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Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. vulgaris) to remediate uranium contaminated groundwater

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ABSTRACT

The uranium removal efficiencies of rhizofiltration in the remediation of groundwater were investigated in lab-scale experiments. Sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. vulgaris) were cultivated and an artificially uranium contaminated solution and three genuine groundwater samples were used in the experiments. More than 80% of the initial uranium in solution and genuine groundwater, respectively, was removed within 24 h by using sunflower and the residual uranium concentration of the treated water was lower than 30 μg/L (USEPA drinking water limit). For bean, the uranium removal efficiency of the rhizofiltration was roughly 60–80%. The maximum uranium removal via rhizofiltration for the two plant cultivars occurred at pH 3–5 of solution and their uranium removal efficiencies exceeded 90%. The lab-scale continuous rhizofiltration clean-up system delivered over 99% uranium removal efficiency, and the results of SEM and EDS analyses indicated that most uranium accumulated in the roots of plants. The present results suggested that the uranium removal capacity of two plants evaluated in the clean-up system was about 25 mg/kg of wet plant mass. Notably, the removal capacity of the root parts only was more than 500 mg/kg.

1. Introduction

Uranium is a naturally occurring primordial radionuclide, consisting of four isotopes of mass number 230, 234, 235, and 238. It has half-lives between 10^5 and 10^9 years and is used as a key element of the nuclear fuel cycle [1,2]. It was reported that about 20% of uranium to which humans are exposed originates from anthropogenic sources such as depleted mine tailings, medical wastes, and by-products of weapons testing and the nuclear power industry [3]. Uranium within the Earth's surface is generally considered to be relatively immobile but it is released in nature by the weathering of depleted mine tailings and ore wastes [4]. A critical factor in weathering is the action of bicarbonate ions, which cause readily soluble uranyl complexes to form [5]. Mobile forms in groundwater are transported as divalent uranyl (UO_2^{2+}) ions or hexavalent carbonate complexes in the presence of high concentrations of CO_2 [6]. They are also adsorbed by colloidal humic–fulvic acids and other low-molecular-weight complexing agents such as hydroxides and carbonates naturally found in groundwater [7–9].

Uranium contamination of soil and groundwater has become a serious problem in the second half of the twentieth century, raising public health concerns, especially in areas where accidental spills and emissions from the typical operational steps of the nuclear fuel cycle, such as mining and milling, have taken place [10–13]. The chemical toxicity of uranium compounds in the human body has been found to affect renal functions leading to kidney failure and its main pathways into the human body are inhalation of dust and ingestion of water contaminated uranium [14].

In Korea, anomalous uranium contents were first detected in the graphitic rocks of the Okcheon Group in the late-1950s [15]. From a systematic geological investigation, uraniferous black shale layers of the Okcheon Group were discovered and their uranium concentration (U_3O_8) ranged from less than 0.02% to 0.05% [16]. Exploration for uranium ores around the Okcheon Group in Korea commenced in the mid-1970s and finished at the end of the 1990s. Depleted uranium mine tailings and ore wastes around abandoned mines have been wasted in ruin, acting as continuous sources of contaminated soils and groundwater. Several groundwater samples taken from around the Okcheon Group were reported to have very high uranium content [17]. Thus, it is essential to effectively remove these radionuclides from groundwater used for municipal supply.

Like other heavy metals, radionuclide contaminants in water can be removed by chemical processes such as ion exchange, reverse osmosis, precipitation, and flocculation [18,19]. However, these processes may be difficult to carry out and can be prohibitively expensive for large water volumes, low metal concentrations, high total salt content, and low discharge limits [2,20]. Rhizofiltration, an

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Table 1
Properties of groundwater samples from three different sites.

Sampling site	Temp. (°C)	EC (mS/m)	NaCl (%)	pH	Metal concentration (μg/L)						
					U	As	Cr	Cu	Ni	Pb	Zn
Deogpyeoungri	4.2	44.2	0.02	7.5	21.7	0.3	3.8	0.3	9.0	0.5	17.1
Wadong	11.1	30.2	0.01	7.4	81.4[a]	1.4	2.5	1.4	13.9	0.3	27.4
Oesamdong	7.6	31.1	0.02	6.8	241.7[a]	0.3	3.1	1.0	1.1	0.1	8.0

[a] Over US EPA drinking water limit (30 μg/L).

emerging technology implementing the use of plant roots to absorb, concentrate, and precipitate heavy metals from water, may provide a cost-effective method to treat uranium at concentrations that are too low for efficient removal by conventional methods, while too high to allow discharge to the environment [21–24]. Roots of many hydroponically grown terrestrial plants such as Indian mustard, sunflower, and various grasses can be used to remove toxic metals such as Cu^{2+}, Cd^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} from aqueous solutions [2,23].

