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Influences of U Sources and Forms on Its Bioaccumulation in Indian Mustard and Sunflower

Steven L. Larson and John H. Ballard

Environmental Laboratory U.S. Army Engineer Research and Development Center 3909 Halls Ferry Road Vicksburg, MS 39180

Fende Meng, Decheng Jin, Kai Guo, Liangmei Chen, Zikri Arslan, Jeremy R. White, Fengxiang X. Han

Department of Chemistry and Biochemistry Jackson State University Jackson, MS 39217

Lixiang Zhou and Youhua Ma

College of Environmental Sciences Nanjing Agricultural University Nanjing 210095, China Guodong Yuan

Environmental and Chemical Engineering Zhaoqing University Zhaoqing 526061, China

Charles A. Waggoner

Institute for Clean Energy Technology Mississippi State University Starkville, MS 39759

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Abstract

Anthropogenic activities, such as ore mining and processing, nuclear power generation, and weapon tests, have generated uranium (U) contamination to soils and waters. The mobility and bioavailability of U are influenced by its sources, speciation, and plant species. Phytoremediation has emerged as an environmentally friendly, cost-effective green technology to remediate radioisotope-and metal contaminated soils. The main objective of this study was to explore the feasibility using sunflower (Helianthus annuus) and Indian mustard (Brassica juncea) in cleaning up soils with UO2, UO3, and UO2(NO3)2. Uranium was found to be bioaccumulated in plant roots more than plant shoots. Uranium uptake by both plant species was significantly higher from the UO3- and uranyl-contaminated soils than from UO2- contaminated soils. UO3- and UO2(NO3)2-contaminated soils showed higher exchangeable, weak acid extractable, and labile U than the UO2-contaminated soils. After a growing season, three U forms decreased as redistribution/transformation of U resulted in U species with lower extractability. This study indicates the importance of U speciation in soil with regard to the potential use of sunflower and Indian mustard for phytoremediation of U-contaminated soils.

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Preface

This study was conducted for the U.S. Army Corps of Engineers under Project 458170, titled, "Depleted Uranium (DU) Clearance from DoD Ranges." The Grant Officer's Technical Representative was Mr. John H. Ballard, Office of the Technical Director for Installations and Operational Environments, ERDC-EL-EZT and the Technical Point of Contact was Dr. Steven L. Larson, Environmental Engineering Branch, ERDC-EL-EPE.

The work was performed by the Environmental Engineering Branch of the Environmental Processes Division, U.S. Army Engineer Research and Development Center, Environmental Laboratory (ERDC-EL). At the time of publication of this Miscellaneous Paper, Ms. Brooke Petery was Acting Branch Chief; Dr. Brandon Lafferty was Acting Division Chief; and Dr. Elizabeth Ferguson was the Technical Director for Installations and Operational Environments. The Acting Deputy Director of ERDC-EL was Dr. Justin Berman and the Acting Director was Dr. Jack Davis.

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The Commander of ERDC was COL Teresa A. Schlosser and the Director was Dr. David W. Pittman.

1 Introduction

Uranium (U) is a naturally occurring toxic heavy metal widely found in soil environment. Radioactivity of U is a result of decay process for three U isotopes (U-238, U-235, and U-234). Its concentration in soil varies from 0.3 to 11.7 mg/kg, with an average of 2.6 mg/kg (Rankin 2008). The presence of mobile, labile and bioavailable U in soil at levels of significant environmental risks is mainly caused by anthropogenic activities, such as ore mining waste, nuclear power waste, and weapon testing. Soil areas with U may reach high levels of 100 of mg/kg and even more than 1000 mg/kg (Larson et al. 2009; Tuovinen et al. 2015; Liu et al. 2017; Dang et al. 2018). For example, U concentrations in sediments from gold mining in South Africa were more than 100 mg/kg in average, reaching up to 1000 mg/kg and U concentration in surface waters was significantly higher than that in the flowing water into the tailing (Winde and van der Walt 2004).

