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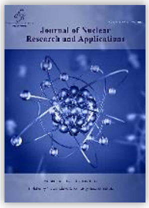

Selection of Appropriate Plant for Remediation of Uranium from Salty Soils

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A B S T R A C T

Phytoremediation is increasingly recognized as an effective method for decontaminating soil and water. In this study, different plants' ability to remediate of uranium from contaminated soils was investigated. The *Amaranthus tricolor, Bassia scoparia, Triticum, Brassica juncea, Conyza canadensis (L) Cronq,* and *Brassica napus* were planted in the sample soils of Isfahan's nuclear area which was contaminated with 300 mg/Kg uranium. The plants were harvested after one and three months, and the uranium content in different parts of the plants was analyzed using the ash method. The results showed higher uranium concentration in the plants cultured in the soil with higher salinity. The addition of fertilizer did not enhance the uranium absorption from the soil. The amount of uranium in different parts of the plants was increased over time. While the maximum accumulation in most of the plants was observed in the roots, the highest uranium concentration in Conyza canadensis (L.) Cronq was found in the stem. Among the plants selected for phytoremediation, Conyza canadensis (L) Cronq indicated the highest uptake of uranium with the average amount of 3233.02 and 3727.81 mg/kg respectively, in the stem after 1 and 3 months. The transfer factor for leaf, stem and root of Conyza canadensis (L) Cronq was determined as 7.40, 12.43 and 6.02; the translocation factor was also calculated as 1.75 after 3 months. Based on these results, Conyza canadensis (L) Cronq can be a promising candidate for phytoremediation of soils contaminated with uranium.

Keywords: Bassia scoparia; Brassica juncea; Conyza canadensis (L) Cronq; Phytoremediation.

1. Introductions

The rapid development of new technologies has been accompanied by environmental problems, including soil, water, and air pollution. While contamination can arise from a wide range of inorganic and organic compounds, inorganic contaminants, particularly heavy metals, pose

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more serious challenges [1]. Heavy metals are of particular concern due to their non-biodegradability. Most heavy metals interfere with the biological processes of living cells and can even inhibit certain reactions. Additionally, heavy metals can become integrated into the body's metabolism, disrupting enzyme activity [2].

The toxicity of heavy metals including nephrotoxicity, neurotoxicity, hepatotoxicity, skin and cardiovascular toxicity has been well established and depends on factors such as dose, route of exposure, and chemical species [3,4]. Uranium, as a heavy metal, exhibits both chemical and radiotoxicity. It can cause renal failure, impaired bone growth, cancer, and a shortened lifespan due to its low-level radioactivity [5]. As part of the nuclear fuel cycle, uranium mining and processing can release varying amounts of uranium into the ecosystem particularly in the soil. Given the hazards and toxicity of uranium, decontamination of contaminated soils is of critical importance.

Various physical, chemical, and biological methods have been proposed to treat contaminated areas; these methods are often expensive and uneconomical [6]. Phytoremediation is considered an effective, economical, and environmentally friendly method and is defined as the use of plants to degrade or extract foreign substances from soil or water by rhizosphere filtration, absorption, stabilization, degradation, and volatilization [7]. Plants provide a sustainable, in-situ, non-disruptive, self-sufficient, and independent method for the remediation of pollutants from soil [8].

This technology dates back to 1948, when Minguzzi and his colleagues reported the high accumulation of nickel in the Italian spiral plants Alyssum bertolonii [9]. Since then, extensive studies have been conducted on the ability of plants to absorb heavy metals and radionuclides. To date, more than 400 plant species belonging to 45 families, such as Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, and Lamiaceae, as having the genetic potential to absorb and tolerate high amounts of heavy metals in their aerial parts have been identified [10].

Several studies have also reported on uranium removal from soil. According to these studies, B. juncea and Helianthus annuus can absorb more uranium than the other plants and are considered uranium hyperaccumulators [11,12,13]. However, the phytoremediation efficiency is influenced by various factors, especially soil properties such as pH, water content, soil texture, and organic matter content [14]. Therefore, selecting the appropriate plant should be based on the soil and weather conditions of the area under study.

This study aimed to select an appropriate plant for uranium uptake from the soil of Isfahan's nuclear area for the first time. Two soil samples were selected for this purpose, each contaminated with 300 mg/kg of uranium. The plants were grown in the greenhouse for three months. The effects of the harvesting time and the fertilizer addition on uranium absorption were investigated. For this purpose, The uranium content in different parts of the plants was determined using the ash method.

2. Material and methods

Two soil samples from Isfahan's nuclear area were selected for planting. ICP-AES, optima 7300 DV (USA), was used for the measurement of cations and anions. XRD, Philips pw 1800 (Netherlands), and XRF, Philips 2404 (Netherlands), were utilized to determine the main minerals in the soil.

