

## RESPONSE OF *LANTANA CAMARA* PLANTS TO FOLIAR APPLIED CITRIC ACID FOR DECREASING THE HARMFUL EFFECT OF HEAVY METALS POLLUTION IN THE IRRIGATION WATER (B) EFFECT OF NICKEL

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**ABSTRACT:** The present study was carried-out at Antoniadis Research Branch, Horticultural Research Institute, Agriculture Research Center (ARC), Alexandria, Egypt during two successive seasons of 2018 and 2019. The aim of this study was to evaluate the effects of irrigation water polluted with nickel on the growth of *Lantana camara* plants and the possibility of using citric acid spray treatments to overcome the effects of nickel pollution. Transplants of *Lantana camara* were planted individually in plastic pots (20 cm diameter) filled with 5 kg of sandy soil. The nickel polluted irrigation water treatments were 0, 100, 200 and 300 mg/l. The plants were monthly sprayed with citric acid at concentrations of 0, 250 and 500 mg/l. The results showed that for vegetative growth parameters there were non significant differences in the interaction between nickel concentrations and foliar spray with citric acid, while significant reduction was observed in all parameters after irrigation with nickel polluted water and significant increase in vegetative growth parameters were observed after 500 mg/l citric acid application. For total chlorophyll and carbohydrate content, the highest significant values were obtained in plants irrigated with tap water and sprayed with 250 mg/l citric acid while the highest significant level of nickel content in the leaves, stem and roots was obtained due treatment by 300 mg/l nickel without application of citric acid.

**Key words:** *Lantana camara*, polluted irrigation water, nickel, citric acid.

### INTRODUCTION

Phytoremediation has become an effective and affordable technological solution used to extract or remove toxic metals from polluted soil. Phytoremediation is the use of plants to clean up a pollution from soils, sediments and water. This technology is environment friendly and potentially cost effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants (Choruk *et al.*, 2006). Plants need trace amount of heavy metals but their excessive availability may cause plant toxicity (Sharma *et al.*,

2006). Phytotoxic concentration of 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 raw material in the metallurgical and electroplating industries, and from sewage sludge and compost (Karam, 1998). Nickel is essential for plants (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 (Küpper and Kroneck, 2007). Therefore, nickel plays a role in various important metabolic processes, including ureolysis, hydrogen metabolism, methane biogenesis and acetogenesis (Mulrooney and Hausinger, 2003). Nickel has a particular toxicity due to its increasing industrial use, with increasing nickel pollution, excess nickel rather than a deficiency, is more commonly found in plants (Salt, 2000). 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).

Endogenous organic acids are the source of both carbon skeleton and energy for cells and are used in the respiratory cycle and other biochemical pathways (Da Silva, 2003). Citric acid (CA) is an organic acid mainly served as antioxidant and involved in plant metabolism through its role in Krebs cycle during the respiration in mitochondria which produces cellular energy by oxidative phosphorylation (Taize and Zaiger, 2002). This energy is required for different physiological processes and development the mechanisms of resistance systems under different stressful factors. It is created by addition of acetyl-CoA to oxaloacetic acid that is converted to succinate and malate in next steps (Wills *et al.*, 1981).

*Lantana camara* plant is found mostly in South India, in sub-tropical and tropical America and in Africa. The plant named *Lantana camara* Linn. Family: Verbenaceae is commonly known as wild sage or red sage and lantana weed. It is a large scrambling evergreen, strong smelling shrub with stout prickles, its leaves are opposite on both sides. Lantana flowers are small, generally orange but often vary in colors from white to

dark red, which are prominently capitates in heads; bracts conspicuous and persistent. Lantana fruits are small, 5 mm diameter, drupaceous and shining, blue, greenish, blackish, with two nutlets in it. Its seeds were germinated easily throughout central and South India in most dry stony hills and black *Lantana camara* is a small perennial shrub which can grow to around 2m in height and forms dense thickets in a variety of habitats. Due to extensive selective breeding throughout the 17<sup>th</sup> and 18<sup>th</sup> Century for use as an ornamental plant there are now many forms of *Lantana camara* present throughout the world (Ashwini *et al.*, 2014).

In this study *Lantana camara* was selected due to its characteristics as non-edible plant which can grow in tropical areas and it has many uses in landscaping. Therefore, the objectives of this study were to determine the potential of *Lantana camara* in removing nickel from soil affected by nickel contaminated irrigation water and to investigate on the ability of *Lantana camara* in removing nickel.

## MATERIALS AND METHODS

The present study was carried-out at Antoniadis Research Branch, Horticultural Research Institute, Agriculture Research Center (ARC), Alexandria, Egypt during two successive seasons of 2018 and 2019.

On 15<sup>th</sup> of February, 2018 and 2019 in the first and second seasons, respectively, uniform transplants of *Lantana camara* (15-20 cm height and 10-15 leaves per plant) were planted individually in plastic pots (20 cm diameter) filled with 5 kg sandy soil. The chemical characteristics of the soil were measured as described by Jackson (1958) and presented in Table (1).

