Bioremediation of Heavy Metals by Using Some Shrubs in Three Different Locations of Alexandria City. (B) *Nerium oleander* Plant

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ABSTRACT

The present study was carried-out in three different locations in Alexandria city [El-Ebrahimeya zone (traffic area), El-Dekhela zone (industrial area) and Antoniadis Park (control area)] during two successive seasons 2015 and 2016. Homogeneous seedlings of *Nerium oleander* were planted individually in plastic pots (30 cm diameter) filled with mixture of sand and clay. 90 plants were planted in three locations in Alexandria city (30 plants each zone). Samples were collected during spring and autumn in both seasons.

The obtained results showed the effect of different locations on vegetative growth of *Nerium oleander* plants. In both seasons, plants planted in Antoniadis Park had the highest leaves, stem and roots parameters in the first and second seasons, respectively. While, plants planted in El-Dekhela had the lowest vegetative growth rate in both seasons. The growth (leaves, stem and root) was also significantly affected by different periods during both seasons. Accordingly, it can be seen that the data were significantly increased gradually in the autumn, while, the lowest growth in the spring.

The results of chemical analysis for plant parts showed the effect of different locations on lead, cadmium and zinc contents in plant parts. In both seasons, plants planted in El-Dekhela had the highest heavy metals content, while, those planted in Antoniadis Park had the lowest lead, cadmium and zinc contents in the first and second seasons, respectively. Chemical analysis of heavy metals content in plant parts was also significantly affected by different periods during both seasons. Accordingly, it can be seen from the data that heavy metals were significantly increased gradually in the spring, while, the lowest heavy metals content (lead, cadmium and zinc) in plant parts was found in autumn in the first and second seasons, respectively.

Transfer factor (TF) indicates the efficiency of *Nerium oleander* plants to transfer metals from the soil to the plant parts. It can be seen that the transfer factor in the lead, cadmium and zinc content in *Nerium oleander* plants was increased steadily with different locations. We found that, plants in Antoniadis Park had the highest transfer factor with respect to the heavy metals content, while, those planted in El-Dekhela had the lowest transfer factor in the first season. On the other hand, the highest transfer factor in plant parts, was found in the stem for lead and cadmium, while it was in the leaves for zinc compared with different parts of plant.

Key word: Bioremediation, *Nerium oleander*, Lead, Cadmium, Zinc

INTRODUCTION

Pollution is the introduction of contaminants into the environment that cause harm or discomfort to humans or other living organisms, or that damage the environment" which can come "in the form of chemical substances, or energy such as noise, heat or light". "Pollutants can be naturally occurring substances or energies, but are considered contaminants when in excess of natural levels (UN ECE, 2008). Generally speaking, there are many types of environmental pollution, but the most important ones are: Air, soil and water pollution.

The recognition of air pollution as a problem in Egypt dates only to the early fifties. The problem came into existence because of the rapid growth of industrialization and urbanization. The consistent in crement in human populace, vehicular movement and commercial enterprises had brought about high concentration of gaseous and particulate pollutants (Joshi *et al*., 2009). Expansive measure of pollutants has been transmitted as a consequence of this urbanization and industrialization which invariably has its many environmental issues such as air pollution as well as waste management (Bang, 2012).

Heavy metals is considered one of the air pollutants, although it is a loosely defined term (Duffus, 2002), it is widely recognised and usually applies to the widespread contaminants of terrestrial and freshwater ecosystems (Duce and Tindale, 1991). The heavy metal can be taken up also from the air or by precipitation directly via the leaves (Alaimo *et al.,* 2000). Also, (Granati. *et al.,* 2007) found that in leaf surface of *Quercus ilex* the mean metal concentration is 74% of the total concentration, this agree with the information about the

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pollution coming from the atmosphere by deposition of particulate matter on the surface.

For example, lead as elevated Pb in soils may decrease soil productivity, and a very low Pb concentration may inhibit some vital plant processes, water absorption with toxic symptoms of dark green such as photosynthesis, mitosis and leaves, wilting of older leaves, stunted foliage and brown short roots (Bhattacharyya *et al*.,2008). As for the effect of cadmium on plant , it was found that root uptake of cadmium from contaminated soils induces physiological changes such as a decrease in plant growth and that elevated concentrations of cadmium led to accumulation of cadmium in the shoot and roots, intervein chlorosis of leaves and loss of pigments (Wahid *et al*., 2008). The phytotoxicity of zinc on plant was showed as high doses of Zinc (65 and 130 mg L^{-1}) can trigger growth depression, dark green leaves, decreased root number and length and sharp depression in the mitotic activity of roots from sugarcane (Jain *et al*., 2010).

Phytoremediation is one of the effective and fordable technological solutions used to extract or remove inactive metals and metal pollutant from contaminated soil, water and air (El-Shanhorey and El-Sayed , 2017). The advantage of this kind of technology not only limited to reduce them, but also effecting the mechanisms of heavy metal uptake as well as one of the effective factors in phytoremediation technology, other advantage is that , it also considered to be friendly and potentially cost effective (Bieby *et al*., 2011).

Plants with exceptional metal accumulating capacity are known as hyperaccumulator plants (Choruk *et al*., 2006). So, plants need trace amount of heavy metal, but their excessive availability may cause plant toxicity (Sharma *et al*., 2006). Heavy metals are potentially toxic and phytotoxicity for plants resulting in chlorosis, weak plant growth, yield depression, and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and reduced ability to fixate molecular nitrogen in leguminous plants (Guala *et al*., 2010).