2.1. Soil sampling and analysis

Soil sampling from Isfahan's nuclear area was conducted at the depth of 0 to 20 cm. The soil characteristics were examined using the ASTM standard method (ASTM D4972, ASTM D1125, ASTM D7263-21, ASTM D422-63, ASTM D7503, and ASTM D2974). Soil texture analysis was performed using the hydrometric method, determining the proportions of clay, silt, and sand. The amounts and types of constituent minerals were determined through X-ray diffraction (XRD) and X-ray fluorescence (XRF) analysis.

2.2. Soil contamination

The soil was passed through a 2-mm stainless steel sieve and then treated with 300 mg/Kg of uranium. For this purpose, uranium nitrate salt was used [12,15], dissolved in 50 ml of distilled water and sprayed on the soil. Uranium nitrate was dissolved in distilled water and sprayed on the soil surface. To stabilize and balance the soil and to assimilate the artificial pollution conditions with the natural pollution conditions, the contaminated soil was left exposed to the air for two weeks. Then the contaminated soils were reanalyzed to confirm the presence of uranium and to assess the stabilization of the pollution.

2.3. Planting and harvesting

In this research, Amaranthus Tricolor, Bassia scoparia, Triticum, Brassica juncea, Conyza canadensis (L) Cronq, and Brassica napus were selected. The seeds were obtained from the Seed and Plant Improvement Institute (Karaj, Iran). The selection of the plants was performed based on their tolerance to salinity and high uranium absorption capacity. The plants were planted in plastic pots (three plants per pot) with a diameter of 18 cm and a height of 15 cm and kept in the greenhouse (temperature of 23±2 °C and light/darkness: 12h/12h cycle). Some pots were filled with 3.2 kg of the contaminated soil, the others were filled with 3.150 kg of the mentioned soil, 63 g of manure, 0.63 g of urea, and 0.4 g of ammonium phosphate to investigate the effect of fertilizer. The pots were irrigated twice a week.

Plants were harvested at two intervals: one month and three months after planting. The plant roots were thoroughly rinsed with distilled deionized water to remove soil and prevent any contamination that could affect the measurement of uranium absorption. The different plant organs (leaf, stem, and root) were then separated for further analysis.

2.4. Sample analysis

The samples were dried at 80 °C for 24 hours, milled and sieved through a 1 mm mesh to achieve homogeneity. The samples were weighed with high-precision balance, and then digested using the ash method. The furnace temperature was gradually raised to 500 °C and maintained for 5 to 6 hours. Then 10 ml of 65% nitric acid was added to each sample and the samples were transferred to a 50 ml Erlenmeyer flask. The samples were placed in an oil bath on a heater at 120 ° C. The amount of uranium in each sample was determined using an ICP-OES spectrometer.

2.5. Calculation of the transfer factor and translocation factor

The amount of uranium transferred from the soil to the plant is calculated for each sample as a transfer factor (TF) using Eq. 1 [16].

$$\mathrm{TF} = \frac{\text{Uranium concentration in plant parts}}{\text{uranium concentration in soil}} \qquad (1)$$

The translocation factor was also determined using Eq. 2 [17].

$$\text{Translocation factor} = \frac{\text{metal concentration in aerial organs}}{\text{metal concentration in the underground organs}}$$

2.6. Statistical analysis

To analyze the data statistically, three pots were maintained under identical conditions, with at least three plants cultivated in each pot. Student's T-test was used to compare the data and all values were presented as mean ± standard deviation. The statistical significance was considered $P<0.05$.

3. Results and discussion

3.1. Soil characteristics

The results of soil analysis are presented in Table 1. As indicated, both types of soils are calcareous with a high percentage of lime and with an alkaline pH. As seen in Fig. 1, according to the results of XRD analysis of soil samples, the main constituent minerals are Calcite (01-086-2334) and Quartz (01-079-1906) shown in θ2 spectrum marked with * and ● respectively. In soil sample 1, in addition to the main soil compounds, small amounts of Dannemorite (00-023-0302), and Ferroglaucophane (00-027-0714) were observed, which are marked with ▼ and ■ symbols, respectively. In soil sample 2, while observing a small amount of Dannemorite composition, Potassium Oxide composition (00-023-0493) is also observed, which is indicated by the ♦ symbol. The results of XRF analysis show that the principal oxides of the soils include (CaO, SiO_2, Fe_2O_3 and Al_2O_3) (Table 2).

Table 1. The characteristics of the soil used for planting.

Soil number	SP	EC(ds/m)	pH	TNV%	OM%	Sand%	Silt%	Clay%	texture
1	28.0	13.41	7.88	51.19	1.27	70	10	20	S.C.L
2	20.11	8.0	7.82	56.42	0.89	78	14	8	S.L

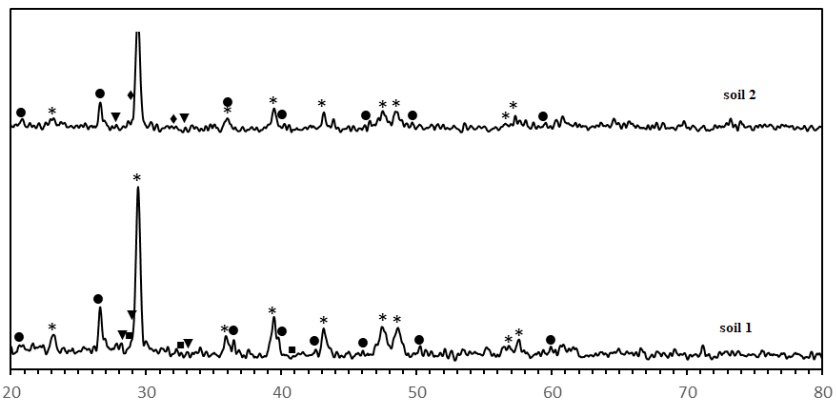

Fig. 1. XRD analysis of soil samples.

