The Use of *Senecio cineraria* Plants Sprayed with Citric Acid for Cadmium Pollution Phytoremediation

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ABSTRACT

This study was carried out at Antoniades Research Branch, Horticulture Research Institute, A.R.C., Alexandria, Egypt during 2015 and 2016 seasons. The study aim was to investigate the ability of using *Senecio cineraria* plants sprayed with citric acid as cadmium phytoremediation. The effect of four levels of cadmium (0, 100, 200 and 300 mg.L^{-1}) in irrigation water and different concentrations of citric acid foliar application (0, 250 and 500 mg.L^{-1}) and their combinations on the vegetative growth and chemical composition of *Senecio cineraria* plants was studied.

The results showed that for vegetative growth parameters there was no significant difference in the interaction between cadmium concentrations and foliar spray by citric acid. While a significant reduction was observed in all parameters after irrigation with cadmium contaminated water and a significant increase in vegetative growth parameters was observed after 500 mg.L^{-1} citric acid application. For chlorophyll and carbohydrate content the highest significant value was obtained from plants irrigated with tap water and sprayed without citric acid while the highest significant amount of cadmium content in leaves and roots was obtained from the treatment 300 mg.L^{-1} cadmium with no application of citric acid. Also, 300 mg.L^{-1} cadmium combined with 500 mg.L^{-1} citric acid caused higher cadmium content in soil after plantation.

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

INTRODUCTION

Phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. 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 amount of heavy metal but their excessive availability may cause plant toxicity (Sharma *et al*., 2006). Cadmium is a toxic heavy metal that has an environmental concern (Mahler *et al.,* 1981). There are many sources of environmental cadmium pollution, including fuel combustion, industrial sludges, phosphate fertilizers, and mine tailings (Unhalekhana and Kositanont, 2008). Cadmium can be absorbed by the human body through respiration and consumption, and cadmium then accumulates in the liver and kidney, causing acute and chronic symptoms such as nausea, abdominal pain, diarrhea, kidney dysfunction, and osteomalacia (Simmons *et al.*, 2005).

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. Silver groundsel or 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* (Aster Family). This family includes members that have cadmium hyperaccumulators which reflect their potential to survive and sequester high level of cadmium in tissues (from several thousands of mg/ kg up to 5% of dry biomass) without exhibiting phytotoxicity (Prasad, 2005). The plant height reaches from 40 to 60 centimeters. 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 were to study the growth of *Senecio cineraria* plants irrigated with cadmium contaminated water, study the possible effect of citric acid spray on alleviating cadmium pollution stress, *and to test Senecio cineraria* as a phytoremediator plant.

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 2015 and 2016.

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On 1st April, homogenous plants 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).

The irrigation treatments started on 1st May in both seasons. Four concentrations of cadmium acetate dehydrate [(CH3COO)2 Cd.2H2O] 0,100,200 and 300 mg.L^{-1} 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 90KPa.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 Tables (2), every plant received about 36 liters per pot of contaminated water. The field capacity of the sandy soil was determined by the pressure Cooker method at 1/3 atm., as described by Israelsen and Hansen (1962). The plants also were sprayed with citric acid at concentrations of 0, 250, 500 mg.L^{-1}, monthly from 1st June till the 1st September (Four times per season). Control plants were sprayed with tap water. The plants were harvested on 1stNovember 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 15th May till 15th September). 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), leaves 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 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 leaves was determined according to Dubios *et al.*(1956).
- Determination of cadmium 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 was determined using an atomic absorption spectrophotometer.
- Available cadmium 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 of a split plot with three replicates. Each replicate contained three plants. The main plot was cadmium contaminated water concentration, while the subplot was citric acid treatments. The means of the individual factors and their interactions were compared using the "Least Significant Difference (L.S.D.)" test at 5% level of probability according to Snedecor and Cochran (1989).

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

Table 2. Total amount of the water used for each plant (L/pot) in each treatment during the growing two seasons of 2015 and 2016

Field Capacity	Months of first and second seasons						
	May	June	July	August	September	October	Total
90 KPa	4.50	5.50	6.50	7.50	6.50	5.50	36.00

RERSULTS

1. Leaves characteristics:

1.1. Number of leaves per plant

Data presented in Table (3) showed that plants irrigated with tap water had the highest number of leaves 44.60 and 63.60 leaves per plant in the first and second seasons, respectively. On the other hand, the lowest number of leaves was 39.77 and 59.99 leaves per plant in the first and second seasons, respectively, was obtained from plants irrigated with water spiked with cadmium concentration 300 mg.L^{-1}. Concerning the effect of citric acid treatments on the number of leaves, Table (3) showed that citric acid treatment at 500 mg.L^{-1} caused a significant increase of 45.33 and 65.28 leaves per plant in the first and second seasons, respectively, compared with control plants that recorded 37.83 and 58.78 leaves per plant in the two seasons, respectively.

