Response of *Jatropha curcas* Plants to Foliar Applied Ascorbic Acid for Decreasing The Harmful Effect of Nickel Pollution in The Irrigation Water

Nader A. El-Shanhorey[1]and Khaled A. Emam[2]
[1]Botanical Gardens Research Department, Horticultural Research Institute, ARC, Alexandria, Egypt.
[2]Botanical Gardens Research Department, Horticulture Research Institute, ARC, Geza, Egypt.

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ABSTRACT

The present study was carried-out at Antoniades Research Branch, Horticultural Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons 2014 and 2015. The aim of this study was to evaluate the effects of irrigation water contaminated with nickel on *Jatropha curcas* plants grown in sandy soil, the possibility of using ascorbic acid spray treatments to overcome the effects of nickel pollution. Seedlings of *Jatropha curcas* were planted individually in plastic pots (30 cm diameter) filled with 8 kg of sandy soil. Four concentrations of nickel 0,100, 200 and 300 ppm were applied in the irrigation water. The plants were treated with ascorbic acid at concentrations of 0, 250 and 500 ppm by monthly spraying in both seasons.

The results showed that for vegetative growth parameters, there was no significant interaction between nickel concentrations and foliar spray by ascorbic acid, while a significant reduction was observed in all parameters after irrigation with contaminated water contained nickel and a significant increase in vegetative growth parameters was observed after 500 ppm ascorbic acid application. For chlorophyll and carbohydrate content the highest significant value was obtained from plants irrigated with tap water and sprayed with 500 ppm ascorbic acid while the highest significant nickel content in leaves, stem and roots was obtained in the treatment 300 ppm without application of ascorbic acid.

Key words: ***Jatropha curcas*** **- nickel - ascorbic acid.**

INTRODUCTION

Planting Jatropha in Egypt started in 2004 (SWERI, 2009). It is still in the experimental stage, but it has been proved that the potential to plant this tree is high in the marginal areas and desert. The planting of this tree has accomplished in Upper Egypt. Nevertheless the growth and blooming periods in this area is shorter than that in other countries; it produces flowers after 18 months, while in the other countries, it needs three years. The industrial effluents often contain large quantity of toxic heavy metals (Ghavri and Singh, 2010). These metals are non bio-degradable and persistent and can be differentially toxic to microbes (Giller *et al*., 2009), plants (Ghavri *et al*., 2010 and Sharma *et al*., 2010), animals (Rainbow, 2007) and human (Lim and Schoenung, 2010). *Jatropha curcas* L. (Family: Euphorbiaceae) is a potential biodiesel plant (Gunaseelan, 2009), which can survive in harsh environments of semi-arid agro-climatic conditions, wastelands (Mangkoedihardjo and Sunahmadia, 2008) and grows fast with little maintenance. It can reach a height of 3-8 m Genus Jatropha contains 172 species having significant economic importance and it is native to Central America and distributed in Africa and Asia (Cano- Asseleih, 1989 and Fairless, 2007). Among the various Jatropha species, *J. curcas, J. glandulifera, J. gossypifolia* (Achten *et al*., 2008), identified as the most suitable oil bearing plant, and has been recommended for plantation on waste land. *Jatropha curcas* L., is a perennial crop with potential such as medicinal and biodiesel crop recently and is recognized as potential oil seed (Effendi *et al*., 2010; Shabanimofrad *et al*., 2011 and Rafii *et al*., 2012). Also the attention on this crop has increased due to high rate of ozone layer depletion and global warming effect caused by increased usage of fossil fuel resulting in environmental pollution. Renewable biofuel feed stocks are perceived to be essential contributors to the energy supply portfolio as they contribute to the world energy supply security, reducing dependency on fossil fuel resources and provide opportunity for mitigating greenhouse gases (Sudhakar and Nalini, 2011). This newly introduced crop, which grows abundantly in wild and abandoned land, has its seed and oil yield unpredictable especially in tropical climate. Favourable environmental conditions that affect its production are yet to be known (Divakara *et al*., 2010 and Ovando *et al*., 2011).

The concentrations of heavy metals increase in the environment from year to year (Govindasamy, 2011). Plants need trace amount of heavy metal but their availability in the excess may cause plant toxicity (Sharma *et al.*, 2006). Phytotoxic concentration of the heavy metals referred in the literature does not always specify the levels (Wua *et* *al*., 2010). Nickel is also released into the environment from anthropogenic activities, such as industrial wastes, fertilizer application and organic manures (Salt, 2000). Nickel is mainly used as a row material in the metallurgical and electroplating industries, and from sewage sludge and compost (Karam, 1998). Nickel is essential for plants (Eskew *et al.*, 1983 and Ragsdale, 1998). It has been identified as a component of a number of enzymes, including glyoxalases, peptide deformylases and a few superoxide dismutases and hydrogenases (Kupper and Kroneck, 2007). Therefore nickel plays a role in various important metabolic processes, including ureolysis, hydrogen metabolism. Methanbiogenes is and acitogensis (Mulrooney and Hausinger, 2003). Toxic effects of high concentrations of nickel in plants have been frequently reported, for example inhibition of mitotic activities (MadhavaRao and Sresty, 2000), reductions in plant growth (Molas, 2002) and adverse effects on fruit yield and quality (Gajewska *et al.*, 2006). Extremely high soil nickel concentrations have left some farmland unsuitable for growing crops, fruits and vegetables (Duarte *et al.*, 2007).

Ascorbic acid is an essential antioxidant in the ascorbate-glutathione pathway, but it also protects enzymes that have prosthetic transition metal ions. Furthermore, it is a cofactor for many enzymes, including those involved in the cell wall synthesis, most notably in the hydroxylation of proline residues (Ishikawa *et al.* 2006). Moreover, alternative oxidase can be induced by H_2O_2 accumulation and, as ascorbate is involved in controlling the intracellular H_2O_2 level. This might provide the means for a concerted interaction to protect the cell against uncontrolled oxidation (Bartoli *et al.*, 2006).

In this study *Jatropha curcas* was selected due to its characteristics as non-edible plant which can grow in tropical areas and its commercial viability for the production of biodiesel, therefore the objective of this study is to evaluate the effects of irrigation water contaminated with nickel on *Jatropha curcas* plants and to investigate the response of these plants to ascorbic acid spray treatments to decrease the harmful effect of nickel pollution in the irrigation water, determine the potential of *Jatropha curcas* in removing nickel from the soil and contaminated irrigated water and to investigate on the ability of Jatropha in removing nickel.

