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Uranium phytoremediation by *Helianthus annuus* L. and *Amaranthus caudatus* L. plants

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Abstract

Uranium contamination presents significant challenges to biological systems due to its chemical toxicity and radiological impacts. Rhizofiltration emerges as a cost-effective strategy for environmental remediation of uranium. This study aimed to compare the uranium uptake capabilities of two plant species, *Helianthus annuus* L. (sunflower) and *Amaranthus caudatus* L. (purple amaranth), in a hydroponic system. The plants were cultivated in nutrient solutions supplemented with 0.5 mM or 1 mM $UO_2(NO_3)_2 \cdot 6H_2O$ without phosphate. After 14 days of growth, we assessed uranium uptake. Our findings revealed that *H. annuus* effectively removed over 95% of the initial uranium concentration from the solution, while *A. caudatus* exhibited a removal efficiency of approximately 65–80%. In both species, uranium accumulation and transport to the upper, harvestable parts were limited. The highest uranium concentration was observed in the roots of *H. annuus* (37,050.8 ± 3,547 mg kg^{-1} DW), whereas *A. caudatus* roots had a noticeably lower concentration (14,944.68 ± 3,278 mg kg^{-1} DW). Interestingly, *A. caudatus* demonstrated greater uranium accumulation in its shoots compared to *H. annuus*. Overall, while *H. annuus* demonstrates superior potential for uranium rhizofiltration, *A. caudatus* emerges as a promising candidate as a hyperaccumulator for uranium.

Keywords
Accumulation, hyperaccumulator, phosphate, remediation, uptake

Introduction

Uranium is the natural radioactive heavy metal. It spreads widely throughout the crust of the earth, and the average concentration of this element is less than 4 mg kg^{-1}. Although in different regions, its natural abundance has been redistributed as a result of anthropogenic activities. Therefore, groundwater and surface soil are contaminated with uranium radionuclide (Favas et al. 2016). Campbell et al. (2015) suggested that various human activities contaminated the soil and water with uranium; the most important of them were: nuclear weapons, nuclear waste disposal, nuclear accidents, and nuclear industries. The mining, milling and processing has been polluting the soils of various parts of the earth. Ibeanu (2003) and Wapwera et al. (2015) suggested that metallic mining produces large amounts of radioactive waste that has been found in tin mining regions in different countries such as Malaysia and Nigeria. In addition, Paschoa and Filho (1995) believed that these wastes were produced by monazite activities in various countries, such as: India, USA, Australia, Brazil, China, and also Thailand.

The studies by Ayotte et al. (2011) confirmed that uranium concentrations in groundwater across the United States exceed the U.S. Environmental Protection Agency (EPA) MCL of 30 μg/L in many areas, including regions without anthropogenic uranium activity. This condition indicates a source of contamination of natural uranium. Pervious examinations (Vicente-Vicente et al. 20217, Neves et al. 2012) showed that drinking uranium-contaminated water has been linked to different disorders, such as nephrotoxicity and osteotoxicity. Therefore, it poses a health risk and is very important to get an effective resurgence of spattered places.

A variety of physicochemical methods for the treatment of radionuclide contamination include soil washing, ion exchange, leaching with chelating agents, flocculation, and reverse osmosis-ultrafiltration. Recently, there has been a spark of interest in biological methods for radionuclide removal (Dushenkov 2003). In this regard, microorganisms and cell cultures were used to remove radionuclides from aqueous streams.

Phytoremediation is a new plant-based remediation technology. It is being applied to a diversity of radionuclide-spattered locations. Phytoremediation is defined as the use of green plants to eliminate pollutants from the environment or make them innocent. Many subsets of radionuclide phytoremediation are being expanded, for example: phytoextraction, rhizofiltration, phytovolatilization, and phytostabilization. Studies have shown that phytoremediation is a rapidly developing field and radionuclide phytoremediation could soon become a complete fragment of the environmental handling and risk depletion process (Favas et al. 2014). The summarization of data on the use of plant species for treatment of ^{3}H, U, Pu, ^{137}Cs, and also ^{90}Sr has been provided by Negri and Hinchman (2000). The species used to radiophytoremediation must have some characteristics such as fast growing, resistant to radiation, as well as a high capacity for radionuclide uptake (Jha et al. 2016; Li et al. 2011; Pratas et al. 2014). In rhizofiltration, the roots of plants are used to precipitate and concentrate radionuclides from polluted effluents. This technology has been examined in the field with uranium-contaminated water at concentrations of 21−874 μg/L at the previous uranium processing facility in Ashtabula, OH. The pilot-scale rhizofiltration system provided final treatment of site source water and decreased uranium concentration to <20 μg/L (EPA Water Quality Standard) before discharge to the environment (Dushenkov et al. 1997).

