Phytoremediation of Lead and Cadmium Contaminated Soil Using Arugula *Eruca sativa* L.

المعالجة النّباتيّة لتربة ملوّثة بالرّصاص والكادميوم باستخدام نبات الجرجير *Eruca sativa* L.

Dr. Mohammad Dikkeh [(1)] Dr. Vienna Hammoud [(2)] Eng. Maissoun Ziadeh [(3)]

د. محمّد دكّه [(1)] د. فينّا حمّود [(2)] م. ميسون زياده [(3)]

maissounziadeh4@hotmail.com

https://doi.org/10.66805/AAE-19.1.183202

Received 18 April 2024; Accepted 22 September 2024

(1) Department of Soil and Water Sciences, Faculty of Agricultural Engineering, Latakia University, Syria.
(1) قسم علوم التربة والمياه، كليّة الهندسة الزراعية، جامعة اللاذقية، سورية.
(2) Department of Biology, Faculty of Science, University of Tartous, Syria.
(2) قسم علم الحياة، كلية العلوم، جامعة طرطوس، سورية.
(3) Ph.D. student, Department of Soil and Water Sciences, Faculty of Agricultural Engineering, Latakia University, Syria.
(3) طالبة دكتوراه، قسم علوم التربة والمياه، كلية الهندسة الزراعية، جامعة اللاذقية، سورية.

Abstract

This study evaluates the uptake and accumulation of cadmium and lead in Arugula *Eruca sativa* to determine its efficiency for use in phytoremediation of lead-and-cadmium-contaminated soil, based on a pot-experiment. The results of the study showed that there was a significant effect of the concentration of lead and cadmium in the soil on the decrease in the wet and dry weight of roots in all treatments compared to the control sample, as well as on the cadmium and lead concentration in the arugula. Meanwhile, there was no significant effect of these concentrations on the germination percentage or the wet and dry weight of the shoots. The arugula plant was able to accumulate cadmium when exposed to soil contaminated with cadmium and lead to a greater extent than being exposed to cadmium contamination only, which indicates the synergy between the two metals. *Eruca sativa* can be relied upon to extract cadmium from soils contaminated with it because it has values of (Bio-Concentration Factor) BCF>1, (Bio-Accumulation Coefficient) BAC>1, and (Transfer Factor) TF>1, despite the fact that it did not behave as a

 ISSN: 2305-5243/ EISSN: 3005-6438. (p: 183-202)

hyperaccumulating plant under the conditions of the current research and the characteristics of the soil used in it. The specific extraction yield percentage SEY% for cadmium was low, and we may need more than 144 plantings of arugula for two months to clean the soil with a total cadmium concentration of 3 mg cadmium/kg of soil. *Eruca sativa* did not show efficiency in extracting lead, as all coefficients of phytoremediation were less than one, and the specific extraction yield percentage SEY% for lead was very low.

Key words: Phytoextraction, Brassicaceae family, Heavy metals, Bioconcentration factor, Bioaccumulation factor, Transfer factor.

الملخص

تناولت هذه الدّراسة تقييم مقدرة نبات الجرجير على مراكمة الكادميوم والرّصاص، لتحديد كفاءته للاستخدام بالمعالجة النّبتيّة لترب ملوّثة بالرّصاص والكادميوم، بالاعتماد على تجربة أصص عُوملت تربتها بنترات الكادميوم بتراكيز 3-15-30 مغ كادميوم /كغ تربة ونترات الرّصاص بتراكيز 100-300-700 مغ رصاص/كغ تربة، وبنترات الكادميوم ونترات الرّصاص معًا بتراكيز 3Cd+100Pb- 15Cd +300Pb- 30Cd + 700Pb مغ/كغ تربة. أوضحت نتائج الدراسة وجود أثر معنوي لتركيز الرّصاص والكادميوم في التّربة على انخفاض الوزن الرّطب والجافّ للجذور في جميع المعاملات مقارنةً بالشّاهد، في حين لم يتأثّر معنويًّا نسبة إنبات بذور الجرجير والوزن الرّطب والجافّ للمجموع الخضريّ، كما كان هناك أثر معنويّ لتركيز الرّصاص والكادميوم في التّربة على محتوى الجذور والمجموع الخضريّ لنبات الجرجير من كلا المعدنين، واستطاع نبات الجرجير مراكمة الكادميوم في جذوره وفي مجموعه الخضري عند تعرّضه لتربة ملوّثة بالكادميوم والرّصاص بشكل أكبر ممّا لو تعرّض للتلوّث بالكادميوم فقط، ممّا يدلّ على التآزر بين المعدنين. يمكن الاعتماد على نبات الجرجير باستخلاص الكادميوم من التّرب الملوّثة به، لامتلاكه قيم معامل تركيز حيوي BCF <1 ، ومعامل تراكم حيويّ BAC <1، ومعامل انتقال TF <1، على الرّغم من كونه لم يتصرّف كنبات فائق المراكمة ضمن شروط البحث الحالي وخصائص التّربة المستخدمة فيه، وكانت قيم عائديّة الاستخلاص للكادميوم (The specific extraction yield percentage SEY%) منخفضة، وقد نحتاج لأكثر من 144 زراعة للجرجير لمدّة شهرين لتنظيف تربة تركيز الكادميوم الكلّي فيها 3 مغ كادميوم/كغ تربة. لم يُظهر نبات الجرجير كفاءة باستخلاص الرّصاص حيث كانت كلّ معاملات كفاءة المعالجة النّباتية أقلّ من الواحد، وقيم عائديّة الاستخلاص للرّصاص SEY% منخفضة جدًّا.

الكلمات المفتاحية: الاستخلاص النّباتيّ، الفصيلة الصّليبية، المعادن الثّقيلة، معامل التّركيز الحيويّ BCF، معامل التّراكم الحيويّ BAC، معامل الانتقال TF.

Introduction

Rapid growth in population and industrialization have added large amounts of toxic waste to the environment, which affects human health around the world.