On 1<sup>st</sup> of March (in both seasons), the polluted of irrigation water were initiated. Four concentrations of nickel chloride (NiCl<sub>2</sub>.6H<sub>2</sub>O): 0,100, 200 and 300 mg/l were dissolved in the irrigation water. The plants were irrigated three times per week. At the end of the experiment every pot had received

about 127 liters of nickel polluted water (Table, 2). In both seasons, the plants received monthly sprayed citric acid from 15<sup>th</sup> May till 15<sup>th</sup> August in both seasons at concentrations of 0, 250 and 500 mg/l. The control plants were sprayed with tap water. On 30<sup>th</sup> of September in the both seasons, the plants were harvested.

In the two seasons, the plants received N, P and K chemical fertilizers in the form of soluble fertilizer (Kristalon 19-19-19) at rate of 1.5 g/pot. Fertilization was repeated every 30 days throughout the growing season (from the 1<sup>st</sup> of March till the 30<sup>th</sup> of September). In addition, weeds were removed manually upon emergence.

**Data recorded:**

**1. Vegetative growth parameters:**

Plant height (cm), number of leaves per plant, leaves dry weight per plant (g), leaves area (cm<sup>2</sup>) according to Koller (1972), branch number per plant, stem diameter (cm), stem dry weight (g), root length (cm), root dry weight (g), flower number per plant and flower dry weight (g) were also measured.

**2. Chemical analysis:**

▪ Total chlorophyll index was measured as a SPAD for the fresh middle 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 carbohydrate percentage in the leaves was determined according to Dubios *et al.* (1956).
- Proline content (mg/g) in the leaves was determined according to Bates *et al.* (1973).
- Determination of nickel content in plant samples was determined as follows: Plant samples were divided into leaves, stem and roots, oven dried at 70 °C for 72 hrs in an oven. The dried plant samples were ground to powder. The oven dried samples were digested for extraction of nickel, using the method described by Piper (1947) and the concentration of heavy metal was assured using an atomic absorption spectrophotometer.
- Available nickel in the soil sample was extracted by DPTA-Solution according to Lindsay and Norvell (1978) and measured by Inductively Coupled Plasma Spectrometry.
- Transfer factor (TF) is calculated as the ratio between the concentrations of metal in the shoots to the concentration of metal in the soil (Chen *et al.*, 2004), as indicates to the efficiency of any plant to transfer any metal from soil to the aerial parts.

The pot experimental design was split plot with three replicates. Each replicate contained three plants. The main plot was

**Table 1. The chemical properties of the used sandy soil for the two seasons 2018 and 2019.**

Season	pH	EC dS/m	Water soluble cations (meq/l)				Water soluble anions (meq/l)		
			Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
2018	7.93	1.55	3.4	3.4	6.5	1.3	3.6	6.7	2.4
2019	7.91	1.52	3.2	3.0	6.3	1.2	3.3	6.5	2.2

**Table 2. Total amount of used irrigation water for each plant (l/pot) in each treatment during the two growing seasons of 2018 and 2019.**

Field Capacity (%)	Months of first and second seasons							Total
	March	April	May	June	July	August	September	
100 %	14.00	15.00	16.00	17.00	20.00	23.00	22.00	127.00

nickel polluted water concentration, while the subplot was citric acid treatments. The obtained data were subjected to analysis of variance (ANOVA) using the SAS program, SAS Institute (SAS Institute, 2002). The Means of the individual factors and their interactions were compared by L.S.D test at 5% level of probability according to Snedecor and Cochran (1989).

## RESULTS

### 1. Vegetative growth:

#### a. Leaves parameters:

Data presented in Table (3) showed that, in both seasons, irrigation water polluted with nickel decreased the tested leaves parameters of *Lantana camara* plants. However, plants irrigated with tap water (control) had the highest mean values of number of leaves per plant (95.50 and 94.99), leaves dry weight (1.71 and 2.06 g) and leaves area (412.54 and 267.21 cm<sup>2</sup>) in the first and second seasons, respectively.

Increasing the nickel concentration significantly reduced in the tested parameters. The highest nickel concentration (300 mg/l) significantly produced the lowest plants mean values of plant number of leaves per plant (84.88 and 85.21), leaves dry weight (1.50 and 1.85 g) and leaves area (232.58 and 191.90 cm<sup>2</sup>) in the first and second seasons, respectively, compared with the other concentrations.

Leaves parameters were also significantly affected by spraying the plants with citric acid. In both seasons, leaves parameters were gradually increased when the citric acid concentration was increased from 0 mg/l (control) to 500 mg/l. Accordingly, Table (3) showed that plants sprayed with 500 mg/l citric acid showed significantly higher mean values of number of leaves per plant (92.24 and 92.41), leaves dry weight (1.65 and 2.01 g) and leaves area (352.59 and 243.89 cm<sup>2</sup>) in the first and second seasons, respectively, compared with

**Table 3. Means of number of leaves per plant, leaves dry weight (g) and leaves area (cm<sup>2</sup>) of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

