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EVALUATION OF OLEANDER PLANTS CONTAMINATION GROWN IN CALCAREOUS SOIL CONTAMINATED WITH SOME HEAVY METALS (NI, CD, PB) ACCORDING TO INTERNATIONAL POLLUTION STANDARDS.

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ABSTRACT

A factorial pot experiment was conducted according to a completely randomized design (CRD) in greenhouse to evaluate contamination of oleander plant with some heavy metals, based on calculation of international environmental pollution indicators and effect of heavy metals on growth of oleander plant , with three factors and six replications. soil was treated with three heavy metals: Pb, Cd, and Ni, and four combinations: Pb + Cd, Cd + Ni, Ni + Pb, and Pb + Ni + Cd, with two levels of each element: 0 and 120 for Lead, 0 and 10 for Cadmium, and 0 and 70 mg kg-1 for Nickel with six time periods used for sampling: 40, 80, 120, 160, 200 and 240 days after planting, using sandy loam soil. Treatments symbolized as T_{Ni}, T_{Cd}, T_{Pb}, $T_{Cd,Ni}$, $T_{Ni,Pb}$, $T_{Cd,Pb}$, $T_{Cd,Ni,Pb}$, and T_O. Results showed an increase in values of bio concentration factor (BCF) of oleander root system for Ni in T_{Ni}, $T_{Cd,Ni}$, $T_{Cd,Ni,Pb}$, and $T_{Ni,Pb}$ treatments, reaching 0.9, 0.43, 0.50, and 0.77, respectively, and decreased in the rest treatments. Highest values were for Cd in treatment C, which reached 1.57, while it was less to other treatments. Values are lower from one for, oleander plant is considered one of plants that accumulate Cd. Values of BCF were low for Pb. Values of BCF increased with increasing time periods. Values of the bioaccumulation factor (BAC) increased with increase in concentrations of available heavy metals in soil. Values of biological transfer factor (TF) for Cd, Ni, and Pb decreased below value of one. Heavy metals added to soil significantly affected values of plant heights, and plant dry weight.

Key words: **contaminated soil; Phytotoxicity, plant pollution standards.**

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INTRODUCTION

Soil contamination with heavy metals is a serious environmental problem due to its non-degradability and long biological half-life in long term (Mapanda, *et,al*,2005; Ahmed, *et,al*,2009 and Ahmadpour, ,*et,al*,2012). Some of them show bioaccumulation, and most of them have toxic effects on living organisms when they exceed a certain concentration in soil. The disposal of municipal, industrial waste, some agricultural practices, use of agricultural chemicals can lead to accumulation of many heavy metals in soil. (8, 20). Release of toxic heavy metals into environment poses a major threat to water quality and aquatic ecosystems, endangering human health (Mapanda, *et,al*,2005; Balali-Mood, *et,al*, 2021,and Türkmen, *et,al*,2022) . Chyad *et al*. (2022) explained the presence of a high percentage of heavy metals such as Lead, Cadmium, Iron and Manganese in irrigation water due to water from hospitals and factories. Hameed and Naser (2023) showed that high concentrations of heavy metals: Cadmium, Zinc, and Nickel in industrial water, exceeding the limits permitted by World Food and Health Organization and irrigation with it leads to the accumulation of

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heavy metals in soil (Jumaa, and Al-Anbari, 2016). Naser (2023) showed that irrigation with water contaminated with heavy metals leads to accumulation of these elements in soil and makes it polluted.Some heavy metals could be absorbed and translocateded to higher parts of some plants and can be in food chains. Lead absorbed by some plants and accumulated in their tissues very quickly (Lehoczky, and Kiss, 2002). Heavy metals accumulate in plants and organisms that enter human food chain (Varol, and Sen, . 2009 and Al-Kshab,2023). Talib and Naser (2023) explained that soil pollution with heavy metals leads to a direct increase in concentrations of these elements in plants (leaves). Odeh and Nasser (2018) pointed out in their study to evaluate efficiency of white radish and carrot plants grown in soil contaminated with some heavy metals in absorbing and accumulating Cadmium, Lead, and Nickel in their tissues. Bio concentration factor (BCF) in plants for Pb, Cd, and Ni has exceeded value of one, and this indicates presence of a transfer of these elements from soil to plant, and their accumulation within root system is higher than shoot system. Value of translocation factor (TF) for Lead in the plant exceeds value of one, which indicates a lack of movement or transfer of Pb from the root system to shoot. The reason for this is the lack of movement of Lead element within plants, as it is an immobile element (semi-mobile) in plant, due to its high atomic weight, which led to its accumulation in root system compared to shoot system. It was observed that values of site TF for Cadmium and Nickel were high for white radish and carrot plants, and they exceeded value of one this indicating the presence of movement and transfer of these element within plant, as the element moved from root system towards vegetative system. Talib and Naser (2023) when studying the process of bio reclamation of soil contaminated with some heavy metals (Lead, Cadmium, and Nickel) using three ornamental plants showed high bio concentration factor (BCF) values to root system in from most to least: Cadmium > Nickel > Lead, which indicates high ability of plants to transfer and accumulate heavy metals in soil to root system. It is also noted that values of bioaccumulation factor (TF) for all plants are higher than value of one and for all heavy metals studied, values of bio transfer factor (TF) from root system to shoot for Cd and Ni are higher than one while it was less than one for Lead. Abdel Karim *et, al,* (2022) showed that high concentrations of Lead in soil increase its concentration in plant grown in it, which leads to higher values of bio concentration factor (BCF) of root system, bio transfer factor (TF) from root system to shoot, and values of bioaccumulation factor for plant. Al-Salmany and Aibrahim (10) showed an increase in values of translocation factor (TF), bioaccumulation factor (BAC), and bioconcentration factor (BCF) for Cadmium and Lead in plants growing in soils contaminated with them. Beautiful oleander shrub (*Nerium oleander*) is one of poisonous and highly toxic ornamental plants that is widely planted in homes, gardens, and on roads for purpose of decoration. The oleander plant is used as a bioaccumulator for trace elements and heavy metals (Türkmen, *et,al*,2022).Oleander plant is also among most diverse plants, as this plant lives in many different climates and tolerates a wide range of conditions, including calcareous soil. Research aims to estimate level of plant pollution with heavy metals based on calculating international environmental pollution indicators and effect of heavy metals on oleander plant growth.