Nerium oleander is a shrub belongs to family Apocynaceae. A common fast growing evergreen shrub, widely cultivated in the Mediterranean area for ornamental purposes. It can reach up to 4 m in height although in parks, gardens and along roadsides it is cutted to keep a rounded smaller shape. Leaves are short-stalked green or grey-green in colour and range from about 10 to 20 cm long, also one of the morphological and physic- chemical characteristics of *Nerium Oleander* leaves that has lanceolate leaves with high cuticle thickness (Ataabadi. *et al*., 2010). The plant produces terminal flower head usually pink or white. Each flower is about 5 cm in diameter and 5 petalled. The plant is supposed to accumulate elements on the leaf surface largely from aerial sources and also by substratum (Seaward and Mashhour, 1991) and (Aksoy and Ozturk, 1997). It was also found that this shrub is a common drought-tolerant and fairly hardy landscaping plant. It is used for hedges, living privacy screens, outdoor planter boxes. (Harrison *et al*., 2012). This species has already been profitably used for investigating the bio-accumulation of trace elements (Matarese *et al*., 2005).

The aim of this study was to evaluate the effects of phytoremediation using *Nerium oleander* plants on reducing the air pollution content with heavy metals (Lead, Cadmium and Zinc) in three locations in Alexandria city (El-Ebrahimeya, El-Dekhela and Antoniadis Park). So it has become necessary to conduct this study to exhibit and determine the kind of environmental pollution and how far they exhibit and efficient as the bioindicator in reducing the degree of pollution in environment.

MATERIALS AND METHODS

The present study was carried-out in three different locations in Alexandria city, during two successive seasons 2015 and 2016, namely:

- El-Ebrahimeya zone which is considered as a traffic area.
- El- Dekhela zone which is considered as an industrial area.
- Antoniadis Garden (Smouha zone) which is considered as a control area.

On March 1$^{st.}$, 2015 and 2016 in the first and second seasons, respectively, homogeneous seedlings of *Nerium oleander* (20-25 cm height, 15-20 leaves per plant on mean and one year ago), seedlings were planted individually in plastic pots (30 cm diameter) filled with 7 kg mixture of sand and clay at the ratio of (1:1 by volume). The chemical constituents of the soil were determined as described by Jackson (1958) in Table (1). 90 seedling were planted in three locations in Alexandria city (30 plants each zone). Samples were collected during spring (April, May and June) and autumn (September, October and November) in both seasons the plants were harvested.

Table 1. Chemical analyses of the used mixture soil for the first season 2015

Season	pH	EC (dSm^{-1})	Soluble cations (meq/l)				Soluble anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
2015	7.91	1.52	3.2	3.0	6.3	1.1	3.3	6.5	2.2

Growth parameters measurements

(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:

- Total chlorophyll content were determined as a SPAD of 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).
- Determinations of heavy metals content (lead, cadmium and zinc) in plant samples while were divided into leaves stem and roots. They were then dried at 70°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 metals was determined using an atomic absorption spectrophotometer.
- Available heavy metals, i.e. (lead, cadmium and zinc) 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 was split plot design with three replicates. Each replicate contained ten plants. The main plots were the three locations while the sub plots were the two periods. 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

Vegetative growth

Leaves parameters

Data presented in Table (2) showed the effect of different locations on the number of leaves per plant, leaves dry weight and leaves area formed on *Nerium oleander* plants. In both seasons, plants of Antoniadis Park had the highest number of leaves (18.10 and 29.50 leaf per plant), leaves dry weight (4.06 and 4.17 g) and leaves area (59.19 and 103.66 cm^2) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest number of leaves (14.10 and 23.92 leaf per plant), leaves dry weight (3.18 and 3.37 g) and leaves area (36.26 and 65.52 cm^2) in the first and second seasons, respectively.

Number of leaves per plant, leaves dry weight and leaves area were also significantly affected by the different periods during both seasons. Accordingly, it can be seen from the data in Table (2) that the data of *Nerium oleander* plants were significantly increased gradually in the autumn, with mean number of leaves of (16.88 and 28.46 leaf per plant), leaves dry weight (3.83 and 4.11 g) and leaves area (52.69 and 94.53 cm^2) in the first and second seasons, respectively. On the other hand, the lowest in the spring, with mean number of leaves (16.03 and 26.02 leaf per plant), leaves dry weight (3.59 and 3.58 g) and leaves area (46.80 and 81.58 cm^2) in the first and second seasons, respectively.

Regarding the interaction the results recorded in the two seasons showed that, the highest values were obtained for the plants of Antoniadis park and the autumn period, with mean number of leaves (18.44 and 30.51 leaf per plant), leaves dry weight (4.21 and 4.44 g) and leaves area (62.36 and 110.04 cm^2) in the first and second seasons, respectively.

Stem parameters:

Data presented in Table (3) showed the effect of different locations on plant height, stem diameter and stem dry weight formed on *Nerium oleander* plants. In both seasons, plants of Antoniadis Park had the highest plant height (27.58 and 29.95 cm), stem diameter (0.88 and 0.93 cm) and stem dry weight (2.33 and 2.79 g) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest plant height (21.34 and 23.04 cm), stem diameter (0.77 and 0.80 cm) and stem dry weight (1.83 and 2.26 g) in the first and second seasons, respectively.

Plant height, stem diameter and stem dry weight was also significantly affected by the different periods during both seasons. Accordingly, it can be seen from the data in Table (3) that the data recorded for *Nerium oleander* plants were significantly increased gradually in the autumn, with mean plant height of (25.84 and 27.72 cm), stem diameter (0.88 and 0.92 cm) and stem dry weight (2.22 and 3.07 g) in the first and second seasons, respectively. On the other hand, the lowest in the spring, with mean plant height of (24.26 and 25.88 cm), stem diameter (0.78 and 0.81 cm) and stem dry weight (2.04 and 2.10 g) in the first and second seasons, respectively.