Uranium poses chemical and biological toxicity. Uranium in soils may be bioaccumulated in plants, posing risks to human health through food chains, such as increasing risks of cancer, kidney toxicity, and birth defect rates (Fetter and von Hippel 2000; Fathi et al. 2013). The mobility of U in soil is significantly related to its speciation and sources (Radenković et al. 2008). In soil environment, U is present usually as U(IV) and U(VI) and hexavalent U is the main form under oxidizing conditions. In reducing environments, the predominant, reduced form, U(IV), forms highly insoluble phases as UO₂ (log K_{sp} = - 53.93 ± 0.2) reducing its mobility and bioavailability (Fujiwara et al. 2003; Abdelouas 2006). However, the reduced form may be transformed to U(VI) under oxidizing conditions in presence of electron acceptors, such as humic substances and Fe(III) (Gu et al. 2005; Sani et al. 2005). Uranium in soil interacts with soil components, such as Fe-Mn oxides, carbonate, organic matter and clay minerals, to form different U complexes with a range of mobility and bioavailability, which in turn influences its redistribution in soil and its uptake by plants (Bednar et al. 2007; Vandenhove et al. 2007; Dang et al. 2016).

The accumulation and distribution of U in plants was reported and shown to be influenced by its speciation in soil (Duquène et al. 2009; Véra-Tome et al. 2009; Laurette et al. 2012a, 2012b; Jagetiya and Sharma 2013; Cordeiro et al. 2016; Favas et al. 2016). Sunflower (*Helianthus annuus*) accumulated U mainly in roots, especially the ionic $UO_2^{2^+}$ (Laurette et al. 2012a; Véra-Tome et al. 2008). Due to high accumulation, Indian mustard (*Brassica juncea*) has been used for phytoremediation of many toxic metals including U in contaminated soil (Qi et al. 2014; Choudhury et al. 2016). A number of studies showed that the phytoremediation of U was enhanced with adding soil amendments (Duquène et al. 2009; Jagetiya and Sharma 2013). However, effects of U forms in soils on its bioaccumulation in plants are not fully understood (Alsabbagh and Abuqudaira 2017).

The objectives of the present study were (1) to examine the effect of U sources (UO₂, UO₃, and UO₂(NO₃)₂) and forms in soils on its bioaccumulation in Indian mustard and sunflower plants; (2) to investigate the relationship between U solid phase distribution in soils as measured with sequential extraction, U bioaccumulation in plants and U partitioning in plant tissues; and (3) to assess the potential of sunflower and Indian mustard in phytoremediation of U-contaminated soils by estimating U removal efficiency. Both plants are widely distributed and planted in the USA and they have shown high bioaccumulation for toxic metals (Duquène et al. 2009; Véra-Tome et al. 2009; Adesodun et al. 2010; Laurette et al. 2012a; Jagetiya and Sharma 2013; Choudhury et al. 2016; Sharma et al. 2016).

2 Materials and Methods

2.1 Chemical Agents and Plant Seeds

We used guarantee reagent (GR) grade chemicals and reagents purchased from Scientific Fisher except for U (UO₂, UO₃, and UO₂(NO₃)₂). The UO₂ and UO₃ were purchased from International Bio-Analytical Industries, Inc. and UO₂(NO₃)₂ was purchased from Poly Scientific R&D Corp. The Indian mustard and Sunflower seeds were purchased from Ferry-Morse (USA). The preparation of nutrient solution according to Hoagland solution and 1 L solution consisted of 2.5 mL 202 g/L KNO₃ + 2.5 mL 472 g/L Ca(NO₃)₂•4H₂O + 1.5 mL 15 g/L Fe-EDTA + 1 mL 493 g/L MgSO₄•7H₂O + 1 mL 80 g/L NH₄NO₃ + 1 mL 2.86 g/L H₃BO₃ + 1 mL 1.81 g/L MnCl₂•4H₂O + 1 mL 0.22 g/L ZnSO₄•7 H₂O + 1 mL $0.051 \text{ g/L} \text{ CuSO}_4 \cdot 5 \text{H}_2 \text{O} + 1 \text{ mL} 0.09 \text{ g/L}$ $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O} + 0.5 \text{ mL} 136 \text{ g/L} \text{ KH}_2\text{PO}_4.$