MATERIALS AND METHODS

Afactorial pot experiment was conducted according to a completely randomized design

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(CRD) in greenhouse with three factors and six replicates: The first factor: soil contaminated with three heavy metals (prepared from sulfate of these elements): Pb, Cd, and Ni and four combinations: Pb + Cd, Cd + Ni, Ni + Pb, and Pb + Ni+Cd and control treatment(0,0,0) .The second factor: levels of adding heavy metals were (slightly higher than its critical levels in soil: 0 and 120 for Lead, 0 and 10 for Cadmium, and 0 and 70 mg $kg^{-1}$for Nickel, thus, coefficients are as follows: T_{Ni}: treatment of adding Nickel at a level 70 mg Ni kg^{-1} soil, T_{Cd}: treatment of adding Cadmium at a level 10 mg Cd kg^{-1} soil , T_{Pb} treatment of adding Lead at a level 120 mg Pb kg^{-1} soil, T_{CN}: treatment of adding Cadmium at a level 10 mg Cd kg^{-1} soil , Nickel at a level 70 mg Ni kg^{-1} soil together , $T_{Ni,Pb}$: treatment of adding Nickel at a level of 70 mg Ni kg^{-1} soil and lead at a level 120 mg Pb kg^{-1} soil together,$T_{Cd,Pb}$: treatment of adding Cadmium at a level 10 mg Cd kg^{-1} soil and lead at a level 120 mg Pb kg^{-1} soil together, $T_{Cd,Ni,Pb}$: treatment of adding both Cadmium at a level 10 mg Cd kg^{-1} soil Nickel at a level 70 mg Ni kg^{-1} soil and Lead at a level 120 mg Pb kg^{-1} soil together, in addition to a comparison T_O (not adding any heavy metal to soil), so number of treatments became: 8 (elements and combinations) x 2 (level) x 6 (replicates) = 96 experimental units. Using soil with a Loamy Sand texture taken from one of college's fields from a depth 0-30 cm. It was dried, grinding, and sieved with 4 mm sieve. A portion of soil was taken and sieved with a 2 mm sieve for determints some chemical, physical, and fertility properties before planting, along with estimating concentrations of heavy metals (Pb, Cd, and Ni) available and total in soil according to methods mentioned in (Jackson, 1958; Black, 1965 and Page, *et,al*,1982) (Table 1) . Soil was treated by some heavy metals (Ni, Cd, Pb)as in solutions of each element separately and in combinations of three elements again at two levels: 0 , 120 for Lead, 0 , 10 for Cadmium, and 0 , 70 mg kg^{-1} for Nickel. 20 kg of it was taken and placed in pots with a capacity of 20 kg, with a diameter of 33 cm, a base diameter of 23 cm, and a height 23 cm. Oleander seedlings (six-month-old young) were planted, one plant per pot, N, P , K fertilizers were added according to fertilizer recommendation (Spray fertilization 5 gL^{-1}) for solution containing NPK complex 20-20-20($N-P_2O_5-K_2O$) in 5gL^{-1} was foliar applied twice in season (Al-Falahi, and Al-Janabi. 2016). Irrigation was with tap water after 50% of plant's available water was exhausted by weighing pot with soil and plant based on loss of available water using gravimetric method. Total heavy metals in soil (Pb^{2+}, Ni^{2+}, Cd^{2+}) were determined at the end of experiment (240 days) after digesting soil using 4 ml of concentrated sulfuric acid and 2 ml of perchloric acid. Determination was made using an atomic absorption device (AAS) and according to method mentioned in (Black, 1965). Plant samples (entire plant, roots, stems, and leaves separately) were taken at the end of experiment (240-day), concentrations of heavy metals (Pb, Cd and Ni) were estimated. These concentrations were compared with their critical concentrations in plant (International determinants), and environmental pollution indicators for plant were calculated to estimate the extent of Plant pollution with these elements.Dry weight of entire plant (1 gm of plant) was calculated by taking a whole plant representing one of replicates of treatment, washing it with running tap water then deionized water to wash off the dirt and dust from plant , air-dried then oven dried for 24 hours for dry weight determination.. Heights of plants were measured using a measuring tape from level of soil surface to the growing apex of plant (Darwish,*et,al*,2016). Total chlorophyll measured according to method of (Harborn, 1973).

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Table 1. Some chemical and physical characteristics of study soil before planting.

	Value	Units
EC 1:1	1.60	dSm^{-1}
pH 1:1	7.40	-----
Soil Organic Matter	4.33	gKg^{-1}soil
$CaCO_3$	220.15	gKg^{-1}soil
CEC	13.01	Cmole + Kg^{-1}
Soluble Cations and Anions	—	$mmol.L^{-1}$
Calcium Ca^{2+}	4.10	=
Magnesium Mg^{2+}	2.66	=
Sodium Na^{+}	2.70	=
Potassium K^{+}	0.28	=
Carbonate CO_3	Nil	=
Bicarbonate HCO_3^-	1.50	=
Chloride Cl^-	9.70	=
Sulphate SO_4	2.21	=
Available Nitrogen	19.00	$mg.kg^{-1}$soil
Available Phosphorus	4.40	=
Available Potassium	69.00	=
Total Lead	10.53	$mg.kg^{-1}$soil
Total Cadmium	0.38	=
Total Nickel	0.54	=
Available Lead	0.20	$mg.kg^{-1}$soil
Available Cadmium	0.10	=
Available Nickel	0.16	=
Sand	840.00	$g.Kg^{-1}$
Silt	64.00	$g.Kg^{-1}$
Clay	96.00	$g.Kg^{-1}$
Soil Texture	Loamy Sand	

Total content of heavy metals (Cd, Pb, and Ni) in plant was estimated after digesting samples (shoot and root system) with a mixture of two acids ($HClO_4$ - H_2SO_4)(Lindsay, 1979), then measurement was carried out to estimate heavy metals with an atomic absorption device (Jones..and,.Beton. 2001). Plant environmental pollution indicators were calculated and are:

1- Bio Concentration Factor (BCF). (Yoon et,al, 2006)

BCF = (Metal)Root / (Metal)Soil.