Regarding the interaction between the effects of different locations and different periods the results recorded in the two seasons showed that, the highest values were obtained for the plants of Antoniadis Park and the autumn period, with mean plant height (28.56 and 31.41 cm), stem diameter (0.93 and 0.98 cm) and stem dry weight (2.43 and 3.29 g) in the first and second seasons, respectively.

Table 2. Means of leaves growth characteristics of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	**Number of leaves per plant**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	17.77	18.44	18.10	28.50	30.51	29.50
El-Dekhala	13.77	14.44	14.10	22.16	25.69	23.92
El-Ebrahimia	16.55	17.77	17.16	27.40	29.18	28.29
Mean	16.03	16.88		26.02	28.46	
LSD 0.05 (Periods)			1.36			2.52
LSD 0.05 (Locations)			0.52			1.80
L.S.D 0.05 (Periods*Locations)			0.42			1.46
Location	**Leaves dry weight per plant (g)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	3.92	4.21	4.06	3.91	4.44	4.17
El-Dekhala	3.09	3.28	3.18	3.12	3.63	3.37
El-Ebrahimia	3.76	4.02	3.89	3.73	4.27	4.00
Mean	3.59	3.83		3.58	4.11	
LSD 0.05 (Periods)			0.10			0.06
LSD 0.05 (Locations)			0.09			0.13
L.S.D 0.05 (Periods*Locations)			0.06			0.10
Location	**Leaves area (cm^2)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	56.03	62.36	59.19	97.28	110.04	103.66
El-Dekhala	34.26	38.26	36.26	58.26	72.78	65.52
El-Ebrahimia	50.12	57.46	53.79	89.21	100.77	94.99
Mean	46.80	52.69		81.58	94.53	
LSD 0.05 (Periods)			2.91			8.28
LSD 0.05 (Locations)			2.63			4.37
L.S.D 0.05 (Periods*Locations)			2.14			3.56

Table 3. Means of stem growth characteristics of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	**Plant height (cm)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	26.60	28.56	27.58	28.49	31.41	29.95
El-Dekhala	20.75	21.94	21.34	22.46	23.62	23.04
El-Ebrahimia	25.43	27.03	26.23	26.70	28.13	27.41
Mean	24.26	25.84		25.88	27.72	
LSD 0.05 (Periods)			0.50			2.89
LSD 0.05 (Locations)			0.55			1.16
L.S.D 0.05 (Periods*Locations)			0.45			0.94
Location	**Stem diameter (cm)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	0.84	0.93	0.88	0.88	0.98	0.93
El-Dekhala	0.72	0.83	0.77	0.74	0.87	0.80
El-Ebrahimia	0.78	0.88	0.83	0.81	0.92	0.86
Mean	0.78	0.88		0.81	0.92	
LSD 0.05 (Periods)			0.12			0.12
LSD 0.05 (Locations)			0.06			0.06
L.S.D 0.05 (Periods*Locations)			0.05			0.04
Location	**Stem dry weight (g)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	2.23	2.43	2.33	2.29	3.29	2.79
El-Dekhala	1.76	1.90	1.83	1.79	2.74	2.26
El-Ebrahimia	2.14	2.35	2.24	2.24	3.18	2.71
Mean	2.04	2.22		2.10	3.07	
LSD 0.05 (Periods)			0.03			0.02
LSD 0.05 (Locations)			0.05			0.04
L.S.D 0.05 (Periods*Locations)			0.04			0.03

Root parameters

Data presented in Table (4) showed the effect of different locations on root length and root dry weight formed on *Nerium oleander* plants. In both seasons, plants of Antoniadis Park had the highest root length (14.88 and 18.31 cm) and root dry weight (1.87 and 1.98 g) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest root length (9.48 and 11.82 cm) and root dry weight (1.19 and 1.23 g) in the first and second seasons, respectively.

Root length and root dry weight were also significantly affected by different periods during both seasons. Accordingly, it can be seen from the data in Table (4) that the data were significantly increased gradually in the autumn, with mean root length of (12.88 and 16.36 cm) and root dry weight (1.67 and 1.71 g) in the first and second seasons, respectively. On the other hand, the lowest in the spring, with mean root length (12.03 and 14.27 cm) and root dry weight (1.45 and 1.53 g) in the first and second seasons, respectively.

Regarding the interaction the results recorded in the two seasons showed that, the highest values were obtained for the plants of Antoniadis Park and the autumn period, with mean root length of (15.38 and 19.34 cm) and root dry weight of (1.99 and 2.07 g) in the first and second seasons, respectively.

Chemical constituents

Total chlorophyll (SPAD) and carbohydrates content (%)

The results presented in Table (5) showed the effect of different locations on total chlorophylls and carbohydrates content formed on *Nerium oleander* plants. In both seasons, plants of Antoniadis Park had the highest total chlorophyll content (75.90 and 71.38 SPAD) and carbohydrates content (6.81 and 4.91 %) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest total chlorophyll content (49.62 and 61.14 SPAD) and carbohydrates content (5.58 and 4.02 %) in the first and second seasons, respectively.

