Bioremediation of Heavy Metals by Using Some Shrubs in Three Different Locations of Alexandria City (A) *Pittosporum tobira* 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 *Pittsporum tobira* 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 that the data show the effect of different locations on vegetative growth on *Pittsporum tobira* 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 *Pittsporum tobira* plants to transfer metals from its soil to the plant parts. It can be seen that the transfer factor in the lead, cadmium and zinc content in *Pittsporum tobira* 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, *Pittsporum tobira*, Lead, Cadmium, Zinc

INTRODUCTION

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. 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).

Lead is widely distributed naturally, but the greatest risks normally arise from emissions to the environment associated with human use of the metal and its derivatives. Fumes and dust come from smelting of lead, manufacture of insecticides, paint, powdery glazes and storage batteries, and from gasoline containing lead additives. Sewage sludge may contain very high levels of lead and its use as a fertilizer may contaminate soils. High levels may occur in urban air as a result of the high traffic density and associated emission of lead from gasoline additives (Harrison and Laxen, 1981). 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). Seed germination was gradually delayed in the presence of increasing concentration of lead (Pb), it may be due to prolong incubation of the seeds that must have resulted in the neutralization of the toxic effects of lead by some mechanisms e. g. leaching, chelating, metal binding or/and accumulation by microorganisms (Ashraf and Ali, 2007).

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

Major uses of zinc are in the production of noncorrosive alloys, brass and in galvanizing steel and iron products. Zinc undergoes oxidation as surface coating, thus protecting the underlying metal from degradation. Zinc oxides are used in rubber and as a white pigment. Zinc is utilized therapeutically in human medicine in the treatment of zinc deficiency. The carbonates, which are organic zinc compounds, are used as pesticides (Friberg *et al*., 2002). 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).

A widespread evergreen ornamental shrub *Pittosporum tobira* (Thunb.), was selected as test plant, as it has been demonstrated that, as a result of their larger leaf area and the turbulent air movements created by their structure, trees and shrubs intercept more air pollutants, including particles, than shorter vegetation (Fowler *et al*., 1989; Beckett *et al*., 2000). The leaves are oval in shape with edges that cruel under and measure up to 10 cm in length. They are leathery, hairless, darker and shinier on the upper surfaces. 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, indoor and 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 *Pittsporum tobira* plants on reducing the air pollution content with heavy metal (Lead, Cadmium and Zinc) in three locations in Alexandria city (El-Ebrahimeya, El-Dekhela and Antoniadis Park).

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 *Pittsporum tobira* (50-60 cm height and 20-25 leaves per plant on average) 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 plants were planted in three locations in Alexandria city (30 plants each zone). Samples were collected during spring and autumn in both seasons the plants were harvested.

Table (1). Chemical analyses of the used mixture soil for the first successive 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

Data recorded :

(1) Vegetative growth parameters:

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

(2) Chemical analysis determination:

- Total chlorophyll content were determined as a SPAD unites from the fresh leaves of plants for the different treatments under the experiment at the end of the season using Minolta (chlorophyll meter) SPAD 502 according to Yadava (1986).
- Total carbohydrates percentage in the leaves was determined according to Dubios *et al.*(1956).
- Determination of heavy metals content (lead, cadmium and zinc). Plant samples 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 Averages 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:

1. Leaves parameters:

The data presented in Table (2) show the effect of different locations on the number of leaves per plant, leaves dry weight and leaves area formed on *Pittsporum tobira* plants. In both seasons, plants of Antoniadis park had the highest number of leaves (11.82 and 23.45 leaves per plant), leaves dry weight (2.54 and 2.68 g) and leaves area (24.80 and 53.56 cm^2) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest number of leaves (8.77 and 19.80 leaves per plant), leaves dry weight (1.78 and 1.91 g) and leaves area (12.89 and 32.91 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 *Pittsporum tobira* plants were significantly increased gradually in the autumn, with Average number of leaves of (10.80 and 22.98 leaves per plant), leaves dry weight (2.34 and 2.68 g) and leaves area (20.35 and 49.58 cm^2) in the first and second seasons, respectively. On the other hand, the lowest in the spring, with Average number of leaves (10.06 and 20.98 leaves per plant), leaves dry weight (2.18 and 2.16 g) and leaves area (18.73 and 40.96 cm^2) in the first and second seasons, respectively.