1.2. Leaves area (cm^2)

The results in Table (3) illustrated that irrigation of *Senecio cineraria* plants with cadmium contaminated water decreased the leaves area compared with control in both seasons, where the control had the largest leaves with mean areas of 695.36 and 937.04 cm^2 in the first and second seasons, respectively. The plant leaves area was decreased steadily with raising the cadmium concentration and the smallest leaves with mean areas of 579.83 and 823.69 cm^2 in the first and second seasons, respectively was obtained from plants irrigated with 300 mg.L^{-1} cadmium contaminated water.

Also, the data presented in Table (3) showed that, the different citric acid treatments had a significant effect on leaves area of *Senecio cineraria* plants. Foliar application of citric acid at 500 mg.L^{-1} caused the largest significant plant leaves area with a mean area of 782.70 and 1046.31 cm^2 in the first and second seasons, respectively.

1.3. Leaves dry weight per plant (g)

Data in Table (3) showed that there was a significant decrease in leaves dry weight after the irrigation with cadmium contaminated water. The heaviest dry weights of leaves were 7.28 and 11.06 g per plant in the first and second seasons, respectively, were obtained from plants irrigated with tap water, while lowest leaves dry weight values of 6.75 and 10.04 g per plant in the first and second seasons, respectively, were obtained from plants irrigated with the highest cadmium concentration 300 mg.L^{-1}.

Table 3. Means of Leaves characteristics of *Senecio cineraria* plants as influenced by cadmiu concentrations in irrigation water, foliar application of citric acid and their interaction in the two seasons of 2015 and 2016

Treatments		Number of leaves per plant		Leaves area (cm^2)		Leaves dry weight per plant (g)	
Cadmium(mg.L^{-1})	Citric acid (mg.L^{-1})	2015	2016	2015	2016	2015	2016
	0	43.33	60.83	560.58	788.65	6.26	9.77
0	250	44.16	63.83	671.04	927.40	7.24	10.83
	500	46.33	66.16	854.46	1095.09	8.34	12.59
Mean		44.60	63.60	695.36	937.04	7.28	11.06
	0	35.50	58.50	473.66	767.39	6.16	9.74
100	250	41.33	62.16	704.84	914.46	7.01	10.31
	500	45.83	65.83	790.62	1094.36	7.84	11.21
Mean		40.88	62.16	656.37	925.40	7.00	10.42
	0	37.83	58.66	447.74	711.45	6.07	9.63
200	250	40.16	61.66	515.42	910.15	7.01	10.03
	500	43.33	65.83	767.34	998.66	7.71	11.02
Mean		40.44	62.05	576.83	873.42	6.93	10.22
	0	34.66	57.16	467.64	669.85	5.85	9.23
300	250	38.83	59.50	553.46	804.10	6.88	10.03
	500	45.83	63.33	718.41	997.13	7.52	10.88
Mean		39.77	59.99	579.83	823.69	6.75	10.04
Mean (Citric acid)	0	37.83	58.78	487.40	734.33	6.08	9.59
	250	41.12	61.78	611.19	889.02	7.03	10.30
	500	45.33	65.28	782.70	1046.31	7.85	11.42
L.S.D. 0.05	Cadmium	2.37	4.71	80.93	48.19	0.67	0.34
	Citric acid	2.11	3.03	77.31	36.67	0.39	0.35
	Cadmium × Citric acid	1.44	3.05	19.84	44.66	0.05	0.04

Table (3) cleared that foliar application of citric acid on *Senecio cineraria* plants at 500 mg.L^{-1} caused the highest significant increase in leaves dry weight (7.85 and 11.42 g per plant in the first and second seasons, respectively), compared with the control (6.08 and 9.59 g per plant in the first and second seasons, respectively).

Table (3) indicated that there was no significant difference in the interaction between cadmium concentrations in water and foliar application of citric acid in all leaves characteristics in the two seasons.

2. Stem characteristics

2.1 Tillers number per plant

Data in Table (4) showed that increasing of cadmium concentration in irrigation water caused a significant reduction in tillers number per plant. The highest significant reduction was obtained from the concentration of 300 mg.L^{-1} which gave 1.72 and 5.22 tillers per plant in the first and second seasons, respectively as compared with control which gave 2.33 and 5.61tillers per plant in the first and second seasons, respectively.

In contrast to the effect of cadmium treatments, citric acid treatments improved tillers number per plant of *Senecio cineraria* plants. The highest significant number was obtained from plants sprayed with 500 mg.L^{-1} citric acid which gave 3.32 and 6.49 tillers per plant in the first and second seasons, respectively.

