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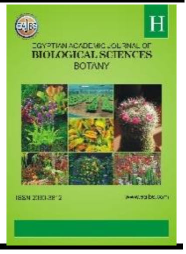

Effect of Foliar Applied Tryptophan on Tuberose Plants for Decreasing the Harmful Effect of Some Heavy Metals Pollution in the Irrigation Water (B) Effect of Lead Treatments

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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 contaminated with lead on the growth of *Polianthes tuberosa* plants and the possibility of using tryptophan spray treatments to overcome the effects of lead pollution. Corms of *Polianthes tuberosa* were planted individually in plastic pots (20 cm diameter) filled with 5 kg of sandy soil. The lead-contaminated irrigation water treatments were 0,100, 200 and 300 mg/L were applied. The plants were also monthly sprayed with tryptophan at concentrations of 0, 250 and 500 mg/L.

The results showed that for vegetative growth parameters there was no significant difference in the interaction between lead polluted water of irrigation and foliar spray by tryptophan, while a significant reduction was observed in all parameters after irrigation with lead polluted water and a significant increase in vegetative growth parameters was observed after 500 mg/L tryptophan application. For chlorophyll and carbohydrate content, the highest significant value was obtained in plants irrigated with tap water and sprayed with 250 mg/L tryptophan while the highest significant level of lead content in leaves and corms was obtained due to treatment by 300 mg/L lead without application of tryptophan.

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 polluted soils, sediments and water. This technology is environmentally friendly and potentially cost-effective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulators (Choruk *et al.*, 2006). Plants need trace amounts of heavy metals but their excessive availability may cause plant toxicity (Sharma *et al.*, 2006). The phytotoxic concentration of heavy metals referred in the literature does not always specify the levels (Wua *et al.*, 2010).

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Lead is a toxic heavy metal that has an environmental concern (Mahler *et al.*, 1981). There are many sources of environmental lead pollution, including fuel combustion, industrial sludges, phosphate fertilizers, and mine tailings (Unhalekhana and Kositanont, 2008).

Lead is one of the main sources of environmental pollution. Many studies have shown that lead inhibits metabolic processes in the plants such as nitrogen assimilation, photosynthesis, respiration, water uptake, and transcription (Kurepa *et al.*, 1997 and Boussama *et al.*, 1999). Lead may inactivate various enzymes by binding to their SH-groups (Rauser, 1995), and can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress (Cuypers *et al.*, 1999; Prasad *et al.*, 1999). In addition, lead can negatively affect mitochondria structure by decreasing the number of mitochondrial cristae, which in turn can lower the capability of oxidative phosphorylation (Malecka *et al.*, 2001).

Tuberose (*Polianthes tuberosa* L.), a member of the Agavaceae family native to Mexico, has long been cherished for the aromatic oils extracted from its fragrant white flowers (Trueblood, 1973). It has recently gained popularity as a cut flower and in a number of countries including Kenya, India and Mexico it is grown commercially for export to the USA, Europe and Japan. Tuberose inflorescences (spikes) bear 10-20 pairs of florets that open acropetally. Commercially, spikes 60-90 cm long are harvested when two or three of the basal florets are open. Less than 50% of the buds normally open after harvest and florets and buds usually abscond after only a few days in the vase. Postharvest performance is worse in tuberose which has been shipped to distant markets. Since tuberose originated from the sub-tropics, this loss of quality might be due to chilling injury induced by exposure to low but non-freezing temperatures during marketing. Alternatively, it might be the result of postharvest desiccation or improper temperature management.

Ramaih *et al.* (2003) confirmed that tryptophan is the major precursor of Indol Acetic Acid (IAA) in most organisms. Plants produce IAA from tryptophan through indole-3-pyruvic acid (Mashiguchi *et al.*, 2011) and (Won, 2011). IAA is also produced from tryptophan through indole-3-acetaldoxime in Arabidopsis (Satoko *et al.*, 2009). El-Bassiouny (2005) demonstrated that tryptophan and nicotinamide increased IAA, Geberellic acid (GA3), cytokinens, and decreased abscicic acid (ABA) in wheat. Nicotinamide is a stress-induced compound to provide a defense mechanism against specific stress. Wyszkowska (1999) and El-Bassiouny (2005) observed an increase in some minerals K^+, Ca^{2+} and Mg^{2+} in wheat plant tissues by tryptophan treatment.

In this study *Polianthes tuberosa* was selected due to its characteristics as non-edible plant, cut flowers and it has many uses in landscaping, therefore the objective of this study is to determine the potential of *Polianthes tuberosa* in removing heavy metals from the soil affected with contaminated irrigation water and to investigate the ability of *Polianthes tuberosa* in removing heavy metals.