MATERIALS AND METHODS

The present study was carried-out at Antoniades Research Branch, Horticultural Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons 2014 and 2015. The aim of this study was to evaluate the effects of irrigation water contaminated with nickel on *Jatropha curcas* plants grown in sandy soil and the possibility of using ascorbic acid spray treatments to overcome the effects of nickel pollution.

On the 7th of February, 2014 and 2015 in the first and second seasons, respectively, homogeneous seedlings of *Jatropha curcas* (70-80 cm height and 20-25 leaves per plant in average) were planted individually in plastic pots (30 cm diameter) filled with 8 kg of sandy soil. The chemical constituents of the soil were determined as described by Jackson (1958) in Table (1).

On the 7th of March in both seasons, the contaminated irrigation water treatments were initiated. Four concentrations of nickel chloride [$NiCl_2.6H_2O$] 0,100, 200 and 300 ppm were applied. The plants were irrigated three times per week. By the end of the experiment every plant has been received about 250 liters per pot of contaminated water (Table 2). In both seasons, the plants were received by monthly spraying from 7th May till 7th August in both seasons. The plants also were sprayed with ascorbic acid at concentrations of 0, 250 and 500 ppm. Control plants were sprayed with tap water. On 30th of September in the both two seasons, the plants were harvested.

In the two seasons, all plants received NPK chemical fertilization using soluble fertilizer (Kristalon 19-19-19) at the rate of 3 g/ pot. Fertilization was repeated every 30 days throughout the growing season (from the 7th of March till the 30th of September). In addition, weeds were removed manually upon emergence.

Table 1: Chemical analyses of the used sandy soil for the two successive seasons 2014 and 2015.

Season	pH	EC (dSm^{-1})	Soluble cations (meq/l)				Soluble anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_2^{--}
2014	7.94	1.57	3.4	3.4	6.5	1.2	3.6	6.7	2.4
2015	7.91	1.52	3.2	3.0	6.3	1.1	3.3	6.5	2.2

Table 2: Total amount of the water used for each plant (l/pot) in each treatment during the growing two seasons 2014 and 2015.

Field Capacity(%)	Irrigation water (L) at months of first and second seasons							
	March	April	May	June	July	August	September	Total
90 %	28.00	30.00	32.00	35.00	40.00	45.00	40.00	250.00

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:

- Total chlorophyll content were determined as a SPAD unites from the fresh leaves of plants for the different treatments under the experiment at the end of the season using Minolta (chlorophyll meter) SPAD 502 according to Yadava (1986).
- Total carbohydrates percentage in the leaves was determined according to Dubios *et al.*(1956).
- Determination of heavy metals content (Nickel). Plant samples were divided into leaves stem and roots. They were then dried at 72°C in an oven until completely dried. The dried plant samples were ground to powder. Element extraction was done according to Piper (1947) method and the concentration of heavy metal was determined using an atomic absorption spectrophotometer.
- Available heavy metal, i.e. (Nickel) in soil samples were extracted by DTPA solution according to Lindsay and Norvell (1978) and determined by Inductively Coupled Plasma Spectrometry.
- Transfer factor (TF) is given by the relation: the ratio of the concentration of metal in the shoots to the concentration of metal in the soil (Chen *et al*., 2004). The transfer factor is a value used in evaluation studies on the impact of routine or accidental releases of pollutant into the environment.

The layout of the experimental design was split plot design with three replicates. Each replicate contained three plants. The main plots were the contaminated irrigation water levels, while the sup plots were the concentrations of ascorbic acid. The means of the individual factors and their interactions were compared by L.S.D test at 5% level of probability according to Snedecor and Cochran (1974).

RESULTS AND DISCUSSION

1. Vegetative growth:

1.1. Plant height (cm)

Data presented in Table (3) show that, in both two seasons, irrigation with contaminated water with nickel decreased the height of *Jatropha curcas* plants, compared to plants irrigated with tap water (control). Plants irrigated with tap water had the highest mean values of plant height 45.49 and 49.38 cm in the first and second season, respectively. Moreover, raising the nickel concentration caused steady significant reductions in plant height, with the highest concentration (300 ppm) giving significantly shortest plants with mean heights of 34.80 and 38.10 cm in the first and second season, respectively, than those received the other nickel concentration.

Plant height was also significantly affected by spraying the plants with ascorbic acid. In both seasons, plant height was increased gradually when the ascorbic acid concentration was raised from 0 ppm (control) to 500 ppm. Accordingly, it can be seen from the data in Table (3) that *Jatropha curcas* plants sprayed with 500 ppm ascorbic acid were significantly taller with mean plant heights of 41.47 and 44.53 cm in the first and second seasons, respectively, than plants sprayed with any other ascorbic acid concentration.

Regarding the interaction between the effects of irrigation with contaminated nickel water and ascorbic acid treatments on growth rate of the plant height of *Jatropha curcas* plants, the highest values were obtained in the plants irrigation with tap water and sprayed with ascorbic acid at 500 ppm (with mean heights of 46.91 and 50.66 cm in the first and second seasons, respectively). On the other hand, the shortest plants with mean heights of 31.50 and 35.33 cm in the first and second seasons, respectively, were resulted in the plants irrigated with the highest nickel concentration 300 ppm without ascorbic acid treatment. It can also been seen from the data presented in Table (3) that in many cases, spraying the plants with ascorbic acid reduced the undesirable effect of contaminated water with nickel.

1.2. Number of leaves per plant

The data presented in Table (3) show the effect of contaminated water with nickel on number of leaves formed on *Jatropha curcas* plants. In both seasons, plants irrigation with tap water had the highest number of leaves 24.05 and 25.77 leaves per plant in the first and second seasons, respectively. Accordingly, the lowest number of leaves 19.21 and 20.38 leaves per plant in the first and second seasons, respectively, was formed by the plants that were irrigated with highest nickel concentration 300 ppm.

Concerning the effect of ascorbic acid treatments on the number of leaves, the data recorded in Table (3) show that the treatment of ascorbic acid 500 ppm caused a significant increase in the number of leaves giving mean values of 22.28 and 23.33 leaves per plant in the first and second seasons, respectively, compared to that of the control plants (19.95 and 21.87 leaves per plant in the two seasons, respectively).