BCF: Bioconcentration Factor., (Metal)Root: Concentration of element in root (mg kg^{-1} dry matter), (Metal)Soil: Total concentration of element in e soil (mg kg^{-1}).

If BCF value is greater than one, this indicates plant's high ability to absorb and accumulate heavy metal in its tissues, but if value is less than one, this indicates plant's inability to absorb heavy metal from soil in sufficient quantity. (Cui et,al,2007).

2- Bio Accumulation Coefficient (BAC) (Cui, et,al,2007and , Li, et,al,2007) .

BAC = (Metal) Shoot / (Metal)Soil.

Where BAC: bioaccumulation coefficient, (Metal) Shoot: concentration of element in vegetative part (mg kg^{-1} dry wt.of plants), (Metal) Soil: total concentration of element in soil (mg kg^{-1})

3- Translocation Factor (TF). (Cui,et,al,2007and , Li, et,al,2007) .

TF = $(Metal)_{Leaves}$ / $(Metal)_{Root}$

Where TF: translocation factor, (Metal) Leaves: concentration of element in the leaves (mg kg^{-1} dry matter), Root (Metal):

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concentration of element in root part (mg kg^{-1} dry wt.of plants).

RESULTS AND DISCUSSION

Plant contamination Standards with heavy metals

Bio Concentration Factor (BCF).

Nickel (BCF-Ni)

Results of statistical analysis of Table (2) show that there are significant differences in values of bio concentration factor of Ni for oleander plant , for different treatments and time periods, as highest values in treatments T_{Ni}, $T_{Cd,Ni}$, $T_{Cd,Ni,Pb}$, and $T_{Ni,Pb}$ reached 0.77, 0.50, 0.43, and 0.39, respectively, while values were lower in other treatments, reaching 0.17, 0.15, 0.16, and 0.19 for treatments T_{Cd}, T_{Pb}, $T_{Cd,Pb}$, and control treatment O, respectively. It is noted from above that factor values for treatments in which Nickel and Cadmium are present are greater than values of treatments where Lead was present. This may be due to fact that Cadmium and Nickel are more mobile in soil than Lead, which leads to increased absorption by plant roots. This is consistent with what was indicated by Odeh and Naser (2018), who showed that Nickel is a mobile element in soil and plants. The time periods significantly affected values of Nickel bio concentration factor, as values began to gradually increase with increasing time periods giving 0.13, 0.19, 0.26, 0.34, 0.46, and 0.67 for time periods of 40, 80, 120, 160, 200 and 240 days, respectively. This may be due to increased plant absorption of Nickel with increasing periods of time, increased plant growth, and increased root biomass, thus increasing values of this factor. The binary interaction had a highly statistically significant effect on values of Nickel bio concentration factor, which were higher values in treatments T_{Ni}, $T_{Cd,Ni}$, $T_{Ni,Pb}$, and $T_{Cd,Ni,Pb}$ reached 1.71, 0.96, 0.70 and 0.80, respectively, for time period of 240 days. They decreased in rest treatments for same time period, as they reached 0.31, 0.27, 0.28, and 0.34 for the treatments T_{Cd}, T_{Pb}, $T_{Cd,Pb}$, and T_O, respectively. When observing all values, we find that they are less than one, except for N treatment for 240 days. This indicates weak absorption and accumulation of Nickel in plant tissues according to values of this indicator, except for N treatment for 240 days, as value exceeded one, Which indicates that oleander plant has a high ability to absorb and accumulate Nickel when it is added alone for a period of 240 days. These results are consistent with findings of Cui *et, al.* (2007), which showed that oleander plant is one of plants that accumulate Nickel.

Table 2. Bioaccumulation factor (BCF) for Nickel for different treatments and time periods.

Treatment	Duration (day)						Average
	40	80	120	160	200	240	
T_{Ni}	0.277	0.394	0.526	0.706	1.008	1.714	0.771
T_{Cd}	0.050	0.088	0.140	0.192	0.245	0.319	0.172
T_{Pb}	0.043	0.084	0.128	0.178	0.223	0.277	0.156
$T_{Cd,Ni}$	0.198	0.289	0.387	0.496	0.679	0.962	0.502
$T_{Pb,Ni}$	0.177	0.253	0.328	0.389	0.504	0.705	0.393
$T_{Cd,Pb}$	0.046	0.086	0.133	0.183	0.228	0.288	0.161
$T_{Cd,Ni,Pb}$	0.189	0.266	0.346	0.435	0.572	0.803	0.435
T_O	0.060	0.106	0.162	0.208	0.268	0.346	0.192
Average	0.130	0.196	0.269	0.348	0.466	0.677	
Treatment LSD5%	0.179*						
Duration LSD5%	0.155*						

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1.2 Cadmium (BCF-Cd)