Nevertheless, there is little information relating uranium speciation to plant uptake, and the forms of uranium taken up by plants and the mechanism by which this occurs have to be identified [25,26]. This study focused on demonstrating the removal of uranium from groundwater by rhizofiltration using sunflower and bean. Lab-scale batch tests were performed and a continuous rhizofiltration clean-up system was employed to demonstrate the feasibility of utilizing rhizofiltration to remove uranium from groundwater.

2. Experimental method

2.1. Plant and materials

Two plant species, sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. vulgaris), which were local landraces in Korea and were not identified with specific cultivar names, were used for the rhizofiltration experiments. All plant seeds were obtained from the National Institute of Agricultural Biotechnology, Korea. Seeds were germinated and then cultivated hydroponically in a glass box with a 5 cm deep layer of silica beads (1 mm in diameter) until the buds came out (in the darkness for 4–7 days). The entire cultivation process was conducted for 2–3 weeks in a growth chamber at 25 °C (80% relative humidity, 16 h of photoperiod/day, and 0.05% of CO_2) and 20 g of each plant cultivar was selected for rhizofiltration. For the batch experiments, deionized-distilled water was titrated with a standard solution of uranium, purchased from Sigma–Aldrich Ltd. (uranium atomic absorption standard solution, 1000 μg/mL in 1% (v/v) HNO_3). This solution was used as artificially contaminated groundwater requiring remediation. Characteristics of genuine groundwater might be quite different from those of artificial groundwater. Batch experiments and a lab-scale continuous rhizofiltration system using genuine groundwater having high uranium concentration (taken from three sites around the Okcheon Group, Korea) were therefore performed. The chemical properties and uranium concentration of groundwater at three sites are shown in Table 1. The precision with which the analyses were measured was determined by measuring duplicate analyses of groundwater samples. Only if duplicate analyses were within 10% of one another, they were averaged and determined as the final concentration in Tables 1 and 2.

2.2. Experiments for rhizofiltration

In this research, the uranium removal efficiencies of sunflower and bean were investigated under various rhizofiltration conditions in batch experiments. The effects of uranium concentration and pH of groundwater on the uranium removal ability were also investigated in batch experiments. Artificially contaminated solution and genuine groundwater having high uranium concentration were used in the batch experiments. A lab-scale pilot rhizofiltration system was designed to continuously clean groundwater. For batch experiments, the rhizofiltration was repeated in three times and their arithmetic mean for the removal efficiency was used only if the deviation were within 30% of the mean (mostly within 15%). For the continuous lab-scale pilot system, the experiment was duplicated and the arithmetic mean was used only if the duplicate results were within 30% of one another.

At the end of the experiments, the amount of uranium accumulated in each plant part (the roots and other parts including leaves) was measured by a wet digestion analysis [27]. Finally, SEM (Scanning Electron Microscope, model: HITACHI S-2400) and EDS (Energy Dispersive X-Ray Spectrometer, model: Kevex Ltd., Sigma) analyses for the roots of each cultivar used in the experiments were conducted to evaluate the structural and compositional changes of the root surface after rhizofiltration.

2.2.1. Batch experiment with artificially uranium contaminated solution

In batch experiments, rhizofiltration was performed using glass jars (12 cm × 12 cm × 8 cm) containing 400 mL of artificially uranium contaminated solution. Seeds of sunflower and bean were germinated on moistened filter paper for 4–7 days and cultivated in a growth chamber for 3 weeks. It is very important that the plant used for the rhizofiltration develops tolerance to the toxicity of uranium in groundwater. To determine the tolerance limit of uranium in groundwater for the rhizofiltration of the aforementioned two plants, batch experiments with solutions having five different initial uranium concentrations were performed and their initial uranium concentrations are shown in Table 2. Twenty grams of plant material was hydroponically located in each glass jar with a 5 cm deep layer of plant support, where the roots reached into the solution. All the rhizofiltration experiments were performed in a growth chamber at 25 °C, 80% relative humidity, 16 h photoperiod, and 0.05% of CO_2. For each experiment, 5 mL of solution

Table 2
Initial uranium concentrations of artificial groundwater and genuine groundwater used in experiments.

Plant used in experiments	Initial uranium concentration (μg/L)							
	Artificially contaminated groundwater					Genuine groundwater		
Sunflower	30	80	136	287	543	22	81	242
Bean	30	80	116	375	646	–	–	–

(–) Not used in experiments.