Table 2. The results of XRF analysis of soil samples.

Sample number	Oxides in soil and the weight percentages					
	CaO	SiO_2	Fe_2O_3	Al_2O_3	MgO	P_2O_5, SO_3, K_2O, TiO_2, MnO, SrO
1	68.52	19.36	5.54	2.80	< 1.0	< 1.0
2	80.14	12.06	3.61	1.60	< 1.0	< 1.0

3.2. Planting

Brassica juncea did not grow in the soil, likely due to salinity and soil texture. Regarding the Amaranthus tricolor, it should be noted that the seeds were planted in at least eight pots (each pot containing 3 to 5 plants). But in these pots, all but one plant dried up and only one remained, and its absorption after one month was investigated. Among the plants selected, Brassica napus, Triticum, Bassia scoparia, and Conyza canadensis (L) Cronq had the best growth, respectively.

3.3. Uranium absorption by plants

The amounts of uranium in different parts of the plants for various soils, harvesting times, and two conditions of with or without fertilizer are presented in Figs 2 to 10. As shown, in most of these plants, the highest uranium absorption was observed in the roots, which is not favorable for the phytoremediation process. Straczek and his colleagues in 2010 also reported the highest absorption of uranium in the roots of Triticum [18]. Only in Brassica napus (after three months) and Conyza canadensis (L) Cronq did the amounts of uranium in the leaves and stems exceed those in the roots which shows the ability of these plants to transfer uranium to the aerial part of the plant.

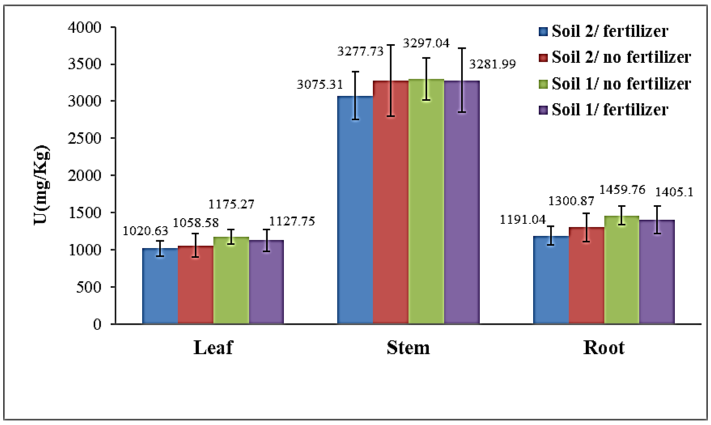

Fig. 2. The amounts of uranium (mg/kg) in different parts of Conyza canadensis (L) Cronq after one month.

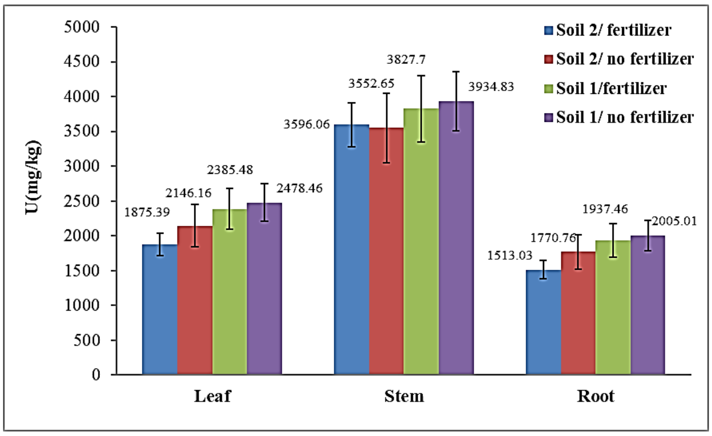

Fig. 3. The amounts of uranium (mg/kg) in different parts of Conyza canadensis (L) Cronq after three months.

