DOI: 10.21608/alexja.2023.249819.1050

The Use of *Gazania rigens* L. Plants Spayed with Citric Acid for Heavy Metals Pollution Phytoremediation: (A) Effect of Lead

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ARTICLE INFO
Article History
Received: 25/11/2023
Revised: 23/12/2023
Accepted: 29/12/2023

Key words:
Gazania rigens; Lead; Citric acid; Phytoremediation.

ABSTRACT

During the 2021 and 2022 growing seasons, this study was conducted at the nursery of the Department of Flowers, Ornamental Plants, and Landscape Gardens, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt. The purpose of the study was to determine whether lead phytoremediation could be accomplished by spraying *Gazania rigens* plants with citric acid and its effects on the vegetative growth and chemical composition of *G. rigens* plants. This study used four levels of lead in irrigation water (0 - 100 - 200 - 300 mg/l) and three concentrations of citric acid (0- 250- 500 mg/l) sprayed on leaves and the interaction between them on vegetative growth, chemical content of Gazanis plants, and soil content were studied. The results showed a considerable difference between lead concentrations and foliar citric acid spray for vegetative growth metrics. While all parameters significantly decreased following irrigation with water containing lead, vegetative growth parameters significantly improved with the application of 500 mg/l citric acid. The highest significant amounts of lead content in leaves and roots were found in plants treated with 300 mg/l lead without the application of citric acid, whereas the highest significant amounts of chlorophyll and carbohydrates were found in plants irrigated with tap water and sprayed without citric acid. A higher lead concentration in the soil after planting was also generated by the combination of 500 mg/l citric acid and 300 mg/l lead.

INTRODUCTION

In order to extract or eliminate metal contaminants from polluted soil, phytoremediation has emerged as an efficient and reasonably priced technological alternative. Phytoremediation, an environmentally beneficial and possibly cost-effective approach, uses plants to remove contaminants from soils, sediments, and water. hyperaccumulator plants are those that have a high capability for accumulating metals (Choruk *et al.*, 2006). Heavy metals are necessary for plants in trace amounts, but their overabundance can be hazardous to plants (Sharma *et al.*, 2006). According to Mahler *et al.* (1981), lead is a hazardous heavy metal that poses a threat to the environment. Fuel combustion, industrial wastes, phosphate fertilisers, and mine tailings are just a few of the sources of environmental lead pollution (Unhalekhana and Kositanont, 2008).

One of the biggest contributors of environmental pollution is lead. According to numerous research (Kurepa *et al.*, 1997; Boussama *et al.,* 1999), lead suppresses metabolic activities such nitrogen absorption, photosynthesis, respiration, water uptake, and transcription. Lead can inhibit a number of enzymes by attaching to their SH-groups (Rauser, 1995), and it can speed up the processes that produce reactive oxygen species (ROS), which cause oxidative stress (Cuypers *et al.*, 1999; Prasad *et al.,* 1999). Lead can also have a deleterious impact on mitochondrial structure by reducing the amount of mitochondrial cristae, which can therefore reduce oxidative phosphorylation capacity (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).

A species of flowering plant belonging to the Asteraceae family is called *Gazania rigens* L. It is a group of 16 species of herbal plants that are all indigenous to South Africa. It can be grown as a perennial plant in temperate climates and planted annually in frigid climates. Due to its decorative and therapeutic qualities, this plant has recently become quite appealing to home producers. Although it is the source of just a small number of items, it is quite significant in both the economy and medicine (Vujosevic *et al.* 2007, Youssef Moustafa *et al.* 2007). Capitula are vividly coloured in orange, yellow, and red. White, blue, brown, and black are among the brilliant or dark hues that surround the bases of the petals. Ongoing flower blooming.

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

MATERIALS AND METHODS

The current study was conducted over the course of the two succeeding growing seasons of 2021 and 2022 at the nursery of the Department of Flowers, Ornamental Plants, and Landscape Gardens, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt.

On April 1st, homogeneous seedlings of *Gazania rigens* with an average leaf number of 25–27 each were planted in plastic pots with a 14 cm diameter that were half filled with sand and half with clay. According to Jackson (1958), the physical and chemical characteristics of the utilized soil are displayed in Table (1).

In both seasons, irrigation treatments began on May (1). There were four different lead (II) acetate concentrations used: 0, 100, 200, and 300 mg/l. Three irrigation sessions per week were utilized to water the plants, with one irrigation level being employed to maintain 100% soil moisture at the field capacity of the mixed soil. During the growth season, the Moisture Tester Model KS-DI (Gypsum Block) was used to measure the decrease in moisture levels. Each plant got approximately 39 liters of polluted water per pot at the conclusion of the experiment, which was computed and given in Tables (2). According to Israelsen and Hansen (1962), the pressure Cooker method at 1/3 atm was used to calculate the field capacity of the combination soil.

Using a fertilizer (Milagro Amino leaf 20-20-20) at a rate of 2g/pot, the plants' NPK requirements were met. Every 30 days during the growing season (from 15 May to 15 September), fertilization was repeated. All other agricultural procedures were also carried out as usual.