Treatments		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm <sup>2</sup> )	
Ni (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019
0	0	93.50	94.08	1.71	2.06	391.00	272.44
	250	95.50	95.08	1.67	2.01	360.35	251.86
	500	97.50	95.83	1.77	2.13	486.27	277.33
Mean (Ni)		95.50	94.99	1.71	2.06	412.54	267.21
100	0	91.50	92.58	1.64	2.01	349.93	231.86
	250	90.50	91.58	1.62	1.97	332.13	226.02
	500	95.50	95.58	1.72	2.06	350.24	236.47
Mean (Ni)		92.50	93.25	1.66	2.01	344.10	231.45
200	0	88.83	89.83	1.57	1.92	307.15	236.43
	250	86.00	85.83	1.53	1.91	301.72	206.61
	500	89.83	90.58	1.60	1.95	309.14	256.33
Mean (Ni)		88.22	88.74	1.56	1.92	306.00	233.12
300	0	85.00	85.08	1.50	1.85	223.58	189.34
	250	83.50	82.91	1.48	1.82	209.46	180.93
	500	86.16	87.66	1.53	1.90	264.72	205.45
Mean (Ni)		84.88	85.21	1.50	1.85	232.58	191.90
Mean (CA)	0	89.70	90.39	1.60	1.96	317.91	232.51
	250	88.87	88.85	1.57	1.92	300.91	216.35
	500	92.24	92.41	1.65	2.01	352.59	243.89
L.S.D. at 0.05	Ni	1.68	1.92	0.03	0.03	49.37	26.34
	CA	1.95	1.70	0.04	0.04	26.80	15.79
	Ni × CA	2.25	1.95	0.04	0.05	30.80	18.15

the other concentrations.

Regarding the interaction between the irrigation with nickel polluted water and citric acid treatments on the tested leaves parameters, the recorded results in the two seasons showed that, the highest values were obtained from plants irrigated with tap water (control) and sprayed with citric acid at 500 mg/l with mean values of number of leaves per plant (97.50 and 95.83), leaves dry weight (1.77 and 2.13 g) and leaves area (486.27 and 277.33 cm<sup>2</sup>) in the first and second seasons, respectively, compared with the other concentrations. On the other hand, the lowest values of number of leaves per plant were (83.50 and 82.91), for leaves dry weight were (1.48 and 1.82 g) and for leaves area were (209.46 and 180.93 cm<sup>2</sup>) in the first and second seasons, respectively, were resulted when the plants were irrigated using the highest nickel concentration (300 mg/l) and sprayed with citric acid at 250 mg/l

treatment. It can be also showed that in many cases, spraying plants with citric acid reduced the adverse effect of polluted water with nickel (Table 3).

**b. Stem parameters:**

Data presented in Table (4) showed that irrigation with nickel polluted water decreased stem parameters, compared to those of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the thickest stem, with mean plant height (32.99 and 38.61 cm), number of branch per plant (6.33 and 11.33), stem diameter (0.49 and 0.66 cm) and stem dry weight (3.01 and 5.03 g) in the first and second seasons, respectively. Increasing the nickel concentration in irrigation water caused a steady reduction in stem parameters. This reduction in stem parameter was significant (compared to the control), even at the highest nickel concentration (300 mg/l), which gave plant height (29.02 and

**Table 4. Means of plant height (cm), branch number per plant, stem diameter (cm) and stem dry weight (g) of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

Treatments		Plant height (cm)		Branch number per plant		Stem diameter (cm)		Stem dry weight (g)	
Ni (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019	2018	2019
<b>0</b>	<b>0</b>	33.33	38.50	6.16	11.33	0.49	0.65	2.85	4.87
	<b>250</b>	32.00	37.83	6.00	10.83	0.48	0.63	2.73	4.75
	<b>500</b>	33.66	39.50	6.83	11.83	0.51	0.70	3.46	5.47
<b>Mean (Ni)</b>		32.99	38.61	6.33	11.33	0.49	0.66	3.01	5.03
<b>100</b>	<b>0</b>	32.00	37.16	5.83	9.83	0.46	0.62	2.47	4.02
	<b>250</b>	31.00	36.83	5.66	9.66	0.44	0.61	2.43	3.95
	<b>500</b>	32.66	38.50	6.00	10.66	0.47	0.66	2.61	4.40
<b>Mean (Ni)</b>		31.88	37.49	5.83	10.05	0.45	0.63	2.50	4.12
<b>200</b>	<b>0</b>	29.75	35.91	5.66	9.16	0.44	0.61	2.38	3.79
	<b>250</b>	29.50	35.33	5.33	8.83	0.42	0.60	2.25	3.61
	<b>500</b>	30.75	36.58	6.00	9.50	0.45	0.62	2.55	4.26
<b>Mean (Ni)</b>		30.00	35.94	5.66	9.16	0.43	0.61	2.39	3.88
<b>300</b>	<b>0</b>	28.83	35.00	5.16	8.66	0.39	0.55	2.41	3.68
	<b>250</b>	28.66	34.50	4.83	7.83	0.37	0.54	2.06	3.58
	<b>500</b>	29.58	35.75	5.33	9.00	0.41	0.56	2.53	4.25
<b>Mean (Ni)</b>		29.02	35.08	5.10	8.49	0.39	0.55	2.33	3.83
<b>Mean (CA)</b>	<b>0</b>	30.97	36.64	5.70	9.74	0.44	0.60	2.52	4.09
	<b>250</b>	30.29	36.12	5.45	9.28	0.42	0.59	2.36	3.97
	<b>500</b>	31.66	37.58	6.04	10.24	0.46	0.63	2.78	4.59
<b>L.S.D. at 0.05</b>	<b>Ni</b>	0.75	0.48	0.41	1.81	0.04	0.03	0.22	0.79
	<b>CA</b>	0.65	0.58	0.27	0.74	0.02	0.03	0.12	0.44
	<b>Ni × CA</b>	0.74	0.66	0.31	0.85	0.02	0.03	0.13	0.50

35.08 cm), number of branch per plant (5.10 and 8.49), stem diameter (0.39 and 0.55 cm), and stem dry weight (2.33 and 3.83 g) in the first and second seasons, respectively, compared with the other concentrations.