Metals, metalloids, radionuclides, and other inorganic substances are the most widespread forms of environmental pollutants and treating them in soil and sediments is a difficult task. Concerns about the toxicity of these substances led to a focus on developing effective techniques to evaluate the presence and mobility of metals in soil, water, and sewage (Dar *et al.*, 2015). In recent decades, attention has turned to studying dangerous heavy metals in soil, such as cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), lead (Pb), and zinc (Zn), because they are not biodegradable. They persist in the environment for long periods, making the soil unsuitable for agriculture. Some of these metals are highly toxic to humans, even in low concentrations, such as cadmium and lead (Bortoloti and Baron, 2022). Phytoremediation is considered an affordable and an environmentally friendly technique for removing or reducing toxic pollutants from various components of the environment. In phytoremediation technology, accumulating plants are used to extract, isolate, and detoxify pollutants. The term phytoremediation comes from the Ancient Greek word "phyto" meaning "plant" and the word "remedium" meaning "to treat or remove evil." The unique and selective absorption capabilities of plant roots systems, effective transport, bioaccumulation and decomposition of pollutants by hyper-accumulating plants are used in phytoremediation technology. One method of plant treatment is known as phytoextraction technology, and it can be used to remove heavy metals from the soil that is found within the biomass of the plants grown in it. After removing these metal-rich-plants from the site and disposing of them, it is necessary to recover the metals accumulated in the plants and recycle them if possible (Dar *et al.*, 2015, Raz *et al.*, 2020).

The harvested biomass can be safely processed through drying, incineration, storage in a landfill, anaerobic digestion, or through microbial, physical, or other chemical techniques. (Ginneken *et al.*, 2007). It is essential that the plants selected for phytoremediation are suitable for agricultural practices and able to produce sufficient biomass with maximum uptake of mineral compounds for them to be effective. Hyperaccumulator plantae can accumulate at least 0.1% of the dry weight of their leaves of any heavy metal (Bortoloti and Baron, 2022). Plants are considered hyperaccumulators when minerals are accumulated in the leaves in concentrations greater than the following: 100 ppm Cd, 1000 ppm Co, Cu, Ni, Pb, 10000 ppm Mn, Zn (Kabata-Pendias and Pendias, 2001).

Many species of the Brassicaceae (cruciferous) family could accumulate heavy metals such as cadmium, arsenic, lead, and zinc, making them a potential source for expanding research into them in the future. (Roy *et al.*, 2020), More than 500 plant species belonging to 101 families have been classified as hyperaccumulators of heavy metals, most of which belong to the following families: Compositae, Brassicaceae, Leguminous, Lamiaceous, and Poaceae. (Sarma, 2011). Many plant species belonging to the genus Brassica tolerate the toxic effects of heavy metals (HMs), and are, therefore, important for use in phytoremediation. Cruciferous species can perform phytoremediation of heavy metals through several physiological mechanisms, such as: Phytovolatilization, phytostabilization, and phytoextraction (Bortoloti and Baron., 2022). The phytoremediation process is successful in slightly to moderately polluted sites and is not suitable for highly polluted sites (Thangavel and Subbhuraam., 2014).

Arugula *Eruca sativa* L. is a plant of the Brassicaceae family. It is native to the Mediterranean basin, and it spreads from southern Europe to North Africa, Iran, India and Pakistan. It is an annual winter (spring) herb. Its height ranges between 10-100 cm, and it has a slender, upright taproot with a highly branched root system. The leaves are simple and pinnate; the lower ones are petiolate, and the upper leaves are sessile with an elongated terminal lobe that is fully ovate or discrete and is rarely shiny and slightly fleshy with a distinctive pungent odor. It has multiple medical uses and has been known since ancient times in folk medicine in areas where it is found naturally (Garg and Sharma., 2014).

E. sativa is a species used to remediate contaminated soils because it absorbs zinc, lead, and cadmium (especially zinc) and produces high biomass yields. (Ghaderian and Nosouhi., 2015). The importance of this study comes from the increase in environmental pollution with heavy metals as a result of human activities, and because soil is the primary entrance for heavy metals into the food chain, it must be rid of pollution in an effective and economical way, such as phytoremediation, and this study is a continuation of previous studies in Syria that proved the existence of several areas that exceed the permissible limits, reaching the warning limits for some heavy metals in the soil, such as agricultural lands close to industrial facilities as paint and battery factories and tanneries, which are considered industries that add a lot of metals to the surrounding environment.

The Arugula *Eruca Sativa* L., was chosen for its ability to accumulate minerals and edibility. Therefore, we can benefit from this research in two directions: the first is to use arugula in phytoremediation, and the second is to warn against planting it in contaminated areas for the purpose of human consumption in case its efficiency in biological extraction of one of the heavy metals studied is proven. In addition to the high biomass that it produces, which is a desirable characteristic in crops used in phytoremediation. The seeds of the arugula plant are also available to farmers in the Mediterranean regions that are its original habitat. If they are advised to use it for phytoremediation, there will be no obstacle in securing seeds.

The current research aims to study the accumulation and distribution of lead and cadmium in plant parts (roots - shoots) of arugula plants that grow in contaminated soil to determine which plant parts are most effective in accumulating the pollutants. Then, the efficiency of the arugula plant in phytoremediation will be evaluated.

Materials and Methods

1. Pot-Experiment and Laboratory Work

This research was conducted during the period between September 2021 - October 2022 in Tartous. Soil tests and analysis were conducted in the laboratories of the Faculty of Agriculture, Latakia University, and the laboratories of the Faculty of Science, University of Tartous, and the Higher Institute for Environmental Research, Latakia University.

1-1. Preparing Pots and Taking Samples

The soil was collected from the surface layer of an agricultural land at a depth of 0 - 10 cm in which the total concentration of lead 0.48 mg $kg^{-1}$and the total concentration of cadmium 0.035 mg kg^{-1} (Table 1). The soil was collected from the suburbs of the City of Tartus away from the highroad, on September 9, 2021 AD. It was left to air-dry for two days, after which the soil to be studied was sieved with a 2-mm sieve then mixed well, and about 1 kg was taken and placed in a closed plastic container for standard soil analysis.

Every 2 kg (the capacity of the pot used is 2 kg of soil) was treated with a solution containing lead nitrate, cadmium nitrate, or both, as shown:

3 pots for the control sample Cd0 + Pb0: without treatment, 2 kg of soil for each pot.
3 pots of Cd1: with a concentration of 3 mg kg^{-1} cadmium.
3 pots of Cd2: 15 mg kg^{-1}cadmium.
3 pots of Cd3: with a concentration of 30 mg kg^{-1} cadmium.
3 pots of Pb1: with a concentration of 100 mg kg^{-1} lead.
3 pots of Pb2: with a concentration of 300 mg kg^{-1} lead.
3 pots of Pb3: with a concentration of 700 mg kg^{-1} lead.
3 pots of Cd1 + Pb1: 3 mg kg^{-1} cadmium + 100 mg kg^{-1} lead.
3 pots of Cd2 + Pb2: 15 mg kg^{-1} cadmium + 300 mg kg^{-1} lead.
3 pots of Cd3 + Pb3: 30 mg kg^{-1} cadmium + 700 mg kg^{-1} lead.
The number of treatments was ten, with three replicates per treatment, making a total of 30 pots.

