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Response Foliar Applied with Ascorbic Acid to Mitigate the Detrimental Impact of Lead Pollution in Water Irrigation on *Jatropha curcas* Plants

Nader A. El-Shanhorey[1*], Ashraf M. Shehata[2]

[1]*Department of Botanical Gardens Research- Antonuades, Horticultural Research Institute, Agriculture Research Center, Alexandria, Egypt.*

[2]*Department of Floriculture, Ornamental Horticulture and Landscape Design - Faculty of Agriculture, Alexandria University, Alexandria, Egypt.*

*Corresponding author: dr_shanhorey@yahoo.com

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ABSTRACT

The current investigation was conducted throughout two consecutive seasons in 2022 and 2023 at the Antoniades Research Branch in Alexandria, Horticultural Research Institutes, A.R.C. This study sought to determine whether lead pollution might be mitigated by spraying ascorbic acid (AA) on *Jatropha curcas* plants planted in sandy soil. It also sought to assess the effects of contaminated irrigation water with lead on these plants. Individual *J. curcas* seedlings were placed in plastic pots with a diameter of 30 cm and 8 kg of sand soil. There were four lead concentrations administered to the contaminated irrigation water treatments: 0, 100, 200, and 300 mg/l. Additionally, the plants were sprayed monthly in both seasons with AA at doses of 0, 250, and 500 mg/l. The findings demonstrated no discernible difference in the way AA foliar spray and lead concentrations interacted with vegetative growth indices. While a substantial rise in vegetative growth metrics was noted following the application of 500 mg/l of AA. As noted a considerable decrease in all parameters was noted following irrigation with lead-contaminated water. The plants that were irrigated with tap water and sprayed with 500 mg/l of AA had the highest significant value for chlorophyll and carbohydrate content, while the plants that received 300 mg/l of lead without AA application had the highest significant amount of lead content in their leaves, stems, and roots. The findings demonstrated that there was no statistically significant difference in the interaction between AA foliar spray and lead concentrations concerning vegetative growth indices. After 500 mg/l of AA was added, metrics about vegetative development significantly increased; however, irrigation with lead-contaminated water caused a considerable decline in all indices. In contrast, the plants that were irrigated with tap water and sprayed with 500 mg/l of AA had the highest significant values for chlorophyll and carbohydrate content. The plants that received 300 mg/l of lead without AA application had the highest significant amount of lead content in their leaves, stems, and roots.

INTRODUCTION

Planting Jatropha started in Egypt in 2004 (SWERI, 2009). It has been shown that planting this tree in marginal and arid settings has great potential, even though it is still in the experimental stage. Upper Egypt has successfully planted this tree. The growth and flowering seasons in this location are shorter than in other countries, though; blooms are generated here in 18 months as opposed to three years elsewhere. Industrial effluents often contain high amounts of dangerous heavy metals (Ghavri and Singh, 2010). These metals can pose varied degrees of risk to humans (Lim and Schoenung, 2010), animals (Rainbow, 2007), plants (Ghavri *et al.*, 2010; Sharma *et al.*, 2010), and microbes (Giller *et al.*, 2009). They are also persistent and non-biodegradable. *J. curcas* is a promising plant for biodiesel production that can withstand tough semi-arid agroclimatic conditions and wastelands (Mangkoedihardjo and Sunahmadia, 2008). It also grows quickly and requires minimal care (Gunaseelan, 2009). It has a height range of 3–8 meters. Originating in Central America, the genus Jatropha has 172 species with noteworthy commercial significance and is now found in Africa and Asia (Fairless, 2007).

The best oil-bearing Jatropha species, according to (Achten, 2008), are *J. curcas, J. glandulifera, and J. gossypifolia*. These plants have been suggested for planting on wasteland. *J. curcas* is a perennial crop that has lately gained recognition as a possible oil seed and has the potential to be used as a biodiesel and medicinal crop (Effendi *et al*., 2010; Rafii *et al*., 2012; Shabanimofrad *et al*., 2011). This plant has demonstrated its significant value as a medicinal herb in the treatment of tropical dermatological illnesses (Igbinosa *et al*., 2009). Additionally, the high rate of ozone layer depletion and the effects of global warming brought on by rising fossil fuel consumption that pollutes the environment have drawn more attention to this crop. Because they increase global energy supply security, lessen reliance on fossil fuel resources, and present a chance to reduce greenhouse gas emissions, renewable biofuel feedstocks are viewed as crucial components of the energy supply portfolio (Sudhakar and Nalini, 2011). This recently introduced crop has an unpredictable seed and oil production, especially in tropical climates. It thrives abundantly in wild and abandoned terrain. Unknown are the favorable environmental factors that influence its production (Ovando *et al.*, 2011; Divakara *et al.*, 2010). According to Ginwal *et al.* (2005).

Jatropha has evolved to a wide range of ecological and environmental conditions, which implies that there is a significant quantity of genetic diversity that has not yet been discovered but may exist (Rao *et al.*, 2008). Although heavy metals are necessary in trace amounts for plants, their availability in excess can be hazardous to them (Sharma and Katyal, 2006). The thresholds of phytotoxic concentrations of heavy metals cited in the literature are typically not specified, according to (Wua *et al.*, 2010). According to (Unhalekhana and Kositanont, 2008), there are several sources of lead pollution in the environment, including mine tailings, phosphate fertilizers, industrial sludges, and fuel combustion. The amount of heavy metals in the environment increases yearly (Govindasamy, 2011).

Therefore, decontaminating soils and water contaminated with heavy metals is essential for maintaining environmental health and restoring biological systems. Lead can enter the human body through the mouth and lungs. Subsequently, lead accumulates in the kidney and liver, where it can result in both acute and chronic symptoms, including nausea, diarrhoea, pain in the abdomen, renal failure, and osteomalacia (Simmons *et al.*, 2005).

