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Nerium oleander L. as a Phytoremediation of Heavy Metals in Diyala Governorate – Iraq

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Abstract
The study samples were collected during the months of April and May 2022, samples were collected from different regions in Diyala Governorate – Iraq. The study aimed to assess the role of oleander plant in the phytoremediation of heavy metals (lead , cadmium , arsenic and selenium) within Diyala Governorate by estimating their concentration in the leaves of the plant and the soil on which it grows as well as calculating the bioaccumulation factor (BAF). Al-Muqdadiyah Industrial area was one of the most polluted areas with lead, the highest concentration of lead appeared in the leaves of the oleander plant grown in this area, as well as the soil on which it grows (2.452 and 0.904) ppm respectively. The highest concentration of cadmium was recorded for oleander leaves in the old city of Baqubah (2.208ppm), while the highest concentration appeared in the soil on which it grows in the Al-Muqdadiyah Industrial area (0.363ppm). The Al-Muqdadiya industrial area also recorded the highest concentration of arsenic in the leaves of the oleander plant (0.559 ppm) ,but the highest concentration in the soil on which it grows was in the central city of Al-Muqdadiyah (0.218ppm) , followed by the industrial city off Al-Muqdadiyah.(0.132ppm) . The highest concentration of selenium appeared in the industrial city of Al-Muqdadiya in both the leaves of the oleander plant and the soil on which it grows (0.441 and 0.104) ppm. The value of the bioaccumulation factor (BAF) shows the efficiency of the oleander plant in the phytoremediation of cadmium, followed by lead, arsenic and selenium in varying proportions.
Keywords : Phytoremediation , *Neium oleander* , Heavy metals
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1. Introduction

In recent years , Iraq was exposed to abnormal conditions that led to the absence of control over factories of all kinds , sewage disposal, the use of toxic chemicals such as pesticides, the quality of fuel used in cars, , the spread of electric generators in residential areas due to poor electrical power supply and many other activities that lead to air, water and soil pollution , Heavy metals are one of the pollutants that cause environmental and health problems, and according to their concentrations in the bodies of living organisms. (Bours et al. ,2005 ; Weaver *et al.* ,2017) Global industrial processes are believed to be the reason for global heavy metals pollution (Shah and Daverey, 2020) .The danger of heavy metals is their high stability and ability to transfer long distances from their areas of origin, and their concentrations can increase through the food chain (Shutzendubbel *et al.* , 2022) . Many plants are used to assess air water and soil pollution with heavy metals due to the property of their accumulation in the different parts of the plant, especially the leaves (Belguidoum *et al.* 2020; Vannucchi *et al.* 2020) . Various methods have been used to reduce the concentrations of these elements in the environment, including phytoremediation, which uses plants to adsorb or absorb these elements from the air, water and soil (Al-Obaidy *et al.*, 2016) . The concentrations of pollutants can be detected both from soil and atmospheric by trees as an effective bio monitors (Mohamed et al., 2021) . It is possible by determining the concentrations of heavy metals in parts of certain plants in different areas, we can first know the extent of contamination with heavy metals in that area, as well as knowing the efficiency of the plant in getting rid of these elements through Biological concentration (Abdulhay and Rathi , 2017) . *N. oleander* is a perennial evergreen species of the Apocynaceae family , it can survive in soils polluted by heavy metals and showing a good capacity for bioaccumulation of heavy metals (Santos *et al*.,2019; Ibrahim and El Afandi , 2020).

The oleander plant is one of the plants that are widely cultivated in Iraq on roadsides and in public gardens. In this research, the efficiency of oleander will be tested in reducing soil contamination with heavy elements by chosen seven different sites in Diyala Governorate. This study provides information about the danger and spread of heavy metal pollution on the environment.