The incubation process continued for 15 days. On the first day, the pots were irrigated in accordance with the field capacity (about 700 ml of water for each pot), then irrigation was done at a rate of one day of watering and one or two days without, with approximately 200 ml of water. When leaching occurred, the leached water was returned to the pot again.

All three pots representing the same replicate were mixed well on the ninth and tenth days of incubation, meaning, 6 kilograms were mixed from three pots bearing the same name of the treatment (Cd1 treatment, replicate 1, 2, 3, for example) and then redistributed among the three pots with the aim of giving the soil of the replicates a uniform texture.

Twenty seeds of arugula were planted in each pot on 15/11/2021, and after germination, the plants were reduced to two in each pot. Planting continued for two months, and harvesting took place on 15/1/2022 AD.

1-2. Soil properties

Table No. (1) shows some of the physical and chemical properties of the studied soil. The soil texture was determined using the Hydrometer Method, Bouyoucos (1962). The organic matter is estimated using the wet digestion method, Walkly and Black (1934). The cation exchange capacity is estimated using the ammonium acetate, Chapman (1965). Estimating the total calcium carbonate is achieved through the volumetric method Gupta (2000), The degree of acidity and electrical conductivity within a 1:5 extract (soil: distilled water), and estimation of field capacity by Gravimetric Method with Oven Drying after 24 hours of saturating the soil with water.

Table 1. Some properties of the soil used in the pot experiment

Soil Properties	
Soil texture	Sandy Clay
Clay percentage (%)	40
Silt percentage (%)	14
Sand percentage (%)	46
CEC ($cmol_{(+)}$/kg)	36.85
OM (%)	2.72
$pH_{(1:5)}$	7.11
$EC_{(1:5)}$ (dS/m)	0.35
Total calcium carbonate (%)	8.41
Total soil lead concentration (mg kg^{-1})	0.48
Total soil cadmium concentration (mg kg^{-1})	0.035

1-3. Preparation of Plant Samples

Immediately after harvesting, the plants were washed with running water several times. The roots were separated from the shoots. The plants were washed with distilled water three times in the laboratory and left to air-dry. The wet weight was measured. Then the samples were dried with an electric dryer at a temperature of 80°C for 7-8 hours and more, until the weight was stable. The dry weight was measured, then the dried samples of the roots and shoots were ground with an electric grinder, sieved with a 1 mm sieve and kept in tightly sealed plastic containers.

The plant samples were digested using high-purity nitric acid, by placing 0.5 g of the ground and dried plant sample in a test tube, adding 5 ml of HNO_3 65% to it, and leaving it until the next day, then placing it in a water bath for two hours. After it cooled, it was transferred to an Erlenmeyer flask. The volume was completed to 50 ml with distilled water, then cadmium and lead were estimated in the extracts using a Shimadzu AA-6800 Atomic Absorption Spectrophotometer. (Gupta, 2000)

2. Determining the Tolerance Index (TI)

The tolerance index (TI) is the ratio between growth parameters (root length, length of shoots or leaves, wet and dry weight of roots, wet and dry weight of leaves) of plants in contaminated soil to the growth ratio of plants in uncontaminated soil, according to (Chen *et al.*, 2011)

$$\text{(1) Tolerance index} = \frac{\text{(Growth parameter) Plant in contaminated pots}}{\text{(Growth parameter) Plant in control pots}}$$

3. Phytoremediation efficiency

3-1. Parameters that determine the efficiency of the plant in extracting each mineral

The translocation factor (TF) of Cd and Pb from root to shoot, bioconcentration factor (BCF), and accumulation in tissues (BAC) were calculated as follows (Amin *et al*, 2018, Malik *et al*, 2010, Yoon *et al*, 2006).

$$\text{(2)} \qquad \text{BCF} = \frac{\text{concentration of the element in the plant roots}}{\text{concentration of the element in the soil}}$$

$$\text{(3)} \qquad \text{BAC} = \frac{\text{concentration of the element in the plant shoots}}{\text{concentration of the element in the soil}}$$

$$\text{(4)} \qquad \text{TF} = \frac{\text{element concentration in the plant shoot}}{\text{element concentration in the plant roots}}$$

3-2. The Specific Extraction Yield Percentage SEY (%)

The SEY% is calculated through the equation (Audet and Charest, 2006, Audet and Charest, 2007):

$$(5)\quad SEY(\%) = \frac{metal\ content\ in\ roots + metal\ content\ in\ the\ shoot}{Total\ metal\ concentration\ in\ soil} \times 100$$

Metal content in roots = metal concentration in roots (mg g^{-1})× dry weight of roots.

Metal content in shoot = metal concentration in shoot (mg g^{-1})× dry weight of the shoot

4. Statistical Analysis

Statistical analysis was done using the software Statistical Program for Social Science (SPSS), The experimental design was Randomized Complete Block Design. One -way-ANOVA was applied, and the differences between means were studied using Duncan, and were significant at $P \leq 0.05$. The regression equations and R-squared (determination coefficient) was calculated using Excel.

Results and Discussion

1. Bioindicators of Arugula

1-1. Germination percentage

Germination percentage values ranged between87.5% and 100%, and the lowest germination values were observed in the two treatments containing lead with the highest value (700 mg kg^{-1} soil) whether lead was present alone in the soil, or if lead was present with cadmium as it was 87.5% and 90% respectively. This indicates a decrease in the germination rate with increasing lead, although this decrease did not reach the level of statistical significance, with p value>0.05 (Table 2).

This is consistent with what was mentioned about the contribution of the cruciferous species to the phytoremediation process, that lead is found in the soil as salts in soluble and insoluble forms, and it is known that contamination of the soil with lead hinders seed germination. (Anjum *et al.*, 2012).

1-2. The Wet and dry weight of the shoots

The wet weight values of the shoots ranged between 22.11 and 16.19 g/plant. These were the corresponding values to the control sample and the highest treatment for both lead and cadmium respectively.

While the dry weight values of the shoots ranged between 2.2 and 1.33 g/plant, and these values were consistent with the control and the highest cadmium treatment (30mg kg^{-1}) respectively.