In the ascorbate-glutathione pathway, ascorbic acid (AA) is a crucial antioxidant that also shields enzymes containing prosthetic transition metal ions. In addition, it functions as a cofactor for a wide range of enzymes, including those involved in the formation of cell walls, particularly those that hydroxylate proline residues (Ishikawa *et al.*, 2006; El-Shanhoreyand Ahmed, 2016). Furthermore, H_2O_2 buildup can stimulate alternative oxidase. Since ascorbate regulates the intracellular H_2O_2 level, this could offer a coordinated mechanism to shield the cell from unchecked oxidation (Bartoli *et al.*, 2006; El-Shanhorey and Khaled, 2016).

Because *J. curcas* is a non-edible plant that grows in tropical climates and is commercially viable for the production of biodiesel, it was chosen for this study. As a result, the study's goal is to ascertain whether *J. curcas* can remove heavy metals from irrigation water contaminated by affected soil and to conduct research on this capacity.

MATERIALS AND METHODS

The current study was conducted over the course of two consecutive seasons in 2022 and 2023 at the Horticultural Research Institute's Antoniades Research Branch in Alexandria, A.R.C. Egypt. This study sought to determine whether lead pollution might be mitigated by spraying AA on *Jatropha curcas* plants planted in sandy soil. It also sought to assess the effects of contaminated irrigation water on these plants.

Uniform *J. curcas* seedlings (height 70–80 cm, average leaf count 20–25) were planted one by one in plastic pots (30 cm in diameter) that were filled with 8 kg of sandy soil on February 15, 2022, and 2023 (first and second seasons, respectively). The chemical component measurements of the soil were conducted in Table(1) described by Jackson, (1973).

The contaminated irrigation water treatments started on March 1st (in both seasons). Lead (II) acetate [(Pb $(CH_3COO)_2$] 0,100, 200, and 300 mg/l were administered in four different concentrations. The plants underwent three weekly irrigations; after the experiment, each plant received approximately 274 liters of contaminated water per pot, as shown in Table(2). Monthly spraying from May 15 through August 15 was used to provide the plants with the appropriate levels of contamination in both seasons. Additionally, AA at doses of 0, 250, and 500 mg/l was sprayed on the plants. Tap water was sprinkled on the control plants. In both seasons, the plants were harvested on September 30.

All plants were chemically fertilized with NPK over the two growing seasons at a rate of 3 g/pot using soluble fertilizer (Kristalon 19-19-19). Throughout the growing season, fertilization was done every 30 days (from March 1st to September 30th). Furthermore, weeds were hand-pulled as soon as they appeared.

Table 1: Chemical analyses of the used sandy soil for the two successive seasons 2022 and 2023.

Season	pH	EC (dSm^{-1})	Soluble cations (mg/l)				Soluble anions (mg/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_2^{--}
2022	7.99	1.52	3.3	3.5	6.6	1.3	3.5	6.7	2.5
2023	7.96	1.47	3.4	3.1	6.4	1.2	3.4	6.5	2.3

Table 2: Total amount of water used for each plant (L/pot) in each treatment during the growing two seasons of 2022 and 2023.

Field Capacity(%)	Months of first and second seasons							
	March	April	May	June	July	August	September	Mean
100 %	31.00	33.50	35.00	35.50	44.50	50.00	44.50	274.00

Data recorded:

(1) Vegetative growth parameters:

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

(2) Chemical analysis determination:

- Using a Minolta (chlorophyll meter) SPAD 502 at the end of the growing season, the total chlorophyll content was ascertained as a SPAD units from the fresh leaves of plants for the various treatments under the experiment according to Yadava (1986).
- The percentage of total carbohydrates in the leaves was calculated using Dubios *et al*. (1956).
- Based on Bates *et al.*, (1973), the proline content (mg/g) of the leaves was computed.
- Ascertaining the lead content. Plant samples were separated into root systems, stems, and leaves. After that, they were baked at 72°C to dry them fully. The samples of dried plants were pulverized into a powder. After that, the dried samples were digested by Piper, (1947) method for extracting lead, and an atomic absorption spectrophotometer was used to measure the concentration of heavy metal.
- According to Lindsay and Norvell (1978), available lead in soil samples was removed using DPTA solution, and its concentration was measured using inductively coupled plasma spectroscopy.
- The ratio of the metal concentration in the shoots to the metal concentration in the soil yields the transfer factor (TF) (Chen *et al.,* 2004). In evaluation studies on the effects of regular or unintentional emissions of pollutants into the environment, a value known as the transfer factor is employed. shows how effectively a plant can move metal from the earth to its aerial parts.

Split plot design with three duplicates was the experimental arrangement. Three plants were present in each replication. The quantities of AA were found in the support plots, but the main plots showed the levels of contaminated irrigation water. According to (Snedecor and Cochran, 1989), the means of the individual components and their interactions were compared using the L.S.D test at the 5% level of probability.

RESULTS

1. Vegetative growth:

1.1. Leaves characteristics

Table (3) presents data demonstrating that, in both seasons, plants irrigated with lead-contaminated irrigation water had lower leaf characteristics per plant of *Jatropha curcas* plants than plants irrigated with tap water (control). In the first and second seasons, respectively, plants irrigated with tap water had the highest mean values of number of leaves (25.66 and 27.99), leaves dry weight (40.19 and 42.03 g), and leaves area (2513.93 and 2723.32 cm^2). Additionally, increasing the concentration of lead in irrigation water caused a steady significant reduction in leaf characteristics, with the highest concentration (300 mg/l) producing significantly fewer results in plants with the mean number of leaves (21.05 and 23.44), leaves dry weight (32.11 and 34.38 g), and leaves area (1083.74 and 1206.44 cm^2) in the two seasons. respectively) than those receiving any other lead concentration.

Applying ascorbic acid (AA) spray to the plants had a substantial impact on the features of the leaves as well. When the AA content was increased from 0 mg/l (control) to 500 mg/l, the features of the leaves steadily rose in both seasons. The data in Table (3) thus show that, in comparison to plants sprayed with any other AA concentration, *Jatropha curcas* plants treated with 500 mg/l AA had significantly larger leaf characteristics, with mean number of leaves (23.58 and 25.95), leaves dry weight (36.54 and 38.60 g), and leaves area (2025.20 and 2229.45 cm^2) in the first and second seasons, respectively.