Data gathered:

(1) The following vegetative growth characteristics were measured: tillers per plant, number of leaves per plant, leaves area (cm^2) as calculated by Koller (1972), leaves dry weight per plant (g), stem height (cm), stem diameter (cm), stem dry weight (g), root length (g), and root dry weight (g).

(2) Chemical analysis determination:

- According to Yadava (1986), total chlorophyll content was measured using a Minolta (chlorophyll metre) SPAD 502 at the conclusion of the season for the various treatments included in the experiment.
- The percentage of total carbohydrates in the dried leaves was calculated based on Dubios *et al.* (1956).
- The proline content (mg/g) of the leaves was calculated based on Bates *et al.* (1973).
- Lead content determination. Roots, stems, and leaves were separated from plant samples. They were then fully dried in an oven at 70 °C. The samples of dried plants were pulverized into a powder. The Piper (1947) method was used to extract the elements, and an atomic absorption spectrophotometer was used to measure the concentration of heavy metals.
- 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.

Three replicates were used in the split plot experimental design. Three plants were present in each replication. The concentration of lead-contaminated water served as the main narrative, while treatments using citric acid served as the subplot. ANOVA was performed on the data using the SAS programme from the SAS Institute (SAS Institute, 2002). According to Snedecor and Cochran (1989), the Means of the individual components and their interactions were compared using the LSD test at a 5% level of probability.

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^{--}
8.15	2.57	18.20	14.20	23.91	4.49	7.30	21.10	27.20

Soil particles	Clay	Silt	Sand	Soil texture
%	50.90	19.65	29.45	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 2021 and 2022.

Field Capacity (%)	Months of the first and second seasons						
	May	June	July	August	September	October	Total
100 %	5.0	6.0	7.0	8.0	7.0	6.0	39.00

RESULTS

1. Leaves characteristics:

The information in Table 3 demonstrated that in the first and second seasons, respectively, plants that were irrigated with tap water had the greatest number of leaves (58.60 and 87.16 leaves per plant), leaves area (518.77 and 1100.63 cm^2), and leaves dry weights (8.71 and 12.50 g/plant). Conversely, plants watered with water containing 300 mg/l of lead had the lowest leaf counts (51.33 and 81.83 leaves per plant), lowest leaf areas (430.80 and 946.28 cm^2), and lowest leaf dry weights (8.18 and 11.47 g/plant) in the first and second seasons, respectively.

Additionally, data in Table 3 demonstrated that *Gazania rigens* plants were significantly impacted by the various citric acid treatments. In comparison to control plants, which recorded the lowest number of leaves (48.41 and 79.87 leaves per plant), leaves area (371.90 and 891.35 m^2), and leaves dry weight (7.51 and 11.02 g/plant) in the two seasons, respectively, foliar application of citric acid at 500 mg/l caused a significant increase in the number of leaves (59.66 and 89.62 leaves per plant), the leaves area (561.73 and 1164.04 cm^2), and leaves dry weight (9,28 and 12.86 g/plant) in the first and second seasons, respectively.

Regarding the interaction between the effects of irrigation with water contaminated with lead and citric acid concentrations on the characteristics of the leaves, the data in Table 3 showed that in the first and second seasons, respectively, the plants irrigated with 300 mg/l contaminated lead water and sprayed with tap water had the lowest mean values in the number of leaves (43.66 and 77.50 leaves/ plant), leaves area (329.94 and 834.89 m^2), and leaves dry weight (7.29 and 10.66 g/plant).

Table 3. Means of leaves characteristics of *Gazania rigens* 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 2021 and 2022.

Treatments		Number of leaves per plant		Leaves area (cm^2)		Leaves dry weight per plant (g)	
Lead (mg/l)	Citric acid (mg/l)	2021	2022	2021	2022	2021	2022
0	0	56.66	83.00	445.55	933.28	7.69	11.20
	250	58.00	87.66	505.55	1076.12	8.68	12.27
	500	61.16	90.83	605.21	1292.51	9.77	14.03
Mean (Lead)		58.60	87.16	518.77	1100.63	8.71	12.50
100	0	44.83	79.33	345.35	891.68	7.59	11.18
	250	53.83	85.00	458.65	1011.69	8.46	11.74
	500	60.33	90.33	564.85	1149.45	9.27	12.64
Mean (Lead)		52.99	84.88	456.28	1017.60	8.44	11.85
200	0	48.50	79.66	366.79	905.56	7.50	11.06
	250	52.00	84.16	444.53	973.00	8.44	11.46
	500	56.66	90.50	536.12	1143.12	9.15	12.48
Mean (Lead)		52.38	84.77	449.14	1007.22	8.36	11.66
300	0	43.66	77.50	329.94	834.89	7.29	10.66
	250	49.83	81.16	421.71	932.89	8.31	11.46
	500	60.50	86.83	540.75	1071.08	8.95	12.31
Mean (Lead)		51.33	81.83	430.80	946.28	8.18	11.47
Mean (Citric acid)	0	48.41	79.87	371.90	891.35	7.51	11.02
	250	53.41	84.49	457.61	998.42	8.47	11.73
	500	59.66	89.62	561.73	1164.04	9.28	12.86
L.S.D. at 0.05	Lead	3.53	7.22	57,43	95.80	0.67	0.35
	Citric acid	3.14	4.53	30.12	79.25	0.39	0.35
	Lead × Citric acid	3.62	5.21	34.62	91.08	0.45	0.41

