbohydrates in dried leaves of *Senecio cineraria* plants was decreased steadily with increasing lead concentration in the irrigation water. The highest percentages of carbohydrates were (9.41 and 9.41 %) in the first and the second seasons, respectively, were found in the control plants, whereas the lowest mean values (6.98 and 6.90 %) in the first and the second seasons, respectively, were found in plants irrigated with 300 ppm lead concentration in water.

Table (6) showed that citric acid treatments had a clear positive effect on the percentage of total carbohydrates. Among the plants receiving the different citric acid treatments, plants sprayed with 250 ppm citric acid had the highest carbohydrates percentage in their leaves of (8.38 and 8.37 %) in the first and the second seasons, respectively, compared with the control (8.10 and 8.07 %) in the first and the second seasons, respectively.

Concerning the interaction effect between irrigation with lead contaminated water and citric acid treatments on the percentage of carbohydrates in leaves, Table (6) showed that the highest percentages of total carbohydrates (9.60 and 9.67%) were formed by plants irrigated with tap water and sprayed with citric acid at 250 ppm. On the other hand, the lowest percentages of total carbohydrates were (6.78 and 6.72%) in the first and the second seasons, respectively, were obtained on plants irrigated by the highest lead concentration at 300 ppm combined with citric acid at 0 ppm.

3.4.3. Lead content in leaves, stem and root (ppm)

The results presented in Table (6) showed that lead content in *Senecio cineraria* plants was increased steadily with increasing lead concentration in the irrigation water. The lowest lead content in leaves of (0.115 and 0.128 ppm), stem (0.087 and 0.097 ppm) and root (0.012 and 0.014 ppm) in the first and the second seasons, respectively, were found in the control plants, whereas the highest contents in leaves were (0.378 and 0.407 ppm), stem (0.322 and 0.352 ppm) and root (0.102 and 0.117 ppm) in the first and the second seasons, respectively, were found in plants irrigated with water containing the highest lead concentration of 300 ppm.

Table (6) illustrated that plant content of lead was slightly reduced by spraying the plants with 500 ppm citric acid which gave a lead content in leaves of (0.163 and 0.177 ppm), stem (0.132 and 0.144 ppm) and root (0.032 and 0.035 ppm) in the first and second seasons, respectively, while the highest values in leaves of (0.369 and 0.396 ppm), stem (0.318 and 0.345 ppm) and root (0.104 and 0.119 ppm) in the first and the second seasons, respectively were recorded for plants unsprayed with citric acid.

For the interaction effects between plant irrigation with lead contaminated water and citric acid treatments data presented in Table (6) showed that the highest lead values in leaves were (0.567 and 0.620 ppm), stem (0.481 and 0.543 ppm) and root (0.151 and 0.191 ppm) in the first and the second seasons, respectively, were obtained from plants irrigated with lead contaminated water at 300 ppm and unsprayed with citric acid. On the other hand, the lowest values in leaves were (0.082 and 0.088 ppm), stem (0.060 and 0.066 ppm) and root (0.004 and 0.008 ppm) in the first and the second seasons, respectively, were obtained from plants irrigated with tap water and sprayed with citric acid at 500 ppm.

3.4.4.. Lead content in soil samples (ppm)

Data in Table (7) showed that the lowest average of lead content was observed in untreated soil, while the highest average of lead content was observed in soil reprieving 300 ppm lead and 500 ppm citric acid.

Table (7): Average of lead content in soil samples as influenced by lead concentrations in irrigation water and foliar application of citric acid on *Senecio cineraria* leaves at the end of both seasons (2017 and 2018).

Treatments		Lead content in soil (ppm)	
Lead (ppm)	**Citric acid (ppm)**	**2017**	**2018**
0	**0**	1.423	1.546
	250	1.546	1.665
	500	1.609	1.732
100	**0**	1.584	1.705
	250	1.665	1.788
	500	1.738	1.862
200	**0**	1.696	1.819
	250	1.768	1.883
	500	1.850	1.977
300	**0**	1.785	1.908
	250	1.888	2.011
	500	1.945	2.066

3.4.5. Transfer factor to leaves (TFL), stem (TFS) and root (TFR)

From the data presented in Table (8), it can be seen that the transfer factor in *Senecio cineraria* plants was decreased steadily with increasing the lead concentration in the irrigation water. Accordingly, the lowest lead value in the leaves (0.075 and 0.078), in the stems (0.057 and 0.059) and in the root the (0.007 and 0.008) in the first and second seasons, respectively, were found in plants irrigated with water containing lead concentration 0 ppm, whereas the highest value in the leaves (0.204 and 0.206), in the stem the (0.174 and 0.178) and in the roots (0.054 and 0.059) were found in plants irrigated with water containing lead concentration at 300 ppm, in the first and the second seasons, respectively.