The information in Table (3) demonstrates that there was a significant interaction between the effects of irrigation with contaminated lead water and AA treatments on the rate of growth of leaves on *J. curcas* plants. The results of the two seasons indicate that the plants that were irrigated with tap water and sprayed with AA at 500 mg/l had larger values concerning the mean number of leaves (27.33 and 29,33), leaves dry weight (43.12 and 44.27 g), and leaves area (2840.13 and 3032.07 cm^2) in the first and second seasons, respectively. Conversely, the features of the leaves with the smaller mean number of leaves (20.66 and 23.00), leaves dry weight (31.41 and 33.64 g) and leaves area (729.13 and 818.63 cm^2) in the first and second seasons, respectively, were the outcome of irrigating the plants with the maximum concentration of lead (300 mg/l) without treating them with AA. The data shown in Table (3) further demonstrate that, in many instances, spraying AA on the plants lessened the negative effects of lead-contaminated water.

1.2. Stem characteristics

Table (4) displays data demonstrating that, in both seasons, *J. curcas* plants watered with lead-contaminated water had less stem characteristics per plant as compared to plants irrigated with tap water (control). The highest mean values of stem height (61.67 and 67.08 cm), stem diameter (3.76 and 4.17 cm), and stem dry weight (45.17 and 50.16 g) were observed in plants that were irrigated with tap water in the first and second seasons, respectively.

Table 3: Means of leaves characteristics of *Jatropha curcas* plants as influenced by Lead (Pb), Ascorbic acid (AA) and their combinations (Pb× AA) in the two seasons of 2022 and 2023.

Treatments		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm^2)	
Pb (mg/l)	**Ascorbic acid (mg/l)**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
000	000	23.33	25.83	36.08	38.39	2331.87	2542.10
	250	26.33	28.83	41.38	43.43	2369.80	2595.80
	500	27.33	29.33	43.12	44.27	2840.13	3032.07
Mean (Lead)		25.66	27.99	40.19	42.03	2513.93	2723.32
100	000	26.00	28.33	40.80	42.59	2779.93	3078.93
	250	20.50	22.50	31.09	32.79	901.52	990.52
	500	22.66	24.66	34.92	36.43	1471.67	1602.47
Mean (Lead)		23.05	25.16	35.60	37.27	1717.70	1890.64
200	000	24.33	26.66	37.88	39.79	1877.53	2058.60
	250	22.16	24.50	34.05	36.15	969.03	1072.17
	500	18.16	20.50	27.01	29.44	637.82	721.12
Mean (Lead)		21.55	23.88	32.98	35.12	1161.46	1283.96
300	000	20.66	23.00	31.41	33.64	729.13	818.63
	250	21.83	24.16	33.47	35.60	1111.47	1238.17
	500	20.66	23.16	31.45	33.92	1410.62	1562.52
Mean (Lead)		21.05	23.44	32.11	34.38	1083.74	1206.44
Mean (AA)	000	22.20	24.41	34.12	36.01	1419.68	1543.57
	250	22.70	24.99	34.99	36.99	1412.74	1555.25
	500	23.58	25.95	36.54	38.60	2025.20	2229.45
L.S.D. at 0.05	Pb	1.07	0.96	1.79	1.56	69.96	57.79
	AA	0.45	0.34	0.80	0.56	25.73	30.40
	Pb * AA	1.26	0.97	2.25	1.59	71.19	78.23

Furthermore, plants with mean stem heights of (50.67 and 56.42 cm), stem diameters of (3.13 and 3.49 cm), and stem dry weights of (37.71 and 41.98 g) in the two seasons showed significantly fewer results at the highest concentration of lead (300 mg/l). Raising the lead in irrigation water concentration also caused steady, significant reductions in leaf characteristics. respectively) than those receiving any other lead concentration.

The AA spraying had a considerable impact on the stem properties of the plants as well. When the AA content was increased from 0 mg/l to 500 mg/l, the features of the leaves steadily rose in both seasons. The data in Table (4) thus shows that, in comparison to plants sprayed with any other AA concentration, *J. curcas* plants sprayed with 500 mg/l AA had significantly larger leaves with mean stem height (56.70 and 62.19 cm), stem diameter (3.49 and 3.85 cm), and stem dry weight (42.00 and 46.25 g) in the first and second seasons, respectively.

The results recorded in the two seasons show that significant interaction with mean stem height (65.60 and 71.03 cm), stem diameter (4.01 and 4.37 cm), and stem dry weight (48.20 and 52.52 g) in the first and second seasons, respectively, the plants irrigation with tap water and sprayed with AA at 500 mg/l obtained the bigger values. Data in Table (4) indicate that a significant interaction was detected in both seasons between the effects of irrigation with contaminated lead water and AA treatments on the growth rate stem of *J. curcas* plants. Conversely, the plants irrigated with the highest lead concentration of 300 mg/l without AA treatment had stem characteristics with mean less small stem height (49.83 and 55.20 cm), stem diameter (3.04 and 3.38 cm), and stem dry weight (36.61 and 40.66 g) in the first and second seasons, respectively. The data shown in Table (4) further demonstrate that, in many instances, spraying AA on the plants lessened the negative effects of lead-contaminated water.

1.3. Root characteristics

When compared to plants watered with tap water (control), *J. curcas* plants irrigated with lead-contaminated water showed less root characteristics per plant in both seasons, as shown by the results in Table (5). Plants that were irrigated with tap water had the highest mean values of root length (38.44 and 42.02 cm) and root dry weight (42.30 and 45.96 g) in the first and second seasons. Furthermore, consistent, notable decreases in root properties were seen when the concentration of lead in irrigation water was increased. With mean root lengths of 31.58 and 35.10 cm and root dry weights of 34,90 and 38.67 g throughout the two seasons, respectively, the plants exposed to the highest concentration of lead (300 mg/l) yielded noticeably worse results than those exposed to any other concentration.