Results of statistical analysis of Table (3) show that there are highly statistically significant differences in values of Cadmium bio concentration factor for different time periods, as highest values in treatment T_{Cd} reached 1.57, which exceeded value of one , therefore, oleander plant is one of plants that has a high ability to absorb Cadmium and adapt to it without causing damage or poor growth in plant, while values were less than one for rest parameters, reaching 0.68, 0.77, 0.98, 0.58, 0.64, 0.50 for T_{Ni}, T_{Pb}, $T_{Cd,Ni}$, $T_{Ni,Pb}$, $T_{Cd,Pb}$, $T_{Cd,Ni,Pb}$ and T_O treatments sequentially. Time periods significantly affected values of bio concentration factor for cadmium. highest values were for time periods of 200 and 240 days, reaching 1.07 and 1.36, its value exceeded one, so plant in these time periods is an accumulator of Cadmium because it can absorb and accumulate Cadmium in its tissues without causing damage, while values were less than one for other time periods, reaching 0.41, 0.55, 0.71, and 0.88, and for time periods 40 and 80, 120 and 160 days, respectively. When observing overlap between transactions and time periods, we find that treatment T_{Cd} is superior in values of this factor for all time periods except first time period. It is also noted that most of transactions exceeded value of one in last time period, reaching 1.28, 1.41, 1.53, 1.02 and 1.52 for T_{Ni}, T_{Pb}, $T_{Cd,Ni}$, $T_{Cd,Pb}$ and T_O treatments sequentially. From above, it clear that oleander plant, can be consider as an accumulator of Cadmium as a result of its increased absorption from soil and its accumulation in plant tissues to above-mentioned treatments. This is consistent with what was indicated by Mostafa *et,al*1996.and Talib and Naser ,2023) who showed increase in size and weight of plant's roots and shoots with passage of time causes an increase in absorption and accumulation of Cadmium in plant tissues. These results are consistent with the findings of AL.Aboudi *et,al*.(2022), who indicated that the oleander plant is one of plants that accumulate Cadmium.

Table 3. Bioaccumulation factor (BCF) for Cadmium for different treatments and time periods Lead (BCF-Pb)

Treatment	Duration (day) 40	80	120	160	200	240	Average
T_{Ni}	0.296	0.410	0.520	0.675	0.937	1.280	0.686
T_{Cd}	0.895	1.029	1.470	1.623	2.005	2.414	1.573
T_{Pb}	0.350	0.490	0.613	0.823	0.968	1.416	0.777
$T_{Cd,Ni}$	0.445	0.695	0.821	1.130	1.308	1.530	0.988
$T_{Pb,Ni}$	0.290	0.385	0.510	0.619	0.736	0.968	0.585
$T_{Cd,Pb}$	0.333	0.441	0.502	0.729	0.857	1.022	0.647
$T_{Cd,Ni,Pb}$	0.299	0.390	0.439	0.519	0.616	0.748	0.502
T_O	0.442	0.577	0.805	0.937	1.137	1.521	0.903
Average	0.419	0.552	0.710	0.882	1.071	1.362	
Treatment LSD5%	0.155*						
Duration LSD5%	0.135*						

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Lead (BCF-Pb) Results of statistical analysis of Table 4 indicate that there are significant differences in values of Lead bioconcentration factor for different treatments and time periods. Treatments varied in their values regarding this factor, treatment in which Lead alone (T_{Pb}) was added was superior to rest treatments which value reached 0.47 compared to other parameters, which are T_{Ni}, T_{Cd}, $T_{Cd,Ni}$, $T_{Ni,Pb}$, $T_{Cd,Pb}$, $T_{Cd,Ni,Pb}$, and T_O, values of which reached 0.20, 0.15, 0.14, 0.27, 0.21, 0.18, and 0.25, respectively. It is noted that all values were less than one, thus according to this factor, plant is considered to have little absorption of Lead. This may be due to fact that Lead is one of immobile elements in soil and is slow-moving, so its absorption by plant is reduced (Marchiol,*et,al*,2004), this is consistent with what was indicated by (Odeh, and Naser. 2018), who showed the lack of movement or transfer of Lead from soil to root system and then to vegetative system, because it is an immobile element (semi-mobile) in plant due to its high atomic weight, which led to its accumulation in root system compared to vegetative system. Time periods also had a highly significant effect on values of Lead bioconcentration factor, as it is noted that there is a significant increase in factor values with increasing time periods. However, all values are less than one, so the plant with these time periods is considered to have little absorption of Lead, as the values reached 0.102, 0.153, 0.207, 0.267, 0.313, and 0.387 for time periods of 40, 80, 120, 160, 200, and 240 days, respectively.

Table 4. Bioconcentration factor (BCF) of lead for different treatments and time periods.

Treatment	Duration (day)						Average
	40	80	120	160	200	240	
T_{Ni}	0.056	0.105	0.181	0.228	0.272	0.387	0.205
T_{Cd}	0.050	0.081	0.114	0.183	0.213	0.270	0.152
T_{Pb}	0.225	0.299	0.420	0.549	0.628	0.750	0.479
$T_{Cd,Ni}$	0.044	0.084	0.111	0.158	0.199	0.259	0.143
$T_{Pb,Ni}$	0.150	0.197	0.242	0.299	0.350	0.407	0.274
$T_{Cd,Pb}$	0.117	0.159	0.185	0.235	0.271	0.310	0.213
$T_{Cd,Ni,Pb}$	0.099	0.138	0.168	0.202	0.238	0.271	0.186
T_O	0.073	0.160	0.231	0.284	0.334	0.438	0.253
Average	0.102	0.153	0.207	0.267	0.313	0.387	
Treatment LSD5%	0.053*						
Duration LSD5%	0.046*						

2. Bioaccumulation Coefficient (BAC)

2.1. Nickel (BAC-Ni): Table (5) indicate that there are highly statistically significant differences in values of Nickel bioaccumulation coefficient for different treatments and time periods. Highest values reached 0.31 for treatment of adding Nickel alone (T_{Ni}), then came treatments TCd, $T_{Ni,Pb}$, and $T_{Cd,Ni,Pb}$, where values reached 0.202 and 0.155 and 0.167 respectively, while T_{Cd}, T_{Pb}, and $T_{Cd,Pb}$ treatments reached 0.067, 0.060 and 0.062 respectively. Increase in value of bioaccumulation coefficient in N treatment may be attributed to addition of Nickel alone to soil, which led to an increase in its availability and absorption by plant and its accumulation in its tissues, these results are consistent with the findings of Mohson and Mohson (2008).It is noted from values that all of them did not exceed value of one, so plant is considered a poor absorber of Nickel, this may be due to low concentrations of element added to soil or the deposition of element in soil due to increase in pH value in calcareous soil used, this is consistent with what was found by Farhan(2020) and Liu *et,al*. (2018), who showed that Nickel deposition increases in soil with an increase in value of soil reaction degree, due to its deposition or adsorption on surfaces of soil particles.