Data in Table (3) showed significant interaction in both seasons between the effects of irrigation with contaminated nickel water and ascorbic acid treatments on number of leaves formed by *Jatropha curcas* plants. Combination between irrigation using tap water and spraying the plants with ascorbic acid at 500 ppm gave the highest number of leaves per plant 24.66 and 26.33 leaves per plant in the first and second seasons, respectively. On the other hand, the lowest number of leaves of 17.66 and 19.16 leaves per plant in the first and second seasons, respectively, were obtained in plants irrigated with the highest nickel concentration 300 ppm and sprayed without ascorbic acid.

1.3. Leaf dry weight (g) per plant

The results recorded in the two seasons in Table (3) showed that the heaviest dry weights of leaves (20.83 and 22.66 g) in the first and second seasons, respectively, were obtained in plants irrigated with tap water. Moreover, the recorded values were decreased steadily with raising the nickel concentration. Accordingly, the lowest values 15.97 and 17.47 g per plant in the first and second seasons, respectively, were obtained in plants irrigated with the highest nickel concentration 300 ppm.

Data presented in Table (3) also show that spraying *Jatropha curcas* plants with 500 ppm ascorbic acid significantly increased the dry weight of leaves 19.01 and 20.39 g per plant in the first and second seasons, respectively, compared to the control which recorded 16.77 and 18.88 g per plant in the first and second seasons, respectively.

Regarding the significant interaction between the effects of irrigation with water contaminated with nickel and ascorbic acid treatments on the dry weight of leaves of *Jatropha curcas*, presented in Table (3) showed that the heaviest dry weights of leaves 21.51 and 23.22 g in the first and second seasons, respectively, were obtained in plants irrigated with tap water and sprayed with 500 ppm ascorbic acid, whereas the lowest dry weights of leaves of 14.98 and 16.20 g in the first and second seasons, respectively, were obtained when the plants were irrigated using the highest nickel concentration 300 ppm with 0 ppm ascorbic acid.

1.4. Leaf area (cm^2)

The results recorded in the two seasons in Table (3) showed that the irrigation with nickel contaminated water has decreased leaf area of *Jatropha curcas* plants, compared to plants irrigated with tap water (control). In both seasons, plants irrigated with tap water (control) had the largest leaves area with means of 1208.50 and 1322.64 cm^2 in the first and second seasons, respectively. The leaf area was decreased steadily with raising the nickel concentration. Accordingly, the smallest leaves area with means of 721.50 and 787.40 cm^2 in the first and second seasons, respectively, were formed in plants that were irrigated using the highest nickel concentration (300 ppm).

Data presented in (Table 3) showed that, in most cases, the different ascorbic acid treatments had no significant effect on leaf area of *Jatropha curcas* plants in terms of leaves area. The only exception to this general trend was recorded in the first and second seasons, for plants sprayed with ascorbic acid at 500 ppm forming significantly larger leaves area with a mean of 1060.97 and 1124.32 cm^2 in the first and second season, respectively, than those formed by control plants (754.425 and 850.70 cm^2, respectively).

Data presented in (Table 3) also showed that significant interaction was detected between the effects of irrigation with water contaminated with nickel and ascorbic acid treatments on the area of *Jatropha curcas* leaves. The largest leaf area 1417.23 and 1528.35 cm^2 in the first and second seasons, respectively, was formed by plants irrigated with tap water and sprayed with 500 ppm ascorbic acid. On the other hand, the smallest leaves area of 603.35 and 674.37 cm^2 in the first and second seasons, respectively, were obtained in plants irrigated using the highest nickel concentration 300 ppm using the lowest concentration 0 ppm ascorbic acid.

1.5. Stem diameter (cm)

The data recorded for stem diameter of *Jatropha curcas* plants in the two seasons in Table (4) showed that irrigation with water contaminated with nickel decreased stem thickness, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the thickest stems, with mean diameters of 5.44 and 5.92 cm in the first and second seasons, respectively. Raising the nickel concentration in irrigation water caused significant reduction in stem diameter, even at the lowest nickel concentration 300 ppm, which gave stem diameters of 4.17 and 4.56 cm in the first and second seasons, respectively.

In contrast to the effect of nickel treatments, ascorbic acid treatments improved stem diameter of *Jatropha curcas* plants, compared to the control. Moreover, plants sprayed with 500 ppm ascorbic acid had significantly thickest stems with mean diameters of 4.97 and 5.32 cm in the first and second seasons, respectively, compared to those of the control plants, or plants sprayed with any other ascorbic acid concentration.

Regarding the significant interaction between the effects of irrigation with water contaminated with nickel and ascorbic acid treatments on stem diameter of *Jatropha curcas* plants, the results recorded in the two seasons in Table (4) showed that significant differences were detected between the values obtained from plants receiving the different treatment combinations. The highest values of 5.63 and 6.07 cm in the first and second season, respectively were obtained in the plants irrigated with tap water and sprayed with 500 ppm ascorbic acid.

Table 3: Means of vegetative growth characteristics of *Jatropha curcas* plants as influenced by Nickel (Ni), Ascorbic acid (AA) and their combinations (Ni × AA) in the two seasons of 2014 and 2015.

Treatments		Plant height (cm)		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm^2)	
Ni (ppm)	Ascorbic acid (ppm)	2014	2015	2014	2015	2014	2015	2014	2015
0	0	44.33	48.50	23.50	25.33	20.27	22.27	971.71	1071.39
	250	45.25	49.00	24.00	25.66	20.71	22.51	1236.56	1368.18
	500	46.91	50.66	24.66	26.33	21.51	23.22	1417.23	1528.35
Mean		45.49	49.38	24.05	25.77	20.83	22.66	1208.50	1322.64
100	0	35.08	42.41	19.16	22.33	16.12	19.39	733.59	885.69
	250	38.83	44.08	21.16	23.00	17.80	20.15	886.69	990.00
	500	41.58	45.83	22.50	23.83	19.07	20.98	1057.57	1110.24
Mean		38.49	44.10	20.94	23.05	17.66	20.17	892.61	995.31
200	0	35.41	38.58	19.50	20.66	16.24	17.68	709.03	771.36
	250	38.00	41.66	20.66	22.16	17.40	19.03	827.27	910.62
	500	40.00	42.00	21.66	22.16	18.31	19.19	919.99	960.68
Mean		37.80	40.74	20.60	21.66	17.31	18.63	818.76	880.88
300	0	31.50	35.33	17.66	19.16	14.48	16.20	603.35	674.37
	250	35.50	39.33	19.66	21.00	16.28	18.04	712.03	789.83
	500	37.41	39.66	20.33	21.00	17.15	18.19	849.12	898.02
Mean		34.80	38.10	19.21	20.38	15.97	17.47	721.50	787.40
Mean (AA)	0	36.58	41.20	19.95	21.87	16.77	18.88	754.42	850.70
	250	39.39	43.51	21.37	22.95	18.04	19.93	915.63	1014.65
	500	41.47	44.53	22.28	23.33	19.01	20.39	1060.97	1124.32
L.S.D. at 0.05	Ni	1.93	1.42	0.87	0.73	0.85	0.65	48.05	36.72
	AA	0.86	0.91	0.43	0.45	0.38	0.41	23.14	17.39
	Ni * AA	1.99	2.10	1.00	1.03	0.89	0.95	53.29	40.03