Significant effects of the AA treatment were also observed in the root characteristics of the plants. Both seasons saw a steady improvement in root characteristics when the AA concentration was raised from 0 mg/l (control) to 500 mg/l. *J. curcas* plants treated with 500 mg/l AA exhibited significantly larger root characteristics: mean root length (35.37 and 38.99 cm) and root dry weight (38.96 and 42.91 g) in the first and second seasons, respectively, compared to plants sprayed with any other AA concentration, according to the data in Table (5).

The data presented in Table (5) indicate that a significant interaction was found in both seasons between the effects of AA treatments and contaminated lead water irrigation on the growth rate roots of *J. curcas* plants. The results of the two seasons indicate that the plants sprayed with AA at 500 mg/l and irrigation with tap water had larger values with mean root lengths of 41.00 and 44.08 cm and root dry weights of 45.10 and 48.34 g in the first and second seasons, respectively. Conversely, the plants irrigated with the highest lead concentration of 300 mg/l without AA treatment had root characteristics with a mean less small root length (32.75 and 34.41 cm) and root dry weight (34.32 and 37.82 g) in the first and second seasons, respectively. The data shown in Table(5) demonstrate that spraying AA on the plants lessened the negative effects of lead-contaminated water.

2. Chemical constituents

2.1. Total chlorophyll content (SPAD unites)

The data in Table (6) show that during the first and second seasons, plant irrigation with tap water generated the highest levels of total chlorophyll (54.60 and 54.92 SPAD), respectively. As the lead concentration in irrigation water rose, the total chlorophyll content gradually and noticeably dropped; in the first and second seasons, the plants with the highest lead concentration, 300 mg/l, had the lowest SPAD values, 51.48 and 51.80, respectively.

Table 4: Means of stem characteristics of *Jatropha curcas* plants as influenced by Lead (Pb), Ascorbic acid (AA) and their combinations (Pb × AA) in the two seasons of 2022 and 2023.

Treatments		Stem height (cm)		Stem diameter (cm)		Stem dry weight (g)	
Pb (mg/l)	**Ascorbic acid (mg/l)**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
000	000	56.08	61.53	3.49	3.88	41.88	46.60
	250	63.35	68.70	3.79	4.27	45.45	51.36
	500	65.60	71.03	4.01	4.37	48.20	52.52
Mean (Lead)		61.67	67.08	3.76	4.17	45.17	50.16
100	000	62.55	68.00	3.84	4.26	46.07	51.18
	250	49.28	54.08	3.05	3.35	36.65	40.26
	500	54.48	59.26	3.34	3.60	40.14	43.34
Mean (Lead)		55.43	60.44	3.41	3.73	40.95	44.92
200	000	58.35	64.03	3.62	3.88	43.47	46.58
	250	53.20	58.88	3.48	3.74	41.88	44.98
	500	43.60	49.51	2.70	3.05	32.50	36.64
Mean (Lead)		51.71	57.47	3.26	3.55	39.28	42.73
300	000	49.83	55.20	3.04	3.38	36.61	40.66
	250	52.40	58.33	3.25	3.58	39.10	43.08
	500	49.80	55.73	3.11	3.51	37.42	42.20
Mean (Lead)		50.67	56.42	3.13	3.49	37.71	41.98
Mean (AA)	000	53.37	58.88	3.29	3.63	39.56	43.67
	250	54.55	59.99	3.39	3.73	40.77	44.92
	500	56.70	62.19	3.49	3.85	42.00	46.25
L.S.D. at 0.05	Pb	2.58	2.53	0.16	0.14	1.74	1.78
	AA	1.07	0.93	0.06	0.07	0.73	0.84
	Pb * AA	3.03	2.62	0.17	0.18	2.06	2.38

Table 5: Means of root characteristics of *Jatropha curcas* plants as influenced by Lead (Pb), Ascorbic acid (AA) and their combinations (Pb × AA) in the two seasons of 2022 and 2023.

Treatments		Root length (cm)		Root dry weight (g)	
Pb (mg/l)	Ascorbic acid (mg/l)	2022	2023	2022	2023
000	000	35.00	39.08	38.52	42.99
	250	39.33	42.91	43.28	46.56
	500	41.00	44.08	45.10	48.34
Mean (Lead)		38.44	42.02	42.30	45.96
100	0000	39.00	42.41	42.88	46.76
	250	30.25	33.75	33.62	37.27
	500	34.00	37.16	37.46	40.89
Mean (Lead)		34.41	37.77	37.98	41.64
200	000	36.50	40.08	40.13	44.10
	250	33.25	36.75	36.66	41.16
	500	27.25	30.75	29.99	34.55
Mean (Lead)		32.33	35.86	35.59	39.93
300	000	32.75	34.41	34.32	37.82
	250	31.00	36.33	36.02	39.97
	500	31.00	34.58	34.36	38.22
Mean (Lead)		31.58	35.10	34.90	38.67
Mean (AA)	000	33.31	36.64	36.72	40.50
	250	33.89	37.43	37.39	41.24
	500	35.37	38.99	38.96	42.91
L.S.D. at 0.05	Pb	1.43	1.65	1.53	1.59
	AA	0.67	0.49	0.76	0.61
	Pb * AA	1.91	1.38	2.16	1.73

The results of the chemical analysis of the leaves in Table (6) show that the AA treatments under investigation significantly affected the total amount of chlorophyll. Plants treated with AA at a concentration of 250 mg/l showed mean values that ranged from 53.77 and 53.95 SPAD in the first and second seasons, respectively, to 52.14 and 52.41 SPAD in the first and second seasons.

Concerning the interaction between the effects of irrigating contaminated water with lead and AA treatments, the data displayed in Table (6) demonstrated that the leaves of plants irrigated with tap water and sprayed with AA at 500 mg/l had the highest total chlorophyll contents of (57.32 and 54.92 SPAD) in the first and second seasons, respectively, while the plants irrigated with lead water at 100 mg/l and sprayed with AA at 500 mg/l had the lowest values of (47.31 and 48.08 SPAD) in the first and second seasons, respectively.