Regarding the interaction between the effects of different locations and different periods on growth rate of the number of leaves per plant, leaves dry weight and leaves area of *Pittsporum tobira* plants, the results recorded in the two seasons show that, the highest values were obtained for the plants of Antoniadis park and the autumn period, with Average number of leaves (12.10 and 24.45 leaves per plant), leaves dry weight (2.69 and 2.94 g) and leaves area (26.26 and 58.24 cm^2) in the first and second seasons, respectively.

2. Stem parameters:

Data presented in Table (3) show the effect of different locations on plant height, stem diameter and stem dry weight formed on *Pittsporum tobira* plants. In both seasons, plants of Antoniadis park had the highest plant height (17.96 and 20.69 cm), stem diameter (0.86 and 0.92 cm) and stem dry weight (1.48 and 1.94 g) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest plant height (12.60 and 16.48 cm), stem diameter (0.58 and 0.64 cm) and stem dry weight (1.01 and 1.47 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 *Pittsporum tobira* plants were significantly increased gradually in the autumn, with Average plant height of 15.18 and 21.89 (cm), stem diameter 0.75 and 0.80 (cm) and stem dry weight 1.33 and 2.19 (g) in the first and second seasons, respectively. In the other hand, the lowest in the spring, with Average plant height of 14.18 and 15.28 (cm), stem diameter 0.72 and 0.75 (cm) and stem dry weight 1.23 and 1.26 (g) in the first and second seasons, respectively.

Regarding the interaction between the effects of different locations and different periods on growth rate of the plant height, stem diameter and stem dry weight of *Pittsporum tobira* plants, the results recorded in the two seasons show that, the highest values were obtained for the plants of Antoniadis park and the autumn period, with Average plant height (18.15 in the first season and 23.65 cm) in El-Ebrahimia and autumn period in the second season, stem diameter (0.88 and 0.92 g) and stem dry weight (1.53 and 2.41 g) in the first and second seasons, respectively.

Table (2). Averages of vegetative growth characteristics (leaves) of *Pittsporum tobira* 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						Leaves Dry Weight per plant (g)						Leaves Area (cm^2)					
	2015			2016			2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	11.55	12.10	11.82	22.45	24.45	23.45	2.50	2.69	2.59	2.42	2.93	2.67	23.35	26.26	24.80	48.88	58.24	53.56
El-Dekhala	8.44	9.10	8.77	18.80	20.80	19.80	1.72	1.85	1.78	1.65	2.17	1.91	13.68	12.11	12.89	29.24	36.59	32.91
El-Ebrahimia	10.21	11.21	10.71	21.71	23.71	22.71	2.32	2.49	2.40	2.41	2.94	2.67	19.18	22.68	20.93	44.76	53.91	49.33
Average	10.06	10.80		20.98	22.98		2.18	2.34		2.16	2.68		18.73	20.35		40.96	49.58	
LSD 0.05 (Periods)			0.525			1.520			0.087			0.154			2.088			6.557
LSD0.05(Locations)			1.369			1.193			0.203			0.118			4.230			2.753
L.S.D 0.05 (Periods*Locations)			1.935			1.688			0.287			0.475			5.982			1.688