MATERIALS AND METHODS

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

On 1st of May, 2018 and 2019 in the first and second seasons, respectively, Tuberose (*Polianthes tuberosa*, L. cv. "Double") corms with an average diameter of 3.8 cm and 70.0 g fresh weight were obtained from a commercial nursery in Alexandria and planted individually in plastic pots (20 cm diameter) filled with 5 kg of sandy soil. The chemical constituents of the soil were measured as described by Jackson (1958) and the results obtained are shown in Table (1).

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

Season	pH	EC ds/m	Cations (meq/l)				Anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
2018	7.93	1.55	4.4	3.6	6.5	1.3	3.6	6.7	2.4
2019	7.91	1.52	4.2	3.1	6.3	1.2	3.3	6.5	2.2

On the 15th of May (in both seasons), the contaminated irrigation water treatments were initiated. Four concentrations of lead acetate [Pb $(CH_3COO)_2.2H_2O$] 0,100, 200 and 300 mg/L were applied. The plants were irrigated three times per week; at the end of the experiment, every plant received about (120) liters per pot of contaminated water in Table (2). In both seasons, the plants were received by monthly spraying from 15th June till 15th September in both seasons. The plants were also sprayed with tryptophan at concentrations of 0, 250 and 500 mg/L. Control plants were sprayed with tap water. On the 30th of October in both seasons, the plants were harvested.

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

Field Capacity (%)	Months of first and second seasons						
	May	June	July	August	September	October	Total
100 %	17.00	18.00	20.00	23.00	22.00	20.00	**120.00**

In both seasons, all plants received NPK chemical fertilization using fertilizer (Milagro Aminoleaf 20-20-20) at the rate of 3 g/ pot. Fertilization was repeated every 30 days throughout the growing season (from 20th of May till 20th of October). In addition, weeds were removed manually upon emergence.

Data Recorded :

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), flower number per spike, flower dry weight (g), spike dry weight (g), rachis length (cm), corm diameter (cm), corm dry weight (g) and number new cormlets were also measured.

Chemical Analysis Determination:

-Total chlorophyll content was determined as a SPAD 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 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 lead content. Plant samples were divided into leaves stems and roots. They were then dried at 72°C in an oven until completely dried. The dried plant samples were ground to powder. The dried samples were then digested for extraction of lead, using the method described by Piper (1947) method and the concentration of heavy metal was determined using an atomic absorption spectrophotometer.

-Available lead in soil samples was extracted by DPTA 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). This indicates the efficiency of any plant to transfer any metal from soil to the aerial parts.

The experimental design was a split-plot with three replicates. Each replicate contained three plants. The main plot was lead polluted water concentration, while the subplot was tryptophan treatments. 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 a 5% level of probability according to Snedecor and Cochran (1989).

RESULTS

Vegetative Growth:

1-Leaves Parameters:

Data presented in Table (3) Showed that, in both seasons, irrigation water polluted with lead decreased the leaves parameters of *Polianthes tuberosa* plants. Plants irrigated with tap water (control) had the highest mean values of plant height (92.16 and 93.97 cm), the number of leaves per plant (90.08 and 91.87), leaves dry weight (21.03 and 21.25 g) and leaves area (1246.67 and 1259.25 cm^2) in the first and second seasons, respectively. Moreover, raising the lead concentration caused steady significant reductions in leaves parameters, with the highest concentration (300 mg/L) giving significantly the shortest plants mean values of plant height (76.19 and 79.07 cm), number of leaves per plant (73.64 and 76.71), leaves dry weight (17.10 and 17.64 g) and leaves area (1018.40 and 1049.71 cm^2) in the first and second seasons, respectively.

Leaves parameters were also significantly affected by spraying the plants with tryptophan. In both seasons, leaves parameters increased gradually when the tryptophan concentration was raised from 0 mg/L (control) to 500 mg/L. Accordingly, it can be seen from the data in Table (3) that *Polianthes tuberosa* plants sprayed with 500 mg/L tryptophan were significantly mean values of plant height (83.98 and 86.10 cm), the number of leaves per plant (81.89 and 83.95), leaves dry weight (19.08 and 19.42 g) and leaves area (1132.05 and 1148.26 cm^2) in the first and second seasons, respectively.

Regarding the interaction between irrigation with lead polluted water and tryptophan treatments on leaves parameter of *Polianthes tuberosa* plants, the results recorded in the two seasons show that the highest values were obtained in the plants irrigated with tap water (control) and sprayed with tryptophan at 500 mg/L with mean values of plant height (94.26 and 95.98 cm), the number of leaves per plant (92.18 and 93.88), leaves dry weight (21.54 and 21.74 g) and leaves area (1276.09 and 1287.25 cm^2) in the first and second seasons, respectively. On the other hand, the shortest plants with mean values of plant height (80.93 and 83.50 cm), number of leaves per plant (78.76 and 81.28), leaves dry weight (18.25 and 18.70 g) and leaves area (1089.61 and 1113.02 cm^2) in the first and second seasons, respectively, were resulted when the plants were irrigated using the highest lead concentration (300 mg/L) without tryptophan treatment. It can also be seen from the data presented in Table (3) that in many cases, spraying the plants with tryptophan reduced the undesirable effect of contaminated water with lead.