2.Materials and Methods

2.1 Samples collection and study area

The study samples were collected from seven regions belonging to four districts in Diyala governorate ((Table 1 , Figure 1) . Seven sites were chosen in districts (New Baqubah , Old Baqubah , Al- Muqdadiyah center , Al-Muqdadiyah industrial area , Alkhalis center , Khanaqin center and agricultural area as a control) to collect the

samples during the months April and May 2022 (Figure 2) , *N.oleander* leaves were collected during the months April and May 2022 from down , middle and top of the plant . The taxonomic identification of plant was confirmed in Biology department, College of Science, University of Diyala , The leaves were preserved in a glass jar and stored in a refrigerator at 4°C (Tanee and Albert , 2013) . As well as soil samples were taken under 10 cm from the surface (soil samples were taken near the plant) . Samples were weighed then dried in an oven with a fan at 60°C until constant weight was reached, crushed then passed through sieve of 2 mm mesh size to remove all the unwanted particles (Vaird *et al*., 2004) . The samples were placed in plastic bags and then transferred to the refrigerator until the required chemical analysis.

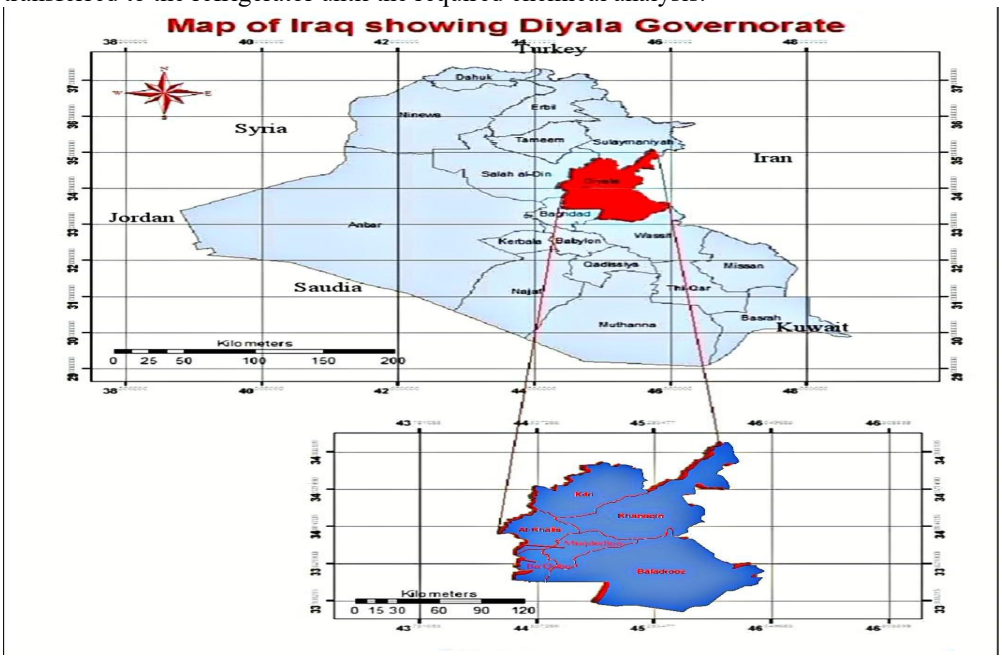

Figure 1 : Map of Iraq showing Diyala Governorate

Figure 2 : Map of Baqubah city showing samples collection locations

Table 1 : The coordinates sampling collection from seven regions of Diyala governorate

Sites	Sites (three replicates for each site)	Samples collection Coordinates	
Old Baqubah (khurasan street)	A1	33.717758	44.647805
New Baqubah (Hospital street)	A2	33.746242	44.622016
Al- Muqdadiyah center	A3	33.978362	44.936920
Al-Muqdadiyah industrial area	A4	33.985950	44.949517
Alkhalis center (Mustafa Jawad Square	A5	33.840131	44.528045
Khanaqin center	A6	34.3445377	45.380358
Control (agricultural area)	A7	33.986725	44.929565

2.2 Determination of heavy metals

One gram dry soil or plant leaves sample was weighed into volumetric flask (50 ml), then 10 ml mixture of analytical grade acids HNO3: HCIO4 in the ratio 5:1 was added. At a temperature of about 190°C for 1.5 h the digestion was performed. The solution were cooled and completed with distilled water to the final volume . A Flame Absorption Atomic Spectrometer (AASF) at Ibn Sina Center, Ministry of Industry and Minerals was used to determine the concentration of Pb , Cd , As and Se. (Walker et. al. 2016). The concentrations of heavy metals were expressed in ppm.