It was observed that both the wet weight and the dry weight of the shoots were significantly reduced in all treatments compared to the control, although the dry and wet weights of the shoots were not significantly affected by the increased concentrations of lead and cadmium in the soil, as the value was ($p > 0.05$). (Table 2). This may be since the arugula plant is moderately tolerant of copper, mercury, chromium, and cadmium, and very tolerant of lead, nickel, and zinc. (Yildirim *et al*, 2019)

1-3. Wet and dry weight of roots

Root wet weight values ranged between 2.71 and 0.56 g/plant, and these were the corresponding values to the control sample and Cd2 treatment (15 mg kg^{-1}) respectively. While the dry weight values of arugula roots ranged between 0.375 and 0.095 g/plant, and these values were consistent with the control and the Cd2 treatment (15 mg kg^{-1}), respectively.

A decrease in both the wet weight and the dry weight of the roots was observed in all treatments compared to the control, more clearly than the decrease in the same treatments in the shoot. The concentrations of lead and cadmium in the soil had a significant effect on both the dry weight and the wet weight of the roots, with a P value <0.01. (Table 2)

Table 2. Effect of Lead and Cadmium pollution on Arugula plant bioindicators

	Germination percentage (%)	Wet weight of shoots (g)	Dry weight of shoots (g)	Wet weight of roots (g)	Dry weight of roots (g)
Control sample	97.50±3.53	22.11±3.27	2.20±0.24	2.71±0.29[a]	0.38±0.02[a]
Cd1	100±0.0	21.78±3.36	1.9±0.15	1.08±0.33[bc]	0.18±0.04[bc]
Cd2	97.50±3.53	17.80±4.38	1.69±0.84	0.57±0.05[c]	0.1±0.02[c]
Cd3	100±0.0	17.27±3.38	1.29±0.44	0.72±0.13[c]	0.13±0.07[bc]
Pb1	100±0.0	19.20±0.007	1.63±.056	1.74±0.55[b]	0.2±0.04[b]
Pb2	95.0±7.07	17.24±3.8	1.66±0.42	1.59±0.59[b]	0.17±0.06[bc]
Pb3	87.5±3.53	17.67±5.96	1.72±0.57	0.68±0.21[c]	0.15±0.06[bc]
Cd1+Pb1	100±0.0	17.48±3.92	1.33±0.3	0.59±0.11[c]	0.10±0.01[c]
Cd2+Pb2	97.5±3.53	18.60±4.78	1.5±0.46	0.84±0.21[c]	0.18±0.03[bc]
Cd3+Pb3	90.0±7.07	16.19±0.3	1.52±0.1	0.81±0.13[c]	0.17±0.04[bc]
P value	0.074	0.808	0.629	0.001	0.001

2. Determining the Tolerance Index (TI).

According to Audet and Charest (2007), if TI values are less than 1, it indicates that the plant has suffered stress due to metal pollution with a net reduction in biomass. In contrast, if TI values are greater than 1, it means that the plants have developed tolerance with a net increase in biomass (hyperaccumulation). If TI values are equal to 1, it means that the plant is not affected by metal contamination.

It is clear from Table (3) that there was a decrease in the biomass of the arugula plant as all values of the tolerance index were less than one, which indicates that the arugula plant was exposed to stress. The roots were more affected than the shoot when exposed to different concentrations of both metals (lead and cadmium). Whether exposed to each mineral alone or both minerals together, we notice a decrease in the wet weight of the shoots between 1-27%, and a decrease in the wet weight of the roots between 68-90%, while the decrease in dry weight for the shoots ranged between 14-41%. As for the roots, it ranged between 52-75%, when compared to the control. (Table 3)

In general, we notice a decrease in the biomass of plants when exposed to contamination with cadmium, lead, or both, compared to control treatments, and this is consistent with many studies (Anjum *et al.*, 2012). A study on hydroponic cultivation of arugula in a nutrient solution demonstrated an increase in dry weight at low concentrations of cadmium and lead. However, the dry weight decreased after a certain limit that was considered the toxicity threshold (Cannata *et al*, 2013)

Table 3. Tolerance Index (TI) for Growth Indicators in Arugula

Pot treatment	Wet weight /g per plant		Dry weight /g per plant	
	shoots	Roots	shoots	Roots
Cd1	0.99	0.2	0.86	0.48
Cd2	0.81	0.1	0.85	0.25
Cd3	0.78	0.13	0.59	0.33
Pb1	0.87	0.32	0.82	0.42
Pb2	0.78	0.29	0.75	0.45
Pb3	0.8	0.13	0.78	0.39
Cd1 + Pb1	0.79	0.11	0.6	0.27
Cd2 + Pb2	0.84	0.15	0.68	0.48
Cd3 + Pb3	0.73	0.15	0.69	0.44

3. The Efficiency of Cadmium phytoremediation

3-1. Cadmium Concentrations in Arugula

Concentrations of cadmium in the roots ranged from 3 to 47.67 mg kg^{-1} dry weight, Higher than the normal range in plants 0.1-2.4 mg kg^{-1} in all treatments (Qunshan *et al.*, 2020), and by comparing pots that contained only cadmium and pots that contained cadmium and lead, it was noted that the arugula plant was able to accumulate larger amounts of cadmium in its roots in the presence of lead contamination and cadmium together, compared to the presence of cadmium alone in the soil.

In contrast, cadmium concentrations in the shoots ranged from 6.75-69.96 mg kg^{-1} dry weight in all treatments, meaning that the arugula plant was able to retain a greater amount of cadmium in its shoots than in its roots, which is a characteristic of the smaller percentage of plant, which makes it a candidate for use in phytoremediation. Although cadmium is retained in most plants in the roots and its concentrations decrease in the following order: roots, stem, leaves, fruits, seeds, its mobility is different within plant species, as it can penetrate the roots of some plants through the cortical tissue to the above-ground tissues. (Benavides *et al.*, 2005). A study conducted by Waheed *et al.*, (2022), showed a significant accumulation of cadmium in the leaves of the Arugula sativa in soils with high cadmium concentrations.

By comparing pots that contained only cadmium and pots that contained cadmium and lead, it was noted that the arugula plant was able to accumulate larger amounts of cadmium in its vegetative system in the presence of both lead and cadmium contamination, compared to the presence of cadmium alone in the soil, consistent with the accumulation of cadmium in the roots. This may be attributed to the synergistic effect between the minerals lead and cadmium. (Kabata Pendias and Pendias, 2001), with the exception of treatment with the minimum amount of cadmium, where it was observed that the shoot was able to retain larger amounts of cadmium when the pollutant was alone in the soil than it was able to retain when it was combined with lead, noting that this difference did not have statistical significance when comparing the average values. values due to the high percentage of clay, and the high percentage of organic matter as well This is explained by the possibility of lead precipitation when it is present within low. (Dede *et al*, 2012 (Table 4)

The arugula plant did not behave as a hyperaccumulating plant of cadmium under the conditions of the current research and the characteristics of the soil used in it, as the cadmium values did not exceed 100 mg cadmium/kg dry weight of the plant under any of the treatments. (Kabata Pendias and Pendias, 2001)

The strong correlation coefficient between cadmium concentrations in the soil and its concentrations in arugula plants in all treatments, which was R=0.93 for the relation between shoots and soil-cadmium concentrations, and R= 0.91 for the relation between roots and soil-cadmium concentrations, when pots treated with cadmium only. and R=0.95 for the relation between shoots and soil-cadmium concentrations, and R= 0.96 for the relation between roots and soil-cadmium concentrations. when pots treated with cadmium and lead together. confirmed the major role played by the total metal concentration in the soil in the accumulation process. (Yildirim *et al*, 2019), (Figures 1,2).