Plants differ dramatically in their ability to accumulate radionuclides. For example, the concentration of uranium in *Brassica chinensis* L. and *Brassica juncea* L. was at least 100 times higher compared to corn. Based on previous investigations (Crawford and Liber et al. 2015; Du et al. 2016; He et al. 2015), the ability of various plant species to absorb radionuclides also depends on the environment and the traits of soil and water. Therefore, it is difficult to normalize and compare the results of the screening of plant species that were achieved under different ecological conditions. The potential of some terrestrial and aquatic plants to accumulate U has been observed in several studies conducted under field or laboratory conditions, supporting their possible use in U biomonitoring (Caldwell et al. 2012) and / or phytoremediation of U-contaminated water or wastewater (Jha et al. 2016), and U-contaminated soil or solid waste (Li et al. 2011). While studying phytoremediation of U-contamination in abiotic matrices, it is revealed that the natural potential of plants can be enhanced by adopting the following measures: (i) combination with fungus (He et al. 2015); (ii) genetic transformation (Eapen et al. 2003); and (iii) use of soil amendments such as chelators (Jagetiya and Sharma 2013).

In fact, the specific process of phytoaccumulation of U depends not only on plant traits but also on certain physicochemical and geochemical processes. In addition, interactions with other organisms, including microorganisms, are conducted to address the entire complexity of the process. For example, in aqueous systems U removal also depends on factors that include microbial biofilms on abiotic substrates and periphyton growth (Stylo et al. 2015).

Helianthus annuus L. is an annual plant of the Asteraceae family that is grown for a wide range of purposes such as oil extraction and ornamental plants (Talebi et al. 2024). Furthermore, *Amaranthus caudatus* L. is widely cultivated in streets and parks as an ornamental herb (Talebi et al. 2016). Our study focused on the evaluation of the accumulation and distribution of uranium in *H. annuus* and *A. caudatus* using a hydroponic experiment setup. Lee and Yang (2010) pointed out that the high growth rate and also the great biomass of these plants make them some of the highest-yielding leafy crops in comparison to previous investigations that may be suitable for phytoremediation. This study aimed to compare the rhizofiltration ability of uranium by these plant species, which also contributes to the understanding of the mechanisms of uranium uptake and its distribution in which plants can enhance the effectiveness of phytoremediation in the removal of sites polluted by uranium. To our knowledge, this is the first report on uranium rhizofiltration using an *A. caudatus* plant.

Materials and methods

Plant material and hydroponic cultivation

Two plant species, sunflower (*H. annuus*) and amaranth (*A. caudatus*), were used for the rhizofiltration experiments. All plant seeds were obtained from the National Institute of Agricultural Biotechnology, Iran. The seeds germinated and then hydroponically cultured in polyvinyl chloride (20 cm tall, 10 cm in diameter) containing modified Hoagland hydroponic solution (pH=6.8), see Hoagland (1920). Each vessel contained two plants supported by cotton plugs. The entire cultivation process was carried out for 6 weeks in a greenhouse at 25 °C (80% relative humidity, 16 h of photoperiod/day).

Experimental Designs

Uranium treatment and effect of phosphate supply

Six-week-old plants in plastic vessels were treated with aerated Hoagland hydroponic medium containing $UO_2 (NO_3)_2{\cdot}6H_2O$ (Sigma) at concentrations 0.5mM and 1 mM without phosphate supply (pH=5). To test the effect of phosphate supply on uranium accumulation, 6-week-old plants in plastic vessels were treated with aerated Hoagland hydroponic medium containing $UO_2(NO_3)_2{\cdot}6H_2O$ (Sigma) at a concentration of 0.5 or 1mM. The supply was tested using a Hoagland solution with phosphates (pH=5).