2.3: Bioaccumulation factor calculator (BAF)

The possibility of the plant in the bioaccumulation of the heavy element in the soil (BAF) can be calculated through the following equation (Mustapha *et al.* , 2018):

$$BAF = C \text{ in plant} / C \text{ in Soil}$$ where :

BAF : Bioaccumulation factor

C : Metal concentration (mg/l)

3. Results and Discussion

Several studies indicated the efficiency of some plants in the phytoremediation of heavy metals (Saini *et al*., 2017 ; Alaboudi *et al*., 2018). This efficacy of *N. oleander* leaves as a phytoremediation for lead, cadmium, arsenic and selenium from seven stations in Diyala Province, Iraq is shown in table 2. In general, the concentrations of Cd in the leaves of all stations exceed the certified standard values (0.2 mg/kg) except for the control group (agricultural area 0.133 mg/kg) . The highest concentration of Cd was recorded in the Old Baqubah area (khurasan street) with 2.208 mg/kg). While concentration of lead of all stations did not exceed the permissible limits (0.3 mg/kg) . Significant differences were observed among the concentrations in oleander leaves of different sites ($P < 0.05$) , The highest concentration of Pb was recorded in the Al-Muqdadiyah industrial area with 2.452 mg/kg . , followed by Al-Khalis center (2.30 ppm) and Khurasan street in old Baqubah (1.98 ppm) . The lowest concentration of lead in oleander leaves was observed in the control group (the agricultural area), as it recorded **0**.133 ppm, and high significant differences ($P < 0.05$) were found between it and all other areas, while the Khanaqin district center recorded the lowest concentration of lead (1.362ppm) in the studied areas. The high concentration of lead in the leaves of the oleander plant planted on the roadsides in the industrial area of Muqdadiya district is due to the nature of the area and its overcrowding with cars, car repair workshops and electric power generation generators that use gasoline and kerosene which leads to the emission of lead. As well as Al-khalis center (Mustafa Jawad Square) and old Baqubah (khurasan street) and other district centers with different concentrations.

Table 2 shows that the center of Khanaqin district recorded the lowest percentages of concentrations of all studied elements in the leaves of oleander (1.362 , 1.011, 0.151, 0.025) ppm for lead, cadmium, arsenic and selenium, respectively, and the reason may be that Khanaqin district belongs to the Kurdistan region, which depends Accurate environmental standards in all fields, including car models, quality of fuel used, maintenance of industrial machines, as well as the capacity of the agricultural area compared to the industrial area .The industrial area in Muqdadiya recorded the highest concentration in oleander leaves of lead, arsenic and selenium (2.452, 0.559, 0.441) ppm respectively, while it was noted that the highest percentage of cadmium was in old Baqubah (2.208 ppm) with significant differences with other sites .

It is clear from Tables 2, 3 and Figures 3, 4, 5 and 6 the role of oleander leaves in the accumulation of heavy metals under study. We find that lead is biologically concentrated in the leaves of the oleander plant in all study areas, as its concentration in the leaves is higher than that of the soil on which it grows. The highest percentage between the level of lead in the leaves of the oleander plant compared to the soil on which it grows in Khanaqin district, as it reached more than twelve times (1.362 : 0.110)ppm , followed by the district of Khalis (Mustafa Jawad Square) and then Old Baqubah (khurasan street) by more than eleven times (2.309: 0.206،1.985: 0.1758) ppm, respectively. Also, the concentration of lead increased by several times in the leaves of oleander

compared to the soil on which it grows in other region (Figure 3) .

