LONG-TERM EFFECT OF SOME HEAVY METAL COMBINATIONS ON GROWTH AND CHEMICAL COMPOSITION OF SOME ORNAMENTAL SHRUBS COMMON IN EGYPT Nº2. – COMMON OLEANDER (*NERIUM OLEANDER* L.)

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Abstract. An experiment was carried out at Orman Botanical Garden, Giza, Egypt during 2019/20 and 2020/21 seasons to reveal the response of *Nerium oleander* L. transplants to Pb, Cd, and Ni in combinations at various concentrations after short and long growth periods. Pollution treatments and growth periods were combined factorially to study the effect of interactions. The results showed that survival % was 100% by the various applied treatments. Plant height, stem diameter, and leaf area traits were improved by heavy metal combinations, whereas No. branches and leaves/plant, root length and top growth, and roots fresh and dry weights were negatively affected. Elongating growth period up to 18 months significantly increased means of the different vegetative and root growth traits compared to 6-months period. So, combining between planting in either control or first treatment combination mixture and the longer growth period significantly improved most of the growth parameters. The percent of pollution resistance index took a similar trend, and it was higher than 88% in the two seasons by fourth treatment combination indicating a high tolerance to HMs toxicity. Thus, *N. oleander* plants can be successfully used for landscaping HMs-polluted sites due to their high survival and tolerance potential.
Keywords: *Nerium oleander L., heavy metals pollution, PRI, survival, growth traits*

Introduction

Nowadays, phytoremediation is one of the most important, cheap, and effective tool to remove different contaminants from the soil and air (Ibrahim and El-Afandi, 2020). Ornamental plants specifically are important bioindicators for environmental pollution, as they are not food-chain crops and remove a considerable amount of pollutants from the soil atmosphere, and water. They act as sink and living filters to minimize environmental pollution by various ways like HMs absorption, adsorption, accumulation, or detoxification, besides their role in improving the air quality by releasing O_2 into the atmosphere and absorb CO_2 (Salih et al., 2017). Among them, *Nerium oleander* may be valid in this concern.

In this regard, Seaward and Mashhour (1991) found that homogenous seedlings of *N. oleander* have a great ability in collecting HMs on the surface of their leaves, mainly from aerial sources and controlled by substratum. This is due to that its leaves characterize by their lanceolate form with a high cuticle thickness (Akosy and Oztürk, 1997). This fact was documented by Houdaji et al. (2010) who reported that it has been known that *N. oleander* assumes an essential role in reducing heavy metals in nature because of its morphological and physic-compound attributes that make it has lanceolate leaves with high cuticle skin thickness. Similar responses were also observed on *N. oleander* by Salih

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et al. (2017), Safari et al. (2018), Salih and Aziz (2019), and Ibrahim and El-Afandi (2020).

On the same line, were those results obtained by Ma et al. (2018) on *Taxodium* hybrid “Zhongshaushan”, Omar (2018) on *Sambucus nigra* and *Bauhinia purpurea*, Eisa (2019) on *Populus nigra* and *Salix mucronata*, Ouf and Gaber (2019) on *Salix mucronata* and Chauhan and Mathur (2020) who found that industrially contaminated soil caused a great reduction in shoot length, root length, fresh weight of shoot/root and leaf area of *Helianthus annuus* vars. PBH and DRSF-108. Chlorophyll a, b and total chlorophyll (a + b) concentrations were decreased, while accumulation of Pb, Cd, Zn, Cu, Fe and as was increased at different range (0.62-158.29, 0.8-59.6, 0.81-166.5, 0.09-101.89, 2.06-53.25 and 0.002-2.55 mg/kg, respectively. Similarly, were those findings of Eid et al. (2020) on four aquatic macrophytes (*Eichhornia crassipes*, *Ludwigia stolonifera*, *Echinochloa stagnina*, and *Phragmites australis*).

However, this work was set out to investigate the effect of Pb, Cd, and Ni heavy metals in combinations at different concentrations on growth performance and chemical composition of common oleander transplants at two different growth periods. This ornamental shrub (*Nerium oleander* L., Fam. Apocynaceae) is an evergreen, erect shrub of 4-4.45 m height; leaves linear to oblong-lanceolate to 20-25 cm long, entire, dark dull green; blooms in summer, showy flowers in terminal branching cymes, yellowish to rose-pink, red-purple or white, sometimes scented; native to Mediterranean region to Japan (Bailey, 1976).