Table 3: Means of plant height (cm), number of leaves per plant, leaves dry weight (g) and leaves area (cm^2) of *Polianthes tuberosa* plants as influenced by Lead (Pb), Tryptophan (T) and their combinations (Pb× T) in the two seasons of 2018 and 2019.

Treatments		Plant height (cm)		Number of leaves per plant		Leaves dry weight (g)		Leaves area (cm^2)	
Lead (mg/L)	**Tryptophan (mg/L)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
0	0	89.96	91.64	87.88	89.54	20.50	20.68	1215.85	1226.58
	250	92.26	94.31	90.18	92.21	21.05	21.33	1248.09	1263.92
	500	94.26	95.98	92.18	93.88	21.54	21.74	1276.09	1287.25
Mean (Pb)		**92.16**	**93.97**	**90.08**	**91.87**	**21.03**	**21.25**	**1246.67**	**1259.25**
100	0	82.16	84.10	80.08	82.10	18.39	18.87	1106.65	1122.33
	250	83.37	84.76	81.29	82.76	18.89	19.01	1123.68	1130.17
	500	86.03	87.09	83.95	84.99	19.54	19.67	1160.92	1162.80
Mean (Pb)		**83.85**	**85.31**	**81.77**	**83.28**	**18.94**	**19.18**	**1130.41**	**1138.43**
200	0	75.93	79.42	73.85	77.32	17.08	17.71	1019.47	1055.46
	250	78.38	81.12	76.30	79.11	17.68	18.16	1053.73	1081.92
	500	79.59	81.98	77.51	79.88	18.07	18.53	1070.76	1091.25
Mean (Pb)		**77.96**	**80.84**	**75.88**	**78.77**	**17.61**	**18.13**	**1047.98**	**1076.21**
300	0	75.70	78.86	73.24	76.17	17.03	17.57	1016.49	1047.71
	250	76.84	79.01	73.78	76.91	17.07	17.61	1018.26	1049.67
	500	76.05	79.36	73.92	77.06	17.20	17.74	1020.45	1051.77
Mean (Pb)		**76.19**	**79.07**	**73.64**	**76.71**	**17.10**	**17.64**	**1018.40**	**1049.71**
Mean (T)	0	80.93	83.50	78.76	81.28	18.25	18.70	1089.61	1113.02
	250	82.71	84.80	80.38	82.74	18.67	19.02	1110.94	1131.42
	500	83.98	86.10	81.89	83.95	19.08	19.42	1132.05	1148.26
L.S.D. at 0.05	**Pb**	**1.32**	**1.62**	**1.46**	**1.64**	**0.42**	**0.27**	**20.48**	**22.56**
	T	**0.85**	**0.65**	**0.78**	**0.67**	**0.17**	**0.17**	**10.99**	**9.41**
	Pb * T	**0.98**	**0.75**	**0.90**	**0.77**	**0.19**	**0.19**	**12.63**	**10.81**

2-Flowering Parameters:

Data presented in Table (4) showed that, in both seasons, irrigation water polluted with lead decreased the flowering parameters of *Polianthes tuberosa* plants. Plants irrigated with tap water (control) had the highest mean values of several flower number per spike (24.89 and 25.14), flower dry weight (5.99 and 6.04 g), spike dry weight (7.60 and 7.66 g) and rachis length (23.18 and 23.39 cm) in the first and second seasons, respectively. Moreover, raising the lead concentration caused steady significant reductions in flowering parameter, with the highest concentration (300 mg/L) giving significantly the smallest flowers mean values of the number of flowers per spike (20.37 and 20.99), flower dry weight (4.79 and 5.11 g), spike dry weight (6.51 and 6.62 g) and rachis length (19.39 and 19.91 cm) in the first and second seasons, respectively.

Flowering parameters were also significantly affected by spraying the plants with tryptophan. In both seasons, plant height increased gradually when the tryptophan concentration was raised from 0 mg/L (control) to 500 mg/L. Accordingly, it can be seen from the data in Table (4) that *Polianthes tuberosa* plants sprayed with 500 mg/L tryptophan were significantly mean values of the number of flowers per spike (22.67 and 22.94), flower dry weight (5.45 and 5.59 g), spike dry weight (7.08 and 7.13 g) and rachis length (21.28 and 21.55 cm) in the first and second seasons, respectively.