Total chlorophylls and carbohydrates content were also significantly affected by the different periods during both seasons. Accordingly, it can be seen from the data in Table (5) that these parameters were significantly increased in autumn, with mean total chlorophylls of (67.93 and 70.52 SPAD), but in the spring, the highest

Table 4. Means of root growth characteristics of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Root length (cm)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	14.38	15.38	14.88	17.28	19.34	18.31
El-Dekhala	9.21	9.76	9.48	10.74	12.90	11.82
El-Ebrahimia	12.52	13.52	13.02	14.80	16.86	15.83
Mean	12.03	12.88		14.27	16.36	
LSD 0.05 (Periods)			2.35			0.14
LSD 0.05 (Locations)			1.27			1.02
L.S.D 0.05 (Periods*Locations)			1.03			0.83

Location	Root dry weight (g)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	1.75	1.99	1.87	1.89	2.07	1.98
El-Dekhala	1.10	1.29	1.19	1.14	1.33	1.23
El-Ebrahimia	1.51	1.74	1.62	1.56	1.74	1.65
Mean	1.45	1.67		1.53	1.71	
LSD 0.05 (Periods)			0.29			0.02
LSD 0.05 (Locations)			0.16			0.10
L.S.D 0.05 (Periods*Locations)			0.13			0.08

Table 5. Means of chemical constituents (chlorophyll content (SPAD) and carbohydrate content (%)) of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	chlorophyll content (SPAD)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	69.75	82.06	75.90	66.10	76.66	71.38
El-Dekhala	45.35	53.90	49.62	59.73	62.56	61.14
El-Ebrahimia	62.77	67.83	65.30	61.60	72.36	66.98
Mean	59.29	67.93		62.47	70.52	
LSD 0.05 (Periods)			0.36			6.21
LSD 0.05 (Locations)			3.36			4.12
L.S.D 0.05 (Periods*Locations)			2.74			3.35

Location	Carbohydrate content of leaves (%)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	7.69	5.94	6.81	5.81	4.01	4.91
El-Dekhala	6.05	5.11	5.58	5.00	3.05	4.02
El-Ebrahimia	7.13	5.58	6.35	5.46	3.43	4.44
Mean	6.95	5.54		5.42	3.49	
LSD 0.05 (Periods)			0.08			2.76
LSD 0.05 (Locations)			0.02			0.62
L.S.D 0.05 (Periods*Locations)			0.01			0.50

carbohydrates content with Mean (6.95 and 5.42 %) in the first and second seasons, respectively. On the other hand, the lowest total chlorophylls content in the spring, with mean (59.29 and 62.47 SPAD) and the lowest carbohydrates content in the autumn, with mean (5.54 and 3.49 %) in the first and second seasons, respectively.

Regarding the interaction the results recorded in the two seasons showed that, the highest values were obtained in the planted plants in Antoniadis Park and the autumn period, with mean total chlorophylls content (82.06 and 76.66 SPAD) and the planted plants in Antoniadis Park and the spring period, with mean highest carbohydrates content of (7.69 and 5.81 %) in the first and second seasons, respectively.

Heavy metals content in plant

Lead (ppm)

Data presented in Table (6) showed the effect of different locations on lead content in different parts of *Nerium oleander* plants. In both seasons, planted plants in El-Dekhela had the highest lead content in leaves (10.930 and 11.344 ppm), lead content in stem (8.725 and 9.030 ppm) and lead content in root (0.459 and 0.622 ppm) in the first and second seasons, respectively. While, planted plants in Antoniadis Park had the lowest lead content in leaves (2.876 and 3.240 ppm), lead content in stem (3.301 and 3.606 ppm) and lead content in root (0.275 and 0.438 ppm) in the first and second seasons, respectively.

Lead content in plant parts was also significantly affected by different periods during both seasons. Accordingly, it can be seen from the data in Table (6) that lead content were significantly increased in the spring, with mean values in leaves (9.403 and 10.317 ppm), in stem (6.381 and 6.653 ppm) and in root (0.402 and 0.567 ppm) in the first and second seasons, respectively.

Table 6. Means of lead content in leaves, stem and root (ppm) of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Lead content in leaves (ppm)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	4.174	1.578	2.876	5.092	1.389	3.240
El-Dekhala	15.976	5.884	10.930	16.889	5.799	11.344
El-Ebrahimia	8.059	3.815	5.937	8.972	3.729	6.350
Mean	9.403	3.759		10.317	3.639	
LSD 0.05 (Periods)			5.496			5.499
LSD 0.05 (Locations)			2.905			2.906
L.S.D 0.05 (Periods*Locations)			2.366			2.367
Location	**Lead content in stem (ppm)**					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	3.397	3.206	3.301	3.602	3.611	3.606
El-Dekhala	8.771	8.679	8.725	9.076	8.984	9.030
El-Ebrahimia	6.976	6.886	6.931	7.281	7.192	7.236
Mean	6.381	6.257		6.653	6.595	
LSD 0.05 (Periods)			0.002			0.004
LSD 0.05 (Locations)			1.857			1.857
L.S.D 0.05 (Periods*Locations)			1.512			1.512
Location	**Lead content in root (ppm)**					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	0.301	0.249	0.275	0.465	0.412	0.438
El-Dekhala	0.501	0.418	0.459	0.664	0.581	0.622
El-Ebrahimia	0.406	0.321	0.363	0.572	0.484	0.528
Mean	0.402	0.329		0.567	0.492	
LSD 0.05 (Periods)			0.046			0.042
LSD 0.05 (Locations)			0.109			0.110
L.S.D 0.05 (Periods*Locations)			0.088			0.090

On the other hand, the lowest lead content plant parts in the autumn, with mean lead content in leaves (3.759 and 3.639 ppm), in stem (6.257 and 6.595 ppm) and in root (0.329 and 0.492 ppm) in the first and second seasons, respectively.

Regarding to the interaction between the effects of different locations and different periods the results recorded in the two seasons showed that the highest values were obtained in the plants planted in El-Dekhela in the spring period, with mean highest lead content in leaves (15.976 and 16.889 ppm), in stem (8.771 and 9.076 ppm) and in root (0.501 and 0.664 ppm) in the first and second seasons, respectively.