On the other hand, the thinnest stems of 3.78 and 4.23 cm in the first and second seasons, respectively, were obtained in the plants irrigated with the highest nickel concentration 300 ppm without ascorbic acid treatment. It can also be seen that in some cases, the ascorbic acid treatments helped to overcome the adverse effect of the nickel treatments on stem thickness.

1.6. Stem dry weight (g)

Data presented in (Table 4) showed that, in both seasons, irrigation with contaminated water with nickel significantly decreased dry weights of stem of *Jatropha curcas* plants, compared to plants irrigated with tap water (control). Plants irrigated with tap water had the heaviest mean dry weight of stems 38.36 and 42.58 g in the first and second seasons, respectively. The dry weight of stems showed a gradual reduction as the nickel concentration was increased. Accordingly, the lowest dry weights of stem 31.06 and 34.57 g in the first and second seasons, respectively, were recorded in plants received the highest nickel concentration 300 ppm.

The results recorded in the two seasons in Table (4) showed that, in both seasons, spraying with ascorbic acid increased the dry weight of stem. In both seasons, spraying plants with 500 ppm ascorbic acid induced the heaviest dry weight of stem 35.11 and 38.59 g in the first and second seasons, respectively. These values were significantly higher than those of control plants, or plants received any other ascorbic acid concentration.

Regarding the significant interaction between the effects of irrigation with contaminated water with nickel and ascorbic acid treatments, the results recorded in the two seasons show that the heaviest stems dry weights 39.70 and 43.27 g in the first and second seasons, respectively, were with plants irrigated with tap water and sprayed without ascorbic acid. On the other hand, the lowest stem dry weights 26.77 and 30.17 g per plant in the first and second seasons, respectively were obtained in plants irrigated using the highest nickel concentrations 200 ppm without ascorbic acid treatment.

1.7. Root length (cm)

Data presented in Table (4) showed that all the tested irrigation with contaminated water with nickel treatments significantly decreased root length (cm) of *Jatropha curcas*, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the highest mean root length 51.51 and 51.08 cm in the first and second seasons, respectively. Raising the nickel concentration caused a steady reduction in the root length, which reached its lowest values 39.50 and 41.57 cm in the first and second seasons, respectively, in plants irrigated using the highest nickel concentration 300 ppm.

The data in Table (4) also indicate that ascorbic acid treatments had a significant effect on the root length, in both seasons on *Jatropha curcas* plants, compared to the control plants. As with the other vegetative growth parameters, spraying the plants with 500 ppm ascorbic acid gave the heaviest root length 47.02 and 47.24 cm in the first and second seasons, respectively.

Regarding the interaction between the effects of irrigation with contaminated water with nickel and ascorbic acid treatments on root length of *Jatropha curcas* plants, the results recorded in the two seasons showed that the highest values were obtained in plants irrigated with tap water and sprayed with 500 ppm ascorbic acid with mean length of 53.19 and 52.21 cm in the first and second seasons, respectively.

1.8. Root dry weight (g)

Data presented in Table (4) show that irrigation of *Jatropha curcas* plants with contaminated water with nickel significantly decreased the dry weights of roots, compared to plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the heaviest dry weight of roots 29.59 and 32.61 g per plant in the first and second seasons, respectively. Steady significant reductions in the dry weight of roots were recorded as the nickel concentration in the irrigation water was increased, with the highest nickel concentration 300 ppm giving the lowest mean values in both seasons 23.51 and 26.02 g per plant in the first and second seasons, respectively.

Regarding the effect of ascorbic acid treatments on the dry weight of roots, data in (Table 4) show that spraying *Jatropha curcas* plants with 500 ppm ascorbic acid significantly increased the recorded values, compared to the control. The highest root weight dry 26.35 and 28.97 g per plant in the first and second seasons, respectively, were those of plants sprayed with 500 ppm ascorbic acid.

Regarding the interaction between the effects of irrigation with contaminated water with nickel and ascorbic acid treatments, the data presented in Table (4) show that the highest values 30.60 and 33.24 g per plant in the first and second seasons, respectively were obtained in plants irrigated with tap water and sprayed with 0 ppm ascorbic acid.

Table 4: Means of vegetative growth characteristics of *Jatropha curcas* plants as influenced by Nickel (Ni), Ascorbic acid (AA) and their combinations (Ni × AA) in the two seasons of 2014 and 2015.

Treatments		Stem diameter (cm)		Stem dry weight (g)		Root length (cm)		Root dry weight (g)	
Ni (ppm)	Ascorbic acid (ppm)	2014	2015	2014	2015	2014	2015	2014	2015
000	0	5.29	5.81	39.70	43.27	50.13	50.23	30.60	33.24
	250	5.41	5.88	37.43	42.31	51.22	50.82	29.07	32.40
	500	5.63	6.07	37.95	42.16	53.19	52.21	29.10	32.21
Mean		5.44	5.92	38.36	42.58	51.51	51.08	29.59	32.61
100	0	4.20	5.07	30.19	33.16	39.87	39.97	23.99	26.65
	250	4.65	5.26	33.06	35.70	44.01	44.01	25.30	28.34
	500	4.98	5.48	34.50	38.38	47.17	46.28	26.06	28.88
Mean		4.61	5.27	32.58	35.74	43.68	43.42	25.11	27.95
200	0	4.24	4.62	26.77	30.17	40.21	36.41	22.54	24.68
	250	4.54	4.97	34.50	37.05	43.03	43.52	25.94	28.02
	500	4.79	5.01	35.80	38.37	45.30	47.46	26.02	28.04
Mean		4.52	4.86	32.35	35.19	42.84	42.46	24.83	26.91
300	0	3.78	4.23	30.15	33.49	35.82	40.75	22.31	24.84
	250	4.25	4.71	30.82	34.75	40.26	40.95	23.98	26.47
	500	4.48	4.75	32.21	35.48	42.43	43.02	24.24	26.76
Mean		4.17	4.56	31.06	34.57	39.50	41.57	23.51	26.02
Mean (AA)	0	4.37	4.93	31.70	35.02	41.50	41.84	24.86	27.35
	250	4.71	5.20	33.95	37.45	44.63	44.82	26.07	28.80
	500	4.97	5.32	35.11	38.59	47.02	47.24	26.35	28.97
L.S.D. at 0.05	Ni	0.22	0.16	1.49	1.66	2.11	1.61	1.14	1.11
	AA	0.10	0.11	0.57	0.60	0.97	0.77	0.22	0.35
	Ni * AA	0.23	0.24	1.32	1.38	2.24	1.77	0.51	0.80