2.2 Soil Sample and Experimental Design

A soil sample was collected from the surface horizon (0-30 cm) of a paddy soil in Mississippi River Delta. After air-drying at room temperature, it was crushed, passed through a 2-mm mesh and homogenized. The soil pH was measured with a pH meter (Oakton, USA) in distilled water (1:5 *w*/*v* ratio), organic matter with K₂Cr₂O₇-H₂SO₄ method (Han et al. 2007), and soil particle size distribution with the hydrometer method (Bouyoucos 1962).

UO₂, UO₃, and UO₂(NO₃)₂ were used as U sources at 100 mg U/kg with 500 g soil in each beaker. Due to the disposal cost, only a small volume of contaminated soil was used for this study. A control without U addition for both plants and a control with 100 mg/kg U added in soil without plants were included. Each treatment was duplicated. After 1 month incubation at 80% of field capacity, 8-9 Indian mustard or sunflower seeds were planted in control and U-contaminated soils (n =16). During cultivation experiments of 3 months, soil water content was maintained at 80% of field capacity with distilled water and watered with nutrient solution twice a week with 10 mL Hoagland solution every time in a greenhouse under natural light at room temperature. At the end, plants were harvested for both shoots and roots and washed in 10 mM Na₂CO₃ and distilled water to remove U from root surface, and cleaned dust from shoot surface by distilled water. Shoots and roots were separated and dried at 85 °C for more than 12 h until constant masses. Soils and rhizosphere soils (near the root surface) in the beakers were air-dried at room temperature. Plant and soil samples were stored in a refrigerator at 4 °C prior to analysis and extraction.

2.3 Chemical Analyses

2.3.1 Extraction of Potentially Bioavailable, Weak-Acid Soluble, and Labile U

These forms of U are potentially bioavailable to plants and microbes in soils. Extraction solutions were prepared as indicated by Smith et al. (2009). The potentially bioavailable, weak acid and labile U were extracted with 0.5 M Ca(NO₃)₂ solution (pH 5.09), 0.44 M CH₃COOH + 0.1 M Ca(NO₃)₂ (pH 2.75), and 0.014 M NaHCO₃ + 0.0028 M Na₂CO₃ (pH 9.64), respectively. Briefly, about 1 g dry soil was weighed into 15 mL centrifuge tubes (Corning, USA) containing 10 mL of one of the prepared solutions and the tubes were shaken at 25 °C for 16 h. Then tubes were centrifuged at 5480 rpm for 15 min (5810R, Eppendorf, Germany). The supernatant was filtered through 0.45- μ m filter (Whatman, England) for U analysis with ICP-MS (Varian 820-MS, US).

2.3.2 U Fractionation in Soils

The sequential selective dissolution procedure (Han et al. 2007) was used for U fractionation.

Step 1 - soluble plus exchangeable fraction (EXC)

Twenty-five milliliters of 1 M NH_4NO_3 with pH adjusted to 7.0 with $NH_3 \cdot H_2O$ was added into 50 mL polytetrafluoroethylene centrifuge tubes containing 1 g air-dried soil. The tubes were shaken for 30 min in a temperature controlled shaker at 25 °C, and then centrifuged at 5480 rpm for 15 min. The supernatant was poured out and filtered through 0.45-µm filter for U analysis with ICP-MS. The soil residue was retained for the next step. The following extraction steps used the same centrifugation-decantation-filtration processes.

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Step 2 - the fraction bound to carbonate (CARB)
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Twenty-five milliliters of 1 M CH₃COONa with pH adjusted to 5.0 with CH₃COOH was added into the soil residue from Step 1. The tubes were shaken for 6 h at 25 °C.