Oleanders are generally grown outdoors in mild climates. They require little attention and are very drought resistant. In cold regions, they are favorite as pot or tub plants, and should be cut back and rested after flowering, then potted in a mixture of loam and rotted manure. It is of easy culture and is well adapted to city conditions. Propagated easily by good-sized cuttings of mature firm wood, sometimes in water. All parts are very poisonous if eaten (Huxley et al., 1992).

Materials and Methods

This investigation was conducted at Orman Botanical Garden, Giza, Egypt under the full sun throughout 2019/20 and 2020/21 consecutive seasons to study the long-term effect of lead (Pb), cadmium (Cd), and nickel (Ni) when applied together at gradual concentrations on survival, growth and chemical composition of *Nerium oleander* L. transplants, as a strong growing ornamental shrub widely used for various landscape purposes in Egypt.

Thus, uniform transplants at a length of about 30-31.5 cm, with two branches carrying about 25-27 leaves were planted on April, 15^{th} for every season in 20-cm-diameter polyethylene black bags (one transplant/bag) filled with about 3 kg/bag of sand and clay mixture at equal volume parts (1:1, v/v). The physical and chemical properties of the sand and clay used in the two seasons were determined and shown in *Table 1*.

Acetate quick thawing salts of Pb, Cd, and Ni, produced by Aldrich Chemical Co., USA were mixed well in combinations through the particles of the used soil mixture before filling the plastic bags at concentrations of 0.00 ppm for each metal as a control, 500 ppm Pb + 50 ppm Cd + 25 ppm Ni for treatment number one (T_1) and 2-, 3- and 4-fold of these concentrations for treatments number two (T2), three (T3) and four (T4), respectively.

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Table 1. *The physical and chemical properties of the sand and clay used in 2019/20 and 2020/21 seasons*

Soil texture	**Seasons**	**Particle size distribution (%)**				**S.P.**	**E.C. (dS/m)**	**pH**	**Cations (meq/L)**				**Anions (Meq/L)**		
		Coarse sand	Fine sand	Silt	Clay				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{--}
Sand	2019/20	18.72	71.28	4.76	5.34	21.83	1.58	8.20	2.65	2.48	21.87	0.78	3.85	13.00	10.93
	2020/21	79.76	9.30	2.50	8.44	23.10	1.76	7.90	19.42	8.33	7.20	0.75	1.60	7.80	26.30
Clay	2019/20	7.46	16.75	34.53	40.89	41.67	2.10	8.33	16.93	9.33	20.44	0.37	3.82	1.46	41.79
	2020/21	7.64	22.50	30.15	39.71	53.36	2.23	7.92	7.50	2.21	15.49	0.75	6.28	8.12	11.05

Immediately after planting, the plastic holeless bags were irrigated with 300 ml of fresh water/bag, but afterward the irrigation was done once day by day with only 250 ml of water/bag during the summer months, while during winter ones the plants were irrigated once every 3 or 4 days to keep the roots from decay. The usual agricultural practices required were done whenever needed. The plants were set out for every season in a complete randomized design and replicated thrice with five plants per replicate (Mead et al., 1993).

Data of the current study were recorded after two periods of growth; the first after 6 months (on October, 15^{th} for every season) and the second after one year (12 months) from the first one (on the next October, 15^{th}) and then expressed in the tables as other factor beside the factor of heavy metals combinations. The recorded data were: survival percentage, plant height (cm), stem diameter at the base (cm), number of branches/plant, number of leaves/ plant, means of root length (cm), as well as fresh and dry weights of top growth and roots (g). Besides, the pollution resistance index as a percentage (PRI %) was calculated from the equation proposed by Wilkins (1957) as follows:

$$\text{PRI \%} = \text{Mean root length of the polluted plants (cm)/mean root length of control x 100} \quad \text{(Eq.1)}$$

In fresh leaf samples taken from the middle parts of the plants, photosynthetic pigments (chlorophyll a, b and carotenoids, mg/g f.w.) and the percent of total soluble sugars were determined according to the methods of Sumantha et al. (2014) and Dubois et al. (1966), respectively, whereas in dry ones, the percentages of nitrogen, phosphorus, and potassium were measured by the methods explained by Chapman and Pratt (1975). Moreover, concentrations of Pb, Cd, and Ni as mg/100 g d.w. in dry samples of both leaves and roots were assessed using a Perkin Elmer 403 atomic absorption spectrophotometer (Jackson, 1973). All chemical analyses were evaluated in the second season only.