Table (3). Averages of vegetative growth characteristics (stem) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Plant Height (cm)						Stem Diameter (cm)						Stem Dry Weight (g)					
	2015			2016			2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	17.07	18.15	17.61	17.96	23.42	20.69	0.84	0.88	0.86	0.87	0.92	0.89	1.42	1.53	1.48	1.48	2.41	1.94
El-Dekhala	11.29	12.21	11.75	12.60	20.36	16.48	0.57	0.60	0.58	0.60	0.64	0.62	0.97	1.05	1.01	1.01	1.94	1.47
El-Ebrahimia	15.52	16.69	16.10	16.47	23.65	20.06	0.76	0.79	0.77	0.79	0.85	0.82	1.32	1.42	1.37	1.30	2.23	1.76
Average	14.18	15.18		15.28	21.89		0.72	0.75		0.75	0.80		1.23	1.33		1.26	2.19	
LSD 0.05 (Periods)			0.645			0.500			0.136			0.142			0.050			0.030
LSD0.05(Locations)			1.389			1.492			0.109			0.115			0.118			0.070
L.S.D 0.05 (Periods*Locations)			1.964			2.111			0.490			0.011			0.167			0.099

3. Root parameters:

Data presented in Table (4) show the effect of different locations on root length and root dry weight formed on *Pittsporum tobira* plants. In both seasons, plants of Antoniadis Park had the highest root length (28.24 and 28.52 cm) and root dry weight (3.45 and 3.28 g) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest root length (17.38 and 18.88 cm) and root dry weight (2.20 and 2.11 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 Average root length of (23.89 and 25.09 cm) and root dry weight (2.92 and 2.79 g) in the first and second seasons, respectively. In the other hand, the lowest in the spring, with Average root length (22.26 and 23.03 cm) and root dry weight (2.71 and 2.70 g) in the first and second seasons, respectively.

Regarding the interaction between the effects of different locations and different periods on growth rate of the root length and root dry weight of *Pittsporum tobira* plants, the results recorded in the two seasons show that, the highest values were obtained for the plants of Antoniadis park and the autumn period, with Average heights root length of (29.16 and 29.55 cm) and root dry weight of (3.58 and 3.32 g) in the first and second seasons, respectively.

Table (4). Averages of vegetative growth characteristics (root) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Root Length (cm)						Root Dry Weight (g)					
	2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	27.31	29.16	28.24	27.49	29.55	28.52	3.32	3.58	3.45	3.24	3.32	3.28
El-Dekhala	16.77	17.99	17.38	17.85	19.91	18.88	2.04	2.20	2.12	2.06	2.16	2.11
El-Ebrahimia	22.71	24.53	23.62	23.75	25.81	24.78	2.77	2.99	2.88	2.81	2.89	2.85
Average	22.26	23.89		23.03	25.09		2.71	2.92		2.70	2.79	
LSD 0.05 (Periods)			0.475			1.382			0.071			0.205
LSD 0.05 (Locations)			1.278			0.454			0.159			0.058
L.S.D 0.05 (Periods*Locations)			1.807			0.642			0.225			0.083

2. Chemical constituents analysis

1. Total chlorophyll (SPAD) and total carbohydrates content (%)

The results presented in Table (5) show the effect of different locations on total chlorophyll and total carbohydrates content formed on *Pittsporum tobira* plants. In both seasons, plants of Antoniadis Park had the highest total chlorophyll content 67.37 and 55.73 (SPAD) and total carbohydrates content 6.71 and 4.68 (%) in the first and second seasons, respectively. While, plants of El-Dekhela had the lowest total chlorophyll content 46.40 and 47.58 (SPAD) and total carbohydrates content 5.34 and 3.59 (%) in the first and second seasons, respectively.

Total chlorophyll and total 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 the data recorded for *Pittsporum tobira* plants were significantly increased gradually in the autumn, with Average total chlorophyll of (64.79 and 53.57 SPAD), but in the spring, the highest total carbohydrates content with Average (7.00 and 4.92 %) in the first and second seasons, respectively. In the other hand, the lowest total chlorophyll content on the spring, with Average (50.78 and 49.14 SPAD) and the lowest total carbohydrates content in the autumn, with Average (5.03 and 3.25 %) in the first and second seasons, respectively.