Step 3 - the fraction bound to easily reducible oxides (ERO)

This U fraction mostly bound to easily reducible oxides, such as Mn-oxides (Shuman 1982; Han et al. 2007). Briefly, 25 mL of 0.1 M NH₂OH·HCl+0.01 M HCl solution (pH 2.0) was added into the residue soil from step 2. The mixture was shaken for 30 min at 25 °C.

Step 4 - the fraction bound to organic matter (OM)

This fraction mainly bound to soil organic matter (e.g., humic acid) and microorganisms. Three milliliters

 $H_2O_2 + 5 \text{ mL } 0.01 \text{ HNO}_3$ was added into the residue soil from step 3. The mixture was put into a water bath at 80 °C. After 2 h, additional 2 mL H_2O_2 were added into mixtures for one more hour in water bath at 80 °C. Then, 15 mL 1 M NH₄NO₃ was added and the tubes were shaken for 10 min.

Step 5 - the fraction bound to amorphous iron oxides (AmoFe)

Twenty-five milliliters of 0.2 M $(NH_4)_2C_2O_4 + 0.2$ M $H_2C_2O_4$ buffer solution (pH 2.89) was added into the residue soil from step 4 and shaken in a temperature controlled shaker for 4 h at 25 °C in dark.

Step 6 - the fraction bound to crystalline iron oxides (CryFe)

Twenty-five milliliters of 0.04 M NH₂OH•HCl + 25% CH₃COOH solution was added into the residue soil from step 5 and the mixture was heated in water bath for 3 h at 95 °C.

Step 7- the residue fraction (RES)

Twenty-five milliliters of 4 M HNO₃ was added into the residue soil from step 6 and the mixture was heated in a water bath for 16 h at 80 °C (Han and Banin 1997).

2.3.3 Pseudo Total U Concentration

The pseudo total U concentration was determined in soil after digestion with HCl-HNO₃ mixture (USEPA SW-846 Test Method 3050B), which may dissolve a majority of U especially potentially available portion (USEPA). HCl-HNO₃ has been widely used to extract the "total" metal in soils (USEPA). Briefly, 0.5 g soil sample was weighed into a 50-mL polytetrafluoroethylene vessel with 4 mL of the mixing acids (3HCl: 1HNO₃, v: v) and the mixture was heated on a hot block (100 °C, Environmental Express Ltd.). At the end, extracts were filtered through 0.45-µm membrane into 50-mL glass flasks and filled to the mark with distilled water for the analysis of U with quadrupole ICP-MS. Uranium is free of polyatomic interference at high m/z ratios. There are no known polyatomic ions that interfere in our solution. The detection limit of ²³⁸U was 5 ng/L. All treatments for each plant and all extractions were performed in duplicates. All extraction and digestion experiments included two blank samples. A series of standards were analyzed every 30 samples to calibrate any shift of the instrument and the standards of U concentration were 0, 5, 10, 25, 50, 75, and 100 μ g/L.

2.4 Determination of U Concentration in Plants

Dried roots and shoots of plant samples were digested on hot block with wet acid digestion (McDaniel 1992; Han and Banin 1997). Briefly, 0.5 g plant samples were weighted into 50 mL polytetrafluoroethylene vessels with 6 mL concentrated HNO₃ (65%) and 2 mL H₂O₂ (30%) for digestion until samples were completely dissolved. The digested solution was diluted to 40 mL with distilled water, filtered through 0.45-µm filter and analyzed for U with ICP-MS, where U concentration should be below 100 µg/ (which was set for ICP-MS measurement). Blanks were prepared in the same way as the samples and each sample was duplicated.