2. Chemical constituents

2.1. Total chlorophyll content (SPAD unites)

The results presented in Table (5) show that the highest content of total chlorophyll was obtained in the plant irrigated with tap water 52.93 and 53.13 SPAD in the first and second seasons, respectively. Raising the nickel concentration in irrigation water resulted in steady significant reductions in the total chlorophyll content, which reached its lowest value 48.02 and 48.43 SPAD in the first and second seasons, respectively, in plants received the highest nickel concentration 300 ppm.

The results of leaf chemical analysis in Table (5) also show that the tested ascorbic acid treatments had clear effect on the total chlorophyll content. The recorded mean values were ranged from 51.48 and 51.65 SPAD in the first and second seasons, respectively, in plants sprayed with 500 ppm ascorbic acid 47.50 and 47.99 SPAD in the first and second seasons, respectively, in plants sprayed with 0 ppm ascorbic acid.

Regarding to the interaction between the effects of irrigation contaminated water with nickel and ascorbic acid treatments, the data presented in Table (5) showed that the highest total chlorophyll contents of 54.55 and 54.65 SPAD in the first and second seasons, respectively, were found in leaves of plants irrigated with tap water and sprayed with ascorbic acid at 500 ppm, the lowest values of 45.02 and 45.76 SPAD in the first and second seasons, respectively, were obtained in plants irrigated with nickel water at 100 ppm and sprayed with tap water.

2.2. Carbohydrates content (%)

Data resulting from chemical analysis in Table (5) show that, the carbohydrates % in the dried leaves of *Jatropha curcas* plants was decreased steadily with raising the nickel concentration in the irrigation water contaminated with nickel. The highest mean carbohydrates content 19.698 and 19.68 % in the first and second seasons, respectively, was found in the leaves of control plants, whereas the lowest mean value 17.62 and 17.94 % in the first and second seasons, respectively, was found in plants irrigated with water containing the highest nickel concentration 300 ppm.

The results in Table (5) also show that most of the tested ascorbic acid concentrations increased total carbohydrates % in the leaves of *Jatropha curcas* plants, compared to the control. Among the plants received the different ascorbic acid treatments, plants sprayed with 250 ppm ascorbic acid had the highest carbohydrate % in leaves 18.98 and 19.25 % in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation water contaminated with nickel and ascorbic acid treatments on the carbohydrates % of leaves, the results presented in Table (5) show that the highest mean values of 20.21 and 20.24 % in the first and second seasons, respectively, were obtained in the leaves of plants irrigated with tap water and sprayed with ascorbic acid at 500 ppm.

2.3. Nickel content in leaves (ppm)

The data resulting from leaves chemical analysis in Table (5) showed that, the nickel content (ppm) in the dried leaves of *Jatropha curcas* plants was raised steadily with raising the nickel concentration in the irrigation water. The lowest mean nickel content 0.116 and 0.129 ppm in the first and second seasons, respectively, was found in the leaves of control plants, whereas the highest values 0.384 and 0.413 ppm in the first and second seasons, respectively, was found in plants irrigated with water containing the highest nickel concentration 300 ppm.

Concerning the effect of ascorbic acid treatments on the nickel content in leaves, the data recorded in the two seasons (Table 5) show that ascorbic acid treatment 500 ppm caused a significant decrease in the nickel content in leaves giving mean values of 0.165 and 0.179 ppm in the first and second seasons, respectively, compared to that of control plants that had the highest nickel content in leaves 0.375 and 0.402 ppm in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation contaminated nickel water and ascorbic acid treatments on the nickel content in leaves. The results in (Table 5) show that the lowest mean values of 0.082 and 0.088 ppm in the first and second seasons, respectively, were obtained in the leaves of plants irrigated with tap water and sprayed with ascorbic acid at 500 ppm. On the other hand, the highest nickel content was obtained in the leaves that treated with nickel at 300 ppm and receiving no ascorbic acid treatment (0.577 and 0.631 ppm in the first and second seasons, respectively).

2.4. Nickel content in stem (ppm)

The data resulting from stem chemical analysis in Table (5) showed that, the nickel content (ppm) in the dried stem of *Jatropha curcas* plants was raised steadily with raising nickel concentration in irrigation water. The lowest mean nickel content 0.064 and 0.073 ppm in the first and second seasons, respectively, was found in the stem of control plants, whereas the highest mean value 0.249 and 0.273 ppm in the first and second seasons, respectively, was found in plants irrigated with water containing the highest nickel concentration 300 ppm.

Concerning the effect of ascorbic acid treatments on the nickel content in stem, the data recorded in the two seasons, Table (5) showed that only one ascorbic acid treatment 500 ppm caused a significant decrease in the nickel content in stem giving mean values of 0.100 and 0.109 ppm in the first and second seasons, respectively, compared to the control plants that had the highest nickel content in stem 0.246 and 0.268 ppm in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation water contaminated with nickel and ascorbic acid treatments on the nickel content in stem, the results in Table (5) show that the lowest mean values of 0.043 and 0.048 ppm in the first and second seasons, respectively, were obtained in the stem of plants irrigated with tap water and sprayed with ascorbic acid at 500 ppm. On the other hand, the highest nickel content was obtained in the stem of plants treated with nickel at 300 ppm and receiving no ascorbic acid treatment (0.374 and 0.424 ppm in the first and second seasons, respectively).