Cadmium (ppm)

Data presented in Table (7) also showed the effect of different locations on cadmium content in plant parts. In both seasons, planted plants in El-Dekhela had the highest cadmium content in leaves (0.559 and 0.548 ppm), in stem (0.527 and 0.736 ppm) and in root (0.412 and 0.509 ppm) in the first and second seasons, respectively. While, planted plants in Antoniadis Park had the lowest cadmium content in leaves (0.249 and 0.316 ppm), in stem (0.207 and 0.348 ppm) and in root (0.183 and 0.276 ppm) in the first and second seasons, respectively.

Cadmium content in plant parts was also significantly affected by the different periods during both seasons. Accordingly, it can be seen from the data in Table (7) that cadmium contents were significantly increased in the spring, with mean contents in leaves (0.428 and 0.479 ppm), in stem (0.408 and 0.606 ppm) and in root (0.327 and 0.424 ppm) in the first and second seasons, respectively.

Table 7. Means of cadmium content in leaves, stem and root (ppm) of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Cadmium content in leaves (ppm)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	0.290	0.209	0.249	0.362	0.271	0.316
El-Dekhala	0.610	0.508	0.559	0.594	0.503	0.548
El-Ebrahimia	0.386	0.305	0.345	0.481	0.390	0.435
Mean	0.428	0.340		0.479	0.388	
LSD 0.05 (Periods)			0.014			0.082
LSD 0.05 (Locations)			0.175			0.092
L.S.D 0.05 (Periods*Locations)			0.142			0.075
Location	**Cadmium content in stem (ppm)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	0.222	0.192	0.207	0.396	0.301	0.348
El-Dekhala	0.574	0.480	0.527	0.783	0.690	0.736
El-Ebrahimia	0.430	0.336	0.383	0.639	0.545	0.592
Mean	0.408	0.336		0.606	0.512	
LSD 0.05 (Periods)			0.045			0.003
LSD 0.05 (Locations)			0.317			0.308
L.S.D 0.05 (Periods*Locations)			0.258			0.251
Location	**Cadmium content in root (ppm)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	0.219	0.148	0.183	0.316	0.237	0.276
El-Dekhala	0.456	0.369	0.412	0.553	0.466	0.509
El-Ebrahimia	0.308	0.223	0.265	0.405	0.320	0.362
Mean	0.327	0.246		0.424	0.341	
LSD 0.05 (Periods)			0.009			0.008
LSD 0.05 (Locations)			0.205			0.200
L.S.D 0.05 (Periods*Locations)			0.166			0.162

On the other hand, the lowest cadmium contents parts plant in the autumn, with mean cadmium content in leaves (0.340 and 0.388 ppm), in stem (0.336 and 0.512 ppm) and in root (0.246 and 0.341 ppm) in the first and second seasons, respectively.

Regarding to the interaction between the effects of different locations and different periods on the cadmium content in plant parts of *Nerium oleander* plants, the results recorded in the two seasons showed that the highest values were obtained in the plants in El-Dekhela and the spring period, with mean cadmium content in leaves (0.610 and 0.594 ppm), in stem (0.574 and 0.783 ppm) and in root (0.456 and 0.553 ppm) in the first and second seasons, respectively.

Zinc (ppm)

Data presented in Table (8) showed the effect of different locations on zinc content in plant parts. In both seasons, planted plants in El-Dekhela had the highest zinc content in leaves (3.758 and 5.261 ppm), in stem (2.597 and 3.548 ppm) and in root (0.501 and 0.607 ppm) in the first and second seasons, respectively. While, planted plants in Antoniadis Park had the lowest zinc content in leaves (1.436 and 2.964 ppm), in stem (1.396 and 2.349 ppm) and in root (0.126 and 0.256 ppm) in the first and second seasons, respectively.

Zinc content in plant parts were also significantly affected by the different periods during both seasons. Accordingly, it can be seen from the data in Table (8) that zinc contents were significantly increased in the spring, with mean zinc content in leaves of (3.170 and 4.707 ppm), in stem (1.977 and 2.930 ppm) and in root (0.380 and 0.469 ppm) in the first and second seasons, respectively.

Table 8. Means of zinc content in leaves, stem and root (ppm) of *Nerium oleander* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Zinc content in leaves (ppm)					
	2015			2016		
	Spring	Autumn	Mean	Spring	Autumn	Mean
Antoniadis Park	1.893	0.979	1.436	3.496	2.432	2.964
El-Dekhala	4.290	3.227	3.758	5.793	4.730	5.261
El-Ebrahimia	3.329	2.277	2.803	4.833	3.781	4.307
Mean	3.170	2.161		4.707	3.647	
LSD 0.05 (Periods)			0.017			0.019
LSD 0.05 (Locations)			1.141			1.140
L.S.D 0.05 (Periods*Locations)			0.929			0.928
Location	**Zinc content in stem (ppm)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	1.444	1.349	1.396	2.398	2.300	2.349
El-Dekhala	2.644	2.551	2.597	3.595	3.502	3.548
El-Ebrahimia	1.845	1.752	1.798	2.797	2.703	2.750
Mean	1.977	1.884		2.930	2.835	
LSD 0.05 (Periods)			0.003			0.001
LSD 0.05 (Locations)			0.553			0.552
L.S.D 0.05 (Periods*Locations)			0.450			0.449
Location	**Zinc content in root (ppm)**					
	2015			**2016**		
	Spring	**Autumn**	**Mean**	**Spring**	**Autumn**	**Mean**
Antoniadis Park	0.162	0.091	0.126	0.217	0.296	0.256
El-Dekhala	0.533	0.470	0.501	0.640	0.575	0.607
El-Ebrahimia	0.446	0.383	0.414	0.552	0.491	0.521
Mean	0.380	0.314		0.469	0.454	
LSD 0.05 (Periods)			0.056			0.054
LSD 0.05 (Locations)			0.114			0.113
L.S.D 0.05 (Periods*Locations)			0.092			0.092

On the other hand, the lowest zinc contents were presented in plant parts in the autumn, with mean zinc content in leaves (2.161 and 3.647 ppm), in stem (1.884 and 2.835 ppm) and in root (0.314 and 0.454 ppm) in the first and second seasons, respectively.

