THE EFFECT OF SOME HEAVY METAL COMBINATIONS ON GROWTH AND CHEMICAL COMPOSITION OF SOME ORNAMENTAL SHRUBS COMMON IN EGYPT Nº1. – HOP BUSH (*DODONAEA VISCASA* L.)

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Abstract. A study was undertaken during 2019/20 and 2020/21 seasons to reveal the impact of lead (Pb), cadmium (Cd), and nickel (Ni) in combinations at various concentrations on the survival, growth, and chemical composition of *Dodonaea viscasa* L. seedlings after two periods of growth (PG): 6- and 18-months from planting. The interaction effect was also studied. The results indicated that survival % was 100% under the different sole or combined treatments, but the mean values of vegetative and root growth traits were gradually decreased with few exceptions as the concentrations of heavy metals were increased to reach minimum by the T4 combination. Elongating growth period up to 18 months significantly improved growth relative to that measured after 6 months. Hence, combining between planting in either unpolluted or polluted mixture with T1 combination and a growing period of 18 months gave the best growth. The gradual increment in HMs concentrations was accompanied by a gradual decrement in pollution resistance index (PRI) percentages, which were higher than 66% at the highest HMs concentrations indicating its high ability to withstand HMs toxicity. Different responses were observed regarding the chemical composition of the leaves and roots. Accordingly, *Dodonaea viscosa* L. plant can be used as a good phytoremediator.
Keywords: *survival, root length, Dodonaea viscasa* L.*, lead, cadmium, nickel, chemical composition*

Introduction

Hop bush (*Dodonaea viscasa* L.) which belongs to the Sapindaceae to Fam., is a very widespread tropical and subtropical bush, used in gardens for hedges and as a solitary specimen on turf. Its height reaches up to 4.5-5 m, has usually sticky shoots and simple, undivided, oblongish alternate leaves up to 7-10 cm long and about 2.5-3.0 cm wide. The flowers are greenish, in short terminal or axillary racemes. The fruits are 3-winged, notched at the apex, and red or purple in colour. It is propagated easily by seeds (Huxley et al., 1992).

Soils polluted with heavy metals (HMs) are still one of the most growing problems facing vegetative in all countries (Adrees et al., 2015). Among various HMs, lead (Pb), cadmium (Cd) and nickel (Ni) are the most serious ones due to their high mobility and toxicity, causing acute biological effects on plant growth and productivity with dangerous disorders to human health (Keller et al., 2015). They are more persistent and not degradable naturally like other organic pollutants, so accumulate in the soils and the different organs of plants (Khudhur et al., 2016). Thus, it is urgent to find out an effective and reasonable way to overcome this problem with the least cost through phytoremediation technology, in which some ornamentals (as non-food chain plants) can be used for remediation of contaminated soils with more cost-effective and fewer side

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effects as well than chemical and physical methods (Tauqeer et al., 2016). Among ornamentals that may serve in this approach *Dodonaea viscasa* as a good bioindicator for pollution in industrial areas due to its high ability to absorb higher concentrations of Fe, Zn, As, Pb, Ni, and Co heavy metals resulting from steel factories irrespective of the lower concentration of chlorophyll a, b, total chlorophyll, carotenoids and total protein contents (Salih and Aziz, 2019). Among *Dodonaea viscasa* L., *Myrtus communis, Platycladus orientalis*, and *Ficus benjamina* ornamentals, Mamand et al. (2020) reported that *Dodonaea viscasa* L., is the most effective phytoremediator.

On other ornamental plants, similar reports were mentioned by Ma et al. (2018) on *Taxoidium* hybrid “Zhong shanshan”, Omar (2018) on *Sambucus nigra* and *Bauhinia purpurea*, Eisa (2019) on *Populus nigra* and *Salix mueronata*, and Ouf and Gaber (2019) on *Salix mucronata*. Likewise, were those results of Dinu et al. (2021) on *Mentha piperita* and Khan et al. (2021) on some wild plants (*Digitaria sanguinalis*, *Hordeum leporinum*, and *Achantherum hymenoides*).