Data were then tabulated and subjected to analysis of variance using the program of SAS Institute (2009), followed by Duncan`s new multiple range t-test (Steel and Torrie, 1980) to detect the significance level among heavy metals combinations, growth periods, and their interactions.

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Results and Discussion

Effect of heavy metal combinations, growth period, and their interactions on:

1- Survival percentage and vegetative and root growth traits

It can be seen from data averaged in *Table 2* that the survival percentage was 100% by the different sole and combined treatments employed in this trial indicating the ability of common oleander plants to withstand the toxicity of HMs, even at high concentrations and with prolonging growth period. however, some growth parameters were not negatively affected by the pollution treatments, where plant height (cm) was significantly improved by HMs combinations compared to control in the two seasons (*Table 2*), with the prevalence of T2 combination in the first season and T1 combination in the second one. Likewise, results of stem diameter reached maximum by T1 combination in both seasons (*Table 2*).

Table 2. *Effect of heavy metal combinations, growth period, and their interactions on survival %, height, and stem diameter of Nerium oleander L. plants during 2019/20 and 2020/21*

Growth period (G.P.) / Pollution treatments	Survival %			Plant height (cm)			Stem diameter (cm)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
	First season; 2019/2020								
Control	100.00a	100.00a	100.00A	44.63f	105.70d	75.17C	0.53e	1.24b	0.89C
T1	100.00a	100.00a	100.00A	51.10w	113.00bc	82.05B	0.70c	1.39a	1.05A
T2	100.00a	100.00a	100.00A	51.67e	118.30a	84.99A	0.63cd	1.27b	0.95BC
T3	100.00a	100.00a	100.00A	48.83e	116.00ab	82.42B	0.60de	1.28b	0.94BC
T4	100.00a	100.00a	100.00A	50.03e	112.00c	81.02B	0.63cd	1.28b	0.96B
Mean	100.00A	100.00A		49.25B	113.00A		0.62B	1.29A	
	Second season; 2020/2021								
Control	100.00a	100.00a	100.00A	49.23e	110.00c	79.62C	0.63e	1.37b	1.00C
T1	100.00a	100.00a	100.00A	56.17d	118.10a	87.14A	0.83c	1.53a	1.18A
T2	100.00a	100.00a	100.00A	56.90d	113.20b	85.05AB	0.73d	1.41b	1.07B
T3	100.00a	100.00a	100.00A	51.13e	114.80b	82.97B	0.70de	1.40b	1.05BC
T4	100.00a	100.00a	100.00A	49.07e	108.60c	78.84C	0.70de	1.38b	1.04BC
Mean	100.00A	100.00A		52.50B	112.94A		0.72B	1.42A	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan's new multiple range t-test at 5 % level

The leaf area (cm^2) was also improved (*Table 3*), where T2, T3, and T4 combinations gave the highest records with non-significant differences among themselves in the two seasons. The other growth parameters were however negatively affected by HMs combinations, especially by T2, T3, and T4 combinations with few exceptions in both seasons (*Tables, 3, 4, 5, and 6*). On the other side, prolonging growth period up to 18 months significantly increased mean values of the different vegetative and root growth attributes measured in the two seasons compared with those measured after only 6

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months. Besides, the interactions of the two factors exerted also a significant effect on various growth criteria, where interacting between planting in either control or T1 combination and the longer growth period (18 months) attained the highest means in most growth characters in the two seasons. However, combining between planting in soil mixture polluted with T3 and T4 combinations and the short period of growth (6 months) achieved the least records with inferiority of planting in T4-polluted soil + 6 months growth treatment which gave the minimal values relative to all the other combinations in both seasons.

Table 3. *Effect of heavy metal combinations, growth period, and their interactions on No. branches, No. leaves and leaf area of Nerium oleander L. plants during 2019/20 and 2020/21 seasons*