On the other hand, the plants irrigated with 0 mg/l contaminated lead water and sprayed with tap water had the highest mean values in the quantities of leaves (61.16 and 90.83 leaves per plant), leaves area (605.21 and 1292.51 m2), and leaves dry weight (9.77 and 14.03g per plant).

Stem characteristics

Table (4) presented data indicating a significant loss in stem properties as the lead concentration in irrigation water increased. When compared to the control, which produced the highest numbers of tillers (3.82 and 7.11 per plant), stem height (6.05 and 8,44 cm), stem diameters (0.70 and 0.89 cm), and stem dry weight (2.19 and 2.19 g per plant) in the first and second seasons, respectively, the concentration of 300 mg/l yielded the highest significant reduction. This concentration produced tillers numbers of 3.21 and 6.71 per plant, stem height (5.63 and 7.49 cm), stem diameter (0.59 and 0.82 cm), and stem dry weight (1.79 and 1.75 g per plant) in the first and second seasons, respectively.

Treatments with citric acid increased the growth of *Gazania rigens* plants, in contrast to the effects of lead treatments. In comparison to the control value, which gave tillers number per plant (2.95 and 5.99), stem height (5.22 and 6.85 cm), stem diameters (0.57 and 0.80 cm), and stem dry weight (1.54 and 1.49 g per plant) in the first and second seasons, respectively, was the highest significant number obtained from plants sprayed with 500 ppm citric acid. These plants had 4.32 and 7.49 tillers per plant, stem height (6.55 and 9.14 cm), stem diameters (0.73 and 0.91 cm), and stem dry weight (2.50 and 2.48 g per plant) in the first and second seasons, respectively.

The data in Table (4) revealed the interaction between the effects of irrigation with water contaminated with lead and citric acid concentrations on the stem characteristics. In the first and second seasons, respectively, plants irrigated with 300 mg/l contaminated lead water and sprayed with tap water had the lowest mean values in the number of tillers per plant (2.66 and 6.16 tiller per plant), stem height (5.08 and 6.58 cm), stem diameter (0.51 and 0.77 cm), and stem dry weight (1.39 and 1.27 g per plant).

Table 4: Means of stem characteristics of *Gazania rigens* 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 2021 and 2022.

Treatments		Tillers number per plant		Stem height (cm)		Stem diameter (cm)		Stem dry weight (g)	
Lead (mg/l)	Citric acid (mg/l)	2021	2022	2021	2022	2021	2022	2021	2022
0	0	3.16	5.83	5.41	7.25	0.67	0.83	1.70	1.64
	250	3.66	7.00	6.08	8.58	0.69	0.85	2.12	2.14
	500	4.66	8.50	6.66	9.50	0.76	1.00	2.75	2.80
Mean (Lead)		3.82	7.11	6.05	8.44	0.70	0.89	2.19	2.19
100	0	3.00	5.83	5.16	6.83	0.56	0.82	1.59	1.57
	250	3.50	7.00	5.75	8.08	0.65	0.85	2.02	2.02
	500	4.66	7.16	6.75	9.25	0.75	0.95	2.66	2.59
Mean (Lead)		3.72	6.66	5.88	8.05	0.65	0.87	2.09	2.06
200	0	3.00	6.16	5.25	6.75	0.54	0.81	1.51	1.51
	250	3.16	6.83	5.66	7.5	0.60	0.84	1.90	1.88
	500	4.33	7.16	6.66	9.16	0.74	0.86	2.34	2.33
Mean (Lead)		3.49	6.71	5.85	7.80	0.62	0.83	1.91	1.90
300	0	2.66	6.16	5.08	6.58	0.51	0.77	1.39	1.27
	250	3.33	6.83	5.66	7.25	0.58	0.83	1.74	1.77
	500	3.66	7.16	6.16	8.66	0.70	0.86	2.25	2.22
Mean (Lead)		3.21	6.71	5.63	7.49	0.59	0.82	1.79	1.75
Mean (Citric acid)	0	2.95	5.99	5.22	6.85	0.57	0.80	1.54	1.49
	250	3.41	6.91	5.78	7.85	0.63	0.84	1.94	1.95
	500	4.32	7.49	6.55	9.14	0.73	0.91	2.50	2.48
L.S.D. at 0.05	Lead	0.38	0.26	0.65	0.39	0.03	0.02	0.17	0.14
	Citric acid	0.39	0.27	0.34	0.27	0.03	0.06	0.13	0.21
	Lead × Citric acid	0.45	0.32	0.38	0.31	0.03	0.07	0.14	0.22

In contrast, the highest mean values in the tillers number per plant (4.66 and 8.50 tiller per plant), stem height (6.66 and 9.50 cm), stem diameter (0.76 and 1.00 cm), and stem dry weight (2.75 and 2.80 g) were found in these plants.