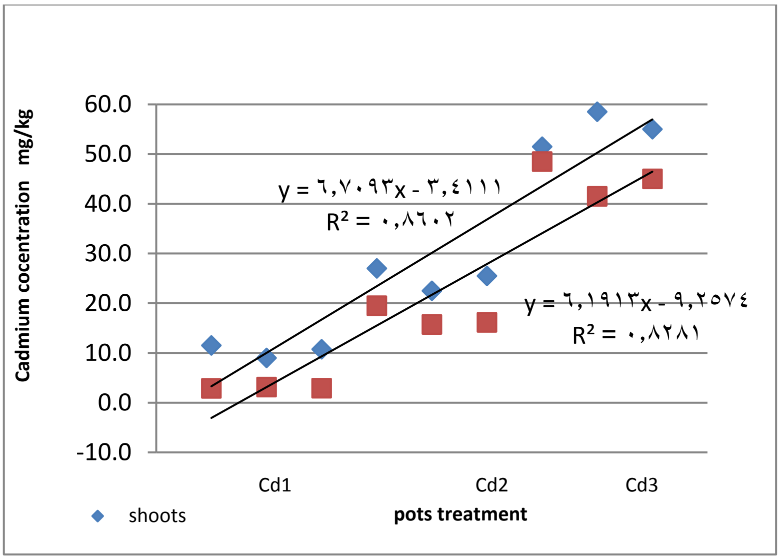

Fig 1. The regression equations and R-squared (determination coefficient) for cadmium concentration in the roots and shoots of arugula plants related to cadmium concentration in the soil/pots treated with cadmium only

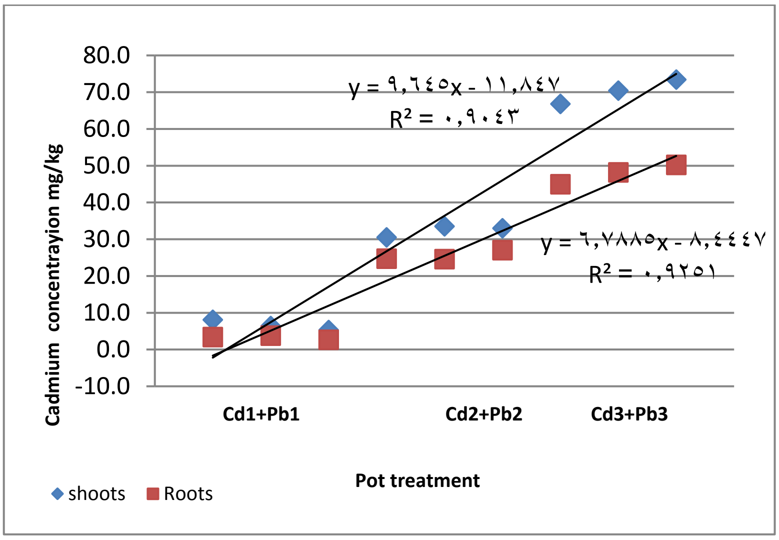

Fig. 2. The regression equations and R-squared (determination coefficient) for the concentration of cadmium in the roots and shoots of arugula plants in related to the concentration of cadmium in the soil/pots treated with cadmium and lead together.

3-2. Transfer Factor, Bioaccumulation and Bioconcentration of Cadmium in Arugula Plants

When calculating the transfer factor (TF), it was found that the ability of arugula to transfer cadmium from the roots to the leaves is high, as the values of the transfer factor in all treatments were greater than one, and the pots which soil was exposed to cadmium contamination with the minimum (3 mg kg^{-1} cadmium in the soil) measured high in the values of the transfer factor (TF). Transplanted onto those exposed to high levels of cadmium soil contamination (15 mg kg^{-1} cadmium in soil and 30 mg kg^{-1} cadmium in soil), which indicates the plant may be exposed to stress.

When conducting a statistical study on the SPSS software on the effect of the concentrations of metals added to the soil (pot treatments) on the accumulation of these metals in the roots and shoots of the studied plants and the biomarkers (bioconcentration factor BCF, bioaccumulation factor BAC, transfer factor TF), it was found that the treatments of the pots significantly affected the concentration of cadmium in both the roots and the shoots, and the plant efficiency parameters, where the values of $P<0.01$. When comparing the average concentrations of cadmium in the roots and the shoots of the arugula plant and the three treatments studied using the Duncan method, for the shoots, there was no significant effect of adding lead to the soil on the concentrations of cadmium in the shoots when treated with the minimum level only. Lead has a significant effect on increasing the cadmium content of shoots in treatments with higher concentrations. (Table 4).

Therefore, arugula can accumulate cadmium in its shoots, which is consistent with the study of Akoumianakis *et al.*, (2008) which states that cadmium accumulates in the edible parts of arugula, and according to Waheed *et al.*, (2022) the significant increase in cadmium accumulation in the leaves is associated with an increase in cadmium stress and indicates that *E.sativa* is a good candidate for phytoremediation of cadmium-contaminated soil.

SEY% decreases when cadmium concentrations increase, as the highest recorded value of SEY% reached 0.69% with the lowest concentration of cadmium at 3mg kg^{-1}, which indicates that the decrease in dry weight of the Arugula plant under the cadmium stress does not compensate for the increase of the concentration of cadmium in Arugula by increasing its concentration in the soil Hence, SEY% was the best when the cadmium concentration was 3 mg cadmium/kg soil, which is about 0.69%, a low percentage, because it indicates that we need to plant arugula more than 144 times, each time for two months, to completely clean the soil of cadmium, and here it is necessary to draw Given that the extraction yield is estimated relative to the total concentration of the metal in the spiked soil, that we added as cadmium nitrate, and since the cadmium available to the plant is the most important part when treating the soil, it is possible that the studied soil will need a much smaller number of times to plant arugula when the goal of the treatment is to get rid of only the available cadmium.