The results of the current study clarify the great role played by the oleander plant in reducing air and soil pollution with lead through its absorption through the roots of the plant leading to its accumulation in the leaves. The difference in the concentration of lead in oleander leaves according to different regions may be due to the age of the plant and the level of air and soil pollution in that region. The oleander leaves recorded impressive results in the accumulation of cadmium, the difference in the level of cadmium between the leaves of the oleander plant and the soil on which it grows in the old area of Baqubah is approximately seventy times (2.208 : 0.032)ppm , while the difference in the city of Khalis was more than forty times (1.612 :0.046) ppm and in the new city of Baqubah more than thirty times (1.287 : 0.042) ppm and so many times over for other regions (Figure 4) .

Although the concentration of lead is higher than the concentration of cadmium in the leaves of the oleander plant in all the study areas except for the Old Baqubah region (khurasan street), we find that the efficiency of the oleander plant in the accumulation of cadmium in its leaves is several times higher than that of lead (Table 2). The same previous results were observed for arsenic and selenium. The concentration of these elements in the leaves of the oleander plant was higher than the soil on which it grows. The highest percentage difference between the concentration of arsenic in the leaves from the soil was in the industrial area of Al-Muqdadiya district by four time(0.559 : 0.132)ppm , then the new Baqubah and old Baqubah area by three times (0.397:0089; 0.369: 0.101)ppm respectively (Figure 5) , while other areas were in varying proportions. Some areas did not record any proportion of selenium in their soil, while the growing leaves of the oleander plant contained a proportion of selenium (Figure 6), and the reason may be the efficiency of the plant in absorbing all the selenium in the soil or the inability of the atomizer to sense very small concentrations of selenium in the soil, generally In all the studied elements, their percentage was more in the leaves than in the soil on which they grow, even in the control group. The obtained results confirm the role of plants and their work as a vital observer of the level of pollution in a particular area, as well as their use in phytoremediation by accumulating them in different parts of the plant and reducing the levels of pollutants in the environment.

It was observed that the level of lead in the leaves of the oleander plant was higher than the levels of the other elements Pb > Cd > As > Se, except for the old Baqubah region, where the concentration of cadmium exceeded the concentration of lead (Cd > Pb > As > Se, and this may be due to the fact that this region is characterized by the presence of Many factories manufacture and sell dyes. All study areas were from the city centers crowded with cars, so it was natural for lead to increase in concentration in those areas, and consequently its transmission to the different parts of the oleander plant, including the leaves, through the air and the soil.

The role of plants in phytoremediation is not limited to heavy metals only, but can include other pollutants such as hydrocarbons. Al-Obaidy *et al*.(2016) found that cotton plants have a significant role in reducing pollution by petroleum hydrocarbons in the soil .

Mohamed *et al*. (2021) found that the oleander plant has a high ability to accumulate heavy metals in its leaves, including lead and cadmium, and it can be used in the biological monitoring of heavy metal contamination. The results reveal a significant difference between control group of all heavy metals and all the stations studied , this is due to the increase in the cultivated area and the large number of plant species that absorb pollutants from the air and soil, in addition to the decrease in the number of cars compared to other areas. Similar results were obtained by using oleander plant in the phytoremediation of copper in wastewater (Zeki and Ridha , 2020) , This study revealed that *N.oleander can* tolerate and treat Cu concentration in wastewater , Their results showed that the highest removal efficiency was 99.9% at 35 days of experiment for the polluted pond with a concentration of 50 mg/L. The lowest removal efficiency was 94% at 65 days of the experiment for the polluted pond at a concentration of 510 mg/l . Table 4 shows the values BAF of *N. oleander* in all studies regions . The BAF values for all sites were higher than one except selenium in Khanaqin center (0.6096) which indicates that *N.oleander* has the capacity to adsorb lead (Pb) , cadmium (Cd) , arsenic (As) and selenium (Se) from the soil . The highest value of the BAF for lead element was recorded in Khanaqin center (12.2855) , followed by Old Baqubah (11.2924) , As for cadmium, it was the highest value in the old Baqubah (70.595) , followed by the Khanaqin center (52.5959) then Alkhalis center (34.4594) , The highest value of the bioaccumulation factor for arsenic was recorded in the city of Al-Muqdadiyah industrial area (4.2278) ,while it was 6.1101 for selenium in old Baqubah city. The results of Table 4 show that the best rate of bioaccumulation factor of oleander plant was for cadmium, then lead, followed by arsenic and selenium with a lesser percentage. This difference between the bioaccumulation factor between the study areas for the same element may be due to the difference in the age of the plant grown in those areas ((Zeki and Ridha , 2020). While the difference in the bioaccumulation coefficient of oleander plant according to the elements may be due to the nature of these elements and their difference in terms of physical characteristics, the most important of which is the solubility of the element in water.