Regarding the interaction between irrigation with lead polluted water and tryptophan treatments on flowering parameters of *Polianthes tuberosa* plants, the results recorded in the two seasons showed that the highest values were obtained in the plants irrigated with tap water and sprayed with tryptophan at 500 mg/L with mean values of the number of flowers per spike (25.48 and 25.69), flower dry weight (6.12 and 6.18 g), spike dry weight (7.76 and 7.80 g) and rachis length (23.6 and 23.85 cm) in the first and second seasons, respectively. On the other hand, the smallest plants with mean values of the number of flowers per spike (20.27 and 20.95), flower dry weight (4.68 and 5.03 g), spike dry weight (6.40 and 6.55 g) and rachis length (19.36 and 19.88 cm) in the first and second seasons, respectively, were resulted in when the plants were irrigated using the highest lead concentration (300 mg/L) without tryptophan treatment. It can also be seen from the data presented in Table (4) that in many cases, spraying the plants with tryptophan reduced the undesirable effect of polluted water with lead.

Table 4: Means of flower number per spike, flower dry weight (g), spike dry weight (g) and rachis length (cm) of *Polianthes tuberosa* plants as influenced by Lead (Pb), Tryptophan (T) and their combinations (Pb × T) in the two seasons of 2018 and 2019.

Treatments		Flower number per spike		Flower dry weight (g)		Spike dry weight (g)		Rachis length (cm)	
Lead (mg/L)	**Tryptophan (mg/L)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
000	0	24.28	24.50	5.84	5.89	7.45	7.51	22.67	22.86
	250	24.92	25.23	6.02	6.07	7.61	7.69	23.22	23.47
	500	25.48	25.69	6.12	6.18	7.76	7.80	23.67	23.85
Mean (Pb)		**24.89**	**25.14**	**5.99**	**6.04**	**7.60**	**7.66**	**23.18**	**23.39**
100	0	22.12	22.43	5.32	5.39	6.93	7.01	20.86	21.12
	250	22.45	22.58	5.39	5.42	7.01	7.04	21.14	21.27
	500	23.19	23.23	5.57	5.58	7.20	7.20	21.76	21.79
Mean (Pb)		**22.58**	**22.74**	**5.42**	**5.46**	**7.04**	**7.08**	**21.25**	**21.39**
200	0	20.39	21.11	4.89	5.07	6.52	6.69	19.42	20.01
	250	21.07	21.65	5.06	5.21	6.68	6.82	19.99	20.45
	500	21.51	21.81	5.24	5.34	6.76	6.87	20.27	20.61
Mean (Pb)		**20.99**	**21.52**	**5.06**	**5.20**	**6.65**	**6.79**	**19.89**	**20.35**
300	0	20.27	20.95	4.68	5.03	6.40	6.55	19.36	19.88
	250	20.33	20.99	4.79	5.04	6.52	6.66	19.39	19.92
	500	20.53	21.03	4.90	5.26	6.63	6.67	19.43	19.95
Mean (Pb)		**20.37**	**20.99**	**4.79**	**5.11**	**6.51**	**6.62**	**19.39**	**19.91**
Mean (T)	0	21.76	22.24	5.18	5.34	6.82	6.94	20.57	20.96
	250	22.19	22.61	5.31	5.43	6.95	7.05	20.93	21.27
	500	22.67	22.94	5.45	5.59	7.08	7.13	21.28	21.55
L.S.D. at 0.05	**Pb**	**0.33**	**0.44**	**0.06**	**0.14**	**0.10**	**0.09**	**0.34**	**0.36**
	T	**0.22**	**0.19**	**0.06**	**0.06**	**0.05**	**0.05**	**0.17**	**0.15**
	Pb * T	**0.26**	**0.19**	**0.07**	**0.25**	**0.06**	**0.06**	**0.19**	**0.17**

3-Corm Parameters:

The data recorded for the corm parameters of *Polianthes tuberosa* plants in the two seasons Table (5) showed that irrigation with lead polluted water decreased the corm parameter, compared to that of plants irrigated with tap water (control). In both seasons, plants irrigated with tap water had the thickest corm, with mean corm diameter (5.05 and 5.20 cm), corm dry weight (32.57 and 32.90 g) and a number of new cormlets (13.64 and 13.78) in the first and second seasons, respectively. Raising the lead concentration in irrigation water caused a steady reduction in the corm parameter. This reduction in corm parameter was significant (compared to the control), even at the highest lead concentration (300 mg/L), which gave corm diameter (4.08 and 4.31 cm), corm dry weight (26.62 and

27.44 g), and the number of new cormlets (11.00 and 11.36) in the first and second seasons, respectively.

In contrast to the effect of lead treatments, tryptophan treatments improved corm parameters of *Polianthes tuberosa* plants, compared to the control. Moreover, plants sprayed with 500 mg/L tryptophan had significantly mean corm diameter (4.57 and 4.74 cm), corm dry weight (29.59 and 30.01 g), and the number of new cormlets (12.31 and 12.51) in the first and second seasons, respectively.