The results in Table (8) also showed that the transfer factor was reduced steadily with increasing citric acid concentration. Accordingly, the highest lead value in the leaves (0.220 and 0.220), in the stems (0.190 and 0.191) and in the roots (0.062 and 0.065) in the first and the second seasons, respectively, were recorded for the control plants, whereas the plants sprayed with the highest citric acid concentration 500 ppm had the lowest lead value in the leaves (0.0.089 and 0.090), in the stem (0.072 and 0.074) and in the roots (0.016 and 0.017) in the first and the second seasons, respectively.

Regarding the interaction between the effect of irrigation using water contaminated with lead and citric acid concentrations on the transfer factor, the data in Table (8) showed that the highest mean values in the dried leaves (0.317 and 0.324), in the stems (0.269 and 0.284) and in the roots (0.084 and 0.099) were obtained in plants irrigated with 300 ppm contaminated lead water and sprayed with tap water, in the first and the second seasons, respectively, while the lowest mean values in the leaves (0.050 and 0.050), in the stems (0.037 and 0.038) and in the roots (0.002 and 0.004were recorded in plants irrigated with lead contaminated water at 0 ppm and sprayed with citric acid at 500 ppm,) in the first and second season, respectively.

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

Table (8): Means of transfer factor to leaves, stems and roots of *Senecio cineraria* plants as influenced by Lead, citric acid and their combinations (Lead × Citric acid) in the two season 2018.

Treatments		Transfer factor to leaves (TFL)		Transfer factor to stem (TFS)		Transfer factor to roots (TFR)	
Lead (ppm)	Citric acid (ppm)	2017	2018	2017	2018	2017	2018
000	0	0.098	0.110	0.076	0.084	0.013	0.014
	250	0.079	0.075	0.059	0.057	0.008	0.006
	500	0.050	0.050	0.037	0.038	0.002	0.004
Mean (Lead)		**0.075**	**0.078**	**0.057**	**0.059**	**0.007**	**0.008**
100	0	0.194	0.182	0.180	0.169	0.073	0.070
	250	0.169	0.153	0.141	0.130	0.039	0.040
	500	0.084	0.088	0.070	0.073	0.018	0.018
Mean (Lead)		**0.149**	**0.141**	**0.130**	**0.124**	**0.043**	**0.042**
200	0	0.271	0.264	0.235	0.230	0.078	0.078
	250	0.189	0.184	0.161	0.161	0.050	0.052
	500	0.122	0.126	0.096	0.100	0.020	0.021
Mean (Lead)		**0.194**	**0.191**	**0.164**	**0.163**	**0.049**	**0.050**
300	0	0.317	0.324	0.269	0.284	0.084	0.099
	250	0.195	0.196	0.167	0.167	0.053	0.052
	500	0.101	0.099	0.086	0.085	0.027	0.026
Mean (Lead)		**0.204**	**0.206**	**0.174**	**0.178**	**0.054**	**0.059**
Mean (Citric acid)	0	0.220	0.220	0.190	0.191	0.062	0.065
	250	0.158	0.152	0.132	0.128	0.037	0.037
	500	0.089	0.090	0.072	0.074	0.016	0.017
L.S.D. at 0.05	Lead	**0.0039**	**0.0050**	**0.0030**	**0.0034**	**0.0016**	**0.0016**
	Citric acid	**0.0019**	**0.0033**	**0.0020**	**0.0027**	**0.0014**	**0.0012**
	Lead×Citric acid	**0.0007**	**0.0038**	**0.0007**	**0.0009**	**0.0004**	**0.0004**

Concerning the treatments and the control samples, 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 translocate 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 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 which led to a statistical 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.

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

CONCLUSIONS

The concentrations of heavy metals increase in the environment from year to year. Therefore decontamination of heavy metal-contaminated water and soils is very important for maintenance of environmental health and ecological restoration. Phytoremediation is a new cleanup concept that involves the use of 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 some ornamental species, have been proven to have noticeable potential to absorb toxic heavy metals.

5. REFERENCES

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

Blaylock M.J. and Huang J.W. (2000). Phytoextraction of Metals. Phytoremediation of Toxic Metals: Using Plants to Clean up the environment. Raskin, I. and B.D. Ensley. (Eds.) (Eds.) John Wiley and Sons, Inc, Toronto,Canada, p. 303.

Boussama N., Ouariti, A., Suzuki A. and Ghorbal, M.H. (1999). Cd-stress on nitrogen assimilation. J. Plant Physiol., 155, 310-317.

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

Chen Y.X., Lin Q., Luo Y.M., He Y.F., Zhen S.J., Yu Y.L., Tian G.M. and Wong M.H. (2003). The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere, 50:507–811.