4. Conclusions

We conclude from the results of the current study that the oleander plant can be used in the biological monitoring of contamination with heavy metals, as well as it can be used in the phytoremediation of heavy metals depending on the type and concentration of the element in the soil.

Table 2 : Mean and S.D of Pb , Cd ,As and Se Concentration in *N. oleander* leaves on different sites in Diyala governorate .

sites / Con. (ppm)	A1 : Old Baqubah (khurasan street)	A2: New Baqubah (Hospital street)	A3 : Al-Muqdadiyah center	A4: Al-Muqdadiyah industrial area	A5: Al-khalis center (Mustafa Jawad Square)	A6: Khanaqin center	A7: Control (agricultural area
Pb	1.985593 ± 0.001779 A	1.597741 ± 0.004284 b	1.612338 ± 0.015563 b	2.452222 ± 0.001637 a	2.30937 ± 0.011967 a	1.362333 ± 0.009866 b	0.133333 ± 0.001528 c
Cd	2.208963 ± 0.002625 aa	1.287815 ± 0.00939 ab	1.284407 ± 0.001772 ab	1.579852 ± 0.019463 ac	1.612704 ± 0.001433 ac	1.011 ± 0.003 ab	0.028778 ± 0.000977 ad
As	0.369889 ± 0.005082 bb	0.397519 ± 0.00105 bb	0.334222 ± 0.003129 bb	0.559481 ± 0.00074 bc	0.295519 ± 0.001806 bb	0.151 ± 0.003606 bd	0.015278 ± 0.000752 be
Se	0.125667 ± 0.001202 cc	0.133778 ± 0.00116 cc	0.301704 ± 0.120251 cb	0.441185 ± 0.000548 cb	0.123926 ± 0.001113 cc	0.025333 ± 0.002517 cd	0.0045 ± 0.00025 ce

Table 3: Mean and S.D of Pb,Cd,As and Se Concentration in soil of different sites in Diyala governorate.

Sites / Con. (ppm)	A1 : Old Baqubah (khurasan street)	A2: New Baqubah (Hospital street	A3: Al-Muqdadiyah center	A4: Al-Muqdadiyah industrial area	A5: Alkhalis center (Mustafa Jawad Square)	A6: Khanaqin center	A7: Control (agricultural area
Pb	0.175833 ± 0.002316 A	0.190633 ± 0.001617 a	0.759667 ± 0.003334 b	0.904889 ± 0.004032 b	0.2061 ± 0.002784 c	0.110889 ± 0.002365 c	0.058667 ± 0.001202 d
Cd	0.032433 ± 0.000702 Aa	0.04236 ± 0.001042 aa	0.289222 ± 0.00222 ab	0.363111 ± 0.004168 ab	0.0468 ± 0.000458 aa	0.019222 ± 0.00077 ac	0.004333 ± 0.000882 ad
As	0.101233 ± 0.000503 Cc	0.098333 ± 0.000603 cc	0.218445 ± 0.003025 ca	0.132333 ± 0.000882 cc	0.084967 ± 0.001365 cc	0.057111 ± 0.000694 cb	Nil
Se	0.020567 ± 0.000611 Da	Nil	0.097889 ± 0.002589 dc	0.104889 ± 0.001262 dc	Nil	0.041555 ± 0.001347 de	Nil

Table 4 : BAF of *N. oleander* plant of different sites in Diyala governorate .