As for the effect of different combinations of cadmium and citric acid treatments the results in Table (4) showed that in the first season there was no significant difference between treatment while in the second one the highest significant number of tillers 7.50 was obtained from the treatment of 0 mg.L^{-1} cadmium and combined with 500 mg.L^{-1} citric acid and the lowest significant number of tillers 4.83 was found after the treatments of 0 mg.L^{-1} cadmium with 0mg.L-1 citric acid and 100 mg.L^{-1} cadmium with 0 mg.L^{-1} citric acid.

2.2. Stem height (cm)

The data recorded in Table (4) show that plants irrigated with cadmium contaminated water significantly decreased stem height. The shortest stem height of 5.13 and 6.99 cm were recorded in the first and second seasons respectively and were obtained from plants irrigated with 300 mg.L^{-1} contaminated water while the tallest stem height was obtained from plants irrigated with tap water 5.55 and 7.94 cm in the first and second seasons, respectively.

On the contrary foliar application of citric acid improved *Senecio cineraria* stem height. The highest significant stem height was obtained from the plants sprayed with 500 mg.L^{-1} which gave 6.05 and 8.64 cm in the first and second seasons, respectively.

2.3. Stem diameter (cm)

Table (4) illustrated that all cadmium concentrations in irrigation water caused significant reduction in stem diameter .The thickest stems was obtained from the untreated control of 0.85 and 1.04 cm in the first and second seasons, respectively while the thinnest stem was obtained from the treatment of 300 mg.L^{-1} cadmium, which gave stem diameters of 0.74 and 0.97 cm in the first and second seasons, respectively.

It is clear from Table (4) that all citric acid treatments caused a significant increase in *Sencio cineraria* stem diameter compared with control plants and the treatment of 500 mg.L^{-1} citric acid caused the thickest stem with mean diameters of 0.88 and 1.07 cm in the first and second seasons, respectively.

2.4. Stem dry weight (g)

Data presented in Table (4) showed that, all cadmium concentrations in irrigation water caused a significant decrease in *Sencio cineraria* stem dry weight in both seasons, the lowest dry weights of stem were 1.48 and 1.68 g per plant in the first and second seasons, respectively, were recorded for plants received the highest cadmium concentration of 300 mg.L^{-1}. While the heaviest mean dry weight of stems were 1.88 and 2.11 g per plant in the first and second seasons, respectively were obtained from plants irrigated with tap water (control).

On the other hand data in Table (4) revealed that foliar application of citric acid on *Sencio cineraria* plants caused a significant increase in stem dry weight, in the both seasons. The plants sprayed with500 mg.L^{-1} citric acid gave the heaviest dry weight of stem 2.19 and 2.41 g per plant in the first and second seasons, respectively.

Data in Table (4) showed that there was no detected significant interaction between the effects of cadmium concentrations in irrigation water cadmium and foliar application of citric acid on stem height, stem diameter and stem dry weight in the both seasons.

3. Root characteristics

3.1. Root length (cm)

Data presented in Table (5) showed that the tested cadmium concentrations in water irrigation significantly decreased the root length (cm) of *Senecio cineraria,* compared with plants irrigated with tap water (control). Plants irrigated with tap water had the highest mean root length of 11.08 and 16.74 cm in the first and second seasons, respectively while lowest root length of 10.35

Table 4. Means of stem characteristics of *Senecio cineraria* plants as influenced by cadmium concentrations in irrigation water, foliar application of citric acid and their interaction in the two seasons of 2015 and 2016

Treatments		Tillers number per plant		Stem height (cm)		Stem diamete (cm)		Stem dry weight (g)	
Cadmium (mg.L^{-1})	Citric acid (mg.L^{-1})	2015	2016	2015	2016	2015	2016	2015	2016
	0	2.16	4.83	4.91	6.75	0.82	0.97	1.39	1.57
0	250	2.66	6.00	5.58	8.08	0.84	1.00	1.81	2.04
	500	3.66	7.50	6.16	9.00	0.91	1.16	2.44	2.72
Mean		2.82	6.11	5.55	7.94	0.85	1.04	1.88	2.11
	0	2.00	4.83	4.66	6.33	0.71	0.97	1.26	1.49
100	250	2.50	6.00	5.25	7.58	0.80	1.00	1.70	1.94
	500	3.66	6.16	6.25	8.75	0.90	1.10	2.35	2.52
Mean		2.72	5.66	5.38	7.55	0.80	1.02	1.77	1.98
	0	2.00	5.16	4.75	6.25	0.69	0.95	1.20	1.43
200	250	2.16	5.83	5.16	7.00	0.75	0.99	1.59	1.81
	500	3.33	6.16	6.16	8.66	0.89	1.01	2.03	2.26
Mean		2.49	5.71	5.35	7.30	0.77	0.98	1.60	1.83
	0	1.66	5.16	4.58	6.08	0.66	0.92	1.08	1.20
300	250	2.33	5.83	5.16	6.75	0.73	0.98	1.43	1.69
	500	2.66	6.16	5.66	8.16	0.84	1.01	1.94	2.15
Mean		2.21	5.71	5.13	6.99	0.74	0.97	1.48	1.68
Mean (Citric acid)	0	1.95	4.99	4.72	6.35	0.72	0.95	1.23	1.42
	250	2.41	5.91	5.28	7.35	0.78	0.99	1.63	1.87
	500	3.32	6.49	6.05	8.64	0.88	1.07	2.19	2.41
L.S.D. 0.05	Cadmium	0.38	0.26	0.65	0.39	0.04	0.03	0.17	0.13
	Citric acid	0.39	0.27	0.34	0.27	0.03	0.06	0.13	0.20
	Cadmium Citric acid	0.05	0.02	0.03	0.02	0.0002	0.0012	0.005	0.013