Regarding the interaction between irrigation with lead polluted water and tryptophan treatments on corm parameter of *Polianthes tuberosa* plants, the results recorded for the two seasons Table (5) showed that significant differences were detected between the values obtained from plants receiving the different treatment combinations. The highest values mean corm diameter (5.19 and 5.32 cm), corm dry weight (33.35 and 33.63 g), and the number of new cormlets (13.99 and 14.12) in the first and second seasons, respectively, were obtained in the plants irrigated with tap water and sprayed with tryptophan at 500 mg/L. On the other hand, the thinnest values mean corm diameter (4.03 and 4.27 cm), corm dry weight (26.58 and 27.39 g), and the number of new cormlets (10.98 and 11.35) in the first and second seasons, respectively, were obtained in the plants irrigated using the highest lead concentration 300 mg/L without tryptophan treatment. It can also be seen from the data presented in Table (5) that in many cases, spraying the plants with tryptophan reduced the undesirable effect of contaminated water with lead.

Table 5: Means of corm diameter (cm), corm dry weight (g) and number new cormalets of *Polianthes tuberosa* plants as influenced by Lead (Pb), Tryptophan (T) and their combinations (Pb × T) in the two seasons of 2018 and 2019.

Treatments		**Corm diameter (cm)**		**Corm dry weight (g)**		**Number new cormalets**	
Lead (mg/L)	**Tryptophan (mg/L)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
000	0	4.90	5.05	31.77	32.05	13.29	13.39
	250	5.06	5.23	32.61	33.04	13.66	13.85
	500	5.19	5.32	33.35	33.63	13.99	14.12
Mean (Pb)		**5.05**	**5.20**	**32.57**	**32.90**	**13.64**	**13.78**
100	0	4.43	4.60	28.93	29.33	12.02	12.20
	250	4.51	4.63	29.38	29.54	12.22	12.29
	500	4.67	4.77	30.34	30.40	12.65	12.68
Mean (Pb)		**4.53**	**4.66**	**29.55**	**29.75**	**12.29**	**12.39**
200	0	4.06	4.30	26.67	27.59	11.01	11.43
	250	4.21	4.42	27.57	28.28	11.41	11.73
	500	4.27	4.47	27.99	28.54	11.61	11.85
Mean (Pb)		**4.18**	**4.39**	**27.41**	**28.13**	**11.34**	**11.67**
300	0	4.03	4.27	26.58	27.39	10.98	11.35
	250	4.05	4.28	26.62	27.44	11.00	11.36
	500	4.16	4.40	26.68	27.50	11.02	11.39
Mean (Pb)		**4.08**	**4.31**	**26.62**	**27.44**	**11.00**	**11.36**
Mean (T)	0	4.35	4.55	28.48	29.09	11.82	12.09
	250	4.45	4.64	29.04	29.57	12.07	12.30
	500	4.57	4.74	29.59	30.01	12.31	12.51
L.S.D. at 0.05	**Pb**	**0.09**	**0.09**	**0.53**	**0.58**	**0.23**	**0.26**
	T	**0.05**	**0.03**	**0.29**	**0.24**	**0.12**	**0.10**
	Pb * T	**0.05**	**0.04**	**0.32**	**0.26**	**0.14**	**0.09**

3.1.Chemical Constituents:

1. Plant Chemical Analysis:

The results presented in Table (6) showed that the highest content of chlorophyll was obtained in plant irrigated with tap water (44.71 and 45.13 SPAD) and carbohydrate (20.95 and 22.17%) in the first and second seasons, respectively, while the highest proline content (2.69 and 2.65 mg/g) was obtained in the plants irrigated with lead water at 300 mg/L. Raising the lead concentration in irrigation water resulted in steady significant reductions in the chlorophyll and carbohydrates content, which reached its lowest value mean chlorophyll (37.13 and 38.14 SPAD) and carbohydrate (16.71 and 18.29 %) in the first and second seasons, respectively, in plants receiving the highest lead concentration 300 mg/L. While proline content (1.53 and 1.57 mg/g) was obtained in the plants irrigated with tap water.

The results of leaf chemical analysis Table (6) also showed that tryptophan treatments had a clear effect on the leaf chemical analysis. The recorded mean values highest mean chlorophyll (40.90 and 41.47 SPAD) and carbohydrate (18.81 and 20.11 %) in the first and second seasons, respectively, in plants sprayed with tryptophan at 250 mg/L. While highest proline content (2.19 and 2.22 mg/g) was obtained in plants sprayed without tryptophan.

Regarding the interaction between irrigation using water polluted with lead and tryptophan treatments, the data presented in Table (6) showed that the highest chlorophyll contents (45.70 and 46.06 SPAD) and carbohydrate (21.49 and 22.69 %) in the first and second seasons, respectively, were found in leaves of plants irrigated with tap water and sprayed with tryptophan at 250 mg/L. While proline content (2.70 and 2.68 mg/g) was resulted in when the plants were irrigated using the highest lead concentration (300 mg/L) without tryptophan treatment.