In contrast to the effect of nickel treatments, citric acid treatments improved stem parameters, compared to the control. Moreover, plants sprayed with 500 mg/l citric acid had significantly mean plant height (31.66 and 37.58 cm), number of branch per plant (6.04 and 10.24), stem diameter (0.46 and 0.63 cm) and stem dry weight (2.78 and 4.59 g) in the first and second seasons, respectively, compared with the other concentrations.

Regarding the interaction between the irrigation with water nickel polluted and sprayed with citric acid on stem parameters of *Lantana camara* plants, the recorded results for the two seasons are presented that Table (4) showed that significant differences were detected between the values obtained from which plants received the different treatment combinations. The highest values of plant height (33.66 and 38.61 cm), number of branch per plant (6.33 and 11.33), stem diameter (0.51 and 0.70 cm) and stem dry weight (3.46 and 5.47 g) in the first and second seasons, respectively, were obtained from the plants irrigated with tap water and sprayed with citric acid at 500 mg/l. On the other hand, the least values of plant height (28.66 and 34.50 cm), number of branch per plant (4.83 and 7.83), stem diameter (0.37 and 0.54 cm) and stem dry weight (2.06 and 3.58 g) in the first and second seasons, respectively, were obtained from plants irrigated with the highest nickel concentration (300 mg/l) and sprayed with citric acid at 250 mg/l treatment. Table (4) showed that, in many cases, spraying the plants with citric acid reduced the adverse effect of nickel polluted water.

### c. Root parameters:

Data presented in Table (5) showed that irrigation with nickel polluted water decreased root parameters, compared to

those of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the highest values of root length (15.65 and 18.97 cm) and root dry weight (1.98 and 3.34 g) in the first and second seasons, respectively. Increasing nickel concentration in irrigation water caused a steady reduction in root parameters. These reductions were significant as compared to the control even at the highest nickel concentration (300 mg/l), which gave root length (13.82 and 17.12 cm) and root dry weight (1.51 and 2.53 g) in the first and second seasons, respectively.

In contrast to the effect of nickel treatments, citric acid treatments improved root parameters, compared to the control. Moreover, plants sprayed with 500 mg/l citric acid had significantly mean root length (15.09 and 18.39 cm) and root dry weight (1.82 and 3.04 g) in the first and second seasons, respectively, compared to either those of control plants, or plants sprayed with any other citric acid concentration.

Regarding the interaction between the irrigation with nickel polluted water and citric acid treatments on root parameters of *Lantana camara* plants, the results showed that significant differences were obtained between the values for plants receiving the different treatment combinations. The highest values of root length (15.99 and 19.32 cm) and root dry weight (2.29 and 3.64 g) in the first and second seasons, respectively, were obtained from the plants irrigated with tap water and sprayed with citric acid at 500 mg/l. On the other hand, the lowest values of root length (14.42 and 17.73 cm) and root dry weight (1.54 and 2.63 g) in the first and second seasons, respectively, were obtained from the plants irrigated using the highest nickel concentration 300 mg/l and sprayed with citric acid at 250 mg/l treatment. It can be seen from Table (5) that in many cases, spraying the plants with citric acid reduced the harmful effect of nickel polluted water.

**Table 5. Means of root length (cm) and root dry weight (g) of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

	Treatments		Root length (cm)		Root dry weight (g)	
	Ni (mg/l)	CA (mg/l)	2018	2019	2018	2019
<b>0</b>		<b>0</b>	15.66	18.97	1.86	3.24
		<b>250</b>	15.30	18.62	1.79	3.16
		<b>500</b>	15.99	19.32	2.29	3.64
<b>Mean (Ni)</b>			15.65	18.97	1.98	3.34
<b>100</b>		<b>0</b>	14.96	18.27	1.61	2.67
		<b>250</b>	14.79	18.10	1.58	2.61
		<b>500</b>	15.65	18.96	1.71	2.92
<b>Mean (Ni)</b>			15.13	18.44	1.63	2.73
<b>200</b>		<b>0</b>	14.49	17.79	1.56	2.51
		<b>250</b>	14.02	17.31	1.47	2.40
		<b>500</b>	14.66	17.96	1.66	2.82
<b>Mean (Ni)</b>			14.39	17.68	1.56	2.57
<b>300</b>		<b>0</b>	13.84	17.13	1.57	2.44
		<b>250</b>	13.58	16.89	1.33	2.36
		<b>500</b>	14.06	17.35	1.65	2.81
<b>Mean (Ni)</b>			13.82	17.12	1.51	2.53
<b>Mean (CA)</b>		<b>0</b>	14.73	18.04	1.65	2.71
		<b>250</b>	14.42	17.73	1.54	2.63
		<b>500</b>	15.09	18.39	1.82	3.04
<b>L.S.D. at 0.05</b>		<b>Ni</b>	0.27	0.28	0.14	0.52
		<b>CA</b>	0.33	0.33	0.08	0.29
		<b>Ni × CA</b>	0.38	0.38	0.09	0.34