and 15.58cm in the first and second seasons, respectively was obtained from plants irrigated by the highest cadmium concentration300 mg.L^{-1}.

Data in Table (5) indicated that citric acid treatments had a significant effect on the root length. Plants sprayed with citric acid at 500 mg.L^{-1} gave the tallest root length of 11.87 and 18.06 cm in the first and second seasons, respectively.

3.2. Root dry weight (g)

Data presented in Table (5) showed that plants irrigated with tap water had the heaviest dry weight of roots of 1.83 and 3.02 g per plant in the first and second seasons, respectively. Steady significant reductions in the dry weight of roots were recorded with increasing of cadmium concentration in the irrigation water. The treatment of 300 mg.L^{-1} cadmium caused the lowest mean values of 1.70 and 2.67 g per plant in the first and second seasons, respectively.

Foliar application of citric acid caused a significant increase in *Senecio cineraria* roots dry weight Table (5). The treatment of 500 mg.L^{-1} caused the highest dry roots of 1.99 and 3.22 g per plant in the first and second seasons, respectively.

Also, there was no significant difference in the interaction between the effect of cadmium concentrations and citric acid treatments in root characteristics in both seasons.

4. Chemical analysis

4.1. Total chlorophyll 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 75.03 and 77.26 SPAD units in the first and second seasons, respectively. Increasing cadmium concentration in irrigation water resulted in steady significant reductions in the total chlorophyll content, which reached its lowest values after treatment with 300 mg.L^{-1} 54.03 and 55.68 SPAD in the first and second seasons, respectively.

Moreover, Table (6) illustrated that citric acid treatments had a clear positive effect on the total chlorophyll content. Mean values ranged from 63.45 and 65.10 SPAD in the first and second seasons,

Table 5. Means of root characteristics of *Senecio cineraria* plants as influenced by cadmium concentrations in irrigation water, foliar application of citric acid and their interaction in the two seasons of 2015 and 2016

Treatments		Root length (cm)		Root dry weight (g)	
Cadmium (mg.L^{-1})	Citric acid (mg.L^{-1})	2015	2016	2015	2016
	0	9.58	14.91	1.60	2.75
0	250	11.33	16.83	1.90	2.96
	500	12.33	18.50	2.01	3.36
Mean		11.08	16.74	1.83	3.02
	0	9.50	14.66	1.50	2.61
100	250	11.16	16.16	1.82	2.89
	500	12.16	18.33	2.01	3.30
Mean		10.94	16.38	1.77	2.93
	0	9.33	14.50	1.44	2.37
200	250	10.00	15.83	1.73	2.89
	500	11.33	18.25	1.97	3.21
Mean		10.22	16.19	1.71	2.82
	0	9.33	13.75	1.43	2.23
300	250	10.08	15.83	1.70	2.77
	500	11.66	17.16	1.97	3.03
Mean		10.35	15.58	1.70	2.67
Mean (Citric acid)	0	9.43	14.45	1.49	2.49
	250	10.64	16.16	1.78	2.87
	500	11.87	18.06	1.99	3.22
L.S.D. 0.05	Cadmium	0.50	1.58	0.09	0.17
	Citric acid	0.60	1.30	0.11	0.09
	Cadmium × Citric acid	0.119	0.564	0.004	0.002

respectively, in plants sprayed with 0 mg.L^{-1} citric acid to 66.47 and 67.53 SPAD units in plants sprayed with 250 mg.L^{-1} citric acid in the first and second seasons, respectively.

Data in Table (6) clearly showed that a significant interaction was detected between the effects of plant irrigation with cadmium contaminated water and citric acid treatments. The highest total chlorophyll content 76.78 and 81.18 SPAD units in the first and second seasons, respectively, were formed by plants irrigated with tap water and sprayed with citric acid at 0 mg.L^{-1}. On the other hand, the lowest chlorophylls content 49.46 and 50.31 SPAD were recorded in the first and second seasons, respectively, for plants irrigated by 300 mg.L^{-1} contaminated water combined with zero citric acid treatment.