2.5 Data Processing

U fraction (Ui) was determined by comparing U concentrations in extractant and soil mass, as follows:

$$U_i (mg/kg) = \frac{C_i V_i}{m}$$

where i was EXC, CARB, ERO, OM, AmoFe, CryFe or RES fractions of U, Ui was the U fraction concentration in soil in mg/kg, C(mg/L) was the U concentration in extractant in mg/L, V was the extractant volume in L, m was the soil mass for extraction in kg, respectively.

U removal efficiency was determined by comparing U concentrations in plants and in soil, as follows:

$$\mathbf{w}(\%) = \frac{w_{\mathrm{r}}m_r + w_s m_s}{CM} \times 100\%$$

where w(%) was the U removal efficiency by plants in %; w_r and w_s were the U concentration in plant roots and shoots in mg/kg, respectively; m_r and m_s were the plant root and shoot weight in kg; C and M were the U concentration in soils in mg/kg and total soil mass in kg, respectively.

Concentration factor (CF) were ratios of U concentration in shoots over U forms or total U in soil and transfer factor (TF) were ratios of U concentrations in shoots over those in roots, as follows: $CF = \frac{w_s}{C_i}$

$$\mathrm{TF} = \frac{w_s}{w_r}$$

where w_s and w_r were the U concentration in plant shoots and roots in mg/kg, respectively; C_i was the soil total U, labile U, weak-acid U or potentially bioavailable U in mg/kg. Microsoft Excel was used to calculate the U fraction content, removal efficiency, U content in plant samples and Origin 9.1 (OriginLab., USA) was used for figure processing. All treatments were duplicated. Average and standard deviations of analytical data were calculated with MS Excel and compared through the text. We used T-test for statistical analysis with MS Excel.

3 Results and Discussion

3.1 Soil Properties

The soil had a slightly alkaline pH of 7.57. After 3 months of the pot experiment, the soil pH increased depending on plant species and U sources (Table 1). pHs in U treated soils after a growing season did not show a significant difference between two plants (Table 1). The soil contained 2% organic matter, 2% sand, 42% silt, and 56% clay (silty clay). The actual U concentrations in soil before the cultivation experiment with UO₂, UO₃ and UO₂(NO₃)₂ as U source were 98, 82 and 72 mg/kg and at the end of growing season with UO₂, UO₃ and UO₂(NO₃)₂ as U source were 96, 77, and 68 mg/kg, respectively. Thus, both UO₃ and UO₂(NO₃)₂ showed a much higher solubility and bioavailability than UO₂, demonstrating the importance of U sources to phytoremediation efficiency.

Table 1 The pH of soil after cultivation of 3 months (n = 16)

Plant species	CK	UO ₂	UO ₃	UO ₂ (NO ₃) ₂
Sunflower	8.72	7.85	8.36	7.92a
Indian mustard	8.38	7.39	8.46	8.67a

Plants with the same letter were not significantly different at the 0.05 probability level

3.2 The Potentially Bioavailable, Weak-Acid Soluble and Labile U in Soils

The potentially bioavailable U refers to U in soils easily available and directly used by plants and microbes, while weak-acid soluble and labile U implies the potentially bioavailable U in soil which is in equilibrium with bioavailable U. These forms of U in soils had a great influence on U bioaccumulation (Tables 2 and 3) as indicated in previous studies (Xian 1989; Roy and McDonald 2015). Changes in three U forms in soils depended on U sources, incubation and cultivation time and plant species (Fig. 1). The potentially bioavailable, weak-acid soluble, and labile U contents decreased with aging time, which may be caused by U adsorption in soil and further U redistribution among solid phase components, namely Fe-Mn oxides, soil organic matter and clay minerals (Han et al. 2007; Kowal-Fouchard et al. 2004; Wei et al. 2007; Dang et al. 2016). Statistical analyses indicated that planting and initial aging had significant effects in UO₃ and uranyl treatments, but not in CK and UO₂ treatments. Both aging and planting significantly decreased the bioavailable U and the labile U as well to some extent the weak acid soluble U in both UO₃ and uranyl treatments compared to U in soils after 1 month with no plants. Significant differences were found among various U species.