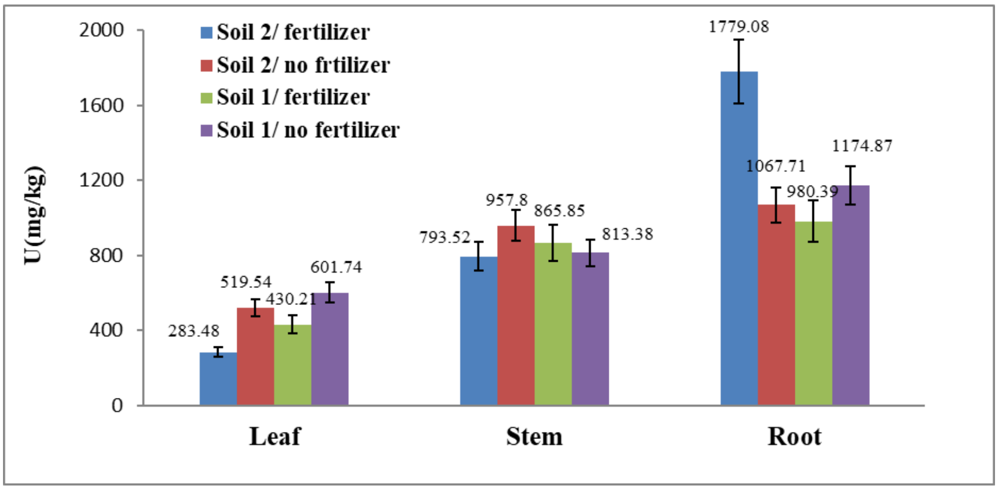

Fig. 4. The amounts of uranium (mg/kg) in different parts of Bassia scoparia after one month.

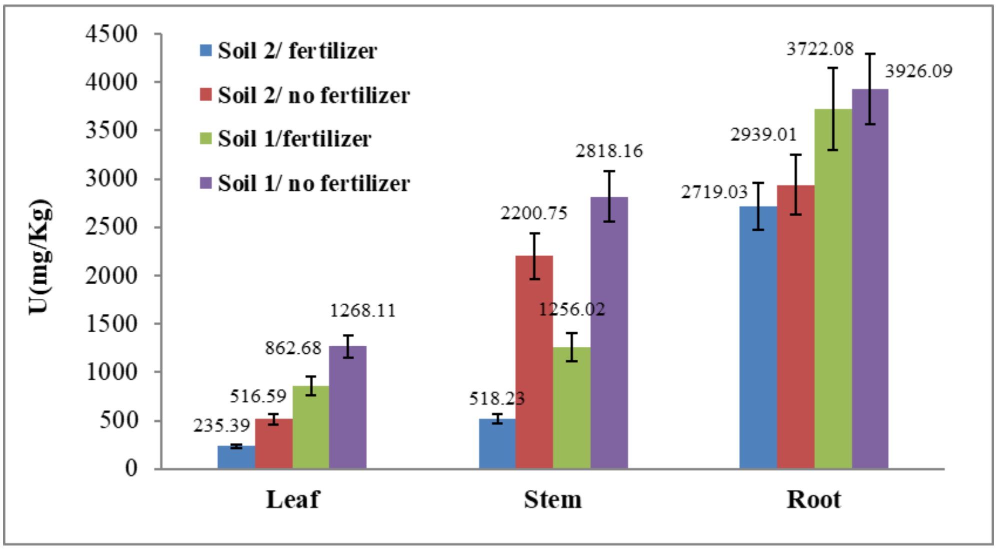

Fig. 5. The amounts of uranium (mg/kg) in different parts of Bassia scoparia after three months.

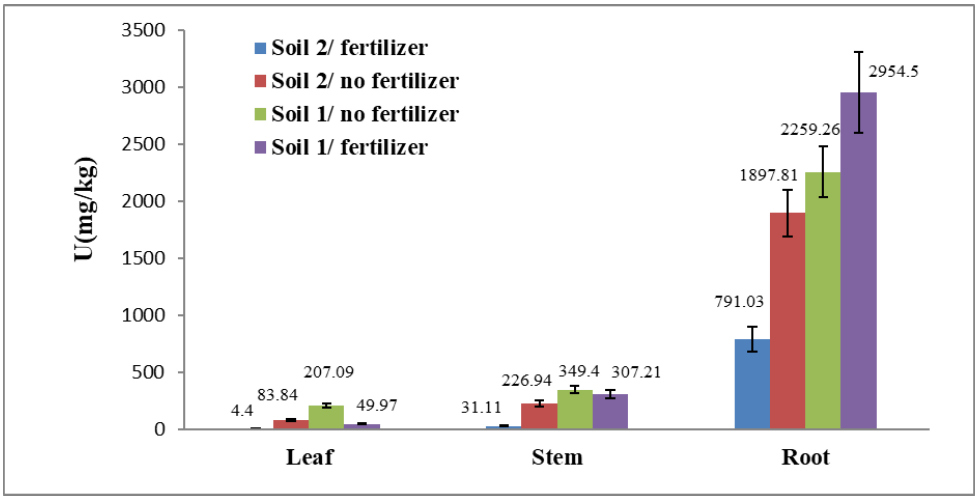

Fig. 6. The amounts of uranium (mg/kg) in different parts of Triticum after one month.