Regarding the interaction between the effects of different locations and different periods on the zinc content in plant parts the results recorded in the two seasons showed that the highest values were obtained in the plants planted in El-Dekhela during the spring period, with mean zinc content in leaves (4.290 and 5.793 ppm), in stem (2.644 and 3.595 ppm) and in root (0.533 and 0.640 ppm) in the first and second seasons, respectively.

Heavy metals content in soil (ppm)

The results of soil chemical analysis presented in Table (9) showed the effect of different locations on heavy metals content in soil. In the first season, soil in El-Dekhela had the highest lead content (31.035 ppm), cadmium content (0.235 ppm) and zinc content (8.609 ppm) in the first season (2015). While, soil in Antoniadis Park had the lowest lead content (2.783 ppm), cadmium content (0.012 ppm) and zinc content (3.299 ppm) in the first season (2015).

Chemical analysis of heavy metals content in soil was also significantly affected by the different periods during first season (2015). Accordingly, it can be seen from the data in Table (9) that heavy metals were significantly increased in the spring, with mean lead content of (15.795 ppm), cadmium content of (0.125 ppm), and zinc content in the autumn (6.148 ppm) in the first season (2015). On the other hand, the lowest heavy metals content was detected in the autumn, with mean lead content of (12.362 ppm) and cadmium content (0.120 ppm), with mean zinc content in the spring (5.093 ppm) in the first season.

Regarding the interaction between the effects of different locations and different periods on the heavy metals content in soil, the results recorded in the first season showed that, the highest values were obtained in El-Dekhela at the spring period, with mean lead content of (35.850 ppm) and cadmium content of (0.239 ppm),

Table 9. Means of lead, Cadmium and Zinc content in soil (ppm) as influenced by locations, periods and their combinations (locations × periods) in the first season (2015)

locations	Lead content in soil (ppm)		
	Spring	**Autumn**	**Mean**
Antoniadis Park	2.780	2.786	2.783
El-Dekhala	35.850	26.220	31.035
El-Ebrahimia	8.756	8.080	8.418
Mean	15.795	12.362	
LSD 0.05 (Periods)			20.660
LSD 0.05 (Locations)			7.583
L.S.D 0.05 (Periods*Locations)			6.175
Location	**Cadmium content in soil (ppm)**		
	Spring	**Autumn**	**Mean**
Antoniadis Park	0.014	0.011	0.012
El-Dekhala	0.239	0.231	0.235
El-Ebrahimia	0.123	0.118	0.120
Mean	0.125	0.120	
LSD 0.05 (Periods)			0.034
LSD 0.05 (Locations)			0.014
L.S.D 0.05 (Periods*Locations)			0.011
Location	**Zinc content in soil (ppm)**		
	Spring	**Autumn**	**Mean**
Antoniadis Park	2.824	3.775	3.299
El-Dekhala	8.028	9.191	8.609
El-Ebrahimia	4.429	5.478	4.953
Mean	5.093	6.148	
LSD 0.05 (Periods)			11.305
LSD 0.05 (Locations)			3.522
L.S.D 0.05 (Periods*Locations)			2.868

while the highest zinc content was obtained in El-Dekhela at the autumn period (9.191 ppm) in the first season (2015).

Transfer factor of heavy metals (Lead, Cadmium and Zinc)

Transfer factor (TF) indicates the efficiency of *Nerium oleander* plants to transfer metals from its soil to the plant parts.

From the data presented in Table (10), it can be seen that the transfer factor for the lead content was increased steadily with different locations. We found that, plants of Antoniadis Park had the highest transfer factor with respect to the lead content (0.772). On the other hand, plants in El-Dekhela had the lowest transfer factor for the lead content (0.215) in the first season, while, the highest transfer factor of the lead content was detected in stem compared with different of parts plant.

From the data presented in Table (10), it can be seen that the transfer factor of the cadmium content in *Nerium oleander* plants was increased steadily with different locations. We found that, plants of Antoniadis Park had the highest transfer factor in the cadmium content with mean (17.750). On the other hand, plants of El-Dekhela had the lowest transfer factor with respect to the cadmium content (2.124) in the first season, while, the highest transfer factor in the cadmium content in leaves compared with different parts of plant.

From the data presented in Table (10), it can be seen that the transfer factor in the zinc content in *Nerium oleander* plants was increased steadily with different locations. We found that, planted plants in El-Ebrahimia had the highest transfer factor in the zinc content with mean (0.337). On the other hand, plants of El-Dekhela had the lowest transfer factor in the zinc content (0.265) in the first season, While, the highest transfer factor in the zinc content in leaves compared with different parts of plant.

DISCUSSION

Environment pollution with toxic metals has increased dramatically since the increasing of the industrial revolution (Zaidi *et al*., 2005). Pollution by heavy metals such as cobalt, cadmium, lead, zinc and chromium etc is a problem of concern (Onder *et al*., 2007). Zn is one of the main elements in many biochemical pathways such as auxin, chlorophyll, carbohydrate and protein synthesis (Broadley *et al*., 2007; McIntosh, 2010). Onder and Dursun (2006) reported that the highest Zn concentrations were detected in cedar needles at an industrial site. Toxic concentrations of Zn for plants range from 300 to 400 mg kg^{-1} depending on plant species (Broadley *et al*., 2007). The low content of heavy metal pollutants in spring and autumn may be closely related to the physiological activities of the trees. In April and May, with the growth of the leaves, cadmium absorbed quickly spreads within the tree body and blades, which results in the lower concentration of heavy metal content.