Regarding the interaction between the effects of different locations and different periods on growth rate of the total chlorophyll and total carbohydrates content of *Pittsporum tobira* plants, the results recorded in the two seasons show that, the highest values were obtained in the planted plants in Antoniadis park and the autumn period, with Average heights total chlorophyll content (74.54 and 56.73 SPAD) and the planted plants in Antoniadis park and the spring period, with Average highest total carbohydrates content of (7.96 and 5.34 %) in the first and second seasons, respectively.

Table (5). Averages of Chemical constituents (Total chlorophyll content (SPAD) and Carbohydrates content (%) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Total Chlorophyll Content (SPAD)						Total Carbohydrates Content of leaves (%)					
	2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	60.20	74.54	67.37	54.73	56.73	55.73	7.96	5.46	6.71	5.34	4.02	4.68
El-Dekhala	38.02	54.78	46.40	43.43	51.73	47.58	6.00	4.68	5.34	4.57	2.62	3.59
El-Ebrahimia	54.13	65.07	59.60	49.26	52.26	50.76	7.05	4.96	6.00	4.85	3.12	3.98
Average	50.78	64.79		49.14	53.57		7.00	5.03		4.92	3.25	
LSD 0.05 (Periods)			1.313			4.755			0.337			1.107
LSD0.05(Locations)			1.571			5.925			0.022			0.659
L.S.D0.05 (Periods*Locations)			2.222			8.380			0.013			0.933

2. Heavy metals analysis

1. Lead content in plants (mg/l):

The results of plant parts chemical analysis Table (6) also show that the effect of different locations on lead content in plant parts formed on *Pittsporum tobira* plants. In both seasons, planted plants in El-Dekhela had the highest lead content in leaves (9.28 and 9.19 mg/l), lead content in stem (10.31 and 10.62 mg/l) and lead content in root (0.47 and 0.63 mg/l) in the first and second seasons, respectively. While, planted plants in Antoniadis Park had the lowest lead content in leaves (2.58 and 2.50 mg/l), lead content in stem (2.48 and 2.78 mg/l) and lead content in root (0.37 and 0.53 mg/l) in the first and second seasons, respectively.

Chemical analysis of 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 the data recorded for *Pittsporum tobira* plants were significantly increased gradually in the spring, with Average lead content in leaves (8.81 and 8.73 mg/l), lead content in stem (6.18 and 6.48 mg/l) and lead content in root (0.45 and 0.61 mg/l) in the first and second seasons, respectively. On the other hand, the lowest lead content plant parts in the autumn, with Average lead content in leaves (3.62 and 3.53 mg/l), lead content in stem (6.09 and 6.39 mg/l) and lead content in root (0.37 and 0.53 mg/l) in the first and second seasons, respectively. Regarding to the interaction between the effects of different locations and different periods on the lead content in plant parts of *Pittsporum tobira* plants, the results recorded in the two seasons show that the highest values were obtained in the planted plants in El-Dekhela and the spring period, with Average highest lead content in leaves (12.70 and 12.61 mg/l), lead content in stem (10.36 and 10.66 mg/l) and lead content in root (0.51 and 0.67 mg/l) in the first and second seasons, respectively.