Table 6: Means of chemical constituents characteristics of *Polianthes tuberosa* plants as influenced by Lead (Pb), Tryptophan (T) and their combinations (Pb×T) in the two seasons of 2018 and 2019

Treatments		**Chlorophyll content (SPAD)**		**Carbohydrates content (%)**		**Proline content (mg/g D.W)**	
Lead (mg/L)	**Tryptophan (mg/L)**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
000	0	43.69	44.05	20.38	21.57	1.59	1.61
	250	45.70	46.06	21.49	22.69	1.45	1.51
	500	44.76	45.28	20.99	22.26	1.56	1.59
Mean (Pb)		**44.71**	**45.13**	**20.95**	**22.17**	**1.53**	**1.57**
100	0	40.06	40.66	18.34	19.64	2.04	2.09
	250	41.86	41.94	19.35	20.38	2.00	2.03
	500	40.62	40.84	18.67	19.79	2.03	2.03
Mean (Pb)		**40.84**	**41.14**	**18.78**	**19.93**	**2.02**	**2.05**
200	0	37.17	38.36	16.73	18.39	2.46	2.51
	250	38.87	39.65	17.68	19.05	2.41	2.47
	500	38.31	39.24	17.37	18.91	2.46	2.48
Mean (Pb)		**38.11**	**39.08**	**17.26**	**18.78**	**2.44**	**2.48**
300	0	37.07	38.10	16.68	18.25	2.70	2.68
	250	37.20	38.25	16.75	18.34	2.70	2.64
	500	37.13	38.07	16.70	18.29	2.69	2.65
Mean (Pb)		**37.13**	**38.14**	**16.71**	**18.29**	**2.69**	**2.65**
Mean (T)	0	39.49	40.29	18.03	19.46	2.19	2.22
	250	40.90	41.47	18.81	20.11	2.14	2.16
	500	40.20	40.85	18.43	19.81	2.18	2.18
L.S.D. at 0.05	**Pb**	**0.68**	**0.83**	**0.37**	**0.43**	**0.06**	**0.04**
	T	**0.36**	**0.31**	**0.20**	**0.17**	**0.02**	**0.02**
	Pb * T	**0.42**	**0.35**	**0.22**	**0.17**	**0.029**	**0.026**

2. Lead Content in Leaves and Corm (mg/L):

Data resulting from plant parts chemical analysis Table (7) showed that the lead content (mg/L) in the dried plant parts of *Polianthes tuberosa* plants was raised steadily with raising the lead concentration in the irrigation water. The lowest mean lead content in leaves (0.119 and 0.125 mg/L) and lead content in corm (0.192 and 0.208 mg/L) in the first and second seasons, respectively, were found in plants irrigated with tap water, whereas the highest mean values lead content in leaves (0.292 and 0.311 mg/L) and lead content in corm (0.707 and 0.762 mg/L) in the first and second seasons, respectively, was found in plants irrigated with water containing the highest lead concentration 300 mg/L.

Concerning the effect of tryptophan treatments on the lead content in plant parts, the data recorded in the two seasons Table (7) showed that only one tryptophan treatment 500 mg/L caused a significant decrease in mean values of lead content in leaves (0.163 and 0.173 mg/L) and lead content in corm (0.322 and 0.355 mg/L) in the first and second seasons, respectively, compared to that of control plants had the highest lead content in leaves (0.224 and 0.236 mg/L) and lead content in corm (0.510 and 0.543 mg/L) in the first and second seasons, respectively.

Concerning the interaction between irrigation using water contaminated with lead and tryptophan treatments on the lead content in plant parts. The results in Table (7) showed that the lowest mean values of lead content in leaves (0.109 and 0.112 mg/L) and lead content in corm (0.140 and 0.150 mg/L) in the first and second seasons, respectively, were obtained in the plant parts irrigated with tap water and sprayed with tryptophan at 500 mg/L. On the other hand, the highest lead content was obtained in the plant parts irrigated with lead water at 300 mg/L and receiving no tryptophan treatment in leaves (0.344 and 0.373 mg/L) and lead content in corm (0.856 and 0.941 mg/L) in the first and second seasons, respectively.

Table 7: Means of chemical constituents characteristics of *Polianthes tuberosa* plants as influenced by Lead (Pb), Tryptophan (T) and their combinations (Pb×T) in the two seasons of 2018 and 2019.