Table 10. Means values of transfer factor (TF) to leaves, stem and roots of *Nerium oleander* plants as influenced by locations and periods in the first season (2015)

locations	Transfer factor of lead			
	Leaves	**Stem**	**Root**	**Mean**
Antoniadis Park	1.033	1.186	0.098	0.772
El-Dekhala	0.352	0.281	0.014	0.215
El-Ebrahimia	0.705	0.823	0.043	0.523
Mean	0.696	0.763	0.051	
Location	**Transfer factor of cadmium**			
	Leaves	**Stem**	**Root**	**Mean**
Antoniadis Park	20.750	17.250	15.250	17.750
El-Dekhala	2.378	2.242	1.753	2.124
El-Ebrahimia	2.875	3.191	2.208	2.758
Mean	8.667	7.561	6.403	
Location	**Transfer factor of zinc**			
	Leaves	**Stem**	**Root**	**Mean**
Antoniadis Park	0.435	0.423	0.038	0.298
El-Dekhala	0.436	0.301	0.058	0.265
El-Ebrahimia	0.565	0.363	0.083	0.337
Mean	0.478	0.362	0.059	

While, in autumn, with the growth of the leaves, the volume growth of leaves slows down; therefore, the cadmium content in leave slowly accumulates to the highest levels. Therefore, the cadmium content is higher in autumn than that in spring (Liu *et al.*, 2015).

In general effect on growth properities (Maria and Tadeusz, 2005) suggested that the inhibitory action of heavy metals on root length, shoot height, shoot diameter, leaf number and leaf area seems principally to be due to chromosomal aberrations and abnormal cell divisions and may also be correlated with the metal-induced inhibition of photosynthetic process and the respiration in the shoot system and protein synthesis in the root, or due to the reduction in cell proliferation and growth. According to Jothinayagi *et al*., (2009) excess amount of heavy metal was toxic for No. of leaves and the less amount of heavy metal was not affected the leaf area but excess amount is harmful for leaves. According to Jadia and Fulekar (2008) suggested that increases the concentration of heavy metal with decreases the shoot and root dry weight, the decrease in biomass in excess heavy metal might be due to low protein formation, resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Manivasagaperumal *et al*., 2011). The plants exposed to metals via root uptake were able to survive in all cases. These results are in agreement with the extremely high ability of resistance against the toxicity of metals reported by (Franco *et al.*, 2012 and 2013) for this plant under field conditions. Preferential accumulation of metals in leaf tissues was only reported in *Nerium oleander* plants grown under extremely high levels of heavy metals (Franco *et al.*, 2012 and 2013). Heavy metals phytoxicity studies reported symptoms of toxicity such as stunted growth, a decrease in pigment content (Bibi and Hussain, 2005). Heavy metals are essential and important for normal growth and development of plants being an essential component of many enzymes and proteins. Plants vary in their ability to absorb and accumulate minerals from the soil solution. Gülser and Erdogan (2008) found that low soil content of heavy metals, lead to a significant increase in the activity of enzymes.

The results of this study showed the phytotoxicity of the three heavy metals (Pb, Cd and Zn) which is apparent from the reduction of chlorophyll content in the leaves of *Nerium oleander*. The studies of photosynthesis can illustrate the direct and indirect effects of Pb. One of the direct effects includes the inhibition of chlorophyll synthesis by heavy metals often manifests as chlorosis. So, the change in structure of chlorophyll indicated that absorption of high levels of Pb affected the uptake of plant nutrients such as Mg (Haider *et al*. 2006). Assessment of leaf chlorophyll content provides information on plant physiological status and might be a valuable tool for agricultural and ecosystem studies, since it is closely linked to nitrogen content and, hence, to photosynthesis (Serrano, 2008). Doganlar and Atmaca, (2011) reported that the lowest chlorophyll and carotenoid contents were found in industrial and urban street areas, respectively. Similarly, chlorophyll content of leaves in urban plants decreased 15–66% as a result of Pb, Zn and Cd pollution. In our study, we generally detected decreasing pigment contents in parallel with increasing pollution levels. Decreased pigment content was most evident in *N. oleander* depending on metal pollution (Baycu *et al.*, 2006).

Total carbohydrates content is an important constituent manufactured during photosynthesis and breakdown during respiration by plants, early studies by Saleh and Al-Garni (2006) indicated that carbohydrates got inhibited if lead and cadmium concentration is more than 5 mg/kg soil. The decrease in total carbohydrates content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. In spring, fluctuations of soluble sugars were significantly different among sites, during the main growth period. Taking into consideration that, *Nerium oleander* shrubs received the same treatment (watering) in the research sites, this difference in fluctuation may be due either to different microclimatic conditions, which does not arise from meteorological data or to different air quality. The elevated values of soluble sugars detected during summer in other Mediterranean shrubs (Palacio *et al.*, 2007) were not distinguished in leaves of oleander, which is in accordance with results from other Mediterranean evergreen sclerophyll species. Starch decreased in spring *(i.e.* during the main growth period), it did not show considerable changes during the prolonged dry season and it was accumulated from November up to March.