U(IV) as UO₂ is sparingly soluble (log $K_{sp} = -53.93 \pm 0.2$), while it may transform into U(VI) under oxidizing conditions in the presence of natural oxidants, such as Fe(III) and O₂: UO₂ + 2Fe³⁺ \rightarrow UO₂²⁺ + 2Fe²⁺; UO₂ + 1/2O₂ \rightarrow UO₂²⁺. Due to its structure, the UO₂ may persist for a long period of time in the oxidizing condition (Finch and Ewing 1992). Zielinski and Meier (1988) showed that the oxidation of UO₂ was extremely slow under natural condition. Low bioavailability of U from soils prepared using UO₂ was evidenced by limited plant uptake (discussed later). For U(VI), the UO₃ and UO₂(NO₃)₂ appeared as ionic forms in soil such as UO₂²⁺ (many more anionic and cationic species) in oxidative conditions.

In comparison with bare soil, plant growth increased potentially bioavailable and extractable U. Labile and weak acid extractable U from soils amended with U(VI) (UO₃ and UO₂(NO₃)₂) especially in rhizosphere soils of Indian mustard increased (Fig. 1). Bioavailable U in soils with UO₃ and UO₂(NO₃)₂ sources under Indian mustard were

Table 2 Ratios of extractable U over soil total U and U concentration in plants tissues (n = 12)

U sources	Ratios of extractable U/soil total U				U concentration (mg/kg) in					
Bioavailable U		le U	Weak-acid U		Liable U		Shoots		Roots	
	Sunflower	Indian mustard	Sunflower	Indian mustard	Sunflower	Indian mustard	Sunflower	Indian mustard	Sunflower	Indian mustard
UO ₂	NA	NA	NA	0.001	0.02	0.02	0.71	2.56a	7.98	3.29a
UO ₃	0.02	0.06	0.15	0.26	0.57	0.62	4.08	19.1b	136	277b
$UO_2(NO_3)_2$	0.004	0.05	0.08	0.16	0.61	0.48	5.06	7.18b	167	184b

NA not available as U concentrations in soil below the detection limit

U concentrations within shoots or roots followed by different letters were significantly different at the 0.05 probability level among U species

higher than those under sunflower and bare soils (Fig. 1). Both weak acid extractable and labile U in soils with UO3 and UO2(NO3)2 sources with both plants were higher than bare soils. This may be caused by root exudates/siderophores, and organic acids, especially in Indian mustard (Mench et al. 1988; Mench and Martin 1991; Yang and Pan 2013; Kim et al. 2010). Root exudate includes low molecular weight organic acids and phenols and high molecular weight polysaccharide and proteins, depending on plant species (Yang and Pan 2013; Baetz and Martinoia 2014). Yang and Pan (2013) reported that root exudates from sunflower had strong complexation propensity for metals, which indicates that root exudates may significantly influence bioavailability, toxicity, and phytoavailability of U in soil. Kim et al. (2010) found that root exudates from Indian mustard increased soluble metals in alkaline soil. However, plant growth did not significantly affect solubility and bioavailability of U(IV) in soils amended with UO₂.

3.3 Redistribution of U Among Solid Phase Components

The sum of U in sequential steps was in agreement with the total U extracted in the single step (Fig. 2). The relative error between the sum and the total was about 4.5%, which indicates that our sequential selective dissolution technique gave a reasonable recovery. The OM (60-70%) and AmoFe (0-30%) fractions were the dominant solid phase fractions in UO2-contaminated soils with both sunflower and Indian mustard while the CARB (70-80%) and OM (10-20%) fractions were the major solid phase fractions for soils with U (VI) $(UO_3 \text{ and } UO_2(NO_3)_2)$ (Fig. 3). The UO₃ polluted soils had 10% more OM and 10% less CARB fractions than soils with $UO_2(NO_3)_2$. This indicates that more soluble U (VI) such as soils with uranyl were bound in the CARB fraction than soils with less soluble UO₃. However, insoluble U (IV) such as UO₂ was bound to the OM fraction. This was reflected by the distribution of reduced, U(IV), species such as UO_2 in soils that more