Choruk K., Kurukote J., Supprung P. and Vetayasuporn S. (2006). Perennial plants in the phytoremediation of lead-contaminated soils. Biotech., 5 (1):1-4.

Christoper B. (2003). RHS A-Z Encyclopedia of Garden Plants. 3rd ed. Dorling Kindersley, London.UK.,ISBN 0-7513-3738-2.

Cuypers A., Vangronsveld J. and Clijsters H. (1999). The chemical behaviour of heavy metals plays a prominent role in the induction of oxidative stress. Free Radical Res. 31, 539-543.

Da Silva J.A.T. (2003). The cut flower: Postharvest considerations, J. Biol. Sci., 3(4): 406-442.

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

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

Jaafari N. and Hadavi E. (2012). "Growth and essential oil yield of basil (*Ocimum basilicum* L.) as affected by foliar spray of citric acid and salicylic acid. Zeitschrift fur Arznei- und Gewurzpflanzen, 17 (2): 80–83.

Jackson N.L. (1958). Soil Chemical Analysis. Constable. Ltd. Co., London, UK, 498 p.

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

Kurepa J., Van Montagu M. and Inze D. (1997). Expression of *sodCp* and *sodB* genes in *Nicotiana tabacum*: Effects of light and copper excess. J. Exp. Bot., 48, 2007-2014.

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. Appli. Sci., 1:72-109.

Malecka A., Jarmuszkiewicz V. and Tomaszewska B. (2001). Antioxidative defense to lead stress in subcellular compartments of pea root cells. Acta Biochem. (3):687–698.

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

Piotrowska A. A., Bajguz B. Godlewska-Zylkiewicz 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.

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.

Prasad K.V.S.K., Paradha S. P. and Sharmila P. (1999). Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environ. Exp. Bota. 42, 1-10.

Prasad M.N.V. (2005). Cadmium ophilous plants and their significance in phytotechnologies. Braz. J. Plant Physiol., 17: 113-128.

Rauser W.R. (1995). Phytochelatins and related peptides, structure, biosynthesis and function. Plant Physiol., 109, 1141-1149.

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 9.0.38. Cary, NC27513, 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. (1989). Statistical Methods, 8th ed. Edition, Iowa State University Press, Iowa, USA.

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.): A Comparative Analysis by ANOVA and Structural Equations Modeling. Adv. Agric. Article ID, 147278: 1-6.

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

Unhalekhana U. and Kositanont C. (2008). Distribution of cadmium in soil around zinc mining area. Thai J. of Toxicol., 23:170-174.

Wills R., Lee T., Graham D., McGlasson W. and Hall E. (1981). Post-harvest: an Introduction to the Physiology and handling of fruit and vegetables. Kensington, NSW, New South Wales Univ. Press, Aust.

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

إستخدام نباتات الشيرانيا المرشوشة بحامض الستريك على العلاج النباتى للتلوث بالرصاص

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ملخص

أجريت هذه الدراسة في فرع أنطونيادس للأبحاث ، معهد بحوث البساتين. الإسكندرية ، مصر خلال عامى 2017 و 2018. تهدف هذه الدراسة إلى معرفة تأثير مستويات مختلفة من الرصاص بتركيز 0،100 ، 200 ، 300 جزء في المليون) في ماء الري، بالأضافة إلى ثلاث تركيزات مختلفة من حامض الستريك (0،250 و 500 جزء في المليون) كرش ورقي على النمو الخضري والتركيب الكيميائي لنباتات الشيرانيا. علاوة على ذلك ، يهدف هذا البحث إلى دراسة قدرة استخدام نباتات الشيرانيا كعالج نباتى لتنقية البيئة من العناصر الثقيلة. أظهرت النتائج انخفاضا كبيرا في جميع القياسات الخضرية تحت تأثير معاملات الرصاص. من ناحية أخرى ، تم اكتشاف زيادة كبيرة في قياسات النمو الخضري باستخدام 500 جزء في المليون من حامض الستريك. فيما يتعلق بمحتوى الكلوروفيل والكربوهيدرات ، تم الحصول على أعلى القيم تحت معاملات الري بماء الصنبور(0و0 مستويات من الحمض وحامض الستريك).علاوة على ذلك، أوضحت النتائج عدم وجود تأثير كبير للمجموعات (التفاعل) بين تركيزات الرصاص وتركيزات حامض الستريك على قياسات النمو الخضري. وفي الوقت نفسه ، أظهرت النتائج أنه تم اكتشاف أعلى كمية للرصاص في الجذور والأوراق المستهدفة تحت المعاملة بتركيز (300 جزء في المليون من الحمض الستريك).

أخيرًا ، أظهرت النتائج أيضًا ، أنه من الممكن استخدام نباتات الشيرانيا كعلاج نباتي باستخدام حمض الستريك (300 جزء في المليون) كتطبيق رش ورقي.

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