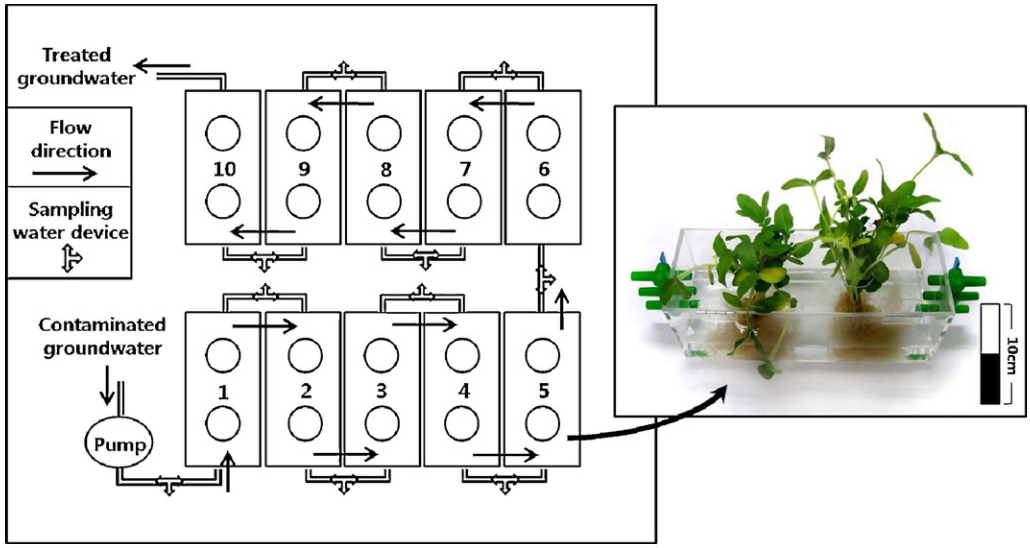

Fig. 1. Schematic and photograph of the continuous clean-up system for rhizofiltration.

was sampled from the jar at 12, 24, 48, and 72 h and the uranium concentrations were analyzed on ICP/MS (PerkinElmer, Elan 6100). Uranium removal efficiency in each rhizofiltration batch experiment was calculated via comparison of the initial uranium concentration to the residual concentration of the solution. Control tests without plant materials were also carried out to consider the non-rhizofiltration effects in batch experiments.

2.2.2. Batch experiment with different pH of solution

Behavior and speciation of uranium and the growth rate of the plant are strongly dependent on the pH of the solution [9,26,28]. Batch experiments for rhizofiltration were repeated with various pH conditions (pH 3, 5, 7, and 9) in solution using two plant cultivars. The pH in solution was adjusted by the addition of 0.1 M HCl or NaOH standard solution (purchased from Sigma–Aldrich Ltd.). Each plant cultivar was exposed to artificially uranium contaminated groundwater (200 μg/L) having different pH of solution during rhizofiltration. The conditions of the growth chamber and the procedures for the batch experiment were the same as employed in the previous batch experiment.

2.2.3. Batch experiment with genuine groundwater

Genuine groundwater having uranium concentration of 22, 81, and 242 μg/L was sampled from three different sites (Deogpyeongri, Wadong, and Oesamdong). Seeds of sunflower were germinated on moistened filter paper for 4–7 days, followed by cultivation in a growth chamber for 3 weeks. Conditions of the growth chamber and the rhizofiltration, and the analytical procedure for the experiment were the same as employed in the previous experiment.

2.2.4. Lab-scale continuous rhizofiltration clean-up system

As a physical model of the continuous rhizofiltration process to clean groundwater, a lab-scale clean-up system having 10 boxes serving as rhizofiltration reservoirs was designed. A schematic and photograph of the continuous clean-up system are shown in Fig. 1. Genuine groundwater having a uranium concentration of 270 μg/L, taken from a site at Oesandong, was used for the experiment. Each glass box (10 cm × 30 cm × 10 cm) was filled with 1.8 L of deionized water and 20 g of sunflower cultivars was placed in the box through two holes (4 cm in diameter) on the top of each box (Fig. 1). Groundwater was then injected from an open tube in the left wall of the first box and drained out through a tube located in the right wall of the box, contacting roots of the cultivars for the rhizofiltration process. Ten boxes in total were connected in a direct series for the continuous rhizofiltration system and groundwater was flowed through the system at two constant rates (5 and 12.5 mL/min) in order to consider the kinetic effect of the system. A water sampling device was equipped between boxes and 5 mL of groundwater was taken at 12 h intervals to measure the uranium concentration in the drained groundwater. The uranium removal rate (%) of each rhizofiltration reservoir (box) and the accumulated removal efficiency of the clean-up system per each pore volume of treated groundwater were calculated to investigate the feasibility of applying rhizofiltration for groundwater remediation.