2.2. Carbohydrates content (%)

Information derived from chemical examination Table (6) demonstrate that, the total carbohydrates % in the dried leaves of *J. curcas* plants was lowered continuously with growing the lead content in the irrigation polluted water with lead. The leaves of control plants had the highest mean content of carbohydrates (20.73 and 20.95 %), respectively) in the first and second seasons, while plants that were irrigated with water that had the highest concentration of lead (300 mg/l) had the lowest mean value (19.55 and 19.75 %), respectively).

The findings in Table (6) further demonstrate that, in comparison to the control, the majority of the AA concentrations that were examined raised the mean total carb% in the leaves of *J. curcas* plants. The plants that were sprayed with 250 mg/l of AA had the highest percentage of carbohydrates in their leaves (20.18 and 20.51%) in the first and second seasons, respectively, among the plants that received the various AA treatments.

Regarding the interplay between the effects of AA treatments and irrigation-contaminated water on the percentage of leaves that contain carbohydrates. The findings displayed in Table (6) demonstrate that the leaves of plants irrigated with tap water and sprayed with 500 mg/l of AA had the highest mean values of (21.86 and 21.90 %) in the first and second seasons, respectively. Conversely, the leaves of plants that were irrigated with 300 mg/l of lead water without AA treatment had the lowest amount of carbohydrates.

Table 6: Means of chemical constituents characteristics of *Jatropha curcas* plants as influenced by lead (Pb), Ascorbic acid (AA) and their combinations (Pb×AA) in the two seasons of 2022 and 2023.

Treatments		**Chlorophyll content (SPAD unites)**		**Carbohydrates content (%)**		**Proline content (mg/g)**	
Pb (mg/l)	**Ascorbic acid (mg/l)**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
000	000	52.55	52.99	19.50	20.21	1.55	1.52
	250	53.94	54.36	20.84	20.74	1.46	1.52
	500	57.32	57.42	21.86	21.90	1.45	1.42
Mean (Lead)		54.60	54.92	20.73	20.95	1.48	1.48
100	000	56.89	56.99	21.20	21.46	1.83	1.81
	250	55.09	55.51	20.72	21.15	1.76	1.84
	500	47.31	48.08	18.04	18.33	1.77	1.85
Mean (Lead)		53.09	53.52	19.98	20.31	1.78	1.83
200	000	50.03	50.12	21.21	19.12	2.14	2.14
	250	54.43	54.53	18.45	19.72	2.04	2.14
	500	53.49	53.59	19.25	20.43	2.07	2.16
Mean (Lead)		52.65	52.74	19.63	19.75	2.08	2.14
300	000	55.62	55.71	18.81	18.73	2.55	2.67
	250	48.37	49.13	20.23	21.25	2.46	2.58
	500	50.47	50.57	19.61	19.28	2.47	2.59
Mean (Lead)		51.48	51.80	19.55	19.75	2.49	2.61
Mean (AA)	000	52.14	52.41	19.69	19.98	2.01	2.01
	250	53.77	53.95	20.18	20.51	1.93	2.02
	500	52.95	53.38	20.06	20.08	1.93	2.02
L.S.D. at 0.05	Pb	0.70	0.89	0.84	0.30	0.015	0.014
	AA	0.86	0.81	0.44	0.37	0.010	0.012
	Pb * AA	2.42	2.28	1.26	1.05	0.015	0.016

2.3. Proline content (mg/g)

Table (6) demonstrates that as the concentration of lead in the irrigation water polluted with lead grew, so did the proline content in the dried leaves of *J. curcas* plants. Plants irrigated with the highest lead concentration of 300 mg/l in the first and second seasons, respectively, had the highest mean proline content (2.49 and 2.61 mg/g), while plants irrigated with the lowest lead concentration of 0 mg/l (control plants) had the lowest mean value (1.48 and 1.48 mg/g) in the first and second seasons, respectively.

In addition, Table (6) results demonstrate that, when compared to the control plants, the majority of the AA concentrations examined raised the average proline content in the leaves of *J. curcas* plants. Plants treated with 250 mg/l AA had the lowest levels of proline (mg/g) in their leaves in the first and second seasons, respectively, out of all the plants that received the various AA treatments.

About the relationship between the proline content (mg/g) of leaves and the effects of irrigation with polluted water and AA treatments. Table(6) presents the results. It can be seen that the plants sprayed with 500 mg/l of AA and irrigated with tap water had the lowest mean values (1.45 and 1.42 mg/g) in the first and second seasons, respectively. Conversely, plants irrigated with 300 mg/l of lead water without AA treatment had the greatest proline content means value (2.55 and 2.67 mg/g) in the first and second seasons, respectively.

2.4. Lead content in leaves (mg/l)

The information derived from chemical examination of leaves Table (7) demonstrates that when the lead concentration in the irrigation water increased, so did the lead content (mg/l) in the dried leaves of *J. curcas* plants. The control plants' leaves had the lowest mean lead content (0.057 and 0.064 mg/l) in the first and second seasons, respectively, while the plants that were irrigated with water that had the highest lead concentration (0.191 and 0.207 mg/l) in the first and second seasons, respectively, had the highest lead concentration 300 mg/l.

In relation to the impact of AA treatments on leaf lead content, data from the two seasons are presented in Table (7). This indicates that only one 500 mg/l AA treatment significantly reduced leaf lead content, with mean values of (0.082 and 0.089 mg/l) in the first and second seasons, respectively, compared to control plants, which had the highest leaf lead content in the first and second seasons, at (0.186 and 0.201 mg/l), respectively.

Regarding the interplay between the effects of AA therapies and irrigation polluted lead water on leaf lead content. According to Table (7) results, plants that were irrigated with tap water and sprayed with 500 mg/l of AA had the lowest mean values in the first and second seasons, respectively, at (0.040 and 0.043 mg/l). Conversely, the leaves of plants that were irrigated with lead water at a concentration of 300 mg/l and did not receive AA treatment had the greatest lead content in the first and second seasons, at (0.287 and 0.316 mg/l), respectively.

2.5. Lead content in stem (mg/l)

Table (7) presents the data obtained from the stem chemical analysis. It indicates that there was a consistent increase in the lead content (mg/l) in the dried stem of *J. curcas* plants when the lead concentration in the irrigation water was raised. In the first and second seasons, the stem of control plants had the lowest mean lead content (0.165 and 0.186 mg/l), while plants that were irrigated with water that had the highest lead concentration (300 mg/l) had the highest mean value (0.555 and 0.600 mg/l) in those same seasons.