2.5. Nickel content in roots (ppm)

The data resulting from roots chemical analysis in Table (5) showed that, the nickel content (ppm) in the dried roots of *Jatropha curcas* plants was raised steadily with raising the nickel concentration in the irrigation water. The lowest mean nickel content 0.010 and 0.013 ppm in the first and second seasons, respectively, was found in the roots of control plants, whereas the highest mean value 0.102 and 0.118 ppm in the first and second seasons, respectively, was found in those irrigated with water contained the highest nickel concentration 300 ppm.

Concerning the effect of ascorbic acid treatments on the nickel content in roots, the data recorded in both seasons, Table (5) show that ascorbic acid treatment 500 ppm caused a significant decrease in the nickel content in roots giving mean values of 0.031 and 0.034 ppm in the first and second seasons, respectively, compared to that of control plants that had the highest nickel content in roots 0.105 and 0.119 ppm in the first and second seasons, respectively.

Concerning the interaction between the effects of irrigation water contaminated with nickel and ascorbic acid treatments on the nickel content in roots, the results in Table (5) show that the lowest mean values of 0.003 and 0.007 ppm in the first and second seasons, respectively, were obtained in the roots of plants irrigated with tap water and sprayed with ascorbic acid at 500 ppm. On the other hand, the highest nickel content was obtained in the roots of plants treated with nickel at 300 ppm and received 0 ppm ascorbic acid treatment (0.152 and 0.193 ppm in the first and second seasons, respectively).

Table 5: Means of chemical constituents of *Jatropha curcas* plants as influenced by Nickel (Ni), Ascorbic acid (AA) and their combinations (Ni ×AA) in the two seasons of 2014 and 2015.

Treatments		Chlorophyll content (SPAD)		Carbohydrates content (%)		Nickel content in leaves (ppm)		Nickel content in stem (ppm)		Nickel content in roots (ppm)	
Ni (ppm)	**Ascorbic acid (ppm)**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
000	0	51.33	51.74	19.26	19.17	0.143	0.173	0.082	0.100	0.018	0.022
	250	52.93	53.02	19.61	19.64	0.124	0.128	0.068	0.071	0.010	0.011
	500	54.55	54.65	20.21	20.24	0.082	0.088	0.043	0.048	0.003	0.007
Mean		52.93	53.13	19.69	19.68	0.116	0.129	0.064	0.073	0.010	0.013
100	0	45.02	45.76	16.67	16.95	0.314	0.315	0.221	0.224	0.117	0.120
	250	54.15	54.23	19.59	19.83	0.287	0.278	0.181	0.180	0.067	0.072
	500	52.43	52.83	19.15	19.55	0.148	0.167	0.092	0.103	0.032	0.035
Mean		50.53	50.94	18.47	18.77	0.249	0.253	0.164	0.169	0.072	0.075
200	0	47.61	47.70	17.38	17.67	0.469	0.490	0.310	0.325	0.134	0.144
	250	51.80	51.89	18.70	18.88	0.340	0.353	0.220	0.232	0.090	0.099
	500	50.91	51.00	18.12	18.23	0.230	0.254	0.137	0.151	0.038	0.042
Mean		50.10	50.19	18.06	18.26	0.346	0.365	0.222	0.236	0.087	0.095
300	0	46.04	46.76	17.05	17.32	0.577	0.631	0.374	0.424	0.152	0.193
	250	50.01	50.43	18.03	18.68	0.376	0.401	0.246	0.261	0.103	0.108
	500	48.03	48.12	17.80	17.82	0.200	0.209	0.129	0.135	0.053	0.055
Mean		48.02	48.43	17.62	17.94	0.384	0.413	0.249	0.273	0.102	0.118
Mean (AA)	0	47.50	47.99	17.59	17.77	0.375	0.402	0.246	0.268	0.105	0.119
	250	52.22	52.39	18.98	19.25	0.281	0.290	0.178	0.186	0.067	0.072
	500	51.48	51.65	18.82	18.96	0.165	0.179	0.100	0.109	0.031	0.034
L.S.D. at 0.05	Ni	0.69	0.79	0.77	0.27	0.006	0.009	0.004	0.005	0.002	0.003
	AA	0.82	0.78	0.41	0.34	0.003	0.005	0.002	0.003	0.002	0.002
	Ni * AA	1.88	1.80	0.96	0.79	0.006	0.013	0.005	0.008	0.005	0.004

3. Transfer factor (TF) of heavy metals

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

3.1. Nickel content in soil samples (ppm)

Data in Table (6) showed that the lowest average of nickel content was observed in soil cultured by untreated plants, while the highest average of nickel content was observed in soil after the treatment 300 ppm nickel and 500 ppm ascorbic acid.

Table 6: Average of nickel content in soil samples as it influenced by nickel concentrations in water irrigation and foliar application of citric acid on *Jatropha curcas* leaves at the end of second season (2015).

Treatments		Nickel content in soil (ppm)
Ni (ppm)	Ascorbic acid (ppm)	
0 ppm	0 ppm	0.045
	250 ppm	0.063
	500 ppm	0.067
100 ppm	0 ppm	0.060
	250 ppm	0.071
	500 ppm	0.084
200 ppm	0 ppm	0.068
	250 ppm	0.081
	500 ppm	0.092
300 ppm	0 ppm	0.076
	250 ppm	0.086
	500 ppm	0.098

3.2. Transfer factor to leaves (TFL)

From the data presented in Table (7), it can be seen that the transfer factor in the dried leaves of *Jatropha curcas* plants was increased steadily with raising the nickel concentration in the irrigation water. Accordingly, the lowest nickel value (2.396) in the second season was found in plants irrigated with water containing 0 ppm nickel (control), whereas the highest value (5.035) in the second season was found in plants irrigated with water contained 300 ppm nickel.

The results in Table (7) also show that the transfer factor in the dried leaves was reduced steadily with raising ascorbic acid concentration. Accordingly, the highest nickel value (6.154) in the second season was recorded in the leaves of control plants, whereas plants sprayed with the highest ascorbic acid concentration 500 ppm had the lowest nickel content (2.049) in the second season.

Regarding the interaction between effect of irrigation contaminated water and ascorbic acid concentrations on the transfer factor in the dried leaves, the data in Table (7) show that the highest mean values (8.307) in the second season, was obtained in plants irrigated with nickel water at 300 ppm and sprayed with tap water, while the lowest mean values (1.313) in the second season, was recorded in plants applied with 0 ppm nickel and sprayed with 500 ppm ascorbic acid.