Sites / Con. (ppm)	A1 : Old Baqubah (khurasan street)	A2: New Baqubah (Hospital street	A3: Al-Muqdadiyah center	A4: Al-Muqdadiyah industrial area	A5: Alkhalis center (Mustafa Jawad Square)	A6: Khanaqin center	A7: Control (agricultural area
Pb	11.2924	8.3812	2.1224	2.7099	11.2050	12.2855	2.2727
Cd	70.5956	30.4016	4.4409	4.3508	34.4594	52.5959	6.6415
As	3.6538	4.0425	1.5300	4.2278	3.4780	2.6439	0.0
Se	6.1101	0.0	3.0821	4.2062	0.0	0.6096	0.0

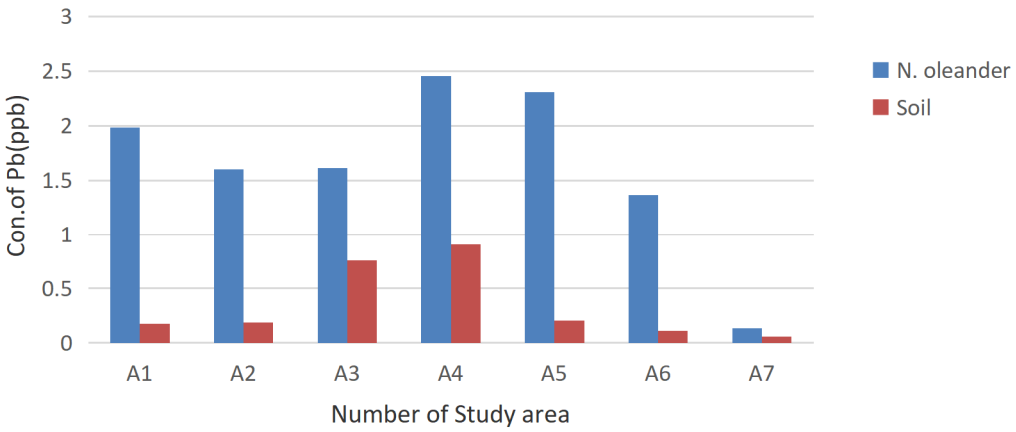

Fig.3: Concentration of **Lead** in *N.oleander* leaves and the soil on which it grows

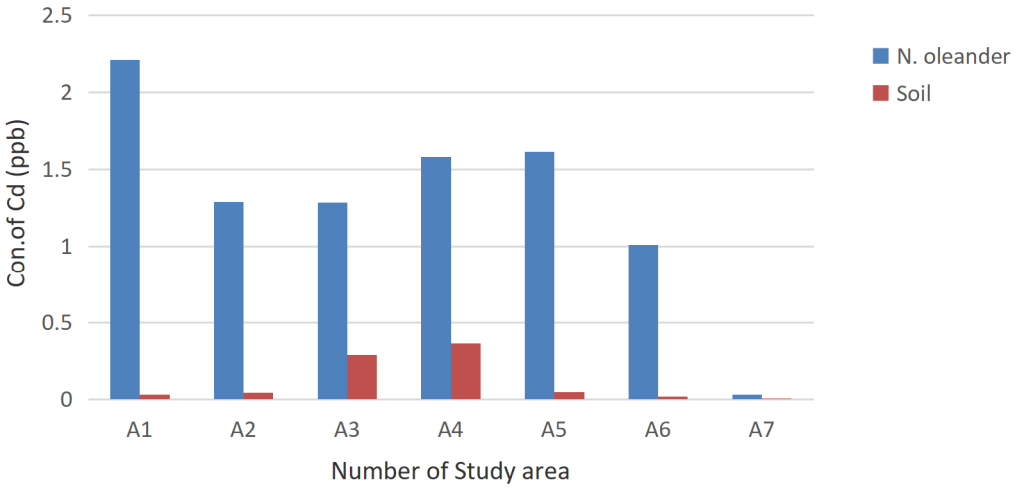

Fig.4: Concentration of **Cadmium** in *N.oleander* leaves and the soil on which it grows