Regarding the impact of AA treatments on the lead content in the stem, Table (7) illustrates the data collected throughout the two seasons. It reveals that a single 500 mg/l AA treatment significantly reduced the lead content in the stem, resulting in mean values of (0.237 and 0.259 mg/l) in the first and second seasons, respectively, when compared to control plants, which had the highest lead content in stem (0.540 and 0.584 mg/l), respectively.

Regarding the interplay between the effects of treatments with AA and irrigation of polluted lead water on the lead content in stems. The findings in Table (7) demonstrate that the stems of plants sprayed with 500 mg/l of AA and irrigated with tap water had the lowest mean values (0.126 and 0.116 mg/l) in the first and second seasons, respectively. Conversely, the stems of the plants that were irrigated with lead water at 300 mg/l of lead water and did not receive any AA treatment had the greatest lead levels (0.832 and 0.916 mg/l), respectively, in the first and second seasons.

2.6. Lead content in roots (mg/l)

The information from the chemical analysis of the roots Table (7) demonstrates that when the lead concentration in the irrigation water increased, so did the lead content (mg/l) in the dried roots of *J. curcas* plants. The roots of control plants had the lowest mean lead content (0.239 and 0.267 mg/l) in the first and second seasons, respectively, while plants that were irrigated with water that had the highest lead concentration (300 mg/l) had the highest mean value (0.791 and 0.852 mg/l) in both seasons.

Table 7: Means of chemical constituents characteristics of *Jatropha curcas* plants as influenced by lead (Pb), Ascorbic acid (AA) and their combinations (Pb×AA) in the two seasons of 2022 and 2023.

Treatments		**Lead content in leaves (mg/l)**		**Lead content in stem (mg/l)**		**Lead content in roots (mg/l)**	
Pb (mg/l)	**Ascorbic acid (mg/l)**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
000	000	0.070	0.086	0.204	0.250	0.294	0.356
	250	0.061	0.063	0.177	0.184	0.256	0.264
	500	0.040	0.043	0.116	0.126	0.169	0.182
Mean (Lead)		0.057	0.064	0.165	0.186	0.239	0.267
100	000	0.156	0.158	0.452	0.458	0.646	0.649
	250	0.142	0.139	0.413	0.404	0.591	0.573
	500	0.073	0.083	0.212	0.241	0.304	0.344
Mean (Lead)		0.123	0.126	0.359	0.367	0.513	0.522
200	000	0.233	0.246	0.675	0.713	0.965	1.010
	250	0.168	0.177	0.488	0.514	0.701	0.728
	500	0.114	0.127	0.331	0.368	0.474	0.523
Mean (Lead)		0.171	0.183	0.498	0.531	0.713	0.753
300	000	0.287	0.316	0.832	0.916	1.188	1.300
	250	0.186	0.201	0.541	0.583	0.775	0.827
	500	0.101	0.104	0.292	0.302	0.411	0.431
Mean (Lead)		0.191	0.207	0.555	0.600	0.791	0.852
Mean (AA)	000	0.186	0.201	0.540	0.584	0.773	0.828
	250	0.139	0.145	0.404	0.421	0.580	0.598
	500	0.082	0.089	0.237	0.259	0.339	0.370
L.S.D. at 0.05	Pb	0.003	0.004	0.009	0.013	0.012	0.019
	AA	0.002	0.003	0.005	0.008	0.007	0.012
	Pb * AA	5.721	0.008	0.016	0.024	0.019	0.034

Regarding the impact of AA treatments on the amount of lead in roots, Table (7) presents data collected over the course of two seasons. It reveals that a single 500 mg/l AA treatment significantly reduced the amount of lead in roots, resulting in mean values of (0.339 and 0.370 mg/l) in the first and second seasons, respectively, when compared to control plants, which had the highest lead content in roots (0.773 and 0.828 mg/l), in the first and second seasons, respectively.

Regarding the interplay between the effects of AA treatments and irrigation polluted lead water on the lead content in roots. The findings in Table (7) demonstrate that the roots of plants irrigated with tap water and sprayed with 500 mg/l of AA had the lowest mean values (0.169 and 0.182 mg/l) in the first and second seasons, respectively. However, the roots of the plants that were watered with lead water at a concentration of 300 mg/l and did not receive any AA treatment had the greatest lead content (1.188 and 1.300 mg/l) in the first and second seasons, respectively.

1. Transfer factor (TF) of heavy metals

Transfer factor (TF) indicates the efficiency of plants in transferring metals from the root to the aerial parts.

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

The data shown in Table (8) indicated that the soil cultivated by untreated plants had the lowest average lead concentration, whereas the soil treated with 300 mg/l lead and 0 mg/l AA had the greatest average lead content.

3.2. Transfer factor to leaves (TFL)

Table (9) displays the findings, which indicates a consistent decline in the transfer factor of dried *Jatropha curcas* plant leaves as the lead concentration in the irrigation water increased. The results indicate that plants irrigated with water containing 0 mg/l of lead had the highest lead mean value (1.083 and 1.410 mg/l) in the first and second seasons, respectively, while plants irrigated with water containing 300 mg/l of lead had the lowest mean value (0.702 and 0.832 mg/l) in the same seasons.

The findings in Table (9) further demonstrate that as the content of AA increased, the transfer factor in the dried leaves decreased gradually. Consequently, plants sprayed with the highest AA concentration (500 mg/l) had the lowest lead mean value (0.586 and 0.878 mg/l) in the first and second seasons, respectively, while the leaves of control plants showed the highest lead values (1.023 and 1.334 mg/l) in the first and second seasons, respectively.

About the interplay between the effects of irrigation-contaminated water and AA concentrations on the transfer factor in the dried leaves of *Jatropha curcas* plants, Table (9) data reveals that plants irrigated with lead water at 0 mg/l and sprayed with tap water had the highest mean values (1.300 and 1.645 mg/l) in the first and second seasons, respectively, while plants irrigated with lead water at 300 mg/l and sprayed with AA at 500 mg/l recorded the lowest mean values (0.491 and 0.610 mg/l) in the first and second seasons, respectively.