In total, 12 plants per treatment (two plants in each vessel and six vessels per each treatment) were placed in a controlled greenhouse (25 °C temperature, 80% relative humidity, 16 hours of photoperiod/day) in a completely random design. Samples for determination of uranium accumulation were harvested after 14 days of treatment.

Analysis of uranium in plant parts and culture solution

After rhizofiltration, the amount of plant organs, the root, and the shoot, of the studied species was measured to specify where the majority of uranium accumulated in these plants.

The roots and shoots, including leaves, of the treated plants of each rhizofiltration examination were separated, rinsed three times with deionized water. The plant material dried at 80 °C in a forced-air convection oven for 2 days. After evaluating their total dry weight, each organ was sliced to less than 2 mm for a wet digestion analysis (ASTM 2011). The plant fragments were mixed with 10 ml of concentrated HNO_3 for 12h, heated at 180 to 200 °C until the dense brown fumes vanished. In this stage, the solutions were boiled until their volumes decreased by approximately 50%. Twenty milliliters of ternary solution (HNO_3:H_2SO_4: HClO 10:1:4) was subjoined to the digested solutions. The solutions obtained were heated anew until it became clear at 180–200 °C.

Finally, the solution was removed from the heating block, filtered by filter paper, and diluted with deionized water to a final volume of 50 ml. Inductively coupled plasma analyzes (ICP, PerkinElmer) were subsequently conducted to measure the uranium content in each plant part. To determine the residual uranium in the culture solution, 5 ml of solution was sampled from the vessel at the end of the experiment period and the uranium concentration was analyzed in ICP.

Data Analysis

Data were analyzed using a one-way ANOVA test to specify if there were significant variations in the uptake of the uranium consequence of metal treatments using SPSS software ver.17. Significant variations between means were distinguished by Duncan's test ($P< 0.05$).

Results and discussion

Morphotoxicity symptoms

Exposure of sunflower and amaranth plants to various concentrations of U induced morphotoxicity symptoms in plants exposed to higher levels (Fig. 1). Symptoms were observed in mature leaves (at 14 days), which indicated signs of chlorosis, necrosis, and early senescence (Fig. 1).

Ernst (2000) reported that the decrease in chlorophyll content and also chlorosis are two injury factors of plant exposure to various metals. The main reason for the reduction of chlorophyll biosynthesis may be the replacement of Mg^{2+} ions by $(UO_2)^{2+}$. However, uranium toxicity can be associated with the disruption of the primary step in glycolysis processes by replacing magnesium by uranyl in the enzyme (Van Horn and Huang 2006). Uranium has no confirmed functional role in plant nutrition, although it is naturally present in plants in small quantities (Stylo et al. 2015).

Shoot dry mass

In the present investigation, U was found to be phytotoxic and significantly reduced the dry mass of the amaranth plant shoot at concentrations of 0.5 and 1 mM (Fig.

2). However, increasing the U level had no significant effect on the dry mass of the sunflower shoot (Fig. 2).

Uranium removal efficiency

The results of the uranium removal efficiencies by rhizofiltration using two plant species are summarized in Table 1. Sunflower removed more than 83% of the uranium from the solution (119 mg/L of uranium in a 0.5 mM solution) and the uranium concentration of the residual solution was reduced to 20 mg/L. For the solution that has 238 mg/L of uranium (1 mM solution), the uranium removal efficiency reached more than 95% and the uranium concentration of the treated solution was reduced to 12mg/L, suggesting that rhizofiltration using sunflower has a powerful capability to remediate water having very high uranium concentrations.

Figure 1. Morphophytotoxicity symptoms in sunflower (*H. annuus*), after exposure to (B) 0.5 and (C) 1mM $UO_2\ (NO_3)_2{\cdot}6H_2O$ for 14 days compared to (A) control plants.