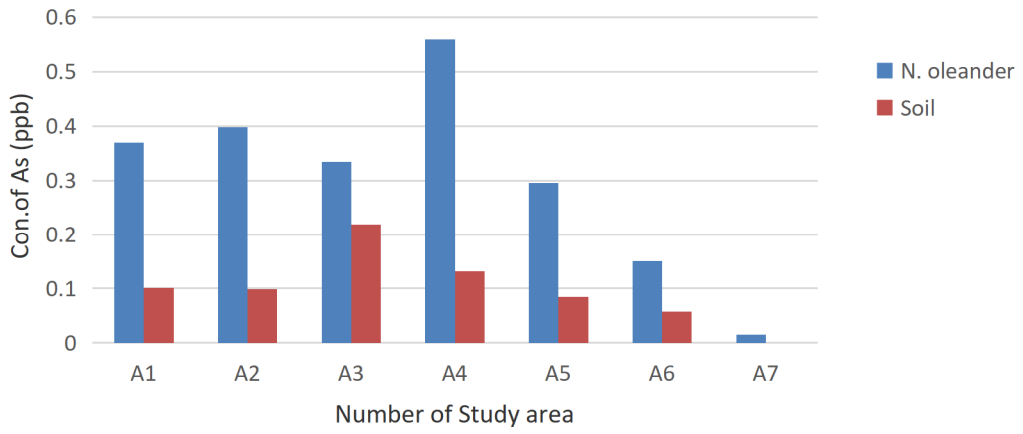

Fig.5: Concentration of **Arsenic** in *N.oleander* leaves and the soil on which it grows

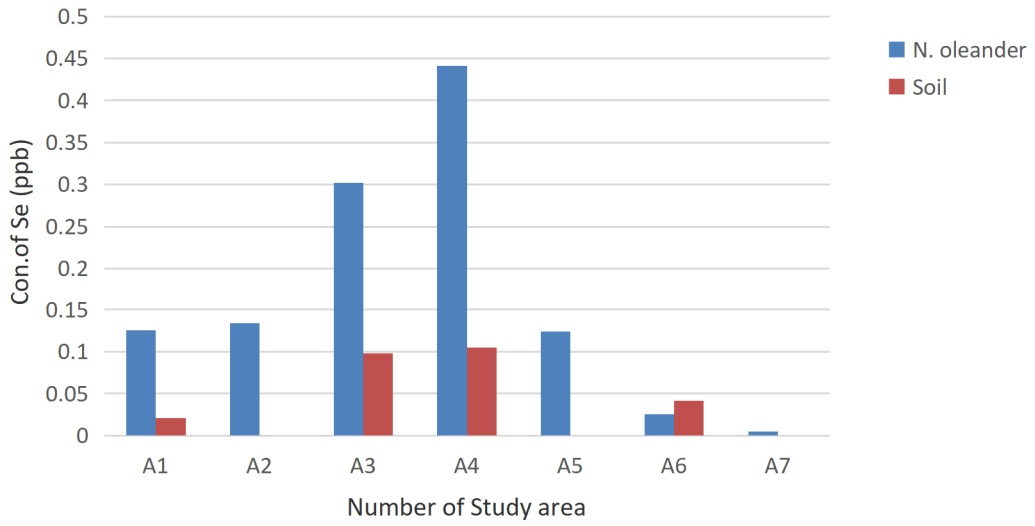

Fig.6: Concentration of **Selenium** in *N.oleander* leaves and the soil on which it grows

References

Abdulhay ,H.S and Rathi ,M.H. (2017) . Lead, Cadmium and Nickel Contamination of Roadside Soils and Plant Leaves in Baghdad City . J. Chem. Pharm. Res. ,9(8): 47-51.