**d. Flowering parameters:**

Data presented in Table (6) showed that, in both seasons, irrigation with nickel polluted water decreased the flowering parameters of *Lantana camara* plants. Plants irrigated with tap water (control) had the highest mean values of number of flowers per plant (20.94 and 27.94) and flower dry weight (1.85 and 3.21 g) in the first and second seasons, respectively. Increasing nickel concentration caused significant reductions in flowering parameters, with the highest nickel concentration (300 mg/l) giving significantly the smallest mean values of number of flower per plant (18.77 and 25.44) and flower dry weight (1.39 and 2.41 g) in the first and second seasons, respectively, compared with the other concentrations.

Flowering parameters were also significantly affected by spraying the plants with citric acid. In both seasons, they gradually increase was noticed with the

increase of citric acid concentration. Accordingly, Table (6) showed that *Lantana camara* plants sprayed with 500 mg/l citric acid gave significantly increased the values of number of flowers per plant (20.33 and 27.16) and flower dry weight (1.70 and 2.92 g) in the first and second seasons, respectively, compared with the other concentrations.

Regarding the interaction between the irrigation with nickel polluted water and citric acid treatments on flowers parameters, the recorded results in the two seasons showed that, the highest values were obtained from the plants irrigated with tap water and sprayed with citric acid at 500 mg/l with values of number of flower per plant (21.33 and 28.50) and flower dry weight (2.17 and 3.51 g) in the first and second seasons, respectively. On the other hand, the plants with values of number of flower per plant (18.33 and 25.16) and flower dry weight (1.21 and 2.24 g) in the

**Table 6. Means of flower number per plant and flower dry weight (g) of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

	Treatments		Flower number per plant		Flower dry weight (g)	
	Ni (mg/l)	CA (mg/l)	2018	2019	2018	2019
<b>0</b>	<b>0</b>		21.00	28.00	1.73	3.10
	<b>250</b>		20.50	27.33	1.66	3.03
	<b>500</b>		21.33	28.50	2.17	3.51
<b>Mean (Ni)</b>			20.94	27.94	1.85	3.21
<b>100</b>	<b>0</b>		20.16	27.00	1.50	2.54
	<b>250</b>		20.00	26.83	1.45	2.49
	<b>500</b>		21.00	28.00	1.58	2.79
<b>Mean (Ni)</b>			20.38	27.27	1.51	2.60
<b>200</b>	<b>0</b>		19.66	26.33	1.45	2.40
	<b>250</b>		19.00	25.66	1.34	2.27
	<b>500</b>		19.83	26.50	1.53	2.69
<b>Mean (Ni)</b>			19.49	26.16	1.44	2.45
<b>300</b>	<b>0</b>		18.83	25.50	1.45	2.30
	<b>250</b>		18.33	25.16	1.21	2.24
	<b>500</b>		19.16	25.66	1.52	2.69
<b>Mean (Ni)</b>			18.77	25.44	1.39	2.41
<b>Mean (CA)</b>	<b>0</b>		19.91	26.70	1.53	2.58
	<b>250</b>		19.45	26.24	1.41	2.50
	<b>500</b>		20.33	27.16	1.70	2.92
<b>L.S.D. at 0.05</b>	<b>Ni</b>		0.24	0.37	0.15	0.53
	<b>CA</b>		0.47	0.47	0.08	0.29
	<b>Ni × CA</b>		0.65	0.65	0.09	0.33

first and second seasons, respectively, obtained found when the plants were irrigated with 300 mg/l nickel concentration water and sprayed with citric acid at 250 mg/l treatment. It can also be seen from Table (6) that in many cases, spraying the plants with citric acid reduced the harmful effect of polluted water with nickel.

## 2. Chemical composition:

### a. Leaf chemical analysis:

Data presented in Table (7) showed that the highest value of total chlorophyll was obtained from plant irrigated with tap water (55.17 and 55.65 SPAD) and of total carbohydrates (18.77 and 18.93%) in the first and second seasons, respectively, while the maximum proline content (2.49 and 2.45 mg/g) was obtained from the plants irrigated with nickel contaminated water at (300 mg/l). Increasing the nickel concentration in irrigation water resulted in steady significant reductions in the chlorophyll and carbohydrates content, which reached its

lowest mean value for chlorophyll (49.76 and 50.47 SPAD) and total carbohydrates (16.92 and 17.16 %) in the first and second seasons, respectively, for plants receiving the highest nickel concentration (300 mg/l), while, proline content values (1.33 and 1.36 mg/g) were obtained from the plants irrigated with tap water.

Data in Table (7) also showed that citric acid treatments had pronounced effect on leaf chlorophyll (54.85 and 55.30 SPAD) and total carbohydrates (18.66 and 18.81 %) in the first and second seasons, respectively, in plants sprayed with citric acid at 250 mg/l, while, proline contents (1.99 and 2.02 mg/g) were obtained in plants non sprayed with citric acid.