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 the Cadmium concentration in the irrigation water. The highest percentage of carbohydrates was 12.47 and 12.47 % in the first and second seasons, respectively, was found in control plants, whereas the lowest mean values 10.03 and 9.96% in the first and second seasons, respectively, were found in plants irrigated with 300 mg.L^{-1} cadmium concentration 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 mg.L^{-1} citric acid had the highest carbohydrates percentage in their leaves of 11.43 and 11.42 in the first and second seasons, respectively.

Concerning the interaction effect between irrigation with cadmium contaminated water and citric acid treatments on the percentage of carbohydrates in leaves Table (6) showed that the highest percentage of total carbohydrates was 12. 65 and 12.72% was formed by plants irrigated with tap water and sprayed with citric acid at 250 mg.L^{-1}. On the other hand, the lowest percentage of total carbohydrate was 9.82 and 9.78% in the first and second seasons, respectively, was obtained on plants irrigated by the highest cadmium concentration at 300 mg.L^{-1} combined with citric acid at 0 mg.L^{-1}.

Table 6. Means of the chemical characteristics of *Senecio cineraria* plants as influenced by cadmium concentrations in irrigation water, foliar application of citric acid and their interaction in the two seasons of 2015 and 2016

Treatments		Total chlorophyll content (SPAD)		Total carbohydrates percentage (%)		Cadmium content in leaves ($mg.L^{-1}$)		Cadmium content in stem ($mg.L^{-1}$)		Cadmium content in roots ($mg.L^{-1}$)	
Cadmium($mg.L^{-1}$)	**Citric acid ($mg.L^{-1}$)**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
	0	76.78	81.18	12.63	12.61	0.150	0.180	0.118	0.141	0.028	0.032
0	250	74.98	75.90	12.65	12.72	0.132	0.136	0.101	0.104	0.020	0.021
	500	73.33	74.71	12.13	12.08	0.091	0.097	0.069	0.075	0.013	0.017
Mean		75.03	77.26	12.47	١٢,٤٧	0.124	0.137	0.096	0.106	0.020	0.023
	0	66.66	67.56	11.58	11.56	0.318	0.319	0.295	0.298	0.125	0.128
100	250	70.78	72.11	11.89	11.86	0.292	0.283	0.245	0.242	0.076	0.081
	500	69.66	70.23	11.73	11.72	0.155	0.174	0.131	0.146	0.042	0.045
Mean		٦٩,٠٣	69.96	11.73	11.71	0.255	0.258	0.223	0.228	0.081	0.084
	0	60.93	61.35	10.61	10.59	0.470	0.491	0.408	0.428	0.142	0.151
200	250	64.40	65.21	11.01	10.98	0.344	0.357	0.294	0.309	0.098	0.107
	500	63.46	64.05	10.82	10.81	0.236	0.259	0.188	0.207	0.047	0.052
Mean		62.93	63.53	10.81	10.79	0.350	0.369	0.296	0.314	0.095	0.103
	0	49.46	50.31	9.82	9.78	0.576	0.629	0.490	0.552	0.159	0.200
300	250	55.73	56.93	10.19	10.15	0.379	0.404	0.326	0.346	0.111	0.116
	500	56.91	59.80	10.09	9.97	0.206	0.215	0.178	0.186	0.062	0.064
Mean		54.03	٥٥,٦٨	10.03	9.96	0.387	0.416	0.331	0.361	0.110	0.126
Mean (Citric acid)	0	63.45	65.10	11.16	11.13	0.378	0.404	0.327	0.354	0.113	0.127
	250	66.47	67.53	11.43	11.42	0.286	0.295	0.241	0.250	0.076	0.081
	500	65.84	67.19	11.19	11.14	0.172	0.186	0.141	0.153	0.041	0.044
L.S.D. 0.05	Cadmium	1.18	0.68	0.06	0.03	0.006	0.009	0.004	0.007	0.002	0.003
	Citric acid	0.91	0.89	0.03	0.05	0.003	0.005	0.003	0.004	0.002	0.001
	Cadmium×Citric acid	0.27	0.26	0.0003	0.0011	0.0037	0.0011	0.0032	0.0062	0.0014	0.0001

4.3. Cadmium content in leaves (mg.L^{-1})

The results presented in Table (6) showed that the cadmium content in the leaves of *Senecio cineraria* plants was increased steadily with increasing the cadmium concentration in the irrigation water. The lowest cadmium content of 0.124 and 0.137 mg.L^{-1} in the first and second seasons, respectively, was found in control plants, whereas the highest content was 0.387 and 0.416 mg.L^{-1} in the first and second seasons, respectively, was found in plants irrigated with water containing the highest cadmium concentration of 300 mg.L^{-1}.