2. Cadmium content in plants (mg/l):

The results of plant parts chemical analysis presented in Table (7) also show the effect of different locations on cadmium content in plant parts formed on *Pittsporum tobira* plants. In both seasons, planted plants in El-Dekhela had the highest cadmium content in leaves (0.59 and 0.69 mg/l), cadmium content in stem (0.50 and 0.71 mg/l) and cadmium content in root (0.32 and 0.42 mg/l) in the first and second seasons, respectively. While, planted plants in Antoniadis Park had the lowest cadmium content in leaves (0.16 and 0.26 mg/l), cadmium content in stem (0.22 and 0.43 mg/l) and cadmium content in root (0.06 and 0.16 mg/l) in the first and second seasons, respectively. Chemical analysis of 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 the data recorded for *Pittsporum tobira* plants were significantly increased gradually in the spring, with Average cadmium content in leaves (0.47 and 0.57 mg/l), cadmium content in stem (0.42 and 0.63 mg/l) and cadmium content in root (0.23 and 0.33 mg/l) in the first and second seasons, respectively. In the other hand, the lowest cadmium content plant parts in the autumn, with Average cadmium content in leaves (0.38 and 0.48 mg/l), cadmium content in stem (0.33 and 0.54 mg/l) and cadmium content in root (0.15 and 0.25 mg/l) 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 *Pittsporum tobira* plants, the results recorded in the two seasons show that the highest values were obtained in the planted plants in El-Dekhela and the spring period, with Average heights cadmium content in leaves (0.64 and 0.73 mg/l), cadmium content in stem (0.55 and 0.76 mg/l) and cadmium content in root (0.37 and 0.46 mg/l) in the first and second seasons, respectively.

Table (6). Averages of Chemical constituents (Lead content in leaves, stem and root (mg/l)) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Lead Content in leaves (mg/l)						Lead Content in stem (mg/l)						Lead Content in root (mg/l)					
	2015			2016			2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	3.68	1.49	2.58	3.59	1.40	2.50	2.52	2.43	2.48	2.83	2.74	2.78	0.41	0.33	0.37	0.57	0.49	0.53
El-Dekhala	12.70	5.86	9.28	12.61	5.77	9.19	10.36	10.27	10.31	10.66	10.58	10.62	0.51	0.43	0.47	0.67	0.59	0.63
El-Ebrahimia	10.07	3.51	6.79	9.98	3.43	6.70	5.65	5.56	5.61	5.96	5.87	5.91	0.43	0.35	0.39	0.60	0.51	0.55
Average	8.81	3.62		8.73	3.53		6.18	6.09		6.48	6.39		0.45	0.37		0.61	0.53	
LSD 0.05 (Periods)			3.078			3.079			2.32			2.32			0.13			0.13
LSD0.05(Locations)			2.324			2.324			2.02			2.01			0.21			0.21
L.S.D 0.05 (Periods*Locations)			3.287			3.287			2.85			2.85			0.29			0.29

Table (7). Averages of Chemical constituents (Cadmium content in leaves, stem and root (mg/l)) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Cadmium Content in leaves (mg/l)						Cadmium Content in stem (mg/l)						Cadmium Content in root (mg/l)					
	2015			2016			2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	0.21	0.12	0.16	0.30	0.22	0.26	0.27	0.18	0.22	0.48	0.39	0.43	0.10	0.03	0.06	0.19	0.12	0.16
El-Dekhala	0.64	0.55	0.59	0.73	0.64	0.69	0.55	0.46	0.50	0.76	0.66	0.71	0.37	0.28	0.32	0.46	0.38	0.42
El-Ebrahimia	0.57	0.48	0.53	0.67	0.58	0.62	0.45	0.36	0.41	0.66	0.57	0.62	0.24	0.15	0.19	0.33	0.25	0.29
Average	0.47	0.38		0.57	0.48		0.42	0.33		0.63	0.54		0.23	0.15		0.33	0.25	
LSD 0.05 (Periods)			0.640			0.640			0.491			0.491			0.012			0.011
LSD0.05(Locations)			0.115			0.115			0.126			0.125			0.087			0.086
L.S.D 0.05 (Periods*Locations)			0.162			0.162			0.178			0.177			0.124			0.122

3. Zinc content in plants (mg/l):

The results of plant parts chemical analysis showed in Table (8) also show the effect of different locations on zinc content in plant parts formed on *Pittsporum tobira* plants. In both seasons, planted plants in El-Dekhela had the highest zinc content in leaves (4.01 and 5.52 mg/l), zinc content in stem (3.20 and 4.15 mg/l) and zinc content in root (0.64 and 0.75 mg/l) in the first and second seasons, respectively. While, planted plants in Antoniadis Park had the lowest zinc content in leaves (1.91 and 3.41 mg/l), zinc content in stem (0.59 and 1.49 mg/l) and zinc content in root (0.15 and 0.26 mg/l) in the first and second seasons, respectively.