Al-Obaidy A. H Jawad.M, Al-Anbari R. H., Hassan Sarah M.(2016) . Phytoremediation of Soil Polluted with Iraqi Crude Oil by Using of Cotton. Plant Mesop. environ. j., 3(.1) :10-16 .

Alaboudi, K.A.; Ahmed, B.; Brodie, G. (2018) . Phytoremediation of Pb and Cd contaminated soils by using sunflower (Helianthus annuus) plant. Ann. Agric. Sci., 63, 123–127.

Belguidoum A, Lograda T, Ramdani M. (2020) . Heavy metals accumulation in Hertia cheirifoliaalong the highway in Setif region, Algeria. Biodiversitas 21(6): 2786-2793.

Bours J., Reitz C,. Strobel J., Breipohi W,. (2005). Detection of secretory IgM in tears during rhino – conjunctivitis. Graefes Arch clin Exp. Ophthalmol. 243(5): 456-63.

Ibrahim N, El Afandi G. (2020). Evaluation of the phytoremediation uptake model for predicting heavy metals (Pb, Cd, and Zn) from the soil using Nerium oleanderL. Environ Sci Pollut Res 27(30): 38120-38133

Mohamed, A. K.; Amina , T .and Messaoud, R. (2021). Heavy metals accumulation in Nerium oleander leaves across urban areas in Setif region, Algeria . Biodiversitas,22(6): 3083-3091.

Mustapha, H. I., H. A. van Bruggen, and P. N. L. Lens. (2018). Vertical subsurface flow constructed wetlands for the removal of petroleum contaminants from secondary refinery effluent at the Kaduna refining plant (Kaduna, Nigeria, Environmental Science and Pollution Research. Environmental Science and Pollution Research. 25 (30): 30451–30462.

Vannucchi F, Traversari S, Raffaelli A, Francini A, Sebastiani L. (2020). Populus albatolerates and efficiently removes caffeine and zinc excesses using an organ allocation strategy. Plant Growth Regul 92(3): 597-606

Viard, B.; Pihan, F. ; Promeyrat,S. (2004) . J Pihan. Chemosphere. 2004, 55, 1349-1359

Saini, V.K.; Suthar, S.; Karmveer, C.; Kumar, K. (2017) .Valorization of Toxic Weed Lantana camara L. Biomass for Adsorptive Removal of Lead. J. Chem. 2017, 5612594.

Santos RS, Sanches FA, Leitão RG, Leitão CC, Oliveira DF, Anjos MJ, Assis JT. (2019) . Multi-elemental analysis in Nerium OleanderL. leaves as a way of assessing the levels of urban air pollution by heavy metals. Appl Radiation Isotopes 152:18-24.

Shah, V.; Daverey, A. (2020). Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil. Environ. Technol. Innov., 18, 100774.

Shutzendubel. A. and Polle. A. (2002) Plant Responses to A biotic Stresses ; heavy metal – induced Oxidative stress and protection by mycorrhization .J.Exp.Bot.53,pp (1351-1365).

Tanee ,F.B. ; Albert,E.(2013) . J Biol Sci., 13, 264-270

Walker, S., Stevenson, B., Peterson, J., Donati, G., Jones, B., Tourne, M., Pollard, D., Kanu, A., & Kanu,A.B. (2016). Determining Micro- and Macro-Elements by Flame Atomic Absorption Spectrometry . Chemial Educator.21: 264- 272.

Weaver Vm, lee bk, Ahn kd, lee gs, Todd ac, Stewart wf, wen j, Simon dj, pArsons pj, schwartz. (2017). association of lead biomarkers with reanal function in korean lead workers. occup environ med, 2003 (60) : 551-562.

Zeki , S.L. and Ridha , M.J.M. (2020) . Phytoremediation of synthetic wastewater containing copper by using native plant . Iraqi Journal of Agricultural Sciences:51(6):1601-1612.