3. Root characteristics

When compared to plants watered with tap water (control), the data given in Table (5) demonstrated that the tested lead concentrations in water irrigation considerably affected the root properties of *Gazania rigens*. In the first and second seasons, plants irrigated with tap water had the highest mean root lengths of 14.08 and 19.74 cm and root dry weights of 2.40 and 3.59 g per plant, respectively. In contrast, plants irrigated with the highest lead concentration of 200 and 300 mg/l had the lowest root lengths of 13.22 cm in the first season and 18.58 cm in the second season, and the highest root dry weights of 2.26 and 3.23 g per plant in the first and second seasons, respectively, were obtained from plants treated with lead at 300 mg/l.

Table (5) presented data indicating a significant influence of citric acid treatments on the root properties. In comparison to the control group (12.43 and 17.45 cm) and its root dry weight (2.06 and 3.05 g per plant) in the first and second seasons, respectively, the plants sprayed with 500 mg/l of citric acid produced the tallest root lengths (14.87 and 21.06 cm) and root dry weights (2.55 and 3.78 g per plant). concentration of 300 mg/l.

In relation to the interplay between the effects of irrigation with water contaminated with lead and citric acid concentrations on the root characteristics, Table (5) data revealed that plants irrigated with 300 mg/l of contaminated lead water and sprayed with tap water had the lowest mean values for root length (12,33 and 16.75 cm) and root dry weight (2.00 and 2.79 g per plant) in the first and second seasons, respectively. On the other hand, plants irrigated with 0 mg/l of lead water and sprayed with 500 mg/l of citric acid had the highest mean values for root length (15.33 and 21.50 cm) and root dry weight (2.58 and 3.93 g per plant) in the first and second seasons, respectively.

4. Chemical analysis

4.1. Total chlorophylls content (SPAD) units

According to Table (6) data, plants that were irrigated with tap water had the highest total chlorophyll content in the first and second seasons, respectively, at 50.80 and 67.35 SPAD. 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 mg/l (47.13 and 60.71 SPAD) in the first and second seasons, respectively.

Table 5: Means of root characteristics of *Gazania rigens* 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 2021 and 2022.

Treatments		Root length (cm)		Root dry weight (g)	
Lead (mg/l)	Citric acid (mg/l)	2021	2022	2021	2022
0	0	12.58	17.91	2.17	3.32
	250	14.33	19.83	2.46	3.52
	500	15.33	21.50	2.58	3.93
Mean (Lead)		14.08	19.74	2.40	3.59
100	0	12.50	17.66	2.07	3.17
	250	14.16	19.16	2.39	3.45
	500	15.16	21.33	2.58	3.86
Mean (Lead)		13.94	19.38	2.34	3.49
200	0	12.33	17.50	2.00	2.94
	250	13.00	18.83	2.29	3.45
	500	14.33	21.25	2.54	3.77
Mean (Lead)		13.22	19.19	2.27	3.38
300	0	12.33	16.75	2.00	2.79
	250	13.08	18.83	2.26	3.33
	500	14.66	20.16	2.53	3.59
Mean (Lead)		13.35	18.58	2.26	3.23
Mean (Citric acid)	0	12.43	17.45	2.06	3.05
	250	13.64	19.16	2.35	3.43
	500	14.87	21.06	2.55	3.78
L.S.D. at 0.05	Lead	0.50	1.58	0.09	0.18
	Citric acid	0.60	1.30	0.11	0.09
	Lead × Citric acid	0.68	1.49	0.13	0.10

Further evidence that citric acid treatments had a positive impact on total chlorophyll content was provided by Table (6), where mean values for plants sprayed with 0 mg/l of citric acid in the first and second seasons, respectively, were 53.78 and 67.81 SPAD, and for plants sprayed with 250 mg/l of citric acid in the same seasons, 46.15 and 61.62 SPAD, 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 content (55.70 and 68.06 SPAD) in the first and second seasons, respectively, were formed by plants irrigated with tap water and sprayed with citric acid at 0 mg/l. On the other hand, the lowest chlorophylls content (43.45 and 56.11 SPAD) were recorded in the first and second seasons, respectively, for plants irrigated by 300 mg/l contaminated water combined with 500 mg/l citric acid treatment.

4.2. Total carbohydrates percentage (%)

Table (6) demonstrated that as the concentration of lead in the irrigation water increased, so did the percentage of total carbohydrates in the dried leaves of *G. rigens* plants. Plants irrigated with 300 mg/l lead concentration in water had the highest percentages of carbohydrates (5.82 and 5.82%) in the first and second seasons, respectively, while plants irrigated with 0 mg/l lead concentration in water had the lowest mean values (4.99 and 4,98%) in the first and second seasons, respectively.