3.3. Transfer factor to stem (TFS)

From the data presented in Table (7), it can be seen that the transfer factor in the dried stem of *Jatropha curcas* plants was increased steadily with raising the nickel concentration in the irrigation water. Accordingly, the lowest nickel value (1.354) in the second season was found in plants irrigated with water containing 0 ppm nickel, whereas the highest value (3.335) in the second season was found in those irrigated with water containing 300 ppm nickel.

The results in Table (7) also showed that the transfer factor in the dried stem was reduced steadily with raising ascorbic acid concentration. Accordingly, the highest nickel value (4.081) in the second season was recorded in the stem of control plants, whereas plants sprayed with the highest ascorbic acid concentration 500 ppm had the lowest nickel value (1.244) in the second season.

Regarding the interaction between effect of irrigation contaminated water and ascorbic acid concentrations on the transfer factor in the dried stem, the data in Table (7) show that the highest mean values (5.583) in the second season, was obtained in plants irrigated with 300 ppm nickel and sprayed with tap water, while the lowest mean values (0.716) in the second season, was recorded in plants irrigated with nickel water at 0 ppm and sprayed with 500 ppm ascorbic acid.

3.4. Transfer factor to roots (TFR)

From the data presented in Table (7), it can be seen that the transfer factor in the dried roots of *Jatropha curcas* plants was increased steadily with raising nickel concentration in the irrigation water. Accordingly, the lowest nickel value (0.255) in the second season was found in plants irrigated with water containing 0 ppm nickel (control), whereas the highest value (1.451) in the second season was found in plants irrigated with water containing 300 ppm nickel.

The results in Table (7) also showed that the transfer factor in the dried roots was reduced steadily with raising ascorbic acid concentration. Accordingly, the highest nickel value (1.784) in the second season was recorded in the roots of control plants, whereas plants sprayed with the highest ascorbic acid concentration 500 ppm had the lowest nickel value (0.384) in the second season.

Regarding the interaction between effect of irrigation contaminated water and ascorbic acid concentrations on the transfer factor in the dried roots, the data in Table (7) show that the highest mean values (2.539) in the second season, was obtained in plants irrigated with 300 ppm nickel and

Table 7: Means of transfer factor to leaves, stem and roots of *Jatropha curcas* plants as influenced by nickel (Ni), ascorbic acid (AA) and their combinations (Ni ×AA) in the second season of 2015.

Treatments		Transfer factor to leaves (TFL)	Transfer factor to stem (TFS)	Transfer factor to roots (TFR)
Ni (ppm)	Ascorbic acid (ppm)	2015	2015	2015
000	0	3.844	2.222	0.488
	250	2.031	1.126	0.174
	500	1.313	0.716	0.104
Mean		2.396	1.354	0.255
100	0	5.255	3.733	2.005
	250	3.920	2.535	1.014
	500	1.992	1.234	0.416
Mean		3.722	2.500	1.145
200	0	7.210	4.789	2.107
	250	4.358	2.864	1.213
	500	2.757	1.648	0.456
Mean		4.775	3.100	1.258
300	0	8.307	5.583	2.539
	250	4.662	3.042	1.255
	500	2.136	1.380	0.561
Mean		5.035	3.335	1.451
Mean (AA)	0	6.154	4.081	1.784
	250	3.742	2.391	0.914
	500	2.049	1.244	0.384
L.S.D. at 0.05	Ni	0.123	0.077	0.043
	AA	0.081	0.053	0.027
	Ni * AA	0.172	0.109	0.059

sprayed with tap water, while the lowest mean values (0.104) in the second season, was recorded in plants irrigated with 0 ppm nickel and sprayed with 500 ppm ascorbic acid.

DISCUSSION

This study revealed that at high heavy-metal concentrations, the plant height was significantly reduced, and the biomass was decreased. Root growth was more sensitive than other parameters, as roots rapidly absorbed water and had higher accumulations of heavy metal elements. The results presented by this study were in agreement with earlier reports on other plants, such as aquatic plant *Wolffia arrhiza* (Piotrowska *et al.*, 2010), barley *Hordeum vulgare* (Tiryakioglu *et al*., 2006) and *Typha angustifolia* (Bah *et al*., 2011). Other studies with woody plant reported a higher inhibition of root elongation (Dominguez *et al*., 2009). In particular, Jatropha plants could bioaccumulate and bioconcentrate toxic heavy metals from an aqueous solution (Mohammad *et al*., 2010) and could be used as phytoremediation candidates in some countries (Juwarkar *et al*., 2008; Kumar *et al*., 2008 and Jamil *et al*., 2009). Additionally, the plant seedling exhibited a high root/shoot ratio throughout the experiment. An alternative explanation might relate to a strong root system with many roots spread out over the entire soil for survival because root/shoot ratio could reflect plant’s response to various environmental factors (Otieno *et al.,* 2005; Lukacova Kulikova and Lux, 2010 and Li *et al.*, 2010).

The physiological responses, such as the gas exchange rate and photosynthetic function, can be ascribed to the different effects of physico-chemical properties of heavy metals on the integrity and function of the photochemical apparatus of plant seedling fronds, as well as the impact on the chlorophyll concentrations in the leaves. The photosynthesis rate, CO2 assimilation rate (Chen *et al*., 2012). The maintenance of an intercellular CO2 concentration is concomitant with the leaf CO2 assimilation rate and reflected photosynthesis function of seedling in the different heavy metal-spiked soils. The chlorophyll and carotenoid contents played a central role in the energy manifestation of green plant. Any significant alteration of their contents possibly resulted in a marked effect on the entire metabolism of the plant (Piotrowska *et al*., 2010).