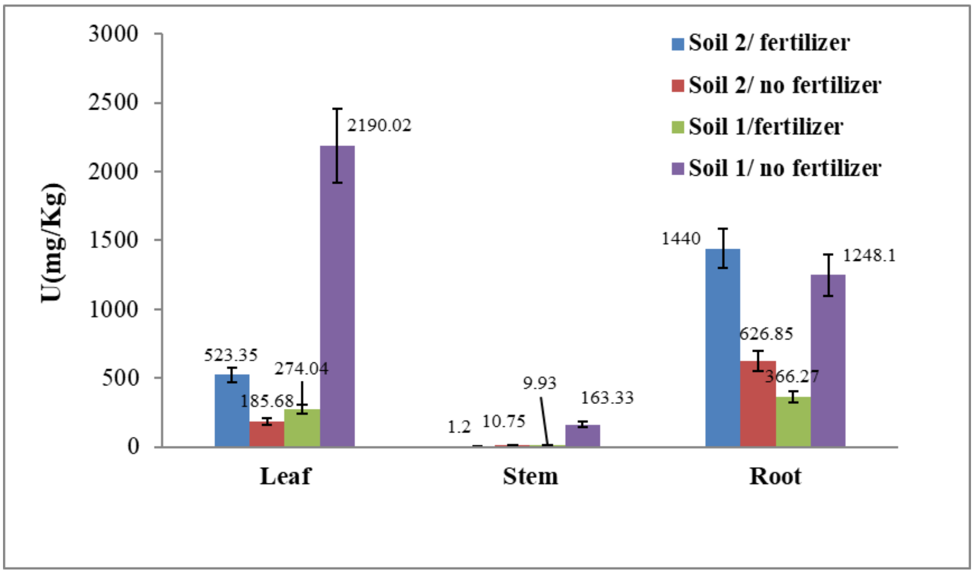

Fig. 7. The amounts of uranium (mg/kg) in different parts of Triticum after three months.

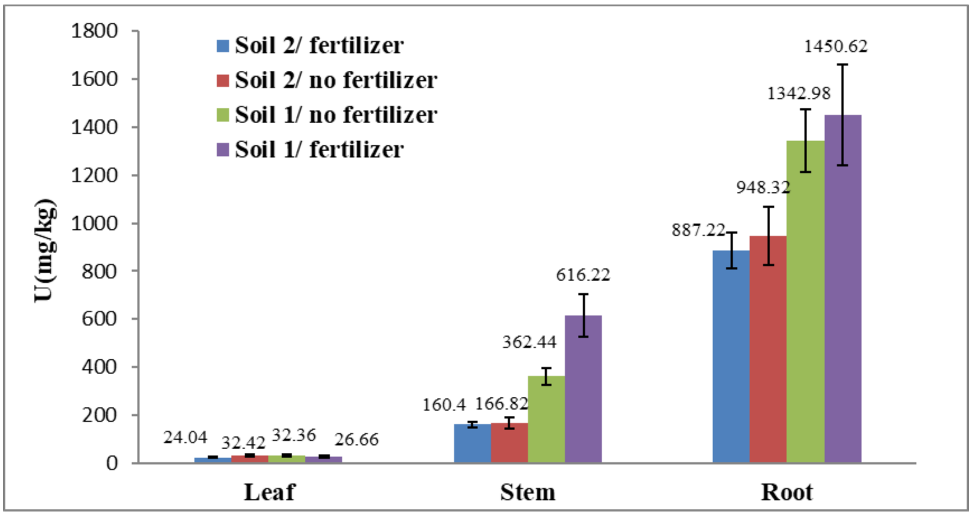

Fig. 8. The amounts of uranium (mg/kg) in different parts of Brassica napus after one month.

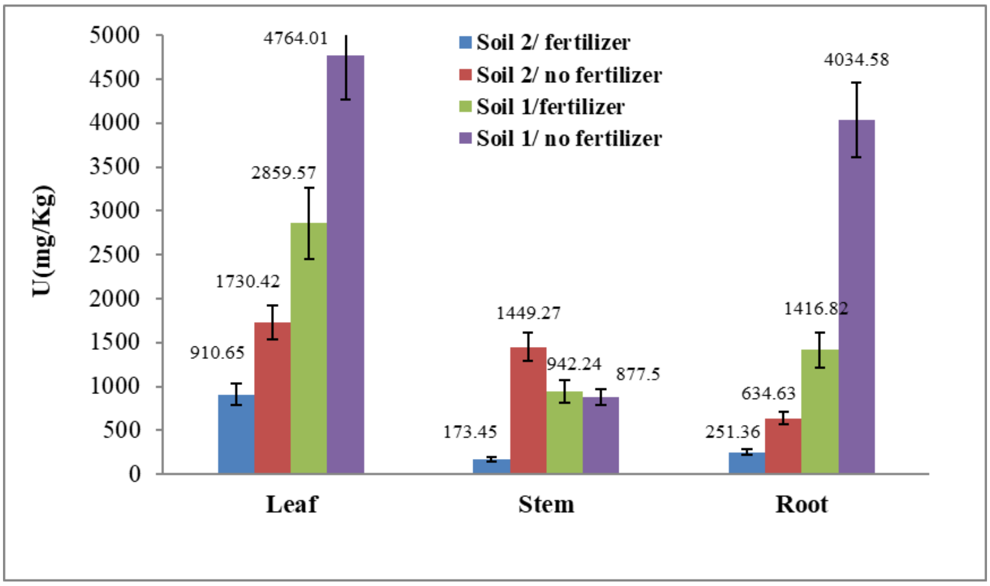

Fig. 9. The amounts of uranium (mg/kg) in different parts of Brassica napus after three months.