Regarding the interaction between the irrigation using nickel polluted water and citric acid treatments, Table (7) showed that the highest total chlorophyll contents (56.83 and 57.20 SPAD) and total carbohydrates (19.34 and 19.46 %) in the first and second

**Table 7. Means of chlorophyll content (SPAD), carbohydrates content (%) and proline content (mg/g d.w.) of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

Treatments		Chlorophyll content (SPAD)		Carbohydrates content (%)		Proline content (mg/g d.w.)	
Ni (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019
<b>0</b>	<b>0</b>	53.50	54.22	18.20	18.44	1.39	1.41
	<b>250</b>	56.83	57.20	19.34	19.46	1.36	1.39
	<b>500</b>	55.18	55.54	18.77	18.89	1.25	1.30
<b>Mean (Ni)</b>		55.17	55.65	18.77	18.93	1.33	1.36
<b>100</b>	<b>0</b>	49.67	50.05	16.90	17.02	1.84	1.89
	<b>250</b>	56.41	56.78	19.19	19.31	1.81	1.83
	<b>500</b>	54.63	55.38	18.58	18.84	1.80	1.82
<b>Mean (Ni)</b>		53.57	54.07	18.22	18.39	1.81	1.84
<b>200</b>	<b>0</b>	48.07	49.08	16.35	16.70	2.26	2.31
	<b>250</b>	54.00	54.38	18.37	18.50	2.24	2.28
	<b>500</b>	53.08	53.45	18.06	18.18	2.21	2.27
<b>Mean (Ni)</b>		51.71	52.30	17.59	17.79	2.23	2.28
<b>300</b>	<b>0</b>	47.03	48.07	15.99	16.35	2.50	2.48
	<b>250</b>	52.16	52.86	17.74	17.98	2.49	2.45
	<b>500</b>	50.10	50.49	17.04	17.17	2.48	2.44
<b>Mean (Ni)</b>		49.76	50.47	16.92	17.16	2.49	2.45
<b>Mean (CA)</b>	<b>0</b>	49.56	50.35	16.86	17.12	1.99	2.02
	<b>250</b>	54.85	55.30	18.66	18.81	1.97	1.98
	<b>500</b>	53.24	53.71	18.11	18.27	1.93	1.95
<b>L.S.D. at 0.05</b>	<b>Ni</b>	0.51	0.48	0.17	0.16	0.05	0.04
	<b>CA</b>	0.38	0.37	0.13	0.12	0.02	0.01
	<b>Ni × CA</b>	0.44	0.43	0.15	0.14	0.02	0.01

seasons, respectively, were found in leaves of plants irrigated with tap water and sprayed with citric acid at 250 mg/l, while, proline contents (2.50 and 2.48 mg/g) were resulted from the plants irrigated with the highest nickel concentration (300 mg/l) without citric acid treatment.

**b. Nickel content in leaves, stem and root (mg/l):**

Data presented in Table (8) showed that, the nickel content (mg/l) in the dried plant parts was increased with increasing the nickel concentration in the irrigation water. Generally, the lowest mean nickel content of the leaves (0.102 and 0.263 mg/l), stem (0.142 and 0.160 mg/l) and root (0.056 and 0.043 mg/l) in the first and second seasons, respectively, were registered in leaves of plants irrigated with tap water, whereas the highest mean values of nickel content of the leaves (0.914 and 0.954 mg/l), stem (0.546

and 0.616 mg/l) and root (0.421 and 0.461 mg/l) in the first and second seasons, respectively, were recorded in plants irrigated with water containing the highest nickel concentration (300 mg/l).

Concerning the effect of citric acid treatments on the nickel content in plant parts, Table (8) showed that citric acid treatment of 500 mg/l caused a significant decrease in the mean values of nickel content in the leaves (0.545 and 0.632 mg/l), stem (0.371 and 0.408 mg/l) and root (0.272 and 0.294 mg/l) in the first and second seasons, respectively, compared to that of control plants had the highest nickel content in the leaves (0.600 and 0.689 mg/l), stem (0.390 and 0.431 mg/l) and root (0.288 and 0.306 mg/l) in the first and second seasons, respectively.

Concerning the interaction between irrigation using nickel polluted water with

**Table 8. Means of nickel content of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

	Treatments		Nickel content in leaves (mg/kg)		Nickel content in stem (mg/kg)		Nickel content in roots (mg/kg)	
	Ni (mg/l)	CA (mg/l)	2018	2019	2018	2019	2018	2019
	<b>0</b>	<b>0</b>	0.154	0.311	0.145	0.168	0.061	0.048
	<b>250</b>	0.092	0.252	0.143	0.166	0.057	0.044	
	<b>500</b>	0.061	0.226	0.139	0.146	0.051	0.038	
<b>Mean (Ni)</b>		0.102	0.263	0.142	0.160	0.056	0.043	
<b>100</b>	<b>0</b>	0.476	0.633	0.346	0.416	0.267	0.321	
	<b>250</b>	0.458	0.615	0.338	0.408	0.261	0.315	
	<b>500</b>	0.414	0.572	0.318	0.388	0.246	0.300	
<b>Mean (Ni)</b>		0.449	0.606	0.334	0.404	0.258	0.312	
<b>200</b>	<b>0</b>	0.848	0.845	0.511	0.511	0.394	0.394	
	<b>250</b>	0.816	0.813	0.497	0.495	0.385	0.383	
	<b>500</b>	0.796	0.802	0.488	0.491	0.377	0.379	
<b>Mean (Ni)</b>		0.820	0.820	0.498	0.499	0.385	0.385	
<b>300</b>	<b>0</b>	0.923	0.969	0.561	0.629	0.433	0.462	
	<b>250</b>	0.912	0.966	0.540	0.613	0.417	0.461	
	<b>500</b>	0.909	0.928	0.539	0.608	0.415	0.460	
<b>Mean (Ni)</b>		0.914	0.954	0.546	0.616	0.421	0.461	
<b>Mean (CA)</b>	<b>0</b>	0.600	0.689	0.390	0.431	0.288	0.306	
	<b>250</b>	0.569	0.661	0.379	0.420	0.280	0.300	
	<b>500</b>	0.545	0.632	0.371	0.408	0.272	0.294	
<b>L.S.D. at 0.05</b>	<b>Ni</b>	0.067	0.056	0.026	0.029	0.029	0.023	
	<b>CA</b>	0.059	0.042	0.029	0.019	0.023	0.019	
	<b>Ni × CA</b>	0.067	0.048	0.032	0.022	0.026	0.022	