Table 4. The effect of lead and cadmium contamination in the soil on the cadmium concentrations of the roots and shoots of arugula plants, and on the efficiency of phytoremediation.

Cd	Cadmium concentration in roots (mg kg^{-1}) D.W	Cadmium concentration in shoots (mg kg^{-1}) D.W	BCF	BAC	TF	SEY%
Cd1	$3±0.18^d$	$10.5±1.41^e$	$1.0±0.059^b$	$3.5±0.47^a$	$3.5±0.68^a$	$0.69±0.14^a$
Cd2	$17.22±1.81^c$	$25.0±0.00^d$	$1.15±0.12^b$	$1.67±0.0^c$	$1.46±0.15^b$	$0.29±0.14^b$
Cd3	$45.0±3.54^a$	$55.0±4.95^b$	$1.50±0.12^a$	$1.83±0.16^{bc}$	$1.23±0.21^b$	$0.25±0.062^b$
Cd1+Pb1	$3.42±0.028^d$	$6.75±0.49^e$	$1.14±0.01^b$	$2.25±0.17^b$	$1.97±0.16^b$	$0.31±0.09^b$
Cd2+Pb2	$25.25±0.78^b$	$32.0±2.12^c$	$1.68±0.05^a$	$2.13±0.14^{bc}$	$1.27±0.05^b$	$0.38±0.08^b$
Cd3+Pb3	$47.67±1.02^a$	$69.6±2.55^a$	$1.59±0.034^a$	$2.32±0.085^b$	$1.46±0.02^b$	$0.38±0.17^b$
P value	$P<0.001$	$P<0.001$	0.001	0.002	0.002	0.036

4. Efficiency of Lead phytoremediation

4-1. Lead concentrations in Arugula Plants

The values of lead concentrations in the roots ranged between 2.675-87.33 mg kg^{-1}dry matter in all treatments, when lead in soil is more than 100 mg kg^{-1}, the root lead concentration has exceeded the natural range of lead in plants 0.2 - 20 mg kg^{-1} (Qunshan *et al.*, 2020). By comparing the pots that contained only lead and the pots that contained cadmium and lead, it was noted that the arugula plant was able to accumulate larger amounts of lead in its roots in the presence of contamination with lead and cadmium together, compared to the presence of lead alone in the soil. (Figure 4)

In contrast, the values of lead concentrations in the shoots ranged between 0.55 - 3.6 mg/kg dry matter in all treatments, within the natural range of lead in plants 0.2 - 20 mg kg^{-1} (Qunshan *et al.*, 2020). and by comparing the pots that contained only lead and the pots that contained both cadmium and lead, it was noted that the arugula plant was able to accumulate larger amounts of lead in its shoots in the presence of both lead and cadmium contamination, compared to the presence of lead alone in the soil, consistent with the accumulation of lead in the plant roots (Table 5). the low bioaccumulation of lead is due to it being highly insoluble and generally unavailable for plant uptake in the normal range of soil pH (Szczygłowska *et al.*, 2011)

It was found that there is a clear correlation between the concentrations of lead in the roots and shoots of arugula and the total concentration of the metal in the soil, whether in the presence of lead alone, with R=0.97 for the relation between shoots and soil-lead concentrations, and R=0.88 for the relation between roots and soil-lead concentrations, Yildirim *et al.*, (2019) or in the case of its presence with cadmium, with R=0.95 for either the relation between shoots or roots and soil-lead concentrations, (Figures 3, 4).

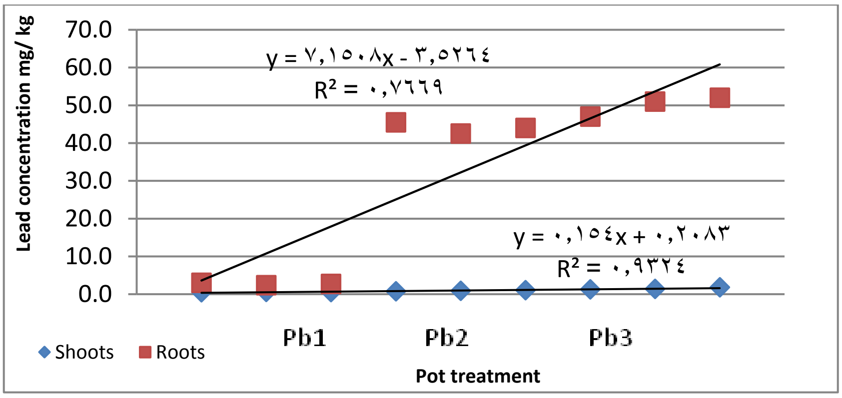

Fig. 3. The regression equations and R-squared (determination coefficient) for the concentration of lead in the roots and shoots of arugula plants and the concentration of lead in the soil/pots treated with lead only

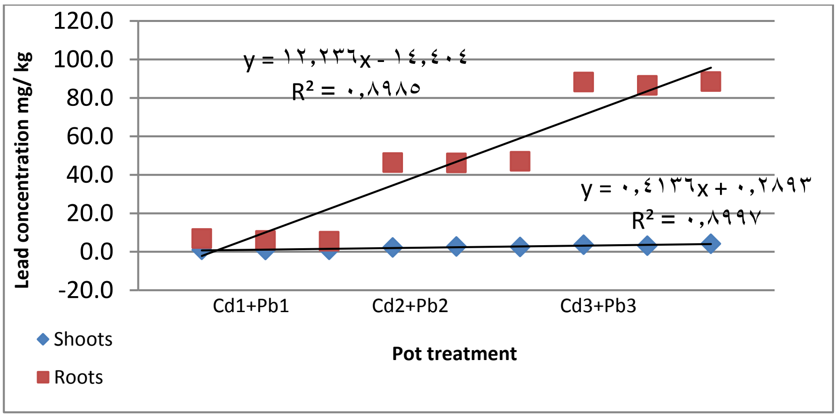

Fig. 4. The regression equations and R-squared (determination coefficient) for the concentration of lead in the roots and shoots of arugula plants and the concentration of lead in the soil/pots treated with lead and cadmium together.