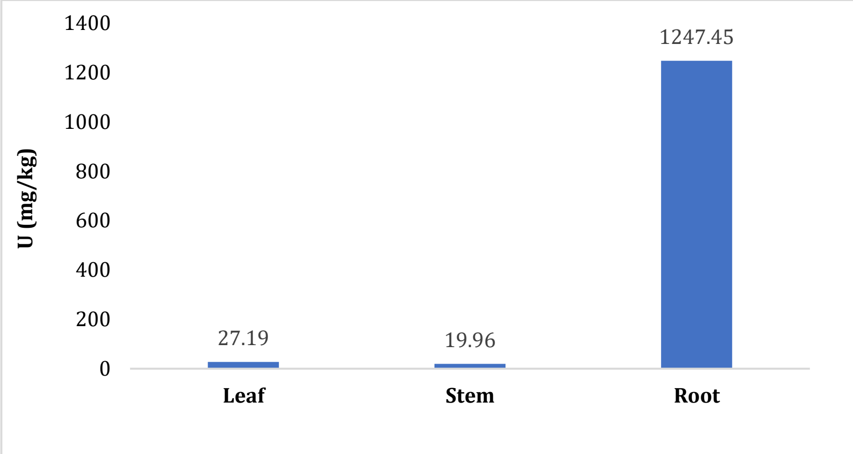

Fig. 10. The amounts of uranium (mg/kg) in different parts of Amaranthus Tricolor after one month (soil 1, fertilizer).

The amount of uranium in different parts of the plants in soil sample1 increased over both one-month and three-month periods compared to soil sample 2 (except for one case, Triticum, with a harvest period of three months). The difference in the amount of uranium absorption in two types of soils can be attributed to various factors, such as soil texture and salinity. The research of Kadkhodaie and his colleagues in 2012 indicated the increment of the absorption rate of heavy metals in all parts of the plant with the enhancement of soil salinity [19]. Therefore, according to the higher EC of soil sample 1 compared to soil sample 2, the increment of uranium absorption in different parts of the plant in soil sample 1 can be related to the higher salinity and consistent with the findings of Kadkhodaie and colleagues.

The absorption of uranium in the dry weight of the plant decreased slightly over the two harvesting periods of one month and three months when the fertilizer was added. This reduction in uranium absorption due to the addition of fertilizer is consistent with the findings of Sevostianova and colleagues. Their research indicated that increasing fertilizer levels reduces uranium uptake by plants, and therefore it is not recommended for the phytoremediation process [20].

The amount of uranium in all parts of the plants increases with the extension of the harvesting period from one month to three months. The only exception was observed in Triticum where uranium levels in the stem and root decreased from 228 mg/kg to 46 mg/kg and from 1975 mg/kg to 1840 mg/kg, respectively. However, the amounts of uranium in the leaves increased from 86 mg/kg to 793 mg/kg. This suggests that uranium may have been translocated from the underground parts of the plant to the aerial parts, specifically from the stem to the leaves.

For better comparison, the average amounts of uranium absorption in different parts of the plants after one and three months are indicated in Fig. 11 and 12, respectively. As shown, Conyza canadensis (L) Cronq exhibits higher uranium absorption in the aerial parts (3233.02 mg/kg in stem compared to 1339.19 mg/kg in root after one month; and 3727.81 in stem than 1806.56 mg/kg in root after three months). Bassia scoparia and brassica napus also showed high uptake: 720.69 and 2566.16 in leaf, 1698.29 and 860.61 in stem, 3326.55 and

1584.34 in root, respectively. A plant capable of accumulating more than 1000 mg/kg of uranium in its dry weight is considered a hyperaccumulating plant [21]. According to the results, Conyza canadensis (L) Cronq, Bassia scoparia and brassica napus have absorbed more than 1000 mg/kg of uranium in their parts after three months.

Conyza canadensis (L.) Cronq is an annual weed that typically grows to a height of about 50 to 100 cm. This plant belongs to the Asteraceae family and is widely distributed across many countries in North America and Asia. It grows in most areas, although it can grow in various environments, it prefers stony, sandy, or loamy soils with high nitrogen content and has good resistance to drought. Due to its strong ecological adaptability, the Conyza Canadensis (L.) Cronq has spread in many places such as rivers, roadsides and agricultural lands [22].