Plants grown in high nickel containing soil showed impairment of nutrient balance and resulted in disorder of cell membrane functions. Thus, nickel affected the lipid composition and H-ATPase activity of the plasma membrane as it was reported in Oryza sativa shoots (Ros *et al.*, 1992). Exposure of wheat to high level of nickel enhanced MDA concentration (Pandolfini *et al.,* 1992). Moreover, Gonnelli *et al.*, (2001) reported an increase in MDA concentration of nickel sensitive plants compared to a nickel tolerant saline. Such changes might disturb membrane functionality and ion balance in the cytoplasm, particularly of K, the most mobile ion across plant cell membrane. Other symptoms observed in nickel treated plants were related with changes in water balance. High uptake of nickel induced a decline in water content of dicot and monocot plant species. The decrease in water uptake is used as an indicator of the progression of nickel toxicity in plants (Pandey and Sharma 2002; Gajewska *et al.*, 2006). However, the information on the effect of excess concentrations of some metals (e.g. Nickel) on anti-oxidative processes is rare (Schickler and Caspi 1999), but they have been found to be useful to plants in lower concentrations while affecting them drastically at elevated concentrations. In addition, the symptoms of nickel toxicity appeared as a reduction in seedling growth. The growth of the main root is considerably affected and as a result, it exhibits the function of fibrous roots.

All vegetative growth parameters showed a significant reduction after treatment with different concentrations of nickel in water irrigation. These may be due to that nickel has some similar characteristics to Ca, Mg, Mn, Fe, Cu, and Zn. Therefore, nickel may compete with these metals in absorption and transpiration processes (Küpper *et al*, 1996). Subsequently, this may affect important physiological processes, and ultimately result in toxic effects (Goncalves, 2007).

A significant decrease in total chlorophyll content was observed after irrigation with nickel contaminated water. Diminished chlorophyll concentration in the leaves of nickel treated plants might be due to replacement of central Mg from chlorophyll molecules by nickel (Küpper *et al.*, 1996). Further, decline in chlorophyll concentration (Gajewska *et al.*, 2006) in leaves of nickel treated plants may also be attributed to increase interruption in pigment synthesis and/or increase in degradation of chlorophyll (Sheoran *et al.*, 1990) and (Molas, 1997) ultimately leading to low photosynthetic rates and lower biomass accumulation.

The results showed that there was a significant decrease in total carbohydrate percentage after irrigation with nickel contaminated water. This decrease may be due to reductions in leaf blade area and leaf density (Molas, 1997). Overall, reductions in plant yield can be attributed to poor plant development (Ahmad *et al*., 2007). These results are in harmony with those were obtained by (Srivastava *et al*., 2012) on *Pisum sativum L*. seedlings.

The increase in nickel content in dried leaves and roots is probably because plant shaves efficient root absorption mechanisms which allow them to specifically accumulate metals from soils and/or water. After root absorption, nickel can be transported quickly into shoots and leaves and then sequestrated in the vacuole (Milner and Kochian, 2008). These results are in agreement with those reported by (Skoula *et al.*, 2003) on chamomile, sage (*Salvia officinalis*) and thymus (*Thymus vulgaris*).

Ascorbic acid is the widely known compound used as an antioxidant and the most effective compound increasing the tolerance of plants to oxidative stresses. Confirmed the role of ascorbic acid in oxidative stress or scavenging freeoxy-radicals (Smith *et al.*, 1989). In addition, ascorbic acid affects the physiological activities of the plants. Also, there is evidence that the tolerance of plants is correlated with the increased amount of ascorbic acid. The antioxidant defense system in the plant cells includes both enzymatic antioxidants such as Superoxide Dismutase, Catalase, Ascorbate Peroxidase and non-enzymatic antioxidants like ascorbic acid, Glutathione and tocopherol. When plants are subjected to environmental stresses, oxidative damage may result because the balance between the production of Reactive Oxygen Substances and their detoxification by the antioxidative system is altered (Gomez *et al.*, 1999). Tolerance to damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify Reactive Oxygen Substances (Foyer *et al.*, 1994). Taking all these observations together, it may be suggested as a hypothetical framework that nickel induces a transient loss in antioxidative capacity perhaps accompanied by a stimulation of oxidant producing enzymes, which results in intrinsic ascorbic acid accumulation. ascorbic acid then would act as a signalling molecule triggering secondary defences.

CONCLUSIONS

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

Phytoremediation of contaminated water and soil with heavy metals using non-edible plant like *Jatropha curcas* offers an environmental friendly and cost-effective method for remediating the polluted soil. The *Jatropha curcas* was found to be able to efficiently remove the heavy metals such as nickel.

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الملخص العربى

إستجابة نباتات الجاتروفا للرش بحمض الأسكوربيك لتخفيض الأثر الضار للتلوث بالنيكل فى ماء الرى

نادر أحمد الشنهورى[١]، خالد عبد المحسن إمام[٢]

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

[٢] فرع بحوث الحدائق النباتية – الجيزة – معهد بحوث البساتين – مركز البحوث الزراعية

أجريت هذه الدراسة في فرع البحوث بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية– الإسكندرية، مصر خلال الموسمين المتتاليين ٢٠١٤ و ٢٠١٥. وكان الهدف من هذه الدراسة تقييم آثار مياه الري الملوثة بالنيكل على نباتات الجاتروفا المزروعة في تربة رملية، كذلك استخدام الرش بحمض الاسكوربيك للتغلب على الآثار الضارة للنيكل. زرعت شتلات الجاتروفا بشكل فردي في أوعية بلاستيكية(قطرها ٣٠ سم) مملوءة ٨ كجم من التربة الرملية. وكانت معاملات مياه الري الملوثة بأربعة تراكيزات من النيكل وهى صفر، ١٠٠، ٢٠٠، ٣٠٠ جزء في المليون. تم معاملة النباتات أيضا بحامض الاسكوربيك في ثلاث تركيزات هى صفر، ٢٥٠ و ٥٠٠ جزء في المليون عن طريق الرش شهريا في كلا الموسمين.

أظهرت النتائج أنة بتقييم معايير النمو الخضري وجد أن هناك اختلاف كبير في التفاعل بين تركيزات النيكل ورش النباتات بحامض الاسكوربيك. لوحظ انخفاض كبير في كافة معاملات الري بالماء الملوث بالنيكل وكذلك لوجظ زيادة كبيرة فى معدلات النموالخضرى بعد الرش بتركيز ٥٠٠ جزء في المليون حمض الاسكوربيك. تم الحصول على أعلى قيمة من محتوى الكلوروفيل والكربوهيدرات فى النباتات المروية بماء الصنبور والرش بتركيز ٥٠٠ جزء في المليون حامض الاسكوربيك في حين أن أعلى تركيز للنيكل كان في الأوراق والساق والجذور كنتيجة للرى بماء ملوث بتركيز ٣٠٠ جزء في المليون دون الرش بحمض الاسكوربيك.