Table (6) demonstrated that the percentage of total carbs was positively impacted by citric acid treatments. When compared to the control (5.17 and 5.15%) in the first and second seasons, respectively, plants sprayed with 250 mg/l of citric acid had the highest percentage of carbohydrates in their leaves (5.30 and 5.30%) among the plants receiving the various citric acid treatments.

Regarding the interaction effect between irrigation with lead-contaminated water and treatments with citric acid on the percentage of carbohydrates in leaves, Table (6) demonstrated that plants irrigated with the highest lead concentration at 300 mg/l and sprayed with 250 mg/l of citric acid had the highest percentages of total carbohydrates (5.92 and 5.95 %). Conversely, plants that were irrigated with the maximum concentration of lead (100 mg/l) in combination with 0 mg/l of citric acid had the lowest percentages of total carbs (4.51 and 4.48 %) in the first and second seasons, respectively.

4.3. Proline content (mg/g)

Table (6) demonstrated that when the concentration of lead in the irrigation water grew, so did the proline content in the dried leaves of *G. rigens* plants. Plants irrigated with 300 mg/l lead concentration in water had the highest percentages of carbohydrates (4.75 and 4.72%, respectively) in the first and second seasons, while plants irrigated with 0 mg/l lead concentration in water had the lowest mean values (3.40 and 3.46%, respectively).

Table (6) demonstrated that the proline content (mg/g) was significantly increased by citric acid treatments. In comparison to the control group (4.02 and 4.07 mg/g) in the first and second seasons, respectively, the plants treated with 500 mg/l of citric acid had the highest proline content in their leaves (4.35 and 4.37 mg/g) among all the plants receiving the various citric acid treatments.

Regarding the interaction effect between irrigation with lead-contaminated water and treatments with citric acid on the proline content in leaves, Table (6) demonstrated that plants irrigated with the highest lead concentration at 200 mg/l combined with 500 mg/l of citric acid spray formed the highest percentages of proline content (4.96 and 4.94 mg/g). On the other hand, plants irrigated with the lowest lead concentration 100 mg/l combined with the lowest amount of citric acid—0 mg/l had the lowest proline content 3.40 and 3.46 mg/g in the first and second seasons, respectively.

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

The findings displayed in Table (6) demonstrated that when the concentration of lead in the irrigation water increased, the lead content in *G. rigens* plants increased gradually. The plants that were irrigated with water that had the highest lead concentration of 300 mg/l had the highest lead contents in their leaves (0.469 and 0.498 mg/l), stem (0.344 and 0.374 mg/l), and root (0.035 and 0.037 mg/l) in the first and second seasons, respectively. In contrast, the plants in the control group had the lowest lead contents in their leaves (0.206 and 0.219 mg/l), stem (0.109 and 0.120 mg/l), and root (0.035 and 0.037 mg/l) in the first and second seasons, respectively.

Table (6) showed that the plants' lead content was slightly reduced by spraying them with 500 mg/l of citric acid. This resulted in lead contents in the leaves of the plants of (0.254 and 0.268 mg/l), stem of (0.154 and 0.167 mg/l), and root of (0.055 and 0.058 mg/l) in the first and second seasons, respectively. On the other hand, plants that were not sprayed with citric acid recorded the highest values in the leaves (0.460 and 0.487 mg/l), stem (0.341 and 0.367 mg/l), and root (0.127 and 0.142 mg/l) in the first and second seasons, respectively.

Table 6 presents the data pertaining to the interaction effects between plant irrigation with lead contaminated water and citric acid treatments.

Table 6: Means of the chemical characteristics of *Gazania rigens* 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 2021 and 2022.