Table 1. Efficiency of uranium removal by sunflower and purple amaranth at varying uranium concentrations

Plants	Initial uranium concentration in solution, mg L^{-1}	Final uranium concentration, mg L^{-1}	Removal in solution efficiency of U, %
Sunflower	238	12.0	95
	119	20.2	83
Purple amaranth	238	47.6	80
	119	41.0	65

For amaranth, more than 65% of the uranium was removed from the solution (119 mg/L of uranium) and the uranium concentration in the solution was reduced to 42mg/L (Table 1). As the initial concentration of uranium in solution was 238mg/L, the uranium removal efficiency exceeded 80%. These results suggest that sunflower plants are more efficient than amaranth plants in removing uranium from solution. The efficiency of metal removal from solution by plants depends on the size and root system (Nakbanpote et al. 2016, Horemans et al. 2016). Terrestrial plants can develop much longer root systems, with adventitious fibrous roots covered with root hairs that create an extremely large surface area. For this reason, the morphology and structure of roots and root systems play a crucial role in plant adaptation to soil and access to water and nutrients (Nakbanpote et al. 2016). Therefore, plants with higher root biomass tend to accumulate more trace elements (Fu Zhong et al. 2011), which have been remarkably evident by the studied species belonging to the family Poaceae (*Agrostis castellana* Boiss. and Reut, *Agrostis curtisii* L., *Holcus lunatus* L., *Vulpia bromoides* (L.) Gray). Members of this family are characterized by an extensive fibrous adventitious root system that allows to absorb water more effectively from the soil, an important feature for achieving the primary requirement of a metal accumulator plant (Sebastian and Prasad 2016). This corresponds to our results. Sunflower plants produce higher root biomass than amaranth plants in hydroponic culture and therefore are more efficient in the removal of uranium from solution.

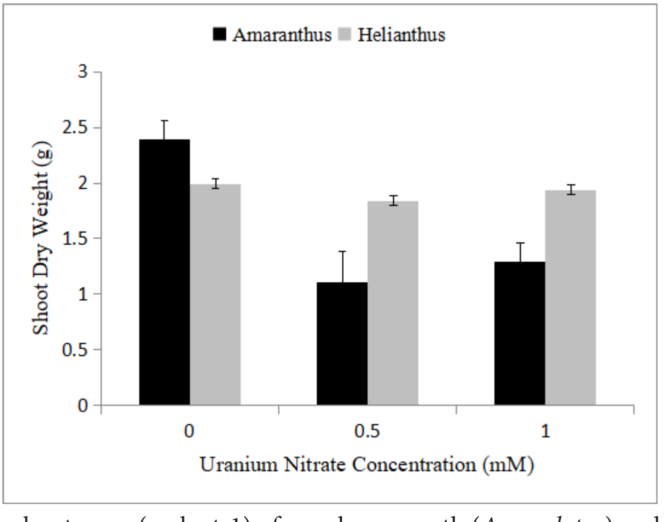

Figure 2. Dry shoot mass (g plant-1) of purple amaranth (*A. caudatus*) and sunflower (*H. annuus*). All values are mean of six replicates ± SD.

Uranium accumulation by plants

The accumulation of uranium increased during the cultivation period in medium supplemented with 0.5 and 1 m Muranyl nitrate. The amount of accumulated uranium was strongly influenced by the concentration of uranium in the medium. In this case, sunflower plants were able to accumulate up to 1.2 times more uranium in roots and 1.5 times more in shoots compared to plants grown at 0.5 mM concentration (Fig. 3). The uptake and accumulation of U in the shoot and root of amaranth plants followed a similar trend (Fig. 4). The highest amount of accumulation of U was detected at a concentration that was 5 and 6 times significantly higher than the concentration of 0.5 mM in root and shoot, respectively (Fig. 4).

Generally, the transport of uranium to the upper parts was very low and the uranium accumulated mainly in the roots (up to 37,050 mg U per kilogram of DW in sunflower and 14,944 mg U per kilogram of DW in amaranth plants, see Figs 3 and 4). This corresponds to the findings of other authors. For example, Straczek et al. (2010) reported that in some plants (*Lathyrus oleraceus* Lam., *Brassica juncea* (L.) Czern, *Zea mays* L. and *Triticum aestivum* L.) the accumulation of uranium in the roots is higher than in the shoots. Their results in maize plants showed that approximately 37% of the total uranium content was found in the first centimeter of the root tips. Furthermore, horseradish, white mustard, and rapeseed autoradiography showed that the transport of uranium from roots to shoots was low (Soudek et al. 2011 a, b).