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Time periods had a significant effect on values of Nickel bioaccumulation. They generally increased with time, reaching 0.115, 0.156, 0.141, 0.137, 0.138 and 0.155 for time periods of 40, 80, 120, 160, 200 and 240 days, respectively. However, values did not reach to stage of sufficient absorption of Nickel into soil, and this may be attributed to reasons mentioned above.

Table 5. Bioaccumulation coefficient (BAC) for Nickel for different treatments and time periods

Treatment	Duration (day)						Average
	40	80	120	160	200	240	
T_{Ni}	0.256	0.317	0.277	0.307	0.331	0.407	0.316
T_{Cd}	0.041	0.078	0.078	0.072	0.068	0.067	0.067
T_{Pb}	0.037	0.073	0.067	0.063	0.061	0.057	0.060
$T_{Cd,Ni}$	0.176	0.221	0.206	0.201	0.209	0.201	0.202
$T_{Pb,Ni}$	0.152	0.193	0.169	0.145	0.140	0.128	0.155
$T_{Cd,Pb}$	0.039	0.074	0.067	0.066	0.063	0.060	0.062
$T_{Cd,Ni,Pb}$	0.161	0.201	0.178	0.161	0.155	0.146	0.167
T_O	0.055	0.090	0.089	0.084	0.078	0.176	0.095
Average	0.115	0.156	0.141	0.137	0.138	0.155	
Treatment LSD5%	0.029*						
Duration LSD5%	0.025*						

2.2. Cadmium (BAC-Ni)

Results of statistical analysis of Table (6) show that there are highly statistically significant differences in values of Cadmium bioaccumulation coefficient for different treatments and time periods, with highest value reaching 1.132 in treatment of Table (6). Bioaccumulation coefficient (BAC) of cadmium for different treatments and time periods. Adding Cadmium alone (T_{Cd}), which differed significantly from rest treatments in which coefficient values were 0.600 and 0.585. 0.667, 0.416, 0.470, and 0.363 for T_{Ni}, T_{Pb}, $T_{Cd,Ni}$, $T_{Ni,Pb}$, $T_{Cd,Pb}$ and $T_{Cd,Ni,Pb}$ respectively. Increase in value of parameter in treatment T_{Cd} may be due to adding Cd alone to soil, and that plant was able to absorb a large amount of it compared to other treatments, comparison treatment in which the value was higher than the other treatments except for T_{Cd}, this may be due to not adding other elements to soil in this treatment.

Plant was able to absorb Cd from soil and accumulate it in its internal tissues without competing with other elements, because Cd is one of mobile elements in soil compared to other elements, this is consistent with what was found by Cui *et,al*. (2007), who showed that oleander plant is one of plants that accumulate Cadmium. Time periods significantly affected values of Cd bioaccumulation factor. Relationship was positive with increasing time periods, reaching 0.371, 0.470, 0.613, 0.721, 0.728, and 0.797 for time periods of 40, 80, 120, 160, 200, and 240 days, respectively.

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Table 6. Bioaccumulation coefficient (BAC) of cadmium for different treatments and time periods

Treatment	Duration (day) 40	80	120	160	200	240	Average
T_{Ni}	0.234	0.321	0.437	0.675	0.812	1.120	0.600
T_{Cd}	0.827	0.941	1.327	1.242	1.259	1.195	1.132
T_{Pb}	0.283	0.372	0.500	0.764	0.718	0.875	0.585
$T_{Cd,Ni}$	0.420	0.568	0.664	0.752	0.791	0.807	0.667
$T_{Pb,Ni}$	0.241	0.298	0.408	0.547	0.500	0.500	0.416
$T_{Cd,Pb}$	0.321	0.412	0.476	0.524	0.539	0.546	0.470
$T_{Cd,Ni,Pb}$	0.273	0.357	0.401	0.390	0.380	0.376	0.363
T_O	0.365	0.488	0.694	0.875	0.827	0.956	0.701
Average	0.371	0.470	0.613	0.721	0.728	0.797	
Treatment LSD5%				0.132*			
Duration LSD5%				0.114*			

3.2. Lead (BAC-Pb)

Results of statistical analysis of Table (7) indicate that there are highly statistically significant differences in values of Lead bioaccumulation coefficient for different treatments and time periods, as highest value reached 0.255 in treatment of adding Lead alone (T_{Pb}), followed by treatments in which Pb was added with other elements, namely NP and $T_{Cd,Pb}$, where value of coefficient reached 0.147 and 0.106, respectively. These coefficients differed significantly from rest coefficients, in which value of factor reached 0.090, 0.070, 0.063, and 0.087 for T_{Ni}, T_{Cd}, $T_{Cd,Ni}$ and $T_{Cd,Ni,Pb}$, treatments respectively. Increase in value of factor in case of adding Lead alone in treatment T_{Pb} is due to increase in availability of Lead in soil and absence of elements another competition for it is in soil solution, which led to an increase in its absorption by plant and its accumulation in its internal tissues. Time periods significantly affected the values of Lead bioaccumulation coefficient. The value increased from first to third time period, reaching 0.084, 0.118, and 0.150. Then values gradually decreased until end, reaching 0.132, 0.112, and 0.107 for time periods of 160, 200 and 240 days, respectively. It is noted that all values are less than one, and this indicates a decrease in absorption of Lead from soil, which may be attributed to this is due to lack of it added to soil, its slow movement in soil, or transformation of available form of it into a precipitate or adsorbent due to conditions of calcareous soil. When value of this factor is greater than one, this indicates that plant is one of plants that accumulate heavy metals, and this allows plant to be used in phytoextraction technique, which is a technique used on site to treat contaminated soil by absorbing heavy metals by roots of plant, then they are attributed to this is due to lack of it added to soil, its slow movement in soil, or transformation of available form of it into a precipitate or adsorbent due to conditions of calcareous soil. When value of this factor is greater than one, this indicates that plant is one of plants that accumulate heavy metals, and this allows plant to be used in phytoextraction technique, which is a technique used on site to treat contaminated soil by absorbing heavy metals by roots of plant, then they are transported and accumulated in parts. other parts of plant (Cristaldi,*et*,*al*,2017).