Chemical analysis of zinc 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 (8) that the data recorded for *Pittsporum tobira* plants were significantly increased gradually in the spring, with Average zinc content in leaves of (3.49 and 4.99 mg/l), zinc content in stem (2.24 and 3.20 mg/l) and zinc content in root (0.93 and 0.50 mg/l) in the first and second seasons, respectively. On the other hand, the lowest zinc content plant parts in the autumn, with Average zinc content in leaves (2.42 and 3.93 mg/l), zinc content in stem (2.15 and 3.10 mg/l) and zinc content in root (0.33 and 0.43 mg/l) in the first and second seasons, respectively.

Regarding to the interaction between the effects of different locations and different periods on the zinc content in plant parts of *Pittsporum tobira* plants, the results recorded in the two seasons show that the highest values were obtained in the planted plants in El-Dekhela and the spring period, with Average heights zinc content in leaves (4.55 and 6.05 mg/l), zinc content in stem (3.25 and 4.20 mg/l) and zinc content in root (0.68 and 0.78 mg/l) in the first and second seasons, respectively.

Table (8). Averages of Chemical constituents (Zinc content in leaves, stem and root (mg/l)) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the two seasons of 2015 and 2016

Location	Zinc Content in leaves (mg/l)						Zinc Content in stem (mg/l)						Zinc Content in root (mg/l)					
	2015			2016			2015			2016			2015			2016		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	2.44	1.38	1.91	3.94	2.88	3.41	0.64	0.54	0.59	1.59	1.49	1.54	0.19	0.12	0.15	0.29	0.23	0.26
El-Dekhala	4.55	3.48	4.01	6.05	4.98	5.52	3.25	3.15	3.20	4.20	4.10	4.15	0.68	0.61	0.64	0.78	0.72	0.75
El-Ebrahimia	3.48	2.42	2.95	4.99	3.92	4.45	2.85	2.76	2.80	3.80	3.71	3.76	0.31	0.25	0.28	0.42	0.36	0.39
Average	3.49	2.42		4.99	3.93		2.24	2.15		3.20	3.10		0.39	0.33		0.50	0.43	
LSD 0.05 (Periods)			0.666			0.666			4.872			3.292			0.190			0.191
LSD 0.05 (Locations)			1.472			1.472			1.930			1.112			0.033			0.032
L.S.D 0.05 (Periods*Locations)			0.124			0.122			2.730			1.573			0.047			0.045

4. Heavy metals content in soil (mg/l):

The results of soil chemical analysis presented in Table (9) also show the effect of different locations on heavy metals content in soil. In the first season, soil in El-Dekhela had the highest lead content (23.36 mg/l), cadmium content (0.21 mg/l) and zinc content (7.25 mg/l) in the first season (2015). While, planted plants in Antoniadis Park had the lowest lead content (2.32 mg/l), cadmium content (0.01 mg/l) and zinc content (2.11 mg/l) 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 gradually in the autumn, with Average lead content of (11.17 mg/l), cadmium content of (0.12 mg/l) and zinc content (5.46 mg/l) in the first season (2015). On the other hand, the lowest heavy metals content was detected in the spring, with Average lead content of (9.93 mg/l), cadmium content (0.10 mg/l) and zinc content of (3.81 mg/l) 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 show that, the highest values were obtained in El-Dekhela at the autumn period, with Average heights lead content of (26.47 mg/l), cadmium content of (0.22 mg/l) and zinc content of (8.46 mg/l) in the first season (2015).