This study, however, aims to reveal the ability of *Dodonaea viscasa* seedlings to withstand the toxicity of Pb, Cd, and Ni heavy metals when applied in combination at ascending concentrations.

Materials and Methods

An experiment was carried out in the open field at Orman Botanical Garden, Giza, Egypt during the two successive seasons of 2019/2020 and 2020/2021 to examine the long-term impact of lead (Pb), cadmium (Cd), and nickel (Ni) in combinations at various concentrations for each metal on survival, growth and chemical composition of hop bush (*Dodonaea viscasa* L.) seedlings.

So, homogenous seedlings of such plant species at a height of about 12.3 cm, with one branch carrying about 12.0 leaves were planted on April, 15th for every season in 20-cm-diameter polyethylene black bags (one seedling/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*).

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

Thawing salts of Pb, Cd, and Ni (acetates), manufactured by Aldrich Chemical Co., Inc., Wisconsin 53233, 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)

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and 2-, 3- and 4- fold of these concentrations for treatments number two (T2), three (T3) and four (T4), respectively.

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 for such plantation 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 from planting (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 besides the factor of heavy metals combinations. These data were: survival percentage, plant height (cm), stem diameter at the base (cm), number of branches/plant, number of leaves/plant, means root length (cm), as well as fresh and dry weights of aerial parts 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 were 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.

Results and Discussion

Effect of lead (Pb), cadmium (Cd), and nickel (Ni) combinations on:

1- Survival percentage and vegetative and root growth traits

As shown in *Table 2*, survival of plants was 100% under the different treatments used in this study although the mean values of their vegetative and root growth parameters were greatly reduced by growing them in polluted soil, especially at the high concentrations of heavy metals (T3 and T4). This may indicate the high ability of plants to tolerate heavy metals toxicity due to their root type, which is fibrous and has a large surface area to cover and absorb more soil metals over time without damaging and affecting any of its tissues (Mamand et al., 2020).

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Table 2. *Effect of heavy metals combinations, growth period, and their interactions on survival, plant height, and stem diameter of Dodonaea viscosa L. plants during 2019/20 and 2020/21 seasons*

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	23.33e	88.33a	55.83A	0.287b	0.640a	0.464A
T1	100.00a	100.00a	100.00A	22.33ef	88.03a	55.18A	0.273b	0.633a	0.453A
T2	100.00a	100.00a	100.00A	20.30f	85.57b	52.94B	0.233b	0.600a	0.417A
T3	100.00a	100.00a	100.00A	15.63g	76.67c	46.15C	0.173b	0.633a	0.403A
T4	100.00a	100.00a	100.00A	14.50g	56.67d	35.59D	0.167b	0.533a	0.350B
Mean	100.00A	100.00A		19.22B	79.05A		0.227B	0.608A	
	Second season; 2020/2021								
Control	100.00a	100.00a	100.00A	25.70e	95.90a	60.80A	0.320d	1.113a	0.717A
T1	100.00a	100.00a	100.00A	24.50ef	95.97a	60.23A	0.303d	0.967b	0.635A
T2	100.00a	100.00a	100.00A	22.37f	91.03b	56.70B	0.260d	0.783c	0.522B
T3	100.00a	100.00a	100.00A	17.40g	85.13c	51.27C	0.207d	0.800c	0.504B
T4	100.00a	100.00a	100.00A	16.03g	64.03d	40.03D	0.193d	0.747c	0.470B
Mean	100.00A	100.00A		21.20B	86.41A		0.257B	0.882A	