Table 3 Concentration factors and transfer factors of U in Sunflower and Indian mustard (n = 12)

Plant species	U sources	Concentration	Transfer factor			
		Soil total U	Liable U	Weak-acid U	Bioavailable U	Shoot-U/Root-U
Sunflower	UO ₂	0.01	0.49	NA	0.01	0.10
	UO ₃	0.05	0.09	0.36	0.05	0.03
	$UO_2(NO_3)_2$	0.07	0.12	0.76	0.07	0.03
Indian mustard	UO ₂	0.03	1.30	7.94	NA	0.78
	UO3	0.25	0.41	0.97	4.46	0.07
	$UO_2(NO_3)_2$	0.11	0.22	0.65	1.90	0.04

NA not available as U concentrations in soil below the detection limit



Fig. 1 Effects of Indian mustard and sunflower plants on U bioavailability in soil (as means of two replicate analyses and error bars were standard deviations)

U (IV) was present in the OM fraction. In part, low solubility of U (IV) (such as UO_2) might be oxidized into U(VI) during the extraction with nitric acids and hydrogen peroxides.

Plant growth affected U redistribution among solid phase components, especially for the EXC fraction of



Fig. 2 Comparison of the total U extracted with a single step acid and the sum of U in solid phase components as extracted with sequential dissolution procedure. ** Significant at the 0.01 probability level

U(VI) in soils prepared with UO_3 and uranyl. Indian mustard growth solubilized more U(VI) than sunflower into the soluble and exchangeable fractions (Fig. 3). Plant root exudates (such as organic acid, polysaccharide, and proteins) increased the fraction of U (VI) in soils as water-soluble and exchangeable forms (Mench et al. 1988; Yang and Pan 2013; Kim et al. 2010). Planting may also change soil properties such as pH (Table 1) which may also shift U distribution among solid phase fractions.

The current study was in agreement with previous reports showing U redistribution among fractions in soil was influenced by both U sources and plant species (Lu et al. 2005; Jalali and Khanlari 2008; Mench et al. 1988; Mench and Martin 1991; Kim et al. 2010). In general, carbonate and organic matter were two important sinks for U in soils (Veeh et al. 1974; Bradl 2004; Weyer et al. 2008; Meng et al. 2017). Meng et al. (2017) reported humic substance extracted from leonardite showed a significant adsorption capacity for uranyl in water.



Fig. 3 The redistribution of U among solid phase components in soils with aging time increased. **a** After 1 month, without plants and **b** 3 months, without plants, and **c** after 3 months with Indian mustard, and **d** sunflower (as means of two replicate analyses)





Fig. 4 The height (cm) of Indian mustard and Sunflower plants in soil with various U sources in the first 11 weeks (each treatment was duplicated. 3–5 plants grew in each pot). Heights were averages of all plants in two replicated pots for each U form. Bars were standard deviations of all these plants

for two replicates. U species within Indian mustard or Sunflower followed by different letters were significantly different at the 0.05 probability level. (Four time series of plant heights for each individual plant species with all times were compared among various U species)



Fig. 5 U uptake and bioaccumulation in shoots and roots of sunflower and Indian mustard from soils with different U sources (as averages of two replicates for each treatment and bars as

3.4 Phyto-Toxicity of U(VI) to Indian Mustard and Sunflower

Two plants showed different tolerances to various U species in soils during their first 3-month growth. Young Indian mustard showed higher U(VI) tolerance than sunflower in soils prepared with U(VI) uranyl (Fig. 4). Sunflower displayed a significant higher phyto-toxicity of U(VI) in uranyl treatment (Fig. 4), as shown by a growth retardation (30-50% decrease) compared to other UO₂ and UO₃ at first 7 weeks. This level of toxicity may be related to the tolerance/sensitivity of plant species and chemical toxicity of U in soil (Sheppard et al. 2005). At first 7-week growth time, sunflower growth had only 50-70% of the plant shoot height in the control. Soils prepared with UO₂ and UO₃ did not show phytotoxicity in sunflower growth. However, phyto-toxicity of U(VI) disappeared in soils during sunflower maturation (Fig. 4), in part attributing to the aging U in soils, which decreased its solubility and bioavailability as well as increased tolerance in older plants (Figs. 1 and 3).