Table (6) illustrated that the leaves content of cadmium was **s**lightly reduced by spraying the plants with 500 mg.L^{-1} citric acid which gave a cadmium content of 0.172 and 0.186 mg.L^{-1} in the first and second seasons, respectively while the highest value of 0.378 and 0.404 mg.L^{-1} in the first and second seasons, respectively was recorded for plants unsprayed with citric acid.

For the interaction effects between plant irrigation with cadmium contaminated water and citric acid treatments data presented in Table (6) showed that the highest cadmium values were 0.576 and 0.629 mg.L^{-1} in the first and second seasons, respectively, were obtained from plants irrigated with cadmium contaminated water at 300 mg.L^{-1} and unsprayed with citric acid. On the other hand, the lowest values were 0.091 and 0.097 mg.L^{-1} in the first and second seasons, respectively, were obtained from plants irrigated with tap water and sprayed with citric acid at 500 mg.L^{-1}.

4.4. Cadmium content in stem (mg.L^{-1})

Data in Table (6) indicated that cadmium content in stem of *Senecio cineraria* plants was increased steadily with increasing the cadmium concentration in the irrigation water. The lowest cadmium content of 0.096 and 0.106 mg.L^{-1} in the first and second seasons, respectively, was found in control plants, whereas the highest cadmium content of 0.331 and 0.361 mg.L^{-1} in the first and second seasons respectively was found in plants irrigated with water containing high cadmium concentration at 300 mg.L^{-1}.

Table (6) also showed that the mean of cadmium content in stem was reduced steadily with increasing citric acid concentration. Accordingly, the highest cadmium content of 0.327 and 0.354 mg.L^{-1} in the first and second seasons, respectively, was recorded in the stem of control plants, whereas plants sprayed with the highest citric acid concentration 500 mg.L^{-1} had the lowest cadmium content of 0.141 and 0.153 mg.L^{-1} in the first and second seasons, respectively.

For the interaction between the irrigation with cadmium contaminated water and citric acid concentrations data presented in Table (6) showed that the highest mean values were 0.490 and 0.552 mg.L^{-1} in the first and second seasons, respectively, were obtained from plants irrigated with contaminated water at 300 mg.L^{-1} and sprayed with tap water, while the lowest mean values were 0.069 and 0.075 mg.L^{-1} in the first and second seasons, respectively, were recorded for plants irrigated with tap water and sprayed with citric acid at 500 mg.L^{-1}.

4.5. Cadmium content in roots (mg.L^{-1})

Data in Table (6) indicated that Cadmium content in roots of *Senecio cineraria* plants was increased steadily with raising the Cadmium concentration in the irrigation water. The lowest Cadmium content of 0.020 and 0.023 mg.L^{-1} in the first and second seasons, respectively, was found in control plants, whereas the highest Cadmium content of 0.110 and 0.126 mg.L^{-1} in the first and second seasons respectively was found in plants irrigated with water containing high Cadmium concentration at 300 mg.L^{-1}.

Table (6) also showed that the mean of Cadmium content in root was reduced steadily with raising citric acid concentration. Accordingly, the highest Cadmium content of 0.113 and 0.127 mg.L^{-1} in the first and second seasons, respectively, was recorded in the roots of control plants, whereas plants sprayed with the highest citric acid concentration 500 mg.L^{-1} had the lowest Cadmium content of 0.041 and 0.044 mg.L^{-1} in the first and second seasons, respectively.

For the interaction between the irrigation with cadmium contaminated water and citric acid concentrations data presented in Table (6) showed that the highest mean values were 0.159 and 0.200 mg.L^{-1} in the first and second seasons, respectively, were obtained from plants irrigated with contaminated water at 300 mg.L^{-1} and sprayed with tap water, while the lowest mean values were 0.013 and 0.017 mg.L^{-1} in the first and second seasons, respectively, were recorded for plants irrigated with tap water and sprayed with citric acid at 500 mg.L^{-1}.

3.6. Cadmium content in soil samples (mg.L^{-1})

Data in Table (7) showed that the lowest average of cadmium content was observed in untreated soil, while the highest average of Cadmium content was observed in soil reprieving 300 mg.L^{-1} cadmium and 500 mg.L^{-1} citric acid.

3.7. Transfer factor to leaves (TFL)

From the data presented in Table (8), it can be seen that the transfer factor in the dried leaves of *Senecio* *cineraria* plants was decreased steadily with increasing the cadmium concentration in the irrigation water. Accordingly, the lowest cadmium value (1.162 in the second season) was found in plants irrigated with water containing cadmium concentration 0 mg.L^{-1}, whereas the highest value (3.007 in the second season) was found in plants irrigated with water containing cadmium concentration 300 mg.L^{-1}.

The results in Table (8) also show that the transfer factor in the dried leaves was reduced steadily with increasing citric acid concentration. Accordingly, the highest cadmium value (3.214 in the second season) was recorded in the leaves of control plants, whereas plants sprayed with the highest citric acid concentration 500 mg.L^{-1} had the lowest cadmium value (1.320 in the second season).