Table (9). Averages of Chemical constituents (Lead, Cadmium and Zinc content in soil (mg/l)) of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the first season (2015)

locations	Lead Content in Soil (mg/l)			Cadmium Content in Soil (mg/l)			Zinc Content in Soil (mg/l)		
	Spring	Autumn	Average	Spring	Autumn	Average	Spring	Autumn	Average
Antoniadis Park	2.64	2.00	2.32	0.01	0.01	0.01	1.54	2.68	2.11
El-Dekhala	20.26	26.47	23.36	0.19	0.22	0.21	6.05	8.46	7.25
El-Ebrahimia	6.90	5.05	5.97	0.12	0.11	0.11	3.86	5.24	4.55
Average	9.93	11.17		0.10	0.12		3.81	5.46	
LSD 0.05 (Periods)			10.385			0.088			4.872
LSD 0.05(Locations)			8.518			0.085			1.930
L.S.D 0.05 (Periods*Locations)			12.046			0.121			2.730

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

Transfer factor (TF) indicates the efficiency of *Pittsporum tobira* 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 (9.704). On the other hand, plants in El-Dekhela had the lowest transfer factor for the lead content (1.433) 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 *Pittsporum tobira* plants was increased steadily with different locations. We found that, plants of Antoniadis Park had the highest transfer factor in the cadmium content with Average (14.666). On the other hand, plants of El-Dekhela had the lowest transfer factor with respect to the lead content (2.237) in the first season, while, the highest transfer factor in the cadmium content in stem compared with different of parts plant.

From the data presented in Table (10), it can be seen that the transfer factor in the zinc content in *Pittsporum tobira* 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 Average (0.441). On the other hand, plants of El-Dekhela had the lowest transfer factor in the lead content (0.360) in the first season, While, the highest transfer factor in the zinc content in leaves compared with different of parts plant.

Table (10). Averages of transfer factor to leaves, stem and roots **of *Pittsporum tobira* plants as influenced by locations, periods and their combinations (locations × periods) in the first season (2015).**

locations	Transfer factor at lead				Transfer factor at cadmium				Transfer factor at zinc			
	Leaves	Stem	Root	Average	Leaves	Stem	Root	Average	Leaves	Stem	Root	Average
Antoniadis Park	1.112	22.000	6.000	9.704	16.000	22.000	6.000	14.666	0.905	0.279	0.071	0.418
El-Dekhala	0.397	2.380	1.523	1.433	2.809	2.380	1.523	2.237	0.553	0.441	0.088	0.360
El-Ebrahimia	1.137	3.727	1.727	2.197	4.818	3.727	1.727	3.424	0.648	0.615	0.061	0.441
Average	0.882	9.369	3.083		7.875	9.369	3.083		0.702	0.445	0.073	

DISCUSSION

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

The physiological responses, such as the gas exchange rate and photosynthetic function, can be ascribed to the different effects of physico-chemical properties of heavy metals on the integrity and function of the photochemical apparatus of plant seedling fronds, as well as the impact on the chlorophyll concentrations in the leaves. The photosynthesis rate, CO_2 assimilation rate (Chen *et al*., 2012). The maintenance of an intercellular CO_2 concentration is concomitant with the leaf CO_2 assimilation rate and reflected photosynthesis function of seedling in the different heavy metal-spiked soils. The chlorophyll and carotenoid contents played a central role in the energy manifestation of green plant. Any significant alteration of their contents possibly resulted in a marked effect on the entire metabolism of the plant (Piotrowska *et al*., 2010).

Heavy metals are essential and important for normal growth and development of plants being an essential component of many enzymes and proteins. On the other hand, it has been found that increasing heavy metals concentrations have led to the emergence of symptoms of poisoning such as inhibiting plant growth (Hall, 2002). 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.