The transfer factor (TF), defined as the ratio of root metal concentration in relation to that in leaves. The last case indicates a low transfer mobility of Zn from roots to leaves. On the other hand, the TF values higher than 1 for Pb indicate higher accumulation in the roots. This suggests that *Nerium oleander* is useful in removing Pb from soil. According to the transfer factor (TF) values the translocation of Pb and Cd from soil to plant leaves in all sampling sites was low and agreement with the low bioavailability of metals in soils. Likewise, a high bioavailability of Cd has also been reported in soils (Kabata-Pendias, 2004). However, it is worth to mention that the most bioavailable metals, Cd (84%) are also those whose substrate concentrations were higher and closer to the reference levels reported in soils (BOA 2008).

Soil contamination by heavy metals is increasing nowadays (Lin and Lin, 2005) as there is a strong correlation between heavy metal concentration in soil and degree of urbanization, this clearly indicates that the origin of metal contamination in the investigated area is related to vehicular traffic (Seshan *et al*., 2010 and Kadi, 2009). Recently, Celika *et al*., (2005) found concentrations of elements at high levels in industrial areas in the order of Zn > Pb > Cd, the significant difference in elemental concentrations of soils between urban, industrial residential, rural, and control areas give some confidence that industrial activities and traffic are major sources of pollution in urban areas, this is in general agreement with similar results (Abou El-Saadat *et al*., 2011). The concentrations of lead was been higher in soil samples collected near roads in urban and industrial areas in the present study than those from rural ones confirm the suggestion that Pb is widespread in urban road dust (Wei and Yang, 2010 and Viard *et al*., 2004). Cadmium is yet not known to have any biological function on the contrary, is said to be highly toxic to plants and animals (Tahar and Keltoum, 2011), compared with the other metals cadmium is more mobile in soil in relation to both leaching and availability to plants(CEC, 2001). Zinc is an essential element in all living organisms and plays a vital role in the biosynthesis of proteins (hormones and enzymes). It was found to be the fourth highest levels in all samples after Fe, Al, and Na in the present investigation. These high levels of zinc would reduce productivity (Bucher and Schenk, 2000 and Celika *et al*., 2005). It usually occurs in low concentrations and does not pose a toxicity problem for plants (Paschke *et al*., 2000), but increased concentrations of zinc in soil can lead to toxic effects in plants.

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الملخص العربي

المعالجة الحيوية للمعادن الثقيلة باستخدام بعض الشجيرات في ثلاثة مواقع مختلفة من مدينة الإسكندرية (ب) نباتات الدفلة

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أجريت هذه الدراسة في ثلاثة مواقع مختلفة في مدينة الإسكندرية [منطقة الابراهيمية (منطقة المرور)، منطقة الدخيلة (المنطقة الصناعية) وحديقة انطونيادس (منطقة الكنترول)] خلال الموسمين المتعاقبين ٢٠١٥ و ٢٠١٦. تم زراعة شتلات من الدفلة المتجانسة بشكل فردي في أصص بلاستيكية (قطرها ٣٠ سم) مليئة بخليط من الرمل والطين. تم زراعة ٩٠ نبات في ثلاثة مواقع في مدينة الإسكندرية (٣٠ نبات لكل منطقة). تم جمع العينات خلال فصلي الربيع والخريف في الموسمين على حد سواء.

أظهرت النتائج التي تم الحصول عليها أن هناك تأثير بين المواقع مختلفة على النمو الخضري لنباتات الدفلة. في كلا الموسمين، كانت النباتات المزروعة في حديقة أنطونيادس (منطقة الكنترول) أعطت أعلى معدل نمو من الأوراق، الساق والجذور في الموسمين الأول والثاني، على التوالي. في حين أن النباتات المزروعة في منطقة الدخيلة (منطقة صناعية) سجلت أدنى معدل للنمو الخضري في كلا الموسمين. كما تأثر معدل النمو بشكل كبير بالفترات المختلفة خلال الموسمين. بالنظر إلى البيانات كانت هناك زيادة كبيرة في فصل الخريف، في حين، أدنى معدل للنمو كان في فصل الربيع.

كما أظهرت نتائج التحليل الكيميائي لأجزاء النبات أن تأثير المواقع المختلفة على الرصاص والكادميوم والزنك في أجزاء النبات كانت واضحة فى موسمى الدراسة، حيث كانت النباتات المزروعة في الدخيلة تحتوي على أعلى نسبة من العناصر الثقيلة، في حين أن النباتات المزروعة في حديقة أنطونيادس كانت أقل محتوى من الرصاص والكادميوم والزنك في الموسمين الأول والثاني على التوالي. كما تأثر التحليل الكيميائي لمحتوى العناصر الثقيلة في أجزاء النبات بشكل كبير بالفترات المختلفة خلال الموسمين. وبناء على ذلك، يمكن أن نرى من البيانات أن العناصر الثقيلة قد زادت بشكل ملحوظ تدريجيا في فصل الربيع، في حين أن أدنى محتوى لأجزاء النبات من الرصاص والكادميوم والزنك كان في فصل الخريف في الموسمين الأول والثاني، على التوالي.

عامل النقل (TF) يشير إلى كفاءة نباتات الدفله لنقل العناصر الثقيلة من التربة إلى أجزاء النبات. يمكن ملاحظة أن معامل التحويل في محتوى الرصاص والكادميوم والزنك في نباتات الدفلة زاد بشكل مطرد مع المواقع المختلفة تحت الدراسة. ووجدنا أن النباتات المزروعة في حديقة أنطونيادس أعطت أعلى عامل للنقل في محتوى العناصر الثقيلة، في حين أن النباتات المزروعة في منطقة الدخيلة كانت أقل عامل للنقل في محتوى العناصر الثقيلة في الموسم الأول. من ناحية أخرى، كان أعلى عامل للنقل في أجزاء النبات، وكان في الساق للرصاص والكادميوم بينما كان في الأوراق للزنك مقارنة مع أجزاء مختلفة من النبات.