Table 7. Average of cadmium content in soil samples as influenced by cadmium concentrations in irrigation water and foliar application of citric acid on *Senecio cineraria* leaves at the end of second season (2016)

Treatments		Cadmium content in soil (mg.L^{-1})
Cadmium(mg.L^{-1})	**Citric acid (mg.L^{-1})**	
0	0	0.111
	250	0.124
	500	0.126
100	0	0.121
	250	0.128
	500	0.139
200	0	0.127
	250	0.138
	500	0.143
300	0	0.133
	250	0.142
	500	0.149

Table 8. Means of transfer factor to leaves, stem and roots of *Senecio cineraria* plants as influenced by cadmium, citric acid and their combinations in the two season 2016

Treatments		Transfer factor to leaves (TFL)	Transfer factor to stem (TFS)	Transfer factor to roots (TFR)
Cadmium (mg.L^{-1})	**Citric acid (mg.L^{-1})**	**2016**	**2016**	**2016**
0	0	1.621	1.270	0.288
	250	1.096	0.841	0.169
	500	0.769	0.600	0.134
Mean		1.162	0.903	0.197
100	0	2.639	2.462	1.060
	250	2.213	1.895	0.632
	500	1.254	1.050	0.323
Mean		2.035	1.802	0.671
200	0	3.868	3.372	1.191
	250	2.586	2.241	0.777
	500	1.813	1.452	0.363
Mean		2.755	2.355	0.777
300	0	4.731	4.152	1.503
	250	2.845	2.436	0.816
	500	1.445	1.248	0.429
Mean		3.007	2.612	0.916
Mean (Citric acid)	0	3.214	2.814	1.010
	250	2.185	1.853	0.598
	500	1.320	1.087	0.312
L.S.D. 0.05	Cadmium	0.069	0.053	0.022
	Citric acid	0.044	0.033	0.013
	Cadmium×Citric acid	0.00062	0.00034	0.000004

Regarding the interaction between effect of irrigation using water contaminated with cadmium and citric acid concentrations on the transfer factor in the dried leaves, the data in Table (8) show that the highest mean values 4.731 in the second season, was obtained in plants irrigated with 300 $mg.L^{-1}$ contaminated cadmium water and sprayed with tap water, while the lowest mean values 0.769 in the second season, was recorded in plants irrigated with cadmium water at 0 $mg.L^{-1}$ and sprayed with ascorbic acid at 500 $mg.L^{-1}$.

3.8. Transfer factor to stem (TFS)

From the data presented in Table (8), it can be seen that the transfer factor in the dried stem of *Senecio cineraria* plants was decreased steadily with increasing the cadmium concentration in the irrigation water. Accordingly, the lowest cadmium value (0.903 in the second season) was found in plants irrigated with water containing cadmium concentration 0 $mg.L^{-1}$, whereas the highest value (2.612 in the second season) was found in plants irrigated with water containing cadmium concentration 300 $mg.L^{-1}$.

The results in Table (8) also show that the transfer factor in the dried stem was reduced steadily with increasing citric acid concentration. Accordingly, the highest cadmium value (2.814 in the second season) was recorded in the stem of control plants, whereas plants sprayed with the highest citric acid concentration 500 $mg.L^{-1}$ had the lowest cadmium value (1.087 in the second season).

Regarding the interaction between effect of irrigation using water contaminated with cadmium and citric acid concentrations on the transfer factor in the dried stem, the data in Table (8) show that the highest mean values 4.152 in the second season, was obtained in plants irrigated with cadmium water at 300 $mg.L^{-1}$ and sprayed with tap water, while the lowest mean values 0.600 in the second season, was recorded in plants irrigated with cadmium water at 0 $mg.L^{-1}$ and sprayed with ascorbic acid at 500 $mg.L^{-1}$.

3.9. Transfer factor to roots (TFR)

From the data presented in Table (8), it can be seen that the transfer factor in the dried roots of *Senecio cineraria* plants was decreased steadily with increasing the cadmium concentration in the irrigation water. Accordingly, the lowest cadmium value (0.197 in the second season) was found in plants irrigated with water containing cadmium concentration 0 $mg.L^{-1}$, whereas the highest value (0.916 in the second season) was found in plants irrigated with water containing cadmium concentration 300 $mg.L^{-1}$.

The results in Table (8) also show that the transfer factor in the dried roots was reduced steadily with increasing citric acid concentration. Accordingly, the highest cadmium value (1.010 in the second season) was recorded in the roots of control plants, whereas plants sprayed with the highest citric acid concentration 500 $mg.L^{-1}$ had the lowest cadmium value (0.312 in the second season).