Several studies demonstrated that heavy metals can function as stressor, causing some physiological constrains that decrease plant vigor and inhibit plant growth (Schutzendubel *et al*., 2001). As the higher concentration of heavy metal has been reported to retard the cell division and differentiation, reduce their elongation and effect plant growth and development (Soares *et al*., 2001). 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.

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 content of heavy metals in plant:

The possibility of using crops as phytoremediants depends on the accumulation and distribution of metals among their morphological organs. According to Vassiliev *et al.* (2002) more detailed information about the biomass crops' ability to withstand metal as well as to accumulate it in the shoots is needed. Metal concentrations in plants vary with plant species (Joonki *et al*., 2006). Under normal growing conditions, plants can potentially accumulate certain metal ions an order of magnitude greater than the surrounding medium (Kim *et al*., 2002). Use of indigenous plant species is generally favoured because they show tolerance to imposed stress conditions, require less maintenance and present fewer environmental and human risks than non-native or genetically altered species (Laghlimi *et al.,* 2015).

Mills (2001) found that the distribution of lead is a passive process and that the level of metal decreased towards the shoot tip of the investigated species. Lead concentration that is toxic to plant is 27 mg/l (Deng *et al*.,2004), as lead bound to cell wall of plant and render lead ineffective in acting as a strong metabolic inhibitor, lead may accumulate in root more than leave and stem because of it relatively low mobility (McBride,2003).

Cadmium is potentially toxic to both plant and has no essential biological function and it excessive concentration is undesirable (Nabulo *et al*., 2011). Cadmium promotes the production of stress ethylene in different species of plant and also rapidly induce synthesis of phytochelatins in plants (Nabulo *et al*.,2011 and Mohammad *et al*.,2009). Cadmium content of shoots increased with increasing soil Cadmium. These results are in accordance with the observations of Jiang *et al*., (2004) and Zhao *et al*. (2003). Also, Vogel-Mikus *et al*., (2005) reported significant hyperaccumulation of Cadmium in shoots and roots with increasing Cadmium concentrations, similary (Singh *et al*.,2011) found that cadmium concentration in leaves and stem is about 1.9 mg/l in a normal plant but the value could be higher in contaminated soil.

Zinc has the next highest concentration in plant samples analyzed after iron though some were below the detection limit of the atomic absorption spectroscopy used, it ranges in the dry weight of the samples was compared to the result that obtained by Murray *et al*., (2000) on plants from contaminated site (Kopittke *et al*., 2008). Zinc is an essential element to plants and studies have shown that total zinc concentration in plant tissues increases as zinc supply increases in both tolerant and non tolerant genotype plant (Murray *et al*., 2000). But it could be higher if the soil is contaminated from the result obtained from the plants we could observe that some of them had concentration beyond the fore mentioned concentration. Studies have shown that increased cadmium application to zinc deficient plant tends to decrease plant zinc concentration but in plant with adequate zinc supply, zinc concentration are either not affected or increased by cadmium (McBride, 2007).

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 *et al*., 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 (Ahmad and Erum, 2010), 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. (أ) نبات البيتسبوروم

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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 البتسبورم المتجانسة بشكل فردي في الأواني البلاستيكية (قطرها ٣٠ سم) مليئة بخليط من الرمال 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 البيانات تظهر تأثير بين المواقع مختلفة على النمو الخضري لنباتات بيتسبورم. 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 تدريجيا 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 النباتات البيتسبورم لنقل العناصر الثقيلة من التربة إلى أجزاء النبات. يمكن ملاحظة أن معامل التحويل في محتوى الرصاص والكادميوم والزنك في نباتات بيتسبورم توبيرا زاد بشكل مطرد مع المواقع المختلفة تحت الدراسة. ووجدنا أن النباتات المزروعة في حديقة أنطونيادس كانت 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 الزنك مقارنة مع أجزاء مختلفة من النبات.