Growth period (G.P.) / Pollution treatments	No. branches/plant			No. leaves/plant			Leaf area (cm^2)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
	First season; 2019/2020								
Control	2.00c	3.27a	2.63A	30.00ef	67.00a	48.50A	35.20d	44.90b	40.05B
T1	2.00c	3.43a	2.72A	33.00d	56.00b	44.50B	37.77c	44.37b	41.07B
T2	1.67d	2.67b	2.17B	31.33de	50.33c	40.83C	39.70c	47.83a	43.77A
T3	1.67d	2.67v	2.17B	28.33f	56.00b	42.17C	37.83c	47.40a	42.62A
T4	1.67d	2.77b	2.22B	27.67f	56.67b	42.17C	39.17c	46.37ab	42.77A
Mean	1.80B	2.96A		30.07B	57.20A		37.93B	46.17A	
	Second season; 2020/2021								
Control	2.33d	3.60b	2.97A	33.70de	71.00a	52.35A	35.93c	45.33a	40.63B
T1	2.40d	3.836a	3.12A	35.53d	62.26bc	48.89B	38.50b	44.97a	41.74AB
T2	2.20d	2.93c	2.57B	34.60d	57.17c	45.89BC	40.23b	46.20a	43.22A
T3	2.20d	2.93c	2.57B	31.63ef	59.20bc	45.42C	38.70b	46.37ab	42.54A
T4	2.17d	2.93c	2.55B	29.60f	59.87b	44.74C	39.57b	45.03a	42.30A
Mean	2.26B	3.25A		33.01B	61.89A		38.59B	45.58A	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan’s new multiple range t-test at 5 % level

Similar results were obtained by Salih et al. (2017) who stated that *Nerium oleander* and neem are found to be more tolerant to air pollution than date palm or Conocarpus. Similarly, Safari et al. (2018) reported that *N. oleander* was found to have the highest absorption capacity for Ni, Pb, Co, and V-metals from the air and soil than *Bugainvillea spectabilis* and *Hibiscus rosa-sinensis* and so, it is a very suitable tool for managing air and soil pollution in highly industrialized area. Furthermore, Ibrahim and El-Afanid (2020) declared that *N. oleander* plants are able to stabilize HMs (Pb, Cd, and Zn) in the soil making them the less available from the soil.

A parallel trend was also revealed in other ornamental plants by Ma et al. (2018) on *Taxodium* hybrid (T118 and T406), Eisa (2019) on *Populus nigra* and *Salix mucronata*, and Chauhan and Mathur (2020) who found that industrially contaminated soil had a significant impeding on *Helianthus annuus* plantlets by reducing shoot and root lengths, leaf area and fresh weight of shoot and root.

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Table 4. *Effect of heavy metal combinations, growth period, and their interactions on root length, No. root branches and PRI of Nerium oleander L. plants during 2019/20 and 2020/21 seasons*

Growth period (G.P.) / Pollution treatments	Root length (cm)			No. root branches/plant			Pollution resistance Index (PRI %)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
	First season; 2019/2020								
Control	33.67d	40.17c	36.92B	12.33g	25.67a	19.00C	100.00cd	100.00cd	100.00B
T1	33.90d	46.37b	40.14A	17.33e	24.17b	20.75A	101.90c	115.40b	108.65A
T2	30.10e	50.33a	40.22A	16.00f	22.83c	19.42B	89.80e	127.30a	108.55A
T3	26.33f	45.57b	35.95B	10.67i	18.97d	14.82D	78.57f	115.90b	97.24B
T4	28.33ef	38.60c	33.47C	11.33h	18.43d	14.88D	85.23cd	96.23d	90.73C
Mean	30.47B	44.21A		13.53B	22.01A		91.10B	110.97A	
	Second season; 2020/2021								
Control	37.03c	43.63a	40.33A	11.10h	22.63b	16.87C	100.00a	100.00a	100.00A
T1	37.20c	45.03a	41.12A	14.60f	23.27a	18.94A	101.60a	103.50a	102.55A
T2	33.20d	44.17a	38.69B	13.77g	21.03c	17.40B	90.07b	101.50a	95.79B
T3	31.17d	43.50a	37.34B	9.77i	17.30d	13.54D	85.17c	99.80a	92.49C
T4	31.13d	40.07b	35.60C	9.60i	16.60e	13.10E	85.17c	91.93b	88.55D
Mean	33.95B	43.28A		11.77B	20.17A		92.40B	99.35A	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan’s new multiple range t-test at 5 % level

Table 5. *Effect of heavy metals combinations, growth period, and their interactions on top growth fresh and dry weights of Nerium oleander L. plants during 2019/20 and 2020/21 seasons*