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, data averaged in *Tables 2, 3, 4, 5, and 6* indicate that means of various growth traits, expressed as: plant height (cm), stem diameter (cm), No. branches and leaves/plant, leaf area (cm^2), root length (cm), No root branches/plant, as well as fresh and dry weights of top growth and roots (g) were gradually decreased with increasing HMs concentrations to reach the minimal values by T4 combination, comparing with records of either control or any other metal combinations in the two seasons. This may be due to the higher accumulation of toxic metals in the leaves and roots (as indicated in *Table 7*), which always leads to depression of vital processes and metabolism, such as photosynthesis, inhibition of some enzymatic systems and blocking the formation of proteins and chlorophylls (Adrees et al., 2015). In this regard, a reduction in glutathione reductase activity in relation to Cd and Pb stress was observed by Chauhan and Mathur (2020) in *Helianthus annuus*. Furthermore, Ma et al. (2018) suggested that the common consequence of heavy metals toxicity is the excessive accumulation of reactive oxygen species (ROS) and methyl glyoxal (MG), both of them can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and/or interact with other vital constituents of plant cells. These hazardous effects were documented by Lajayer et al. (2019) who mentioned that heavy metals may inhibit plant metabolic processes such as water uptake, N assimilation, respiration, photosynthesis and transcription, and may retard the different enzymatic activities via binding to sulfhydryl (SH-) groups and intensifying reactive oxygen species (ROS) production leading to oxidative stress. Besides, Mamand et al. (2020) stated that lead can negatively affect the structure of mitochondria through decreasing mitochondrial cristae and in turn lowering the capability of oxidative phosphorylation.

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

Growth period (G.P.) / Pollution treatments	No. branches/plant			No. leaves/plant			Leaf area (cm2)		
	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	1.00f	4.00a	2.50A	43.33f	481.70a	262.52A	14.57b	14.62b	14.60A
T1	1.00f	3.67b	2.34B	29.67g	453.30c	241.58B	14.47b	14.98a	14.73A
T2	1.00f	3.33c	2.17C	25.67h	450.30b	237.99B	12.90d	14.00c	13.45B
T3	1.00f	3.00d	2.00D	18.67i	385.70d	202.19C	11.83e	13.00d	12.42C
T4	1.00f	2.67e	1.84E	15.33j	280.00e	147.67D	9.87f	12.10e	10.99D
Mean	1.00B	3.33A		26.53B	409.90A		12.83B	13.74A	
	Second season; 2020/2021								
Control	1.00f	4.90a	2.95A	49.10e	438.00a	243.55A	15.00d	16.20b	15.60A
T1	1.00f	4.23b	2.62B	32.83f	436.00a	234.42B	14.77de	16.53a	15.65A
T2	1.00f	4.00c	2.500B	29.27g	410.30b	219.79C	13.70f	15.47c	14.58B
T3	1.00f	3.50d	2.25C	21.33h	360.00c	190.67D	12.63h	14.63e	13.63C
T4	1.00f	2.97e	1.99D	18.03i	295.90d	156.97E	11.03i	13.37g	12.20D
Mean	1.00B	3.92A		30.11B	388.04A		13.43B	15.24A	

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

Growth period (G.P.) / Pollution treatments	Root length			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	29.33d	38.33a	33.83A	14.23b	16.67a	15.45A	100.00a	100.00a	100.00A
T1	25.83f	34.33b	30.08B	11.67e	17.00a	14.34B	88.33c	90.00b	89.17B
T2	23.20g	33.00c	28.10C	10.67f	13.33c	12.00C	79.17f	86.43e	82.80C
T3	19.57h	33.33c	26.45D	10.17g	12.67d	11.42D	66.63h	87.13d	76.88D
T4	18.43i	26.67e	22.55E	10.00g	11.67e	10.84E	62.83i	69.63g	66.23E
Mean	23.27B	33.13A		11.35B	14.27A		79.39B	86.64A	
	Second season; 2020/2021								
Control	32.33e	30.50a	35.92A	12.83c	15.07ab	13.95A	100.00a	100.00a	100.00A
T1	28.77g	37.77b	33.27B	10.63e	15.30a	12.97B	89.17e	95.70b	92.44B
T2	26.37h	35.67d	31.05C	9.60f	14.73b	12.17C	81.63f	90.37d	86.00C
T3	21.87i	36.70c	29.29D	9.33f	11.43d	10.38D	67.57h	92.87c	80.22D
T4	20.87j	29.63f	25.25E	9.17f	10.43e	9.80E	64.53i	75.00g	69.77E
Mean	26.04B	35.85A		10.31B	13.39A		80.58B	90.79A	