3.3. Transfer factor to stem (TFS)

Table (9) displays the findings, which indicate a consistent decline in the transfer factor in the dried stem of *J. curcas* plants as the lead concentration in the irrigation water increased.

Table 8: Average lead content in soil samples as influenced by lead concentrations in water irrigation and foliar application of Ascorbic acid on *Jatropha curcas* leaves at the end of both second seasons 2022 and 2023.

Treatments		Lead content in soil 2022 (mg/l)	Lead content in soil 2023 (mg/l)
Pb (mg/l)	**Ascorbic acid (mg/l)**		
000	000	0.091	0.067
	250	0.073	0.049
	500	0.055	0.031
100	000	0.120	0.096
	250	0.113	0.089
	500	0.105	0.081
200	000	0.252	0.228
	250	0.243	0.219
	500	0.232	0.208
300	000	0.261	0.237
	250	0.245	0.221
	500	0.234	0.211

As a result, plants irrigated with water containing a lead concentration of 0 mg/l had the highest lead mean value (3.146 and 4.094 mg/l) in the first and second seasons, respectively, while plants stems irrigated with water containing a lead concentration of 300 mg/l had the lowest mean value (2.037 and 2.414 mg/l) in the same seasons.

Table (9) displays further results indicating a consistent decrease in the transfer factor in the dried stem with increasing AA content. Consequently, plants sprayed with the highest AA concentration of 500 mg/l had the lowest lead mean value (1.700 and 2.559 mg/l) in the first and second seasons, respectively, while the stems of control plants had the highest lead values (2.968 and 3.873 mg/l) in the first and second seasons, respectively.

In relation to the interplay between the effects of irrigation contaminated water and AA concentrations on the transfer factor in the dried stem of *J. curcas* plants, Table 9 data reveals that plants irrigated with lead water at 0 mg/l and sprayed with tap water had the highest mean values (3.766 and 4.770 mg/l) in the first and second seasons, respectively, while plants irrigated with lead water at 300 mg/l and sprayed with AA at 500 mg/l recorded the lowest mean values (1.426 and 1.769 mg/l) in the first and second seasons, respectively.

3.4. Transfer factor to roots (TFR)

Table (9) displays data that indicates a consistent decline in the transfer factor of dried roots of *J. curcas* plants as the content of lead in the irrigation water increased. Thus, plants irrigated with water containing 0 mg/l of lead had the highest lead mean value (4.502 and 5.814 mg/l) in the first and second seasons, respectively, while plants irrigated with 300 mg/l of lead had the lowest mean value (2.918 and 3.422 mg/l) in the first and second seasons, respectively.

The findings in Table (9) further demonstrate that as the content of AA increased, the transfer factor in the dried root decreased gradually. The plants+ sprayed with the highest AA concentration of 500 mg/l had the lowest lead mean value (2.441 and 3.668 mg/l) in the first and second seasons, respectively, while the roots of the control plants had the highest lead value (4.248 and 5.496 mg/l) in the first and second seasons, respectively.

About the interplay between the effects of irrigation-contaminated water and AA concentrations on the transfer factor in the dried root of *Jatropha curcas* plants, Table (9) data reveals that plants irrigated with lead water at a concentration of 0 mg/l and sprayed with tap water had the highest mean values (5.383 and 6.760 mg/l) in the first and second seasons, respectively, while plants irrigated with lead water at a concentration of 300 mg/l and sprayed with AA at 500 mg/l recorded the lowest mean values (2.043 and 2.514 mg/l) in the first and second seasons, respectively.

Table 9: Means of transfer factor to leaves, stem and roots of *Jatropha curcas* plants as influenced by lead (Pb), ascorbic acid (AA) and their combinations (Pb ×AA) in the two seasons of 2022 and 2023.

Treatments		**Transfer factor to leaves (TFL)**		**Transfer factor to stem (TFS)**		**Transfer factor to roots (TFR)**	
Pb (mg/l)	**Ascorbic Acid (mg/l)**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
000	0	1.300	1.645	3.766	4.770	5.383	6.760
	250	1.256	1.561	3.654	4.539	5.230	6.438
	500	0.695	1.024	2.019	2.975	2.895	4.246
Mean (Lead)		1.083	1.410	3.146	4.094	4.502	5.814
100	0	0.769	1.283	2.241	3.731	3.230	5.313
	250	0.835	1.285	2.424	3.755	3.506	5.387
	500	0.727	1.387	2.109	4.064	3.072	5.870
Mean (Lead)		0.777	1.318	2.258	3.850	3.269	5.523
200	0	1.099	1.333	3.187	3.864	4.551	5.485
	250	0.759	0.909	2.208	2.638	3.163	3.742
	500	0.431	0.492	1.2478	1.431	1.756	2.042
Mean (Lead)		0.763	0.911	2.214	2.644	3.156	3.754
300	0	0.924	1.078	2.678	3.127	3.829	4.429
	250	0.691	0.808	2.008	2.347	2.884	3.324
	500	0.491	0.610	1.426	1.769	2.043	2.514
Mean (Lead)		0.702	0.832	2.037	2.414	2.918	3.422
Mean (AA)	0	1.023	1.334	2.968	3.873	4.248	5.496
	250	0.885	1.140	2.573	3.319	3.695	4.722
	500	0.586	0.878	1.700	2.559	2.441	3.668

DISCUSSION

According to this study, there was a considerable reduction in plant height and biomass at high concentrations of heavy metals (lead). Since roots absorbed water more quickly and accumulated more heavy metal elements than other factors, the growth of the roots was more sensitive than other parameters. The findings of this investigation 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). A greater suppression of root extension was observed in other research with woody plants (Dominguez *et al.,* 2009). Toxic heavy metals from an aqueous solution, in instance, may be bioaccumulated and bioconcentrated by Jatropha plants (Mohammad *et al.,* 2010). In certain nations, these plants may also be employed as candidates for phytoremediation (Juwarkar *et al.,* 2008; Kumar *et al.,* 2008; Jamil *et al.,* 2009). Throughout the trial, the plant seedling also showed a high root/shoot ratio. Since the root/shoot ratio may represent a plant's reaction to different environmental variables, an alternate explanation could involve a robust root system with many roots dispersed throughout the soil for survival (Otieno *et al.,* 2005; Lukacova Kulikova and Lux, 2010; Li *et al.,* 2010).