nickel and citric acid treatments on the nickel content in plant parts, Table (8) showed that the lowest mean values of nickel content in the leaves (0.061 and 0.226 mg/l), stem (0.139 and 0.146 mg/l) and root (0.051 and 0.038 mg/l) in the first and second seasons, respectively, were of found in plants irrigated with tap water and sprayed with citric acid at 500 mg/l. On the other hand, the highest nickel content was obtained in the plant parts irrigated with nickel water at (300 mg/l) and receiving no citric acid treatment nickel content in the leaves (0.923 and 0.969 mg/l), stem (0.561 and 0.629 mg/l) and root (0.433 and 0.462 mg/l).

### c. Transfer factor (TF) of heavy metals:

#### 1. Nickel content in soil samples (mg/kg):

Data presented in Table (9) showed that the lowest average value of nickel content was in untreated soil, while the highest was

observed in 300 mg/l nickel treated soil and 0 mg/l citric acid.

#### 2. Transfer factor to leaves, stem and root:

Data presented in Table (10), showed that the transfer factor (TF) in the dried plant parts of *Lantana camara* plants was steadily decreased with increasing nickel concentration in irrigation water. Accordingly, the lowest nickel values in leaves (1.069 and 2.477 mg/l), stem (1.396 and 1.487 mg/l) and root (0.558 and 0.405 mg/l) were recorded in plants irrigated with water containing nickel concentration of (300 mg/l), whereas the highest values of transfer factor in leaves (0.998 and 0.986 mg/l), stem (0.596 and 0.637 mg/l) and root (0.460 and 0.476 mg/l) were registered in plants irrigated with tap water (control).

**Table 9. Average values of nickel content in soil samples as influenced by nickel concentrations in irrigation water and foliar application of citric acid on *Lantana camara* in the two seasons of 2018 and 2019.**

Treatments		Nickel content in soil (mg/kg)			
Nickel (mg/l)	Citric acid (mg/l)	2018		2019	
		Before	After	Before	After
0	0	0.016	0.085	0.016	0.089
	250	0.017	0.095	0.019	0.104
	500	0.026	0.142	0.028	0.149
100	0	0.078	0.416	0.011	0.439
	250	0.075	0.402	0.079	0.422
	500	0.072	0.388	0.076	0.409
200	0	0.058	0.734	0.065	0.775
	250	0.048	0.681	0.055	0.717
	500	0.038	0.629	0.044	0.664
300	0	0.017	0.943	0.027	0.995
	250	0.012	0.917	0.022	0.968
	500	0.015	0.888	0.016	0.937

**Table 10. Mean values of transfer factor to leaves, stem and roots of *Lantana camara* plants as influenced by nickel (Ni), citric acid (CA) and their combinations (Ni × CA) in the two seasons of 2018 and 2019.**

Treatments		Transfer factor to leaves (TFL)		Transfer factor to stem (TFS)		Transfer factor to root (TFR)	
Nickel (mg/l)	citric acid (mg/l)	2018	2019	2018	2019	2018	2019
		0	0	1.811	3.494	1.705	1.887
250	0.968		2.423	1.505	1.596	0.600	0.423
500	0.429		1.516	0.978	0.979	0.359	0.255
Mean (Ni)		1.069	2.477	1.396	1.487	0.558	0.405
100	0	1.144	1.441	0.831	0.947	0.641	0.731
	250	1.139	1.457	0.840	0.966	0.649	0.746
	500	1.067	1.398	0.819	0.948	0.634	0.733
Mean (Ni)		1.116	1.432	0.830	0.953	0.641	0.736
200	0	1.155	1.090	0.696	0.659	0.536	0.508
	250	1.198	1.133	0.729	0.690	0.565	0.534
	500	1.265	1.207	0.775	0.739	0.599	0.570
Mean (Ni)		1.206	1.143	0.733	0.696	0.566	0.537
300	0	0.978	0.973	0.594	0.632	0.459	0.464
	250	0.994	0.997	0.588	0.633	0.454	0.476
	500	1.023	0.990	0.606	0.648	0.467	0.490
Mean (Ni)		0.998	0.986	0.596	0.637	0.460	0.476
Mean (MA)	0	1.272	1.749	0.956	1.031	0.588	0.560
	250	1.074	1.502	0.915	0.971	0.567	0.544
	500	0.946	1.277	0.794	0.828	0.514	0.512

Data in Table (10) showed also that the transfer factor (TF) in the dried plant parts was steadily reduced with increasing citric acid concentration. Accordingly, the highest nickel values in leaves (1.272 and 1.749 mg/l), stem (0.956 and 1.031 mg/l) and root (0.588 and 0.560 mg/l) were recorded in the leaves of control plants, whereas plants sprayed with the highest citric acid

concentration (500 mg/l) had the lowest nickel values in leaves (0.946 and 1.277 mg/l), stem (0.794 and 0.828 mg/l) and root (0.514 and 0.512 mg/l) of the two seasons, respectively.