Growth period (G.P.) / Pollution treatments	Top growth fresh weight (g)			Top growth dry weight (g)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
	First season; 2019/2020					
Control	37.70e	142.40a	90.05A	9.23de	48.90c	29.07BC
T1	42.93d	129.80b	86.37BC	10.40d	51.33b	30.87A
T2	35.43e	140.20a	87.82AB	8.70de	55.23a	31.97A
T3	34.83e	124.60c	79.72D	8.17ef	49.00c	28.58C
T4	36.93e	131.90b	84.42C	9.13def	52.17b	30.65B
Mean	37.56B	133.78A		9.13B	51.33A	
	Second season; 2020/2021					
Control	41.50e	134.60c	88.05B	9.77e	52.70c	31.24B
T1	45.00e	142.10b	93.55A	10.43e	55.77b	33.10A
T2	38.97f	147.10a	93.04A	9.40e	57.53a	33.47A
T3	38.30f	129.90d	84.10C	9.27e	50.83d	30.05C
T4	37.57f	131.70cd	84.64C	9.00e	51.27d	30.14C
Mean	40.27B	137.08A		9.57B	53.62A	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan’s new multiple range t-test at 5 % level

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Table 6. *Effect of heavy metals combinations, growth period, and their interactions on roots fresh and dry weights of Nerium oleander L. plants during 2019/20 and 2020/21*

Growth period (G.P.) / Pollution treatments	Roots fresh weight (g)			Roots dry weight (g)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
	First season; 2019/2020					
Control	6.33c	59.57a	32.95A	1.58d	23.70bc	12.64BC
T1	7.20c	60.27a	33.74A	1.99d	24.60b	13.30B
T2	6.73c	61.60a	34.17A	2.07d	26.47a	14.27A
T3	5.87c	58.43a	32.15A	1.57d	24.77b	13.17B
T4	6.30c	51.77b	29.03B	1.74d	22.87c	12.31C
Mean	6.49B	58.33A		1.79B	24.48A	
	Second season; 2020/2021					
Control	6.63e	65.37a	36.00A	1.65e	25.80a	13.73AB
T1	7.43e	65.00a	36.22A	1.96e	26.13a	14.05A
T2	6.93e	60.13b	33.53B	1.88e	24.67b	13.28A
T3	6.13e	56.33c	31.23BC	1.59e	23.50c	12.55C
T4	6.37e	52.33d	29.35C	1.66e	22.13d	11.90D
Mean	6.70B	59.83A		1.75B	24.45A	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan's new multiple range t-test at 5 % level

2- Pollution resistance index as a percentage (PRI %)

As shown in *Table 4*, the percentages of PRI of control plants in both seasons were 100% and significantly increased in the first season to 108.65 and 108.55% by T1 and T2 HMs-combinations, respectively, while in the 2nd one raised only to 102.55% by T1 combination with non-significant differences relative to control. Increasing HMs concentrations afterwards in T3 and T4 in the 1st season and in T2, T3, and T4 in the 2nd one significantly decreased the means of such index to percents slightly higher than 90% in both seasons by T4 (which contains the highest concentrations of Pb, Cd, and Ni). This may clearly indicate the high ability of *N. oleander* in tolerating HMs toxicity. In this connection, Salih et al. (2017) found that air pollution tolerance index tends to increase in *Azadirachta indica* and *Nerium oleander* plants grown in the polluted site near Bahrain Oil Refinery. Similarly, Safari et al. (2018) observed that the highest comprehensive bioconcentration index (CBCI) was found in leaf (0.37) and bark (0.12) of *N. oleander*, whereas the maximum metal accumulation index (MAI) was found in leaf of *N. oleander* (1.58) and bark of *H. rose-sinensis* (1.95).

In relation to the effect of growth period, it was noticed that determination of PRI after 18 months from planting significantly recorded higher percentages than those determined after only 6 months of growth in the two seasons as prolonging growth period was accompanied by longer roots irrespective of pollution treatment. Thus, combining between planting in soil mixture polluted with T1, T2, and T3 HMs-combinations and 18-months growth period gave the highest mean values of this index over all the other combinations in the first season, but that was true in the second one by interacting between planting in soil mixture polluted with T1 and T2 HMs combinations and 18-months growth period.

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The forgoing results are in accordance with those postulated by Salih et al. (2017) and Safari et al. (2018) on *N. oleander* and Omar (2018) who cited that PRI % of *Sambucus nigra* grown in contaminated soil with Pb, Cd, and Ni was slightly improved by the low concentrations of HMs, while decreased by the medium and high levels of such metals to be more than 50-55 % by the highest levels showing the ability of *S. nigra* to withstand the high levels of the 3 toxic metals when applied in combinations.