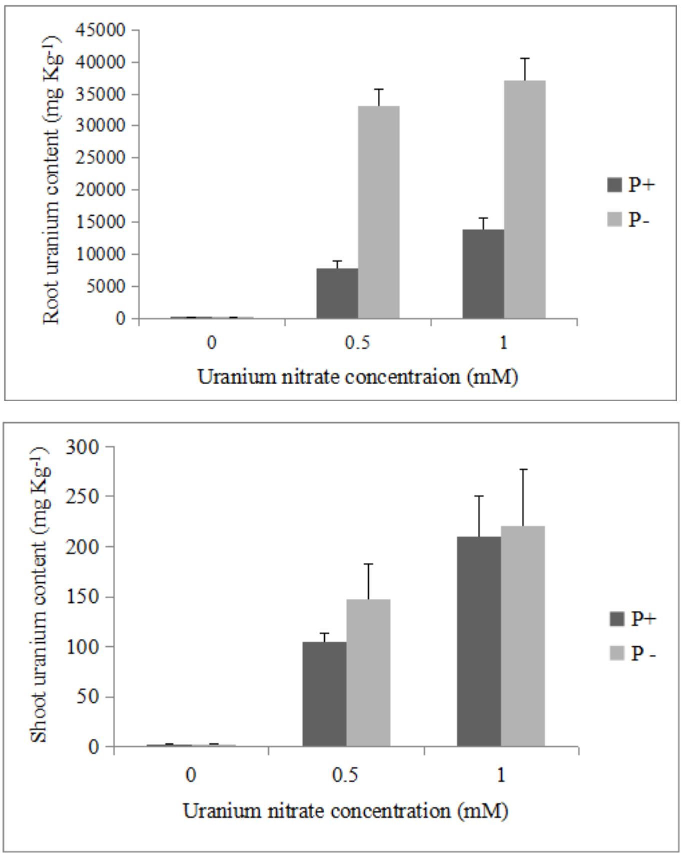

Figure 3. Uranium content (mg kg^{-1} DW) in roots (A) and shoots (B) of sunflower (*H. annuus*). All values are mean of six replicates ± SD.

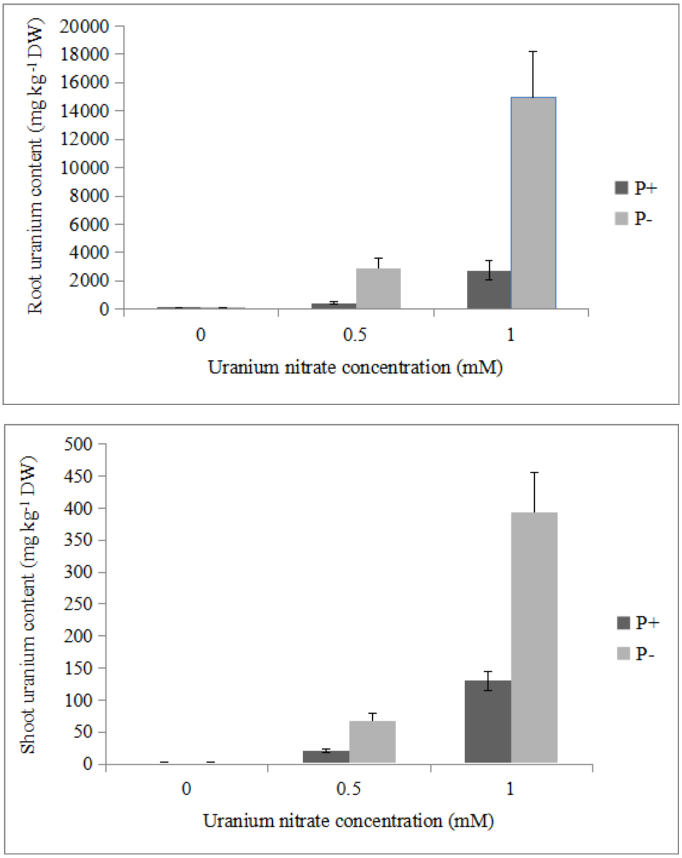

Figure 4. Uranium content (mg kg^{-1} DW) in roots (A) and shoots (B) of purple amaranth (*A. caudatus*). All values are mean of six replicates ± SD.