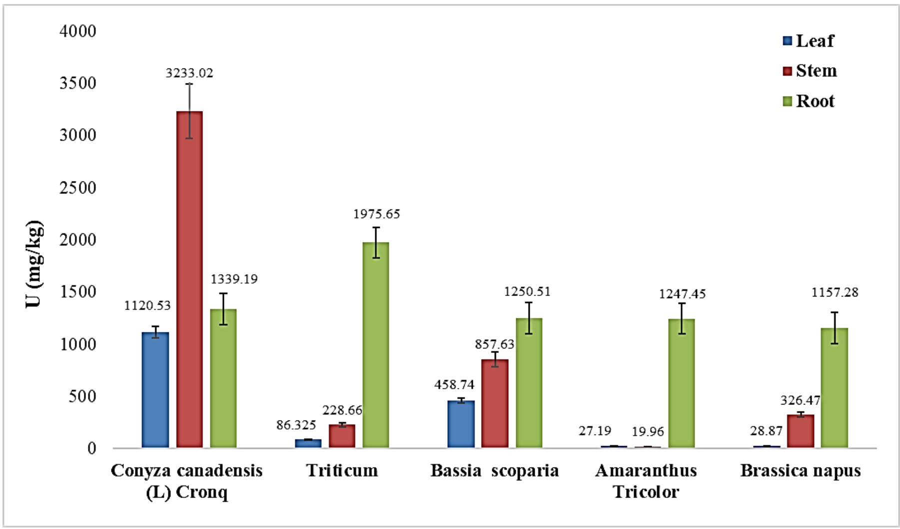

Fig. 11. The average amounts of uranium (mg/kg) in different parts of the plants after one month (for both soil samples and conditions: with or without fertilizer).

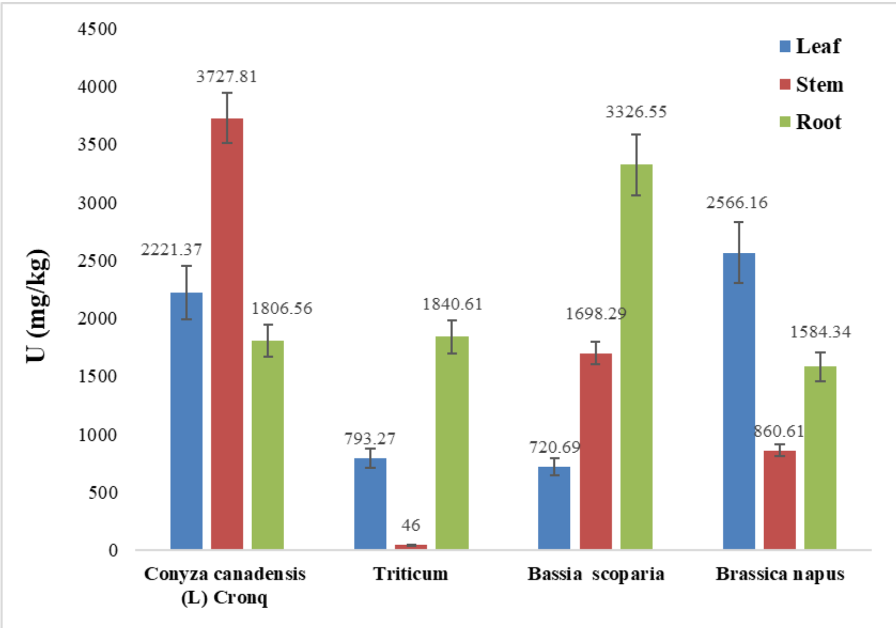

Fig. 12. The average amounts of uranium (mg/kg) in different parts of the plants after three months (for both soil samples and conditions: with or without fertilizer).

In the recent research by Xie and his colleagues on the uranium absorption in 31 plant species from a uranium-contaminated area in China, more than 600 mg/kg of uranium in the dry weight of the plant was observed in only three species, including Conyza canadensis (L) Cronq. This group of researchers recognized Conyza canadensis (L) Cronq as an effective plant in the phytoremediation of uranium-contaminated soils, however, the effect of various factors on the absorption of uranium by the plant has not been investigated. As a result, these researchers suggested more studies on the ability of Conyza canadensis (L) Cronq to remediate soils contaminated with uranium [25]. The results of this study also show the uranium content of more than 1000 mg/kg in each part of the plant, which is in agreement with the results of Xie and his colleague's research.

The average values of the transfer factor are presented in Tables 3 and 4 for one and three months, respectively. As expected, the transfer factor values increase with the duration of harvesting. The highest quantities of transfer factor were observed in Conyza canadensis (L) Cronq. The Transfer factors for hyperaccumulator plants should exceed one". However, all plants had transfer factors greater than one after three months, only Conyza canadensis (L) Cronq and Bassia scoparia indicated the values of higher than 1 after one month.

Although various studies have investigated phytoremediation and the removal of uranium from soil by plants, it is important to note that the amount of uranium absorbed by plants depends on several factors, including soil type, the initial concentration of uranium in the soil, the addition of fertilizers and chelating agents, and harvesting time. As a result, the authors tried to compare the results of this study with the research with high similarities. Table 5 indicates the values of uranium transfer factor for Brassica juncea and Helianthus annuus reported in the research conducted by Chang and his colleagues [26].