4-2. Transfer Factor, Bioaccumulation and Bioconcentration of Lead in Arugula Plants,

We note from Table (5) that there is no significant concentration of lead in the root tissues compared to the soil content, and that it does not accumulate within the vegetative tissues above the soil surface. Even the values that accumulated within the roots were retained in them and not transferred to the vegetative system, which is attributed to the low mobility of lead in comparison with cadmium

(KabataPendias *et al.*, 2001) in addition to the soil characteristics that encouraged the deposition of both metals (relatively high clay content and high organic matter percentage). Shah *et al.*, (2010)

Even when lead is absorbed by plants, due to its relatively low mobility in plants, most of the lead absorbed by plants is confined to the roots, and only a small amount is transported to the shoots. (Gupta *et al.*, 2013)

When conducting a statistical study on the SPSS software on the effect of the concentrations of minerals added to the soil (potting treatments) on the accumulation of these metals in the roots and shoots of the studied plants and the biomarkers (bioconcentration factor BCF, bioaccumulation factor BAC, transfer factor TF), it was found that there was a significant effect of the treatments of the pots on the concentration of lead in both the roots and the shoots, and the plant efficiency parameters, with P values <0.01. When comparing the average concentrations of lead in the roots and the shoots of the arugula plant and the three treatments studied using Duncan method, adding cadmium to the soil led to an increase in the lead concentration. Significantly in the roots of arugula plants in all treatments, this was accompanied by a significant increase in the concentration of lead in the shoots. (Table 5)

Table 5. The effect of lead and cadmium contamination in soil on the lead concentrations of roots and shoots of arugula plants, and on the efficiency of phytoremediation.

Pb	lead concentration in roots (mg kg^{-1}) D.W	lead concentration in shoots (mg kg^{-1}) D.W	BCF	BAC	TF	SEY%
Pb1	2.67±0.5^{e}	0.55±0.07^{d}	0.027±0.005^{d}	0.0055±0.0007^{b}	0.21±0.06^{a}	0.0014±0.00009^{b}
Pb2	44.0±2.12^{c}	0.89±0.01^{d}	0.15±0.07^{a}	0.003±0.00004^{c}	0.02±0.0007^{b}	0.003±0.0011^{ab}
Pb3	49.5±2.12^{bC}	1.47±0.12^{c}	0.07±0.003^{c}	0.002±0.0001^{c}	0.03±0.0002^{b}	0.0014±0.0006^{b}
Cd1+Pb1	6.25±0.49^{d}	0.94±0.08^{d}	0.06±0.005^{c}	0.0094±0.0008^{a}	0.15±0.001^{a}	0.0019±0.0005^{b}
Cd2+Pb2	46.25±0.14^{b}	2.47±0.38^{b}	0.15±0.0005^{a}	0.008±0.001^{a}	0.053±0.053^{b}	0.0039±0.0006^{a}
Cd3+Pb3	87.33±1.23^{a}	3.6±0.07^{a}	0.13±0.0018^{b}	0.005±0.0001^{b}	0.041±0.0002^{b}	0.0028±0.0011^{ab}
P value	P<0.001	P<0.001	P<0.001	P<0.001	0.001	0.034

Plants with BCF, BAC, and TF > 1 are considered a promising plant extract suitable for the extraction and stabilization of heavy metals, while plants with bioconcentration factor and transfer factor of <1 are not suitable for plant extraction or plant stabilization, while plants with values of BCF > 1 and TF <1 are suitable for stabilization only (Amin *et al*, 2018).

Plants that have a ratio of metal concentration in the shoots to the total metal concentration in the soil greater than one, or a transfer factor greater than one, are considered potential candidates for use in phytoremediation using the bioextraction method (Mendez and Maier, 2008, Cruzado-Tafur *et al*, 2021).

By applying the above to the results of this study, we find that it is possible to use the arugula plant as a plant extract for cadmium, due to its BCF, BAC, andTF values being greater than one, while it did not show efficiency in extracting lead. and the specific extraction yield percentage SEY% for lead was very low, did not exceed 0.0039% (Table5).

Conclusions and Recommendations

No significant effect was observed of the concentration of lead and cadmium in the soil on the germination rate of arugula plants, nor was the dry or wet weight of the shoots significantly affected by increasing concentrations of lead and cadmium in the soil, while the dry weight and wet weight of the roots were significantly affected, The biomass of arugula plants decreases when exposed to increasing concentrations of lead and cadmium, as all values of the tolerance index (TI) were less than one, , and the roots were affected more than the shoots, and it was able to concentrate quantities of lead in its roots, but it was unable to transfer the metal to the shoots in significant quantities, and to concentrate cadmium in its roots, accumulate it in its shoots, and transfer cadmium from the roots to the shoots as all phytoremediation efficiency factors (BCF, BAC, TF) were greater than one.

The arugula plant did not exhibition ability to concentrate lead in the roots, accumulate it in the shoots, or transfer it from the roots to the shoots, as all phytoremediation efficiency factors were less than one It is possible that the arugula plant is considered a promising plant extract suitable for extracting cadmium from soils contaminated with it, because it possesses a BCF, BAC, and TF$>$1, We recommend continuing the study on the ability of the arugula plant to carry out the bio-extraction process in soils with properties that encourage the transfer of heavy metals, such as a lower percentage of clay, a lower percentage of organic matter, and even on other heavy metals, because the arugula plant demonstrated a good ability to extract cadmium under the conditions of this research.

References

- Akoumianakis, K.A., H.C. Passam, P.E. Barouchas, and N.K. Moustakas. (2008). Effect of cadmium on yield and cadmium concentration in the edible tissues of endive (*Cichorium endivia* L.) and rocket (*Eruca sativa*). Journal of Food, Agriculture and Environment, 6 (3-4): 206 - 209.