## DISCUSSION

All vegetative growth parameters showed significant reduction after treatment

with the different concentrations of nickel in irrigation water. These may be due to that Ni 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). For example, Ni can decrease Mg (or Fe) uptake and then induce deficiencies of these elements in plants. This may result in the retardation of germination, growth suppression, and reductions in yields (Seregin and Kozhevnikova, 2006). These results are in harmony with those obtained by MadhavaRao and Sresty (2000) on *Cajanus cajan*, Pandey and Sharma (2002) on cabbage and Eman *et al.* (2007) on *Hibiscus sabdariffa*.

A significant decrease in total chlorophyll content was observed after irrigation with nickel polluted 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. Along with the interference in chlorophyll biosynthesis, nickel may also damage the thylakoid membrane and chloroplast grana structure (Molas, 2002), reducing the size of grana (Seregin and Kozhevnikova, 2006). These results are in agreement with the results reported by (Prasad *et al.*, 2005) on *Glycine max* (L.) under nickel stress.

The results showed that there was a significant decrease in total carbohydrates 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 sequestered 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*).

For the effect of citric acid, it is observed that there is a significant increase in all vegetative parameters, chlorophyll content, carbohydrate percentage, significant decrease in nickel content in leaves and roots and decrease in nickel content in soil samples. This may be due to that application of citric acid with any of the concentrations of nickel led to a statistically decrease in the uptake of nickel. This decrease in the uptake of nickel in the presence of citric acid resulted in the formation of citric acid–nickel complexes that inhibited the uptake (Chen *et al.*, 2003). The decrease in nickel uptake helped to overcome the negative effects of Ni on the previous studied parameters. These results are in agreement with those mentioned by (Talebi *et al.*, 2014) on *Gazania* plants and (Jaafari and Hadavi, 2012) on *Ocimum basilicum* L.

Concerning treatments, at a preliminary stage, one should note that the transfer factor (TF) of most treatments is lower than one for nickel; which means that the physiological need of the plant for this element is rather limited. Translocation from roots to shoots via a number of physiological processes, including metal unloading into root xylem cells, long-distance carrying from the xylem to the shoots and metal reabsorption, by leaf mesophyll cells, from the xylem stream.

Once the nickel has been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream (Blaylock and Huang, 2000). Confirming the previous results (El-Shanhorey and EL-Sayed, 2017) on *Senecio cineraria* and (El-Shanhorey and Emam, 2016) on *Jatropha curcas*.

## CONCLUSION

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.

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

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### إستجابة نباتات اللانتانا للرش بحمض الستريك لتقليل الأثر الضار للتلوث بالعناصر الثقيلة في ماء الري (ب) تأثير النيكل

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أجريت هذه الدراسة في فرع البحوث بأنطونيداس، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر خلال الموسمين المتتاليين ٢٠١٨ و ٢٠١٩. وتهدف الدراسة إلى تقييم آثار الري بمياه ملوثة بالنيكل على نمو نباتات اللانتانا المزروعة في تربة رملية، كذلك إمكانية استخدام الرش بحمض الستريك للتغلب على الآثار الضارة للنيكل. ولتحقيق ذلك زرعت شتلات اللانتانا بشكل فردي في أوعية بلاستيكية (قطرها ٢٠ سم) مملوءة ٥ كجم من التربة الرملية. وكانت معاملات مياه الري الملوثة بأربعة تراكيزات من النيكل وهي صفر، ١٠٠، ٢٠٠، ٣٠٠ ملليجرام/لتر. تم رش النباتات شهريا أيضاً بحامض الستريك باستخدام ثلاث تراكيزات هي صفر، ٢٥٠، ٥٠٠ ملليجرام/لتر في كلا الموسمين. أظهرت نتائج الدراسة أن هناك إختلاف كبير في التفاعل بين تراكيزات النيكل ورش النباتات بحامض الستريك. وقد وجد انخفاض كبير في كافة معاملات الري بالماء الملوث بالنيكل وكذلك لوحظ زيادة كبيرة في معدلات النمو الخضري بعد

الرش ٥٠٠ مليجرام/لتر حمض الستريك. تم الحصول على أعلى قيمة من محتوى الكلوروفيل والكربوهيدرات من النباتات المروية بماء الصنبور والرش بتركيز ٢٥٠ مليجرام/لتر حامض الستريك في حين أن أعلى تركيز من النيكل في الأوراق والساق و الجذور من خلال الري بماء ملوث بتركيز ٣٠٠ مليجرام/لتر من النيكل بدون الرش بحمض الستريك.