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 5. *Effect of heavy metals combinations, growth period, and their interactions on top growth fresh and dry weights of Dodonaea viscosa 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.B.	Mean	1st G.P.	2nd G.B.	Mean
	First season; 2019/2020					
Control	5.02f	99.70a	52.36A	1.75f	35.26a	18.50A
T1	3.46fg	84.70c	44.08C	1.23f	30.26c	15.75B
T2	2.99fg	90.27b	46.63B	1.06f	31.43b	16.25B
T3	2.25g	73.67d	37.96D	0.82f	26.03d	13.42C
T4	1.81g	65.83e	33.82E	0.65f	23.37e	12.01D
Mean	3.11B	82.83A		1.10B	29.27A	
	Second season; 2020/2021					
Control	6.04f	109.40a	57.72A	2.18f	38.77a	20.48A
T1	4.22fg	93.57b	48.90B	1.54fg	32.97b	17.25B
T2	3.78fg	84.97c	44.38C	1.38fg	29.23c	15.31C
T3	3.09g	70.40d	36.75D	1.13g	24.83d	12.98D
T4	2.43g	64.70e	33.57E	0.90g	22.60e	11.75E
Mean	3.91B	84.61A		1.43B	29.68A	

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

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	1.60f	19.53a	10.57A	1.09e	8.03a	4.56A
T1	1.45f	17.50b	9.48B	0.99ef	7.40b	4.20B
T2	1.32fg	15.03c	8.18C	0.90e-g	6.33c	3.61C
T3	1.11g	14.43d	7.77D	0.76fg	6.17c	3.46C
T4	1.02g	12.60e	6.81E	0.69g	5.33d	3.01D
Mean	1.30B	15.82A		0.88B	6.65A	
	Second season; 2020/2021					
Control	2.01f	19.71a	10.86A	1.30f	9.93a	5.62A
T1	1.76g	18.10b	9.93B	1.16fg	9.03b	5.10B
T2	1.60g	14.81c	8.21C	1.03fg	7.37c	4.20C
T3	1.37h	13.57d	7.47D	0.92g	6.80d	3.86D
T4	1.40h	12.53e	6.97E	0.87g	6.27e	3.57E
Mean	1.63B	15.75A		1.06B	7.88A	

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 7. *Effect of heavy metals combinations, growth period, and their interactions on pigments and total soluble sugars concentrations in Dodonaea viscosa 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	1.388b	1.345b	1.367B	0.335a	0.368a	0.352A	0.326a	0.363a	0.345A	27.16a	16.10b	21.63A
T1	1.466a	1.446a	1.456A	0.359a	0.344a	0.352A	0.350a	0.315b	0.333A	25.67a	16.81b	21.24A
T2	1.304b	1.403a	1.354B	0.227c	0.328a	0.278B	0.208c	0.303b	0.256C	15.50c	15.78c	15.64B
T3	1.217c	1.363b	1.290C	0.199c	0.301b	0.250B	0.239c	0.340a	0.290B	14.81c	15.18c	15.00B
T4	1.194c	1.309b	1.252C	0.176c	0.298b	0.237C	0.194c	0.338a	0.266C	9.76d	16.89b	13.33C
Mean	1.314A	1.373A		0.259B	0.328A		0.264B	0.332A		18.58A	16.15B	

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 previous results, however are in agreement with those detected by Ma et al. (2018) on two *Taxodium* clones (T.118 and T.406), Eisa (2019) on *Populus nigra* and *Salix mucronata* and Chauhan and Mathur (2020) who found that the different concentrations of Pb, Cd, Cu and As caused morphological irregularities and hampered shoot and root lengths, fresh weight of shoot/root and leaf area of both varieties PBH and DRSF-108 of *Helianthus annuus*. The maximum shoot and root lengths were observed in control plants, while the minimum was recorded by PBH variety plants.