- Amin, H., B.A. Arain, T.M. Jahangir, M. S. Abbasi, and F. Amin. (2018). Accumulation and distribution of lead (Pb) in plant tissues of guar (*Cyamopsis tetragonoloba* L.) and sesame (*Sesamum indicum* L.): profitable phytoremediation with biofuel crops, *Geology, Ecology, and Landscapes*, 2(1): 51-60.
- Anjum, N.A., S. G. Sarvajeet, I. Ahmad, M.E. Pereira, M. Pacheco, A.C. Duarte, Sh. Umar, and N.A. Khan. (2012). The Plant Family *Brassicaceae* - Contribution Towards Phytoremediation, Environmental *Pollution,* 21(1):1-33.
- Audet, P., and C. Charest. (2006). Effects of AM colonization on "wild tobacco" plants grown in zinc contaminated soil. Mycorrhiza, 16: 277-283.
- Audet, P., and C. Charest .2007. Heavy metal phytoremediation from a meta-analytical perspective, *Environmental Pollution*, 147(1): 231-237.
- Benavides, M.P, S.M. Gallego, and M.L. Tomaro. (2005). Cadmium toxicity in plants, Brazilian Journal of Plant Physiology, 17(1): 21-34.
- Bortoloti, G. A., and D. Baron. (2022). Phytoremediation of toxic heavy metals by Brassica plants: A biochemical and physiological approach, Environmental *Advances*, 8.
- Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy journal*, 54(5): 464-465.
- Cannata, M.G., R. Carvalho, A.C. Bertoli, A.S. Augusto, R.R. Bastos, J.G. Carvalho, and M.P. Freitas. (2013). Effects of cadmium and lead on plant growth and content of heavy metals in Arugula cultivated in nutritive solution, *Communications in Soil Science and Plant Analysis*, 44(5): 952-961.
- Chapman, H.D. (1965). Cation exchange. In: C. A. Black (Ed.) Methods of soil analysis - Chemical and microbiological properties. *Agronomy*, 9(2): 891-901.
- Chen, L., X.H. Long, Z.H. Zhang, X.T. Zheng, Z. Rengel, and Z.P. Liu. (2011). Cadmium accumulation and translocation in two Jerusalem artichoke (Helianthus tuberosus L.) cultivars, *Pedosphere*, 21(5): 573-580.
- Cruzado-Tafur, E., K. Bierla, L. Torró, and J. Szpunar .(2021). Accumulation of as, Ag, Cd, Cu, Pb, and Zn by Native Plants Growing in Soils Contaminated by Mining Environmental Liabilities in the Peruvian Andes, Journal *Plants*, 10(241): 1-23.
- Dar, M.I., F.A. Khan, F. Rehman, A. Masoodi, A.A. Ansari, D. Varshney, F. Naushin, and M.I. Naikoo .(2015). Roles of Brassicaceae in Phytoremediation of Metals and Metalloids, Phytoremediation*: Management of Environmental Contaminants,* 1: 201 - 213.

- Dede, G., S. Ozdimir, and O.H. Dede. (2012). Effect of soil amendments on phytoextraction potential of Brassica juncea growing on sewage sludge,*International Journal of Environmental Science and Technology*, 9: 559-564.
- Garg, G., and V. Sharma. (2014). *Eruca sativa* (L.): Botanical Description, Crop Improvement, and Medicinal Properties, Journal of Herbs Spices Plants and Medicinal,20(2): 171-182.
- Ghaderian, M., and S. Nosouhi .(2015). The capability of uptake and removal of toxic heavy metals from the industrial discharge of Mobarakeh Steel Complex by some metal accumulating plants, Journal of Plant Process and Function Iranin Society Of Plant *Physiology*, 4(12): 43- 49.
- Ginneken, L. V., E. Meers, R. Guisson, A. Ruttens, K. Elst, F.M.G. Tack, and W. Dejonghe .(2007). Phytoremediation for heavy metal contaminated soils combined with energy production. *Journal of Environmental Engineering and Landscape Management* ,15(4): 227-236.
- Gupta, D. K., H. G. Huang, and F. J. Corpas .(2013). Lead tolerance in plants: Strategies for phytoremediation, *Environmental Science and Pollution Research*,20: 2150-2161.
- Gupta, P.K. (2000). Soil, plant, water and fertilizer analysis, Agrobios, Second Edition, New Dehli, India, 438.
- Kabata-Pendias, A.,Pendias.H. (2001).*Trace Elements in Soils* and Plant, Third Edition, London:CRC Press.
- Malik, R. N., S. Z. Husain, and I. Nazir .(2010). Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, *Pakistan Journal of Botany*, 42(1), 291-301.
- Mendez, M.O., and, R.M. Maier. (2008). Phytostabilization of mine tailings in arid and semiarid environments-an emerging remediation technology,*Environment Health Perspective*, 116(3): 278-283.
- Qunshan, W.B., Noman, M, Shen, Z, Saba A. K, Ullah,S, Khan,F, Panhwar,K, Emiliy, H, Tasleem,R, Ahmad,J, Ul Haq,I, SubhHanullah h,M, and Ullah, Z. (2020). Phytoremediation of contaminated soil Lead and Cadmium by Brassica júncea (L.) Czern plant, *Journal of Earth Sciences and Environmental Studies,* 5(4): 110-120.
- Raz,A, M. Habib, Sh. Kakavand, Z. Zahid, N. Zahr, R. Sharif, and M. Hasanuzzaman. (2020). Phytoremediation of Cadmium: Physiological, Biochemical, and Molecular Mechanisms, *Biology*, 9(177).
- Sarma, H. (2011). Metal Hyperaccumulation in Plants: A Review Focusing on Phytoremediation Technology, *Journal of Environmental Science and Technology*, 4(2):118-138.
- Shah, F.U.R., N. Ahmad, Kh. R. Masood, J.R. Peralta-Videa, and F.U.D. Ahmad,.2010. Heavy Metal Toxicity in Plants, Ch: 4, Springer.
- Roy, Sh., and S. Mondal . (2020). *Brassicaceae plants response and tolerance to metal/metalloid toxicity-The Plant Family Brassicaceae*, Hasanuzzaman, Singapore: Springer.

- Szczygowska, M., A. Piekarska, p. konieczka, and J. Namiesnik. (2011). Use of Brassica Plants in the Phytoremediation and Biofumigation Processes, I*nternational Journal of Molecular Sciences*, (12): 7760-7771.
- Thangavel, P., and C.V. Subbhuraam .(2004). Phytoextraction: Role of the Hyperaccumulator in Metal Contaminated Soils*, Indian natn.Sci. Acad,*70(1): 109-130.
- Waheed, A., Y. Haxim, W. Islam, M. Ahmad, S. Ali, X. Wen, Kh. Ali Khan, H.A. Ghramh, Z. Zhang, and D. Zhang. (2022). Impact of Cadmium Stress on Growth and Physio-Biochemical Attributes of *Eruca sativa* Mill, *Plants*, 11(2981).
- Walkley, A. and I. A. Black. (1934). An examination of degtjareff method for determination soil organic matter and a propsed modification of the chromic acid titration method. *Soil Science*, 37(1): 29-38.
- Yildirim ,E., M. Ekinci, M. Turan, G. Agar, S. Örs, A. Dursun, R. Kul, and T. Balci. (2019). Impact of Cadmium and Lead Heavy Metal Stress on Plant Growth and Physiology of Rocket (*Eruca* sativa L.),*KSU J. Agric Nat*, 22(6): 843-850.
- Yoon, J., X. Cao, Q. Zhou, and L.Q. Ma. (2006). Accumulation of Pb, Cu, and Zn in Native Plants Growing on a Contaminated Florida Site. *Science of The Total Environment*, 368(2-3): 456-464.

N° Ref: 1188