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Table 7. Bioaccumulation coefficient (BAC) of Lead for different treatments and time periods

Treatment	Duration (day) 40	80	120	160	200	240	Average
T_{Ni}	0.047	0.083	0.141	0.113	0.076	0.081	0.090
T_{Cd}	0.035	0.067	0.097	0.091	0.066	0.065	0.070
T_{Pb}	0.187	0.234	0.299	0.285	0.268	0.255	0.255
$T_{Cd,Ni}$	0.030	0.055	0.088	0.075	0.071	0.061	0.063
$T_{Pb,Ni}$	0.127	0.162	0.189	0.154	0.127	0.121	0.147
$T_{Cd,Pb}$	0.099	0.128	0.116	0.109	0.096	0.088	0.106
$T_{Cd,Ni,Pb}$	0.086	0.117	0.091	0.079	0.076	0.074	0.087
T_O	0.061	0.098	0.175	0.152	0.117	0.108	0.119
Average	0.084	0.118	0.150	0.132	0.112	0.107	
Treatment LSD5%	0.021*						
Duration LSD5%	0.019*						

3.Translocation factor (TF)

3.1. Nickel (TF-Ni)

Table (8) show that there are highly statistically significant differences in values of site translocation coefficient of Nickel for different treatments and time periods, as values ranged between 0.620 and 0.725 for different treatments, except for comparison treatment, which had highest value of 0.757, this may be due to absorption of Nickel from soil by plant without competition with other elements. It is noted that all values do not exceed one thus, transfer of Nickel from roots to leaves is weak, due to lack of absorption by plant due to small amount of it added to soil. Time period significantly affected values of TF for Nickel. Values began to rise gradually with increasing time periods, they reached 0.499, 0.650, 0.693, 0.760, 0.756, and 0.735 for time periods of 40, 80, 120, 160, 200, and 240 days, respectively. Increase in value of TF with time may be due to increased plant growth and increased biomass (root and vegetative), which led to increased absorption of Nickel and increased transfer within plant, these results are consistent with value of Nickel bioaccumulation coefficient (Table 5) and with what was indicated by Kabata-Pendias, and Pendias,(2001), who showed that Ni is a moderately mobile element within plant from roots to upper parts. This is also consistent with what Odeh and Naser(2018) explained, which shows that Nickel is a mobile element within the plant, and thus it reaches the upper parts of plant and its accumulation in plant increases with increasing concentrations in contaminated soil and the aging of the plant parts.

Table 8. Translocation factor (TF) for Nickel for different treatments and time periods

Treatment	Duration (day) 40	80	120	160	200	240	Average
T_{Ni}	0.438	0.639	0.751	0.739	0.714	0.638	0.653
T_{Cd}	0.585	0.717	0.627	0.794	0.796	0.830	0.725
T_{Pb}	0.554	0.658	0.700	0.775	0.821	0.809	0.720
$T_{Cd,Ni}$	0.418	0.613	0.715	0.741	0.705	0.647	0.640
$T_{Pb,Ni}$	0.420	0.579	0.696	0.702	0.701	0.635	0.622
$T_{Cd,Pb}$	0.558	0.687	0.673	0.777	0.811	0.814	0.720
$T_{Cd,Ni,Pb}$	0.409	0.588	0.701	0.717	0.673	0.629	0.620
T_O	0.608	0.717	0.678	0.838	0.824	0.876	0.757
Average	0.499	0.650	0.693	0.760	0.756	0.735	
Treatment LSD5%	0.055*						
Duration LSD5%	0.048*						

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Cadmium (TF-Cd)

Table (9) show that there are highly significant differences in values of TF - Cd for different treatments and time periods. Highest values in T_{Ni}, T_{Cd}, T_{Pb}, and $T_{Ni,Pb}$ treatments were 0.728, 0.725, 0.758, and 0.721 respectively, while other treatments had lower values of 0.603. 0.627 and 0.617 for $T_{Cd,Ni}$, $T_{Cd,Pb}$ and $T_{Cd,Ni,Pb}$ treatments respectively. It is noted that highest value in comparison treatment was 0.778, and this result was consistent with result of bioaccumulation factor for Cadmium (Table 6). This may be attributed to fact that Cd is a mobile element within plant compared to Ni and Pb, which leads to its rapid movement within plant, these results are consistent with what was indicated by Kabata-Pendias, and Pendias,(2001) they showed that Cadmium is one of elements that moves within plant from roots to upper parts of plant. These results are also consistent with what was obtained by Talib and Naser (2023), who showed that Cd is one of elements that moves well within plant and moves from root system to shoot system. Time periods significantly affected values of TF-Cd , it is noted that direct relationship between time periods and coefficient values reached 0.581, 0.601, 0.666, 0.745, 0.769, and 0.806 for time periods of 40, 80, 120, 160, 200, and 240 days, respectively, this may be attributed to increased plant growth over time and increased absorption of Cd, thus increasing its transfer within plant. This result is consistent with results of Cd bioaccumulation factor (Table 6).

It was shown from above results that all values did not exceed one, and this indicates a weak transfer process of element into plant, this may be attributed to small amount available present in soil with added quantity of Cd and its lack of availability due to calcareous soil conditions, this is consistent with what Farhan (2020) showed that increasing degree of soil interaction and influence of carbonate minerals in calcareous soils leads to deposition of Cd in soil in form of Cadmium carbonate ($CdCO3$), which reduces its readiness in soil (Prasad, 2006,and Tahervand, and Jalali, 2016) .

Table 9. Site transfer factor (TF) for cadmium for different treatments and time periods.