Treatments		Total chlorophyll content (SPAD)		Total carbohydrates percentage (%)		Proline content (mg/g)		Lead content in leaves (mg/l)		Lead content in stem (mg/l)		Lead content in roots (mg/l)	
Lead (mg/l)	**Citric acid (mg/l)**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
0	0	55.70	68.16	4.89	4.88	3.71	3.74	0.232	0.262	0.132	0.154	0.042	0.047
	250	48.16	68.06	5.09	5.07	3.47	3.58	0.214	0.218	0.114	0.117	0.036	0.035
	500	48.55	65.85	5.01	5.00	3.40	3.46	0.173	0.179	0.082	0.089	0.027	0.031
Mean (Lead)		50.80	67.35	4.99	4.98	3.52	3.59	0.206	0.219	0.109	0.120	0.035	0.037
100	0	52.80	66.55	4.51	4.48	3.40	3.46	0.400	0.402	0.308	0.311	0.139	0.143
	250	47.18	62.90	4.68	4.67	4.45	4.56	0.374	0.365	0.258	0.255	0.090	0.095
	500	47.60	59.58	4.63	4.57	4.29	4.42	0.237	0.256	0.144	0.159	0.056	0.059
Mean (Lead)		49.19	63.01	4.60	4.57	4.04	4.14	0.337	0.341	0.236	0.241	0.095	0.099
200	0	53.31	68.28	5.38	5.36	4.21	4.34	0.552	0.573	0.421	0.441	0.156	0.165
	250	44.63	57.76	5.53	5.52	4.24	4.36	0.426	0.439	0.307	0.327	0.112	0.121
	500	43.45	56.11	5.46	5.45	4.96	4.94	0.318	0.342	0.201	0.221	0.061	0.066
Mean (Lead)		47.13	60.71	5.45	5.44	4.47	4.54	0.432	0.451	0.309	0.329	0.109	0.117
300	0	53.31	68.28	5.90	5.89	4.77	4.77	0.658	0.712	0.503	0.565	0.174	0.214
	250	44.63	57.76	5.92	5.95	4.74	4.70	0.461	0.486	0.339	0.359	0.125	0.130
	500	43.45	56.11	5.65	5.63	4.76	4.69	0.288	0.297	0.191	0.199	0.076	0.078
Mean (Lead)		47.13	60.71	5.82	5.82	4.75	4.72	0.469	0.498	0.344	0.374	0.125	0.140
Mean (Citric acid)	0	53.78	67.81	5.17	5.15	4.02	4.07	0.460	0.487	0.341	0.367	0.127	0.142
	250	46.15	61.62	5.30	5.30	4.22	4.30	0.368	0.377	0.254	0.264	0.090	0.095
	500	45.76	59.41	5.18	5.16	4.35	4.37	0.254	0.268	0.154	0.167	0.055	0.058
L.S.D. at 0.05	Lead	4.84	2.78	0.03	0.02	0.10	0.06	0.006	0.009	0.004	0.006	0.003	0.002
	Citric acid	2.88	3.82	0.01	0.02	0.05	0.05	0.003	0.006	0.003	0.004	0.002	0.002
	Lead×Citric acid	3.31	4.39	0.01	0.03	0.06	0.05	0.003	0.006	0.003	0.005	2.661	2.307

The results indicate that the plants irrigated with lead contaminated water at a concentration of 300 mg/l and left unsprayed with citric acid had the highest lead values in leaves (0.658 and 0.712 mg/l), stem (0.503 and 0.565 mg/l), and roots (0.174 and 0.214 mg/l) in the first and second seasons, respectively. Conversely, the plants that were irrigated with tap water and sprayed with 500 mg/l of citric acid had the lowest values in leaves (0.173 and 0.179 mg/l), stem (0.082 and 0.089 mg/l) and root (0.027 and 0.031 mg/l) in the first and second seasons, respectively, were obtained from plants irrigated with tap water and sprayed with citric acid at 500 mg/l.

3.6. Lead content in soil samples (mg/l)

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 mg/l lead and 500 mg/l citric acid.

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

It is evident from the results in Table (8) that when the content of lead in the irrigation water increased, the transfer factor in *G. rigens* plants increased gradually. Thus, plants irrigated with water containing lead concentration 0 mg/l had the lowest lead values in the leaves (27.01 and 26.67), in the stem (14.36 and 14.61), and in the root (4.59 and 4.57) in the first and second seasons, respectively. In contrast, plants irrigated with water containing lead concentration at 300 mg/l had the highest values in the leaves (50.23 and 51.26), in the stem (36.94 and 38.53), and in the root (13.38 and 14.47), in the first and second seasons, respectively.

Table (8) results likewise demonstrated a consistent decrease in the transfer factor as the concentration of citric acid increased. Accordingly, the highest lead value in the leaves (54.92 and 54.15), in the stem (40.44 and 40.64) and in the root (15.17 and 15.70) in the first and second seasons, respectively, were recorded of control plants, whereas plants sprayed with the highest citric acid concentration 500 mg/l had the lowest lead value in the leaves (27.92 and 27.98), in the stem (16.86 and 17.32) and in the root (5.99 and 6.07) in the first and second seasons, respectively.

In relation to the interaction between the irrigation method's effects on the transfer factor when using water contaminated with lead and citric acid concentrations, Table (8) data revealed that the plants irrigated with 300 mg/l of contaminated lead water and sprayed with tap water in the first and second seasons, respectively, had the highest mean values in the dried leaves (73.02 and 73.93), in the stem (55.82 and 58.67), and in the root (19.31 and 22.22). In contrast, the plants irrigated with tap water and sprayed with lead contaminated water at 0 mg/l and sprayed with citric acid at 500 mg/l had the lowest mean values in the leaves (21.30 and 20.48), the stem (10.09 and 10.18), and the root (3.32 and 3.54) in the first and second seasons, respectively.

DISCUSSION

This study showed that there was a considerable reduction in biomass at high concentrations of heavy metals. Because leaves absorbed water more quickly and accumulated more heavy metal elements than other parts, their growth was more sensitive than other parts'. The findings of this study were consistent with previous findings on other plants, including *Typha angustifolia* (Bah *et al.*, 2011), barley *Hordeum vulgare* (Tiryakioglu *et al.*, 2006), and aquatic plant *Wolffia arrhiza* (Piotrowska *et al.*, 2010) and *Senecio cineraria* (El-Shanhorey *et al.*, 2019).