The only exception noticed in such work is that some HMs combinations, especially T1 caused either a slight improvement or reduction in some growth traits with non-significant differences relative to control in some cases of the two seasons. This may be referred to that some heavy metals, such as Cd at low concentration may act as co-factor for some metabolic enzymes. In this concern, Tauqeer et al. (2016) reported that the activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) in *Alternanthera bettzickiana* plant tissues increased under lower levels of Cd and Pb (0.5 and 1.0 mM), but decreased at higher ones (2.0 mM). On willow (*Salix mucronata*), Ouf and Gaber (2019) revealed that the HMs-contaminated soil obtained from the Sabaghy El-Baida district in Kafr El-Dawar City was superior than the non-contaminated soil (control) in improving growth parameters (plant height, stem diameter, leaf area, No. leaves, root length, fresh and dry weights of leaves, shoots, and roots) and wood properties (specific gravity and fiber length).

Data in the aforementioned tables indicate also that elongating growth period up to 18 months significantly improved the mean values of the different measured growth characters compared to their means recorded after only 6 months of growth. This may be reasonable because the plants took enough time for good growth and alter their growth behavior to cope with pollution stress. Furthermore, dodonaea plant is considered one of the quick-growing shrubs with high biomass production that can both tolerate and accumulate pollutants. It has also a great ability to restore its growth after pruning or any stress. In this respect, Ouf and Gaber (2019) found that various growth traits of *Salix mucronata* plants potted in MHs-contaminated soil were progressively improved with prolonging growth period from 6- to either 12- or 18 months.

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Accordingly, the best growth of plants under conditions of this trial was attained by combining between planting in either unpolluted soil mixture or T1 polluted one and 18 months growing period, as these two combined treatments gave the highest growth means, which were statistically at par with each other in most cases of both seasons.

These results could be documented by those of Salih and Aziz (2019) who stated that *Dodonaea viscasa* is a good bioindicator for pollution and has a high accumulation ability rendering it suitable for removing HMs from soil and atmosphere. Similarly, Mamand et al. (2020) mentioned that *Dodonaea viscosa* is the most effective phytoremediator among *F. benjamina*, *Myrtus communis*, and *Platycladus orientalis* because of its root type which is fibrous and has a large surface area to cover and absorb more HMs by time without injury. On *Salix mucronata*, Ouf and Gaber (2019) noticed that plant height, stem diameter, leaf area, No. leaves and fresh and dry weights of leaves and shoots mean values were progressively increased with time spanned up to the end of the experiment (18 months).

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

Data averaged in *Table 4* exhibit that PRI of control plants was 100% in the two seasons. However, the gradual increase in HMs concentrations was accompanied by a gradual decrease in the percentages of this index to be minimum by the highest concentrations of such metals (T4 combination), which reduced its means to 66.23% in the 1st season and to 69.77% in the 2nd one. Reduction of PRI to a percent higher than 66% in both seasons plus 100% survival under the highest concentrations of toxic metals clearly show that dodonaea plants are good tolerant for toxicity of Pb, Cd, and Ni metals under the conditions of such work. This may be ascribed to the good distribution of dodonaea fibrous roots in the polluted soil mixture without damage (Mamand et al., 2020). In this respect, Eisa (2019) suggested that *Populus nigra* and *Salix mucronata* are good candidates for remediation of Cd, Cu and Pb contaminated soil due to their high tolerance index. Likewise, Ouf and Gaber (2019) pointed out that growing *Salix mucronata* in HMs-contaminated soil linearly increased their root length with increasing growth period from 6 to either 12 or 18 months, increasing its tolerance to HMs toxicity over time.