Treatments		Lead content in leaves (mg/L)		Lead content in corm (mg/L)	
Lead (mg/L)	**Tryptophan (mg/L)**	**2018**	**2019**	**2018**	**2019**
000	0	0.129	0.143	0.236	0.265
	250	0.121	0.121	0.201	0.209
	500	0.109	0.112	0.140	0.150
Mean (Pb)		**0.119**	**0.125**	**0.192**	**0.208**
100	0	0.200	0.196	0.437	0.428
	250	0.158	0.161	0.316	0.327
	500	0.130	0.141	0.229	0.274
Mean (Pb)		**0.162**	**0.166**	**0.327**	**0.343**
200	0	0.225	0.234	0.513	0.538
	250	0.213	0.216	0.476	0.482
	500	0.171	0.184	0.355	0.392
Mean (Pb)		**0.203**	**0.211**	**0.448**	**0.470**
300	0	0.344	0.373	0.856	0.941
	250	0.290	0.303	0.699	0.738
	500	0.243	0.258	0.566	0.607
Mean (Pb)		**0.292**	**0.311**	**0.707**	**0.762**
Mean (T)	0	0.224	0.236	0.510	0.543
	250	0.195	0.200	0.423	0.439
	500	0.163	0.173	0.322	0.355
L.S.D. at 0.05	**Pb**	**0.004**	**0.003**	**0.009**	**0.014**
	T	**0.001**	**0.003**	**0.005**	**0.008**
	Pb * T	**0.002**	**0.003**	**0.006**	**0.009**

3.Transfer factor (TF) of heavy metals

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

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

Data in **Table (8)** showed that the lowest average of lead content was observed in soil cultured by untreated plants, while the highest average of lead content was observed in the soil after the treatment of 300 mg/L lead and 0 mg/L tryptophan.

Table 8: Average values of lead content in soil samples as influenced by lead concentrations in irrigation water and foliar application of Tryptophan on *Polianthes tuberosa* in the two seasons of 2018 and 2019.

Treatments		Lead content in soil (mg/kg)			
Lead (mg/L)	Tryptophan (mg/L)	2018		2019	
		Before	After	Before	After
0	0	0.093	0.311	0.124	0.331
	250	0.101	0.432	0.135	0.451
	500	0.106	0.495	0.141	0.515
100	0	0.104	0.471	0.138	0.492
	250	0.111	0.553	0.146	0.573
	500	0.115	0.627	0.154	0.647
200	0	0.112	0.584	0.151	0.604
	250	0.117	0.648	0.156	0.668
	500	0.123	0.742	0.164	0.762
300	0	0.118	0.673	0.158	0.693
	250	0.126	0.775	0.168	0.795
	500	0.132	0.831	0.173	0.851

3.2. Transfer factor to leaves and corm

From the data presented in Table (9), it can be seen that the transfer factor in the dried plant parts of *Polianthes tuberosa* plants was increased steadily with raising the lead concentration in the irrigation water. Accordingly, the highest lead value in leaves (0.392 and 0.407) and in corm (0.951 and 0.999) was found in plants irrigated with water containing lead concentration 300 mg/L, whereas the lowest value in leaves (0.304 and 0.298) and in corm (0.501 and 0.518) was found in plants irrigated with tap water (control).

The results in Table (9) also showed that the transfer factor in the dried plant parts was reduced steadily with raising tryptophan concentration. Accordingly, the highest lead value in leaves (0.433 and 0.438) and in corm (0.958 and 0.979) was recorded in the control plants, whereas plants sprayed with the highest tryptophan concentration 500 mg/L had the lowest lead value in leaves (0.237 and 0.244) and in corm (0.451 and 0.485) in the first and second seasons, respectively.

Table 9: Means of transfer factor to leaves, stem and roots of *Polianthes tuberosa* plants as influenced by Lead (Pb), Tryptophan (T) and their combinations (Pb ×T) in the two seasons of 2018 and 2019**.**

Treatments		Transfer factor to leaves (TFL)		Transfer factor to corm (TFC)	
Lead (mg/L)	Tryptophan (mg/L)	2018	2019	2018	2019
000	0	0.414	0.398	0.758	0.800
	250	0.280	0.280	0.465	0.463
	500	0.220	0.217	0.282	0.291
Mean (Pb)		**0.304**	**0.298**	**0.501**	**0.518**
100	0	0.424	0.432	0.927	0.869
	250	0.285	0.268	0.571	0.570
	500	0.207	0.217	0.365	0.423
Mean (Pb)		**0.305**	**0.305**	**0.621**	**0.620**
200	0	0.385	0.387	0.878	0.890
	250	0.328	0.323	0.734	0.721
	500	0.230	0.241	0.478	0.514
Mean (Pb)		**0.314**	**0.317**	**0.696**	**0.708**
300	0	0.511	0.538	1.271	1.357
	250	0.374	0.381	0.901	0.928
	500	0.292	0.303	0.681	0.713
Mean (Pb)		**0.392**	**0.407**	**0.951**	**0.999**
Mean (T)	0	0.433	0.438	0.958	0.979
	250	0.316	0.313	0.667	0.670
	500	0.237	0.244	0.451	0.485

DISCUSSION

This study revealed that at high heavy metal concentrations, the biomass was significantly reduced. The leaves' growth was more sensitive than other parts, as leaves rapidly absorbed water and had higher accumulations of heavy metal elements. The results presented by this study were in agreement with earlier reports on other plants, such as aquatic plant *Wolffia arrhiza* (Piotrowska *et al.*, 2010), barley *Hordeum vulgare* (Tiryakioglu *et al.*, 2006) and *Typha angustifolia* (Bah *et al.*, 2011).