Treatment	Duration (day)						Average
	40	80	120	160	200	240	
T_{Ni}	0.438	0.639	0.751	0.739	0.714	0.638	0.653
T_{Cd}	0.585	0.717	0.627	0.794	0.796	0.830	0.725
T_{Pb}	0.554	0.658	0.700	0.775	0.821	0.809	0.720
$T_{Cd,Ni}$	0.418	0.613	0.715	0.741	0.705	0.647	0.640
$T_{Pb,Ni}$	0.420	0.579	0.696	0.702	0.701	0.635	0.622
$T_{Cd,Pb}$	0.558	0.687	0.673	0.777	0.811	0.814	0.720
$T_{Cd,Ni,Pb}$	0.409	0.588	0.701	0.717	0.673	0.629	0.620
T_O	0.608	0.717	0.678	0.838	0.824	0.876	0.757
Average	0.499	0.650	0.693	0.760	0.756	0.735	
Treatment LSD5%	0.055*						
Duration LSD5%	0.048*						

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Lead (TF-Pb)

Table (10) indicate that there are highly statistically significant differences in values of TF-Pb for different treatments and time periods, as highest values in T_{Ni}, T_{Cd}, and $T_{Ni,Pb}$ treatments amounted to 0.768, 0.744, and 0.712, respectively, while other treatments had lower values than that, it reached 0.652, 0.605, 0.645 and 0.656 for T_{Pb}, $T_{Cd,Ni}$, and $T_{Cd,Pb}$ and $T_{Cd,Ni,Pb}$, respectively. It is noted that all values are less than one, and therefore process of TF-Pb into plant is light. This may be attributed to fact that Pb is a slow-moving within plant, which leads to an increase in its accumulation in roots and a decrease in its transfer to shoot. These results are consistent with what was indicated by .Kabata-Pendias, and Pendias (2001), who showed that Pb is slow to move from roots to other parts of plant, as it is strongly linked to root cells. This is also consistent with what was mentioned by Talib and Naser (2023), who stated that Pb is slow to move inside plant because it a semi-mobile element in plants due to its high atomic weight. Time periods had a significant effect on values of the TF-Pb. Relationship was direct between time periods and values of TF-Pb, reaching 0.552, 0.588, 0.742, 0.784, and 0.785 for time periods of 40, 80, 120, 160, 200 and 240 days, respectively. Values increase with time. It is attributed to increased plant growth, increased biomass, and its ability to absorb Pb from soil and transfer it to other parts of plant.These results are consistent with what was obtained in the Lead bioaccumulation factor (Table 7).

Table 10. Location transfer factor (TF) for lead for different treatments and time periods.

Treatment	Duration (day)						Average
	40	80	120	160	200	240	
T_{Ni}	0.590	0.660	0.727	0.892	0.920	0.817	0.768
T_{Cd}	0.516	0.640	0.754	0.822	0.834	0.896	0.744
T_{Pb}	0.521	0.616	0.708	0.682	0.698	0.687	0.652
$T_{Cd,Ni}$	0.528	0.462	0.636	0.571	0.696	0.739	0.605
$T_{Pb,Ni}$	0.552	0.667	0.790	0.757	0.752	0.754	0.712
$T_{Cd,Pb}$	0.561	0.531	0.643	0.656	0.709	0.769	0.645
$T_{Cd,Ni,Pb}$	0.505	0.566	0.671	0.684	0.740	0.769	0.656
T_O	0.645	0.558	0.666	0.872	0.926	0.850	0.753
Average	0.552	0.588	0.699	0.742	0.784	0.785	
Treatment LSD5%	0.065*						
Duration LSD5%	0.056*						

Growth indicators

Plant lengths (cm).

Table (11) show that there are highly significant differences between different treatments in lengths of plants for different time periods. Treatment T_{Ni} excelled in having highest plant length, reaching 117.50 cm, comparison treatment came after it, then $\mathbf{T_{Cd,Ni,Pb}}$, $\mathbf{T_{Cd}}$, $\mathbf{T_{Pb,Ni}}$, $\mathbf{T_{Cd,Pb}}$, $\mathbf{T_{Cd,Ni}}$, and $\mathbf{T_{Pb}}$ treatments, as lengths plant reached 117.16, 116.83, 115.33, 111.50, 106.58 and 101.50 cm respectively. Superiority of $\mathbf{T_{Ni}}$ treatment may be due to fact that Nickel is one of micronutrients for plant and is therefore beneficial in growth process. Comparison treatment, it had highest lengths due to absence of heavy metals being added to soil, and thus there were no consequential damages. As for time periods only, they had a highly statistically significant effect on lengths of plants, as lengths reached 103.50, 106.68, 110.50, 113.12, 116.29, and 119.25 cm for time periods of 40, 80, 120, 160, 200 and 240 days, respectively. The binary intervention had a highly statistically significant effect on heights of plants, as highest values were in treatment O, which reached 131.00 cm and for 240 days it reached 62.98, then treatments $\mathbf{T_{Ni}}$,

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T_{Cd}, $T_{Cd,Ni,Pb}$, $T_{Cd,Pb}$, $T_{Pb,Ni}$, $T_{Cd,Ni}$ and **T_{Pb}** treatments, as lengths of plants reached 128.00, 122.00 and 120.00. and 118.00, 117.50, 111.00 and 105.50 cm, respectively.

Table 11. Plant lengths (cm) for plants of different treatments and for all time periods.

Treatment	Duration (day) 40	80	120	160	200	240	Average
T_{Ni}	106.000	111.000	116.000	120.000	124.000	128.000	117.500
T_{Cd}	107.000	110.000	115.000	118.000	120.000	122.000	115.333
T_{Pb}	95.500	97.000	102.000	103.000	105.000	106.500	101.500
$T_{Cd,Ni}$	101.000	103.000	105.000	106.000	110.330	111.000	106.055
$T_{Pb,Ni}$	105.000	107.000	111.500	113.000	115.000	117.500	111.500
$T_{Cd,Pb}$	96.500	101.000	104.000	108.000	112.000	118.000	106.583
$T_{Cd,Ni,Pb}$	112.000	115.500	116.500	118.000	119.000	120.000	116.833
T_O	105.000	109.000	114.000	119.000	125.000	131.000	117.167
LSD 5% Enteraction			1.951*				LSD 5% Treatment 0.797 *
Duration average	103.500	106.688	110.500	113.125	116.291	119.250	