3- The chemical composition of leaves and roots

Data listed in *Table 7* exhibit that, concentrations of chlorophyll a, b, and carotenoids (mg/g f. w.), as well as the percentage of total soluble sugars in the leaves were non-constant in response to either HMs combinations or growth periods, as the variations among treatments were narrow in most instances. The effect of interaction treatments took a similar trend.

Table 7. *Effect of heavy metals combinations, growth period, and their interactions on pigments and total soluble sugars concentrations in Nerium oleander L. leaves during 2020/21 season*

Growth period (G.P.) / Pollution treatments	Chlorophyll a (mg/g. f. w.)			Chlorophyll b (mg/g. f. w.)			Carotenoids (mg/g. f. w.)			Total soluble sugars (%)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
Control	0.596a	0.597a	0.597A	0.312b	0.236c	0.274B	0.240b	0.234b	0.237A	17.55d	18.06c	17.81C
T1	0.597a	0.519c	0.558B	0.367a	0.248c	0.308A	0.263a	0.240b	0.252A	21.50a	18.45c	19.98A
T2	0.583a	0.503c	0.543B	0.303b	0.231c	0.267C	0.229b	0.231b	0.230B	19.50b	16.56e	18.03B
T3	0.577a	0.561b	0.569A	0.372a	0.210d	0.291B	0.263a	0.218c	0.241A	16.90d	18.50c	17.70C
T4	0.560b	0.598a	0.579A	0.410a	0.238c	0.324A	0.268a	0.236b	0.252A	14.76f	16.58e	15.67D
Mean	0.583A	0.556A		0.353A	0.233B		0.253A	0.232A		18.04A	17.63B	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan's new multiple range t-test at 5 % level

On the other hand, the percentages of N, P, and K were the highest in control plants but were decreased by HMs combinations with various significance levels compared to those of control treatment (*Table 8*). The concentration of N and P was significantly improved by prolonging growth period (after 18 months) relative to the short growth period (6 months), while the opposite was the right regarding K percentage. In general, combining between planting in non-polluted (control) soil mixture and either short or long growth period scored the utmost high N and K concentrations, whereas interacting between planting in soil mixture polluted with either T1 or T4 HMs combinations and the longer growth period acquired the highest P concentration.

As for Pb, Cd, and Ni concentrations in the leaves and roots (mg/100 g d. w.), data averaged in (*Table 9*) show that a progressive increase in concentrations of these three metals was accompanied to their gradual increment in the polluted soil mixture to reach maximum by T4 combination either in the leaves or in roots. However, Pb concentration in the leaves was significantly decreased with prolonging growth period to 18 months, but the opposite was occurred regarding Cd concentration. In the roots, however concentrations of the three metals were significantly decreased by prolonging growth

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period. In addition, the connecting between planting in soil mixture treated with either T3 or T4 HMs combinations and short growth period attained the highest concentration of Pb in the leaves, but that was true for Cd and Ni concentrations by connecting between planting in T4 combination treated-soil mixture and the longer growth period. In the roots, however combining between planting in soil mixture polluted with T4 HMs combination and the short growth period increased the three metals content to the utmost high concentrations over all the other combinations.

Table 8. *Effect of heavy metals combinations, growth period, and their interactions on nitrogen, phosphorus, and potassium concentrations in Nerium oleander L. leaves during 2020/21 season*

Growth period (G.P.) / Pollution treatments	N (%)			P (%)			K (%)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
Control	2.213a	2.310a	2.262A	0.438c	0.535b	0.487A	2.04a	2.18a	2.11A
T1	1.327c	1.576b	1.452C	0.347d	0.601a	0.474A	1.87b	1.55c	1.71B
T2	1.547b	1.653b	1.601B	0.361d	0.526b	0.444B	1.87b	1.37d	1.62B
T3	1.565b	1.671b	1.618B	0.238e	0.579b	0.409C	1.64c	1.35d	1.50C
T4	1.548b	1.550b	1.549C	0.179f	0.703a	0.441B	1.63c	1.72b	1.68B
Mean	1.640B	1.752A		0.313B	0.589A		1.81A	1.64B	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan's new multiple range t-test at 5 % level

Table 9. *Effect of heavy metals combinations, growth period, and their interactions on lead, cadmium, and nickel concentrations in Nerium oleander L. leaves and roots during 2020/21 season*