Table 3. The average values of transfer factors for different plants after one month (for both soil samples and conditions: with or without fertilizer).

Plants	leaf	stem	root
Conyza canadensis (L) Cronq	3.73 ± 0.42	10.77 ± 1.33	4.46 ± 0.64
Triticum	0.28 ± 0.03	0.76 ± 0.07	6.58 ± 0.59
Bassia scoparia	1.52 ± 0.11	2.85 ± 0.30	4.17 ± 0.54
Amaranthus Tricolor	0.09 ± 0.01	0.06 ± 0.01	4.16 ± 0.35
Brassica napus	0.04 ± 0.00	0.54 ± 0.07	1.92 ± 0.19

Table 4. The average values of transfer factors for different plants after three months (for both soil samples and conditions: with or without fertilizer).

Plants	leaf	stem	root
Conyza canadensis (L) Cronq	7.40 ± 0.86	12.43 ± 1.55	6.02 ± 0.83
Triticum	2.64 ± 0.20	0.15 ± 0.01	6.13 ± 0.62
Bassia scoparia	2.4 ± 0.17	5.66 ± 0.61	11.09 ± 1.55
Brassica napus	8.55 ± 0.91	2.87 ± 0.35	5.28 ± 0.47

Table 5. The values of transfer factor for Indian mustard and Brassica napus after 15-weeks [26].

Plant	leaf	stem	root
Brassica juncea	-	1.02	1.37
Helianthus annuus	0.01	0.02	1.33

In this study, the highest transfer factor values, observed in the stem of Conyza canadensis (L.) Cronq, were 10.72 after one month and 12.43 after three months (Tables 2 and 3), These values are higher than those reported by Chang and colleagues for Brassica juncea and Helianthus annuus (which are known to be among the most uranium-accumulating plants in numerous studies) [11-13].

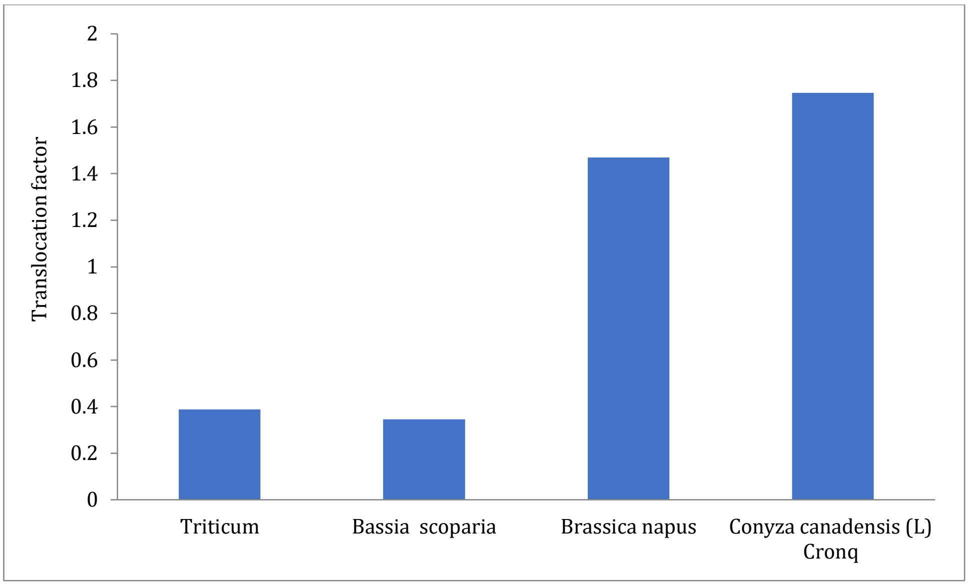

Fig. 13. The translocation factor for the plants after 3 months.

Nevertheless, a plant must also have the ability to accumulate the metal in its aerial parts. To achieve this, the translocation factor should be greater than one [i]. The translocation factor for the plants after 3 months is presented in Fig. 13 which shows the amounts of higher than 1 for Conyza canadensis (L) Cronq and Brassica napus.

5. Conclusion

In this study, the uranium absorption capacities of various plants, including Conyza canadensis (L.) Cronq, Triticum, Bassia scoparia, and Brassica napus, were investigated. The results showed higher uranium absorption in plants grown in soil with higher salinity. The highest uranium concentration was observed in Conyza canadensis (L.) Cronq. The uranium content in different parts of Conyza canadensis (L.) Cronq exceeded 1000 mg/kg even after only one month of planting. The transfer factor for various parts of Conyza canadensis (L.) Cronq was greater than one. Additionally, the translocation factor was determined to be 1.75 after three months, indicating the plant's ability to transfer uranium to its aerial parts. Based on these results, Conyza canadensis (L.) Cronq can be classified as a uranium hyper-accumulator and could be used for the decontamination of uranium-contaminated soils.

Conflict of interest

The authors declare no potential conflict of interest regarding the publication of this work.

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