Regarding the interaction between effect of irrigation using water contaminated with cadmium and citric acid concentrations on the transfer factor in the dried roots, the data in Table (8) show that the highest mean values 1.503 in the second season, was obtained in plants irrigated with cadmium water at 300 $mg.L^{-1}$ and sprayed with tap water, while the lowest mean values 0.134 in the second season, was recorded in plants irrigated with cadmium water at 0 $mg.L^{-1}$ and sprayed with citric acid at 500 $mg.L^{-1}$.

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).

The physiological responses, such as the gas exchange rate and photosynthetic function, can be ascribed to the different effects of physico-chemical properties of heavy metals on the integrity and function of the photochemical apparatus of plant seedling fronds, as well as the impact on the chlorophyll concentrations in the leaves. The photosynthesis rate, CO_2 assimilation rate, and stomatal conductance in response to cadmium heavy metal have been well documented (Chen *et al*., 2012). In this study, cadmium resulted in a significant reduction in the chlorophyll contents, possibly due to the inhibition of chlorophyll biosynthesis or a breakdown of pigments and their precursors (Agrawal and Mishra, 2009). Cadmium might replace the central Mg from chlorophyll molecules and thereby reduce the photosynthetic light-harvesting ability of plant (Agrawal and Mishra, 2009). In contrast, Carotene was less sensitive than Chl a and Chl b in response to both cadmium heavy metals, which probably facilitated the maintenance of photosynthetic apparatus against heavy metal stress (Piotrowska *et al.,* 2010). Carotene stabilized and protected the lipid phase of the thylakoid membrane by serving as the antioxidant to scavenge the free radicals (Polle *et al.,* 1992; Piotrowska *et al.*, 2010).

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 cadmium; 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 cadmium content in leaves and roots and decrease in cadmium content in soil samples. This may be due to that application of citric acid with any of the concentrations of cadmium led to a statistically decrease in the uptake of cadmium. This decrease in uptake of cadmium in the presence of citric acid resulted in the formation of citric acid–cadmium complexes that inhibited the uptake (Chen *et al.,* 2003). The decrease in cadmium uptake helped to overcome the negative effects of cadmium on the previous studied parameters. These results are in agreement with those mentioned by (Talebi *et al*., 2014) on Gazania plants and (Jaafari and Hadavi, 2012) on *Ocimum basilicum* L.

We conclude that we can use *Senecio cineraria* plants as cadmium 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 cadmium we can spray the plants with citric acid to overcome the negative effect of cadmium.

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 woody species, have been proven to have noticeable potential to absorb toxic heavy metals.

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الملخص العربي

استخدام نباتات الشيرانيا المرشوشة بحامض الستريك على العلاج النباتى للتلوث بالكادميوم

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أجريت هذه الدراسة خلال موسمى ٢٠١٥ و ٢٠١٦ في مزرعة الأفرع البحثية بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية، الاسكندرية، مصر. لدراسة استخدام نباتات الشيرانيا المرشوشة بحامض الستريك على علاج التلوث بالكادميوم . تم دراسة اربعة مستويات من الكادميوم (٠ – ١٠٠– ٢٠٠ – ٣٠٠ جزء فى المليون) في ماء الري وثلاثة تركيزات من حمض الستريك (٠ – ٢٥٠– ٥٠٠ جزء في المليون) رشا على الأوراق والتفاعل بينهم على النمو الخضري والمحتوى الكيمائي لنباتات الشيرانيا ومحتوى التربة من الكادميوم بعد الزراعة.

أوضحت النتائج أنه لا يوجد فارق معنوي في التفاعل بين تركيزات الكادميوم والرش بحامض الستريك بينما الري بالماء الملوث بالكادميوم ادى إلى أنخفاض معنوي في كل صفات النمو الخضري وبالنسبة للرش بحامض الستريك اظهرت النتائج أن الرش بحامض الستريك بتركيز ٥٠٠ في المليون ادي إلى زيادة معنوية في النمو الخضري. أعلى محتوى معنوي للنباتات من الكلورفيل والكربوهيدرات تم الحصول عليه من النباتات المروية بماء الصنبور ولم ترش بحامض الستريك .أعلى محتوى للكادميوم في الأوراق والساق والجذور تم الحصول عليه من النباتات المروية بماء يحتوي على ٣٠٠ جزء في المليون كادميوم ولم ترش بحامض الستريك. وكذلك أعلى محتوى للكادميوم فى التربة بعد الزراعة وجد من استخدام ٣٠٠ جزء فى المليون فى ماء الرى.

من النتائج يمكن استخدام نباتات الشيرانيا لتنقية التربة الملوثة بالكادميوم مع استخدام ٥٠٠ جزء فى المليون رشا على النباتات لزيادة أمتصاصها للكادميوم.

كلمات دليلية: الشيرانيا – كادميوم – حمض الستريك – العلاج النباتى للتلوث.