Growth period (G.P.) / Pollution treatments	Pb (mg/100 g d. w.)			Cd (mg/100 g d. w.)			Ni (mg/100 g d. w.)		
	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean	1st G.P.	2nd G.P.	Mean
	In the leaves								
Control	15.517e	11.833f	13.675E	37.551g	54.366e	45.959E	16.003d	8.676f	12.340E
T1	18.633d	13.654e	16.144D	37.978g	68.763d	53.371D	16.886d	11.258e	14.072D
T2	32.689b	26.879c	29.784C	45.600f	137.315c	91.458C	18.500d	16.950d	17.725C
T3	37.245a	28.600c	32.923B	68.403d	204.150b	136.277B	23.210c	25.431c	24.321B
T4	36.583a	32.901b	34.742A	67.526d	235.648a	151.587A	28.086b	38.939a	33.513A
Mean	28.134A	22.774B		51.412B	140.043A		20.537A	20.251A	
	In the roots								
Control	17.690g	23.952f	20.821E	210.0841f	178.243h	194.164D	28.310f	21.129g	24.720D
T1	19.219g	28.389e	23.804D	243.179d	195.984g	219.582C	32.403d	30.956e	31.680C
T2	30.256d	31.240d	30.748C	259.875b	214.500e	237.188B	33.796d	30.967e	32.368C
T3	43.950b	35.000c	39.475B	263.733a	250.130c	256.932A	41.948b	33.598d	37.773B
T4	58.257a	40.756b	49.507A	261.500a	256.431b	258.966A	44.977a	35.736c	40.357A
Mean	33.875A	31.868B		247.674A	219.058B		36.287A	30.477B	

Control: 0.00 ppm for Pb, Cd and Ni, T1: 500 ppm Pb + 50 ppm Cd + 25 ppm Ni; T2: 1000 ppm Pb + 100 ppm Cd + 50 ppm Ni; T3: 1500 ppm Pb + 150 ppm Cd + 75 ppm Ni and T4: 2000 ppm Pb + 200 ppm Cd + 100 ppm Ni. Means followed by the same latter in a column or row are not differ significantly according to Duncan's new multiple range t-test at 5 % level

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The previous results could be supported by those reported by Salih et al. (2017) who found that *Nerium oleander* grown near Bahrain oil refinery accumulated more Cu, Cr, Mn, Mo, and Fe than Neem, Conocarpus, and date palm, but chlorophyll content was decreased. Because oleander plants remove significant amounts of toxic metals from the environment, it is considered an excellent phytoremediator. Likewise, Safari et al. (2018) declared that *N. oleander* absorbed higher amounts of Ni, Pb, Co, V from the air and soil than *Bougainvillea spectabilis* and *H. rosa-sinensis*. So, it is very suitable tool for remediating HMs pollution in highly industrialized areas. Besides, Salih and Aziz (2019) found that pigments and protein contents in leaves of *N. oleander* plants grown near steel factories were lower than those in leaves of plants far away from the factory. Ibrahim and El-Afandi (2020) concluded that Pb was accumulated in the roots, while Cd and Zn were concentrated in the aerial parts of *N.* oleander plants grown in El-Dakhlia (industrial zone), Alexandria, Egypt.

Identical responses were also obtained by Eisa (2019) on *Populus nigra* and *Salix mucronata* and Ouf and Gaber (2019) who revealed that the highest accumulation of Cd, Pb, Zn, N, P and K elements was found in different parts of willow plants grown in polluted soil at different growth periods (16, 12 and 18 months). Chauhan and Mathur (2020) stated that *Helianthus annuus* plantlets grown in industrially contaminated soil accumulated different concentrations of Pb, Cd, Zn, Cu, Fe, and As. Similarly, Eid et al. (2020) cited that *Phragmites australis* accumulated the highest concentrations of Cd and Ni, while *Eichhornia crassipes*, accumulated the highest concentration of Pb in its tissues.

From foregone, it is obvious that common oleander (*Nerium oleander* L.) plants can tolerate Pb, Cd, and Ni metals when applied to the soil in combination at high concentrations without mortality, but with little adverse effects on some growth characters. Because of its higher concentrations uptake from these toxic metals, it can be used as a good phytoremediator for the HMs-polluted soil.

Conclusion

It can be advised to use *Nerium oleander* shrub for beautification and landscaping contaminated areas with toxic or heavy metals.

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