However, the higher concentration of U in roots rather than in shoots of sunflower and amaranth plants also suggests the action of physiological barriers, which could be attributed to the effect of U speciation in translocation. As reported by Laurette et al. (2012a), U complexation with endogenous phosphate residues leads to precipitation and fixation in roots, avoiding its translocation to aerial parts. In addition, studies by Mahdieh et al. (2013) confirmed that the higher amount of metals seen in the roots of *Pelargonium roseum* is extremely related to sequestration and also translocation. Uranium sequestration in roots has been associated with precipitation of uranium crystals on the surface of the root. The transport of uranium to the shoot occurs mainly through xylem tissue. However, the movement of metal ions in the xylem vessels appears to be primarily related to transpiration-driven mass flow (Soudek et al. 2011). The cell walls of the xylem have a high cation exchange valence; they are expected to severely defer severely the upward uranium motion in amaranth and sunflower plants.

The efficiency of uranium accumulation in plants was reduced by the addition of phosphate-containing compounds to the cultivation solution. In our study, the phosphate supplement clearly decreased uptake and accumulation in sunflower and amaranth plants. For example, 76% and 63% significant decreases in root uranium accumulation were observed at concentrations of 0.5 and 1 mM, respectively (Fig. 4). However, in shoots, the decrease in uranium accumulation due to phosphate addition is not significant (P>0.05). This corresponds to the findings of other authors (Laurette et al. 2012). The main reason for the significant variation in the accumulation of uranium in the presence or absence of phosphates has been the fact that the phosphate addition results in precipitation of uranium in the shape of insoluble uranium phosphate complexes. Therefore, it leads to a decrease in the dissolved uranium concentration in the solution. However, there was no significant effect of phosphates on the uptake of uranium in *Phaseolus vulgaris* L. (Laroche et al. 2005). These investigators suggested that plants take up a uranium-phosphate complex or that free uranium depletion caused by uptake resulted in dissociation of the uranium-phosphate complex to produce free uranium in solution. In previous studies (Misson et al. 2009; Yi et al. 2016), a significant decline in phosphate concentration was observed in the solution that used phosphate for the precipitation of uranium. Moreover, in other investigations, phosphorus was completely eliminated from the cultivation medium (Eapen et al. 2003). However, the decrease or exclusion of this anion leads to imbalances in the cultivation medium and has a negative influence on plant growth. In the soil, there are also two fractions of uranium and other elements, soluble and insoluble, which are in balance with each other. Plants absorb uranium from the soluble fraction, which is constantly supplied by uranium from the insoluble fraction.

Our results suggest that there is variability in uranium accumulation at the plant species level and that sunflower plants are more efficient than amaranth plants in uranium bioaccumulation.

Conclusion

The study highlights significant variability in uranium accumulation at the plant species level, with *Helianthus annuus* (sunflower) demonstrating superior efficiency in uranium uptake compared to *Amaranthus caudatus* (purple amaranth). The presence or absence of phosphate in the cultivation medium plays a crucial role in determining uranium uptake. Both species exhibited limited translocation of uranium to their upper harvestable tissues; however, the absence of phosphate appeared to improve this transport in amaranth plants. Further research is needed to investigate the unique translocation capacity of these species for aerial parts and the kinetics of uranium accumulation and speciation. Such insights will be essential for understanding the mobility and toxicity of uranium in plant systems. Notably, a consistent trend of higher uranium accumulation in roots and rhizomes suggests that these plants may possess adaptive strategies to restrict uranium absorption and enhance its retention in the rhizosphere. Overall, the findings confirm that both sunflower and amaranth are effective in removing uranium from solutions, offering an environmentally friendly and cost-effective remediation approach.

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