Table 7: Average of lead content in soil samples as influenced by lead concentrations in irrigation water and foliar application of citric acid on *Gazania rigens* leaves at the end of both seasons (2021 and 2022).

Treatments		Lead content in soil (mg/l)	
Lead (mg/l)	**Citric acid (mg/l)**	**2021**	**2022**
0	0	0.718	0.780
	250	0.780	0.840
	500	0.812	0.874
100	0	0.802	0.861
	250	0.840	0.903
	500	0.877	0.940
200	0	0.856	0.918
	250	0.892	0.951
	500	0.934	0.998
300	0	0.901	0.963
	250	0.953	0.974
	500	0.982	0.991

Table 8: Means of transfer factor to leaves, stem and roots of *Gazania rigens* plants as influenced by Lead, citric acid and their combinations (Lead × Citric acid) in the two season 2022.

Treatments		Transfer factor to leaves (TFL)		Transfer factor to stem (TFS)		Transfer factor to roots (TFR)	
Lead (mg/l)	**Citric acid (mg/l)**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
000	0	32.31	33.58	18.38	19.74	5.84	6.02
	250	27.43	25.95	14.61	13.92	4.61	4.16
	500	21.30	20.48	10.09	10.18	3.32	3.54
Mean (Lead)		27.01	26.67	14.36	14.61	4.59	4.57
100	0	49.87	46.68	38.40	36.12	17.33	16.60
	250	44.52	40.42	30.71	28.23	10.71	10.52
	500	27.02	27.23	16.41	16.91	6.38	6.27
Mean (Lead)		40.47	38.11	28.50	27.08	11.47	11.13
200	0	64.48	62.41	49.18	48.03	18.22	17.97
	250	47.75	46.16	34.41	34.38	12.55	12.72
	500	34.04	34.26	21.52	22.14	6.53	6.61
Mean (Lead)		48.75	47.61	35.03	34.85	12.43	12.43
300	0	73.02	73.93	55.82	58.67	19.31	22.22
	250	48.37	49.89	35.57	36.85	13.11	13.34
	500	29.32	29.96	19.45	20.08	7.73	7.87
Mean (Lead)		50.23	51.26	36.94	38.53	13.38	14.47
Mean (Citric acid)	0	54.92	54.15	40.44	40.64	15.17	15.70
	250	42.01	40.60	28.82	28.34	10.24	10.18
	500	27.92	27.98	16.86	17.32	5.99	6.07

Lead can harm plant cell walls, cell membranes, mitochondria, chloroplasts, nuclei, and ultra structures of organs and tissues. According to Salazar and Pignata (2014), this damage has the potential to impair normal physiological processes such as protein synthesis, respiration, cell division, and photosynthesis in plants by causing a loss of organelle function.

In terms of treatments and the control sample, it is important to first observe that most treatments have a transfer factor that is lower than the lead factor, indicating that the plant's physiological requirement for these components is relatively modest as in the results *Salvia splendens* (El-Shanhorey and Barakat, 2020).

Plants are capable of tolerating lead either through internal tolerance or external exclusion. According to Sharma and Dubey (2005), the external exclusion prevents lead ions from entering plant cells, preventing lead from building up in the organelles and removing extra lead ions from the plant cell. According to Pourrut *et al.* (2011), the internal tolerance of lead is primarily caused by the synthesis of organic lead compounds like cysteine, glutathione, phytochelatin, etc. Ultimately, the lead ions are converted in the cell into chemically bound structures with lower toxicity, which reduces the toxic effect of lead on the tissues of plants.

Many physiological processes, such as metal unloading into root xylem cells, long-distance transport from the xylem to the shoots, and metal reabsorption by leaf mesophyll cells from the xylem stream, facilitate the translocation of trace elements from roots to shoots. The transpiration stream transports the trace metals to the shoots after they have been discharged into the xylem vessels (Blaylock and Huang, 2000).

The transfer factor of the majority of treatments is lower than one for lead, indicating that the physiological requirement of the plant for these elements is relatively low. This is important to keep in mind when comparing treatments with the control sample. The findings of this study were consistent with those of previous studies on several plants, as in the results *Salvia splendens* (El-Shanhorey and Barakat, 2020).

Citric acid has been shown to have a considerable positive impact on all vegetative metrics, including chlorophyll content, carbohydrate percentage, lead content in the leaves and roots, and lead content. This might be because applying citric acid to any lead concentration caused a statistically significant reduction in the absorption of lead. Citric acid hindered the uptake of lead by causing a decrease in lead uptake, which led to the production of citric acid-lead complexes (Chen *et al.*, 2003). Reduced lead uptake made it easier to counteract lead's detrimental effects on the earlier investigated parameters. These findings concur with those made reference to by Talebi *et al.*, (2014) on Gazania plants and Jaafari and Hadavi (2012) on *Ocimum basilicum* L.

We come to the conclusion that *G. rigens* plants can be used as lead phytoremediation plants without the need for citric acid spraying, and that if we want to use *G. rigens* as an ornamental plant and the irrigation water is tainted with lead, we can spray the plants with citric acid to counteract the harmful effects of lead.