The mean values of PRI registered in the second growth period (after 18 months from planting) were higher than those recorded in the first one because the plants gave longer roots in the 2nd growth period than those attained in the 1st one (*Table 4*). So, the highest % of PRI in the two seasons was achieved by combining between planting in soil mixture free from HMs and either of growth period, followed directly by connecting between planting in soil mixture of T1 combination and the longer growth period (18 months). These gains are in harmony with those of Mamand et al. (2020) on *Dodonaea viscasa*, Eisa (2019) on *Populus nigra* and *Salix mucronata*.

3- The chemical composition of leaves and roots

It is obvious from data presented in *Table 7* that chlorophyll a, b, and carotenoids concentrations (mg/g. f.w.) were significantly decreased by T2, T3, and T4 HMs combinations with the inferiority of T4 one that scored the least concentrations, whereas control and T1 combination recorded values closely near together with non-significant differences among themselves, except for T1 combination that raised chlorophyll-a concentration to the highest value, even over control value. Plants also acquired higher concentrations of the three pigments at a growth period of 18 months than those recorded

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at 6 months one. Generally, combining between planting in T1-polluted soil mixture and either 6- or 18-month growth period gave the utmost high concentration of chlorophyll a, while that was true for both chlorophyll b and carotenoids by interacting between planting in either control or T1-polluted soil mixture and either growth period.

The harmful effect of HMs on the photosystem may be referred to as the disturbances caused by the metals in Calvin cycle reactions and down-regulation or even feedback inhibition of electron transport by the excessive amounts of ATP and NADPH (Krupa et al., 1993). Besides, Droppa et al. (1996) found that Cd in greening leaves interferes with chlorophyll biosynthesis, and acts mainly by inhibiting the LHC synthesis into stable complexes required for normal functional photosynthesis activity.

Data presented in *Table 8* exhibit that N % in the leaves was progressively decreased with increasing HMs concentrations. The opposite was the right regarding P and K percentages, as their concentrations have fluctuated with non-significant differences in between, except for P %, which was reduced to the minimum (0.285%) by T2 combination, and K %, which was the least (1.63%) by control (zero HMs). In addition, the percentages of N and P measured after the long period growth was significantly higher than those measured after the short one. Thus, the highest concentration of N was achieved by interacting between planting in unpolluted soil mixture and the 18-months growth period, but that was true for P % by combining between planting in either T1 or T4 polluted soil and 18-months growth period. The highest K %, however was attained by most interaction treatments, especially after the short growth period (6 months).

Table 8. *Effect of heavy metals combinations, growth period, and their interactions on nitrogen, phosphorus, and potassium in Dodonaea viscosa 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	1.991b	2.857a	2.424A	0.259c	0.363b	0.311AB	1.47c	1.78b	1.63B
T1	2.213b	1.993b	2.103B	0.257c	0.451a	0.354A	1.93a	2.07a	2.00A
T2	1.337d	1.659c	1.448C	0.233c	0.337b	0.285C	2.13a	1.88b	2.01A
T3	1.116e	1.327d	1.222D	0.264c	0.418a	0.341A	1.98a	2.04a	2.01A
T4	0.996e	1.548c	1.272D	0.168d	0.452a	0.310AB	2.10a	1.79b	1.95A
Mean	1.531B	1.877A		0.236B	0.404A		1.92A	1.91A	

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

As for Pb, Cd, and Ni concentrations (mg/100 g d.w.), data presented in *Table 9* show that their mean values were gradually increased in the leaves and roots as a result of increasing their levels in the contaminated soil mixture to reach the maximum by T4 HMs-combination, except for Pb concentration that was maximum in the roots by T3 HMs-combination. On the other side, elongating the growth period to 18 months increased only Cd and Ni concentrations in the leaves over those scored in the 6 months one, and the opposite was the correct in the matter of Pb concentration. In the roots, however that was true for only Cd concentration which was 114.142 mg/100 g. d.w. after 18 months from planting against 99.725 mg/100 g d.w. after only 6 months of growth.