The physiological responses, including gas exchange rate and photosynthetic function, can be attributed to the various effects of heavy metal physicochemical properties on the integrity and function of the plant seedling fronds' photochemical apparatus as well as the impact on the leaves' chlorophyll concentrations. The effects of lead heavy metal on photosynthesis rate, CO_2 assimilation rate, and stomatal conductance have been well-documented (Chen *et al.,* 2012). The maintenance of an intercellular CO_2 concentration is concurrent with the leaf CO_2 assimilation rate and reflected the photosynthesis function of seedlings in the various heavy metal-spiked soils. The energy manifestation of green plants was largely dependent on the contents of chlorophyll and carotenoid contents. Any notable changes to their contents could have a noticeable impact on the plant's complete metabolism (Piotrowska *et al.,* 2010). According to Agrawal and Mishra (2009), lead in this study significantly decreased the amounts of chlorophyll, probably because it inhibited the production of chlorophyll or broke down pigments and their precursors. Lead may take the role of magnesium in chlorophyll molecules, which would decrease a plant's capacity to absorb light through photosynthetic processes (Agrawal and Mishra, 2009). Car, on the other hand, responded to both lead heavy metals less sensitively than Chl a and Chl b, which most likely helped to maintain the photosynthetic apparatus against heavy metal stress (Piotrowska *et al.,* 2010). Car acted as an antioxidant to scavenge the free radicals, stabilizing and protecting the lipid phase of the thylakoid membrane (Piotrowska *et al*., 2010).

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.

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

AA is a vital vitamin for plants and is needed for many physiological processes. Ascorbic acid is an enzymatic antioxidant that helps form the glutathione peroxidase enzyme, which eliminates free radicals (Sies and Brigelius, 2016). It also helps protect the enzymatic system of anti-oxidation and increases the enzymatic activity of catalase, peroxidase, and superperoxidase, which are very important in eliminating free radicals. AA, on the other hand, raises the concentration of phenols in plants (Farouk, 2011; Al-Alawy, 2015). Phenols bind to the heavy elements and either make them physiologically ineffective or deposit them in the cell wall (Al-Wahibi, 2007); additionally, AA plays a role akin to that of promotive growth regulators that increase the shoot and root systems (Abdul Adheem, 2017). This increases the absorption of nutrient elements, including microelements that stabilize the process (AL-Jumaily, 2010).

CONCLUSIONS

A novel approach to cleanup called phytoremediation uses plants to either clean up or stabilise damaged areas. The most efficient plant-based technique for eliminating contaminants from damaged areas is phytoremediation of heavy metals. With the use of this green technology, contaminated soils can be cleaned up without endangering the structure of the soil. It has been demonstrated that some particular plants, namely woody species, have a discernible capacity to absorb harmful heavy metals. Phytoremediation of contaminated water and soil with heavy metals using non-edible plant like *Jatropha curcas* offers an environmentally friendly and cost-effective method for remediating the polluted soil.

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الملخص العربي

أستجابة الرش بحمض الأسكوربيك للتخفيف من التأثير الضار لتلوث الرصاص في مياه الري على نباتات الجاتروفا

نادر أحمد الشنهورى[١]، أشرف مصطفى شحاتة[٢]

[١]فرع بحوث الحدائق النباتية بأنطونيادس – معهد بحوث البساتين – مركز البحوث الزراعية – الإسكندرية – مصر

[٢]قسم الزهور ونباتات الزينة وتنسيق الحدائق – كلية الزراعة – جامعة الإسكندرية – الإسكندرية – مصر

أجريت هذه الدراسة في مشتل الأفرع البحثية بحديقة أنطونيادس، معهد بحوث البساتين، A.R.C. الإسكندرية، خلال موسمين متتاليين ٢٠٢٢ و٢٠٢٣. كان الهدف من هذه الدراسة تقييم تأثيرات مياه الري الملوثة بالرصاص على نباتات الجاتروفا المزروعة في التربة الرملية، وإمكانية استخدام الرش بحامض الأسكوربيك للتغلب على آثار التلوث بالرصاص. تمت زراعة شتلات نبات الجاتروفا منفردة في أصص بلاستيكية (قطر ٣٠ سم) مملوءة بـــ ٨ كجم من التربة الرملية. تم أستخدام معاملات مياه الري الملوثة بأربعة تراكيز من الرصاص ٠ و١٠٠ و٢٠٠ و٣٠٠ ملجم/لتر. كما تم رش النباتات بحامض الاسكوربيك بتركيزات ٠ و٢٥٠ و٥٠٠ ملجم/لتر بالرش شهريا في كلا الموسمين.

أظهرت النتائج أنه بالنسبة لمعاملات النمو الخضري لا يوجد فرق معنوي في التفاعل بين تركيزات الرصاص والرش الورقي بحامض الاسكوربيك. بينما لوحظ انخفاض معنوي في جميع المعاملات بعد الري بالمياه الملوثة بالرصاص، كما لوحظ زيادة معنوية في معاملات النمو الخضري بعند الرش بتركيز ٥٠٠ ملجم/لتر من حامض الأسكوربيك. بالنسبة لمحتوى الكلوروفيل والكربوهيدرات والبرولين تم الحصول على أعلى قيمة معنوية من النباتات المروية بماء الصنبور ورشها بحمض الأسكوربيك ٥٠٠ ملجم/لتر بينما تم الحصول على أعلى قيمة معنوية لمحتوى الرصاص في الأوراق والسيقان والجذور من معاملة الرى بتركيز ٣٠٠ ملجم/لتر رصاص بدون تطبيق حمض الاسكوربيك.