That at high heavy-metal concentrations, the plant height was significantly reduced, and the biomass was decreased. The 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 plants 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).

Plants can be tolerated lead either by external exclusion or internal tolerance. By the external exclusion, lead ions are excluded from entering the plant cells and thus lead cannot accumulate in the organelles and excess lead ions are removed out of the plant cell (Sharma and Dubey 2005). The internal tolerance of lead is mainly due to the synthesis of organic lead compounds (cysteine, glutathione, phytochelatin, etc) and eventually, the lead ions are transformed in the cell into chemically bound structures with lower toxicity, alleviating the Pb toxic effect on the plants' tissues (Pourrut *et al.,* 2011). Lead can damage the ultrastructures of the organs, tissues, chloroplast, mitochondria, nucleus, cell wall, and cell membrane in the plants. This damage can cause a loss of organelle function, and can eventually affect the normal physiological functions that include photosynthesis, respiration, protein synthesis, cell division within the plant species (Salazar and Pignata, 2014).

Concerning treatments and the control sample, at a preliminary stage, one should note that the transfer factor of most treatments is lower than one for lead; which means that the physiological need of the plant for these elements is rather limited.

Trace elements translocation from roots to shoots via a number of physiological processes, including metal unloading into root xylem cells, long-distance carrying from the xylem to the shoots and metal reabsorption, by leaf mesophyll cells, from the xylem stream. Once the trace metals have been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream (Blaylock and Huang, 2000).

For the effect of tryptophan it is observed that there is a significant increase in all vegetative parameters, chlorophyll content, carbohydrate percentage, a significant decrease in lead content in leaves. This may be due to that application of tryptophan with any of the concentrations of lead led to a statistically decrease in the uptake of lead. This decrease in uptake of lead in the presence of tryptophan resulted in the formation of citric acid-lead complexes that inhibited the uptake (Chen *et al.,* 2003). The decrease in lead uptake helped to overcome the negative effects of lead on the previously 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. In addition, we can use *Senecio cineraria* plants as lead phytoremediation and if we want to use *Senecio cineraria* as an ornamental plant and the irrigation water is contaminated with the lead we can spray the plants to overcome the negative effect of lead (El-Shanhorey and EL-Sayed, 2017). The decrease in lead uptake helped to overcome the negative effects of lead on the previously 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.

CONCLUSIONS

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 on soil structure. Some specific plants have been proven to have a noticeable potential to absorb toxic heavy metals.

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

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ARABIC SUMMARY

تأثير الرش بالتربتوفان على نباتات التبروز لتقليل الأثر الضار لبعض التلوث بالمعادن الثقيلة فى ماء الرى. (ب) تأثير معاملات الرصاص.

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الملخص العربى

تم إجراء هذه الدراسة في فرع البحوث بأنطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية - الإسكندرية، مصر خلال الموسمين المتتاليين 2018 و 2019. وتهدف هذه الدراسة إلى تقييم آثار الري بالمياة الملوثة بالرصاص على نمو نباتات التبروز المزروعة في تربة رملية , كذلك إمكانية استخدام الرش بالتربتوفان للتغلب على الآثار الضارة للرصاص. ولتحقيق ذلك زرعت أبصال التبروز بشكل فردي في أصص بلاستيكية (قطرها 20 سم) مملوءة 5 كجم من التربة الرملية. وكانت معاملات مياه الري الملوث بأربعة تراكيزات من الرصاص وهى صفر , 100، 200 , 300 مليجرام/ لتر. بينما تم رش النباتات شهريا بالتربتوفان بإستخدام ثلاث تركيزات هى صفر، 250 و 500 مليجرام/ لتر في كلا الموسمين.

أظهرت نتائج الدراسة أن هناك اختلاف كبير في التفاعل بين رى النباتات بتركيزات مختلفة من الرصاص ورش النباتات بالتربتوفان. وقد وجد انخفاض كبير في كافة معاملات الري بالماء الملوث بالرصاص وكذلك لوحظ زيادة كبيرة فى معدلات النموالخضرى بعد الرش500 مليجرام/ لتر تربتوفان. تم الحصول على أعلى قيمة من محتوى الكلوروفيل والكربوهيدرات من النباتات المروية بماء الصنبور والرش بتركيز 250 مليجرام/ لتر تربتوفان في حين أن أعلى تركيز من الرصاص في الأوراق و الكورمات من خلال الرى بالماء الملوث كان بتركيز 300 مليجرام/ لتر من الرصاص بدون الرش بالتربتوفان.