3.5 The Uptake and Bioaccumulation of U by Indian Mustard and Sunflower

The uptake and bioaccumulation of U by plants was significantly influenced by U sources used to prepare the current soil and plant species (Fig. 5). Both plants bioaccumulated more U in both shoots and roots from soils with U(VI) source (UO₃ and UO₂(NO₃)₂) than from soils with U(IV) (UO₂) prepared soil. This was controlled by the solubility of U compounds in soils.



standard deviations). U species within roots or shoots followed by different letters were significantly different at the 0.05 probability level

U(IV) as UO₂ was highly insoluble in soil, while U(VI) such as UO₃ and UO₂(NO₃)₂ had higher solubility (Abdelouas 2006). UO_3 was partly soluble and $UO_2(NO_3)_2$ was soluble in soils. However, both plants took up more U(VI) from partially soluble UO₃ than from soluble uranyl (Fig. 4). This may be affected by two main reasons: (1) root exudates from both plants, which significantly increased solubility of UO_3 ; (2) soil components, such as Fe-Mn oxides, minerals and humic substances, which sorb uranyl ionic (Mench et al. 1988; Kowal-Fouchard et al. 2004; Wei et al. 2007; Yang and Pan 2013; Kim et al. 2010; Dang et al. 2016; Meng et al. 2017). Moreover, Indian mustard has more abundant root systems and thus may have released more exudates than sunflower, resulting in higher solubility of U(VI) under Indian mustard (previous section) and higher uptake of U from soils in this study.

4 Conclusions

In this study, three U sources $(UO_2, UO_3, and UO_2(NO_3)_2)$ were used to produce U-contaminated soils in order to study bioaccumulation processes in Indian mustard and sunflower. Potentially bioavailable, weakacid soluble, and labile U decreased with time during growing seasons because of an aging effect on solubility. Both plant species and U sources had influences on bioaccumulation of U from soils prepared with these three U forms. The redistribution of U among solid phase components was influenced by U sources, time and plant species. Phyto-enhancement of U solubility/ bioavailability in soil may be caused by root exudates, such as small molecular weight organic acids. Indian mustard showed higher shoot biomass, uptake, and bioaccumulation than sunflower, as well as a higher tolerance to uranyl phytotoxicity and an improved U solubility and bioavailability in soils with UO₃. However, due to the disposal cost, only small volumes of contaminated soils were used for this study. Thus plant uptake and phytoremediation efficiency of U may not be directly applied to fields due to sizes of soil amount. Field feasibility of phytoremediation of U soils with these two plants required further study.

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contamination to so	ils and waters. The m	obility and bioavaila	bility of U are influence	ced by its source	s, speciation, and plant species.		
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contaminated soils.	The main objective of	f this study was to ex	plore the feasibility us	sing sunflower (<i>l</i>	Helianthus annuus) and Indian mustard		
(<i>Brassica juncea</i>) in plant shoots Uraniu	m uptake by both pla	in UO ₂ , UO ₃ , and UC	icantly higher from the	e UO ₃ - and uran	vl-contaminated in plant roots more than		
contaminated soils.	UO ₃ - and UO ₂ (NO ₃)	2-contaminated soils	showed higher exchan	geable, weak aci	d extractable, and labile U than the		
UO2-contaminated soils. After a growing season, three U forms decreased as redistribution/transformation of U resulted in U species with							
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