CONCLUSIONS

From year to year, the amount of heavy metals in the environment rises. Decontamination of heavy metal-contaminated water and soils is crucial for ecological restoration and environmental health maintenance. The use of plants to clean or stabilize damaged settings is known as phytoremediation, a new concept in environmental cleanup. The most efficient plant-based technique for removing contaminants from damaged environments is phytoremediation of metals. This environmentally friendly technology can be used to clean up dirty soils without harming the soil's structure. It has been demonstrated that some particular plants, such as some species used as ornamental plants, have a discernible capacity to absorb harmful heavy metals.

REFERENCES

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

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

Blaylock,M, J. and Huang, J.W. **2000**. Phytoextraction of Metals. Phytoremediation of Toxic Metals: Using Plants to Clean up the environment. Eds., Raskin, I. and B.D. Ensley. John Wiley and Sons, Inc, Toronto, 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. Applied Geochemistry **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. Biotechnology, **5 (1)**:1-4.

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.

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

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

El-Shanhorey, N. A. and Barakat A.A. **2020**. Effect of lead and cadmium in irrigation water and foliar applied malic acid on vegetative growth, flowering and chemical composition of *Salvia splens* plants (A) Effect of lead. Scientific J. Flowers & Ornamental Plants, **7(3)**: 321-335.

El-Shanhorey, N.A., A.I. Adam and F.H. EL-Tony **2019**. The use of *Senecio cineraria* plants sprayed with citric acid for Lead pollution phytoremediation. Bull. Fac. Agric. Cairo Univ. **70 (1):**19-29.

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

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, 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.Applied Science, **1**: 72-109.

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

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

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

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

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

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.

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, The Iowa State University Press.Ames, Iowa, USA.

Talebi, M., E. Hadavi and N. Jaafari (**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.

Talebi, M.; Hadavi, E. and Jaafari, N. **2014**. Foliar sprays of citric acid and malic acid modify growth, flowering, and root to shoot ratio of gazania (*Gazania rigens* L.). Adv. Agric. Article ID 147278, 1-6.

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

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

Vujosevic, A.; Lakic, N.; Beatovic. D. and Jelacic, S. **2007**. Influence of slow disintegrating fertilizer rates on quality of gazania (*Gazania rigens* L.) seedlings. Journal of Agricultural Sciences, **52(2)**: 121-130.

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.

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

Youssef Moustafa, A.M.; Ismail Khodair, A. and Hammouda, F.M. **2007**. Phytochemical investigation and α-cellulose content determination of *Gazania splendens* Moore. Research Journal of Phytochemistry, **1(1)**: 21-32.

الملخص العربي

استخدام نباتات الجازانيا المرشوشة بحمض الستريك فى العلاج النباتى للتلوث بالعناصر الثقيلة

1. تأثير الرصاص

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أجريت هذه الدراسة خلال موسمي 2021 و2022 في مشتل قسم الزهور ونباتات الزينة وتصميم وتنسيق الحدائق، كلية الزراعة (الشاطبي)، جامعة الإسكندرية، الإسكندرية، مصر. كان الغرض من الدراسة هو تحديد ما إذا كان من الممكن تحقيق المعالجة النباتية بالرصاص عن طريق رش نباتات الجازانيا بحمض الستريك. وتأثيره على النمو الخضري والتركيب الكيميائي لنباتات الجازانيا "استخدم أربعة مستويات من الرصاص في مياه الري (0– 100– 200– 300 ملجم/لتر) وثلاثة تراكيز من حامض الستريك (0– 250– 500 ملجم/ لتر) الرش على الأوراق ودراسة التفاعل بينها في النمو الخضري والمحتوى الكيميائي لنباتات الجازانيا ومحتوى التربة. من الرصاص بعد الزراعة.

أظهرت النتائج وجود فرق كبير بين تركيزات الرصاص ورذاذ حامض الستريك الورقي لمقاييس النمو الخضري. في حين انخفضت جميع المؤشرات بشكل ملحوظ بعد الري بالمياه التي تحتوي على الرصاص، تحسنت مؤشرات النمو الخضري بشكل ملحوظ عند إضافة 500 ملجم/لتر من حمض الستريك. تم العثور على أعلى كميات كبيرة من محتوى الرصاص في الأوراق والجذور في النباتات المعالجة بـ 300 ملجم/لتر من الرصاص دون إضافة حمض الستريك، في حين تم العثور على أعلى كميات كبيرة من الكلوروفيل والكربوهيدرات في النباتات المروية بماء الصنبور ورشها بدون حمض الستريك. حامض. كما تم توليد تركيز أعلى من الرصاص في التربة بعد الزراعة من خلال الجمع بين 500 ملجم/لتر من حمض الستريك و300 ملجم/لتر من الرصاص.

الكلمات المفتاحية: الجزانيا – رصاص– حمض الستريك– العلاج النباتى للتلوث بالعناصر الثقيلة.