Dry weight (g plant^{-1})

Table (12) show that there are highly significant differences in dry weight of plants for different treatments and for all time periods. Highest weight reached 148.00 g plant^{-1} in control treatment, then T_{Ni}, $T_{Cd,Ni,Pb}$, T_{Cd}, $T_{Pb,Ni}$, $T_{Cd,Pb}$, $T_{Cd,Ni}$, and T_{Pb} treatments reached 132.35, 118.82, 100.59, 96.79 88.86 and 86.21 gm plant^{-1}, respectively. These results were consistent with results of plant heights. Increase in dry weight of plants in comparison treatment may be due to lack of adding heavy metals to soil, and thus there are no harmful effects on plants. N treatment came in second place, then treatments in which Nickel was added, because Ni is one of micronutrients that plant needs for its growth. Time period significantly affected the increase in dry weight of plants for all treatments, as dry weights of plants reached 78.43, 93.16, 105.55, 117.84, 125.99 and 130.62 g plant^{-1} respectively, for time period of 40, 80, 120, 160, 200 and 240 days. Results of Statistical analysis are shown a highly significant bilateral interaction between different treatments and time periods in dry weights of plants. Highest values were in the T_O treatment for 240 days, amounting to 198.70 gm plants^{-1}, while the lowest value was for P treatment during 40 days, amounting to 62.72 g plants^{-1}.

Table 12. Dry weight (gm plant^{-1}) of plants of different treatments for all time periods

Treatment	Duration (day) 40	80	120	160	200	240	Average
T_{Ni}	98.130	118.600	130.100	145.030	150.780	151.480	132.353
T_{Cd}	72.830	87.050	104.040	110.150	113.280	116.200	100.592
T_{Pb}	62.750	74.440	89.850	94.400	96.700	99.130	86.212
$T_{Cd,Ni}$	64.080	70.330	81.930	100.150	106.510	110.200	88.867
$T_{Pb,Ni}$	72.600	88.800	94.630	100.230	110.800	113.680	96.790
$T_{Cd,Pb}$	71.450	86.340	92.200	96.300	111.900	120.360	96.425
$T_{Cd,Ni,Pb}$	92.700	106.000	120.400	127.300	131.300	135.250	118.825
T_O	92.900	113.760	131.300	169.200	186.660	198.700	148.753
LSD 5% Enteraction			2.476*				LSD 5% Treatment 1.011*
Duration average	78.430	93.165	105.556	117.845	125.991	130.625	
LSD 5% Duration			0.875*				

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From the above finding,it can be concluded that:

Heavy metals can be similar to contamination of soil with nearby industrial or sewage effluent or any anthropogenic actively disposal in soil.When such contaminations of such heavy metals can reach soil apollution with such elements can be expected.The ability of Oeander plants (*Nerium oleandet*) to accumulate some heavy metals especially in its aerial parts can suggest its ability to be phytoremediate especially it is an inedible plant and far from food chain.

Conclusions

Soil pollution with some heavy metals led to elevated soil and plant pollution indices. Cadmium had the most significant impact on soil and plant health, unlike nickel and lead. Time periods significantly affected the increase in indices, increase in concentration of available heavy metals in the soil, and decrease in oleander plant growth indices.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

AUTHOR/S DECLARATION

- We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.
- Author/s signature on Ethical Approval Statement.

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تقييم تلوث نبات الدفلة المزروع في تربة كلسية ملوثة ببعض العناصر الثقيلة (Ni , Cd , Pb) وفق معايير التلوث العالمية

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المستخلص

اجريت تجربة اصص عاملية وفق تصميم تام التعشية (CRD) في البيت البلاستيكي لتقييم تلوث نبات الدفلة ببعض العناصر الثقيلة بالاعتماد على حساب مؤشرات التلوث البيئي العالمية وتاثير العناصر الثقيلة في نمو نبات الدفلة, بثلاثة عوامل وستة مكررات وهي تلويث التربة بثلاثة عناصر ثقيلة هي Pb و Cd و Ni واربعة توليفات هي Pb + Cd و Cd +Ni و Pb + Ni و Pb + Ni + Cd. ومستويات العناصر هي 0 و 120 للرصاص و 0 و10 للكادميوم و 0 و70 ملغم كغم$^{-1}$ للنيكل وستة مدد زمنية هي بعد 40 و80 و 120 و160 و200 و 240 يوم من الزراعة للنيكل باستعمال تربة ذات نسجة مزيجة رملية. ورمزت المعاملات كالاتي T_{Ni} وT_{Cd} وT_{Pb} و$T_{Cd,Ni}$ و$T_{Ni,pb}$ و T_{CP} و$T_{Cd,Ni,pb}$ و T_{O}. بينت النتائج ارتفاع قيم عامل التركيز الحيوي (BCF) للمجموع الجذري لنبات الدفلة للنيكل للمعاملات T_{Ni} و$T_{Cd,Ni}$ و $T_{Cd,Ni,Pb}$ و $T_{Ni,Pb}$ بلغت 0.77 ,0.50 ,0.43 ,0.9 بالتعاقب وانخفضت في باقي المعاملات, وكانت اعلى القيم للكادميوم بالمعاملة C بلغت1.57 في حين كانت القيم اقل من الواحد لبقية المعاملات وبذلك يعد نبات الدفلة من النباتات المراكمة للكادميوم. كانت BCF منخفضة للرصاص.اثرت المدد الزمنية معنويا في زيادة قيم BCF مع زيادة المدد الزمنية, زيادة قيم معامل التراكم الحيوي (BAC) مع زيادة تراكيزالعناصر الثقيلة الجاهزة في التربة. انخفاض قيم معامل الانتقال الحيوي (TF) للعناصر Cd و Ni و Pb عن قيمة الواحد. اثرت العناصر الثقيلة المضافة للتربة معنويا في خفض قيم كل من اطوال النباتات والوزن الجاف للنبات.

الكلمات المفتاحية: التربة الملوثة، معايير تلوث النبات.

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