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Hence, combining between planting in T4 polluted soil mixture and growth period of 6 months gave the highest Pb concentration in the leaves, but for the highest Cd and Ni concentration in the leaves, it was attained by connecting between planting in T4 polluted soil and growth period of 18 months. In the roots, however the highest Pb concentration was obtained by planting in T3 polluted soil in the first growth period, while the highest Cd concentration was recorded by planting in T4 polluted soil in the second growth period, and that of Ni concentration was found due to planting in T4 polluted soil in the first growth period. In general, concentrations of Pb, Cd, and Ni in the roots were higher than those in the leaves, with few exceptions.

Table 9. *Effect of heavy metals combinations, growth period, and their interactions on lead, cadmium, and nickel concentrations in Dodonaea viscosa 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	39.341e	26.500h	32.921E	31.143i	73.535f	52.339E	14.187e	13.246e	13.717D
T1	47.635d	27.876h	37.756D	35.256h	80.333e	57.795D	17.146d	16.725d	16.936C
T2	56.495c	30.583g	43.539C	52.951g	91.675d	72.313C	18.500d	18.705d	18.603C
T3	62.138b	36.676f	49.407B	82.075e	120.310b	101.193B	21.893c	26.459b	24.176B
T4	73.663a	36.798f	55.231A	104.640c	152.923a	128.782A	26.376b	29.591a	27.984A
Mean	55.855A	31.687B		61.213B	103.755A		19.621B	20.945A	
	In the roots								
Control	74.548e	11.465h	43.007E	61.548h	74.665g	68.107E	23.290f	25.299e	24.295D
T1	83.436d	13.501h	48.469D	73.860g	87.740f	80.800D	26.795e	23.967f	25.381D
T2	91.742c	16.239g	53.991C	97.399e	115.036d	106.218C	28.932d	25.983e	27.458C
T3	116.501a	33.510f	75.006A	130.807c	139.768b	135.2088B	43.500c	30.786d	37.143B
T4	110.400b	35.102f	72.751B	135.012b	153.500a	144.256A	52.099a	46.179b	49.139A
Mean	95.326A	21.963B		99.725B	114.142A		34.923A	30.443B	

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

Absorption of metals by roots of plants grown in HMs-polluted soil may be necessary for these stressed plants to keep the equilibrium between their concentrations in soil solution and nutrient content in plant tissues. In this respect, Salih and Aziz (2019) observed that higher concentrations of Fe, Zn, As, Pb, Cd, and Co were recorded in the leaves of *Dodonaea viscosa* grown in the garden inside the steel factory (polluted site), which was accompanied with lower concentrations of Ni, chlorophyll a and b, total chlorophyll (a + b), carotenoids and protein. Similarly, Mamand et al. (2020) indicated that the highest bioaccumulation factor (BF) of Pb (39.15) was observed in *Dodonaea viscasa*. So, the maximum values of total Pb (1084.96 mg/kg) were detected in this garden plant. On *Mentha piperita*, Dinu et al. (2021) showed that Cd, Ni, and Pb were accumulated in the different parts of the plant, except for As.

In a biomonitoring study for HMs accumulation in some wild plants, Khan et al. (2021) revealed that the highest Co, Cu, Zn, Fe, and Mn contents were observed in *Digitaria*

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sanguinalis (0.3 mg/kg), *Hordeum leporinum* (15.7 mg/kg), *H. leporinum* (36.5 mg/kg), *Achnatherum hymenoides* (26.1 mg/kg) and *H. Leporinum* (28.3 mg/kg), respectively. One of the maximum dangerous impacts of HMs is the slow disappearance of chlorophyll and yellowing of leaves, which can be associated with a reduction in the photosynthesis potential (Ma et al., 2018).

Conclusion

From the previous results, it can be advised to use Hop bush plants as a good phytoremediator for toxic HMs.

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