2.2.5. Analysis of uranium accumulation in plant parts

The uranium content in parts ('the root' and 'the shoot') of plant cultivars after rhizofiltration was measured to determine where the majority of uranium accumulated in the plant. Roots and shoots (whole other parts including leaves) of plants used in each rhizofiltration experiment were separated, rinsed three times in deionized water, and dried at 80 °C in a forced-air convection oven for 2 days. After measuring its total dry weight, each part was ground to less than 2 mm size for a wet digestion analysis [27]. Ground plant parts were mixed with 10 mL of concentrated HNO_3 for 12 h, heated at 180–200 °C until the dense brown fumes disappeared, and then boiled until the volume was reduced by approximately 50%. Twenty milliliters of ternary solution (HNO_3:H_2SO_4:$HClO_4$ = 10:1:4) was added to the digested solution and the solution was heated again 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 100 mL. Analyses on ICP/MS were subsequently conducted to measure uranium content in each plant part.

SEM is one of the useful equipments to visualize the surface of roots and EDS (Energy Dispersive X-Ray Spectrometer) is a useful instrument to evaluate the compositional characteristics of elements. Rhizofiltration batch experiments using a solution with a uranium concentration of 1000 μg/L were repeated to visualize the change on the surface of the root caused by sorption or precipitation of uranium. After 24 h of rhizofiltration, the surface structure of sunflower roots was analyzed by SEM (model: HITACHI S-2400) coupled with EDS (model: Kevex Ltd., Sigma) and the results were compared to those obtained prior to rhizofiltration.

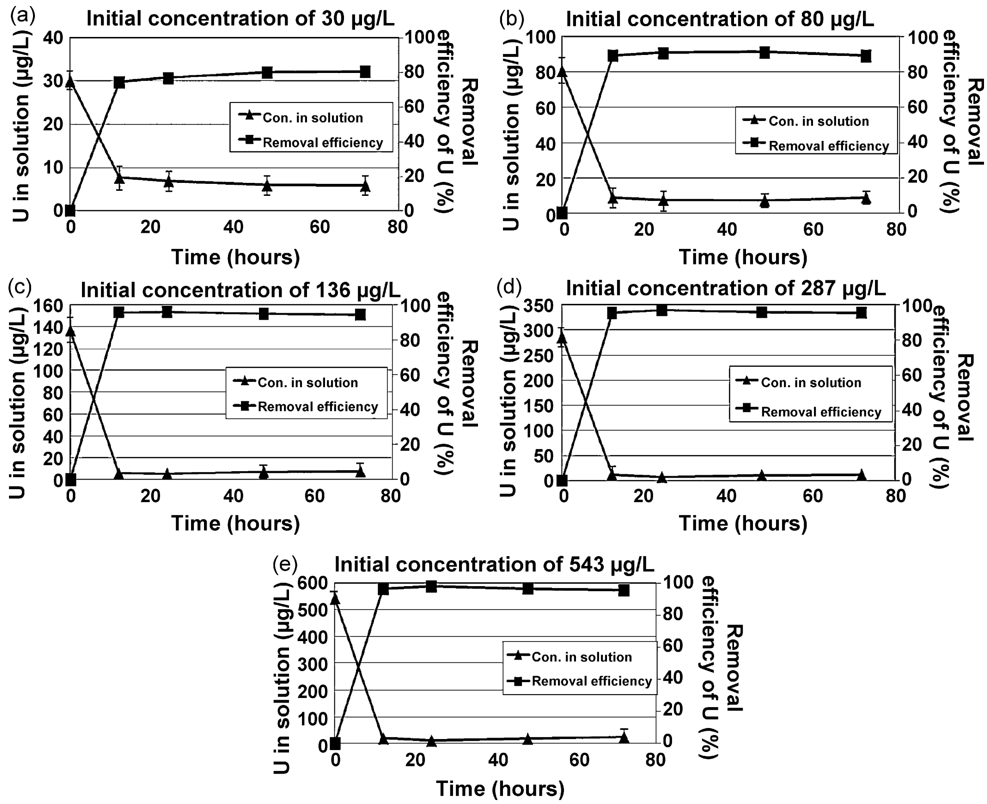

Fig. 2. Results of rhizofiltration batch experiments using sunflower with five different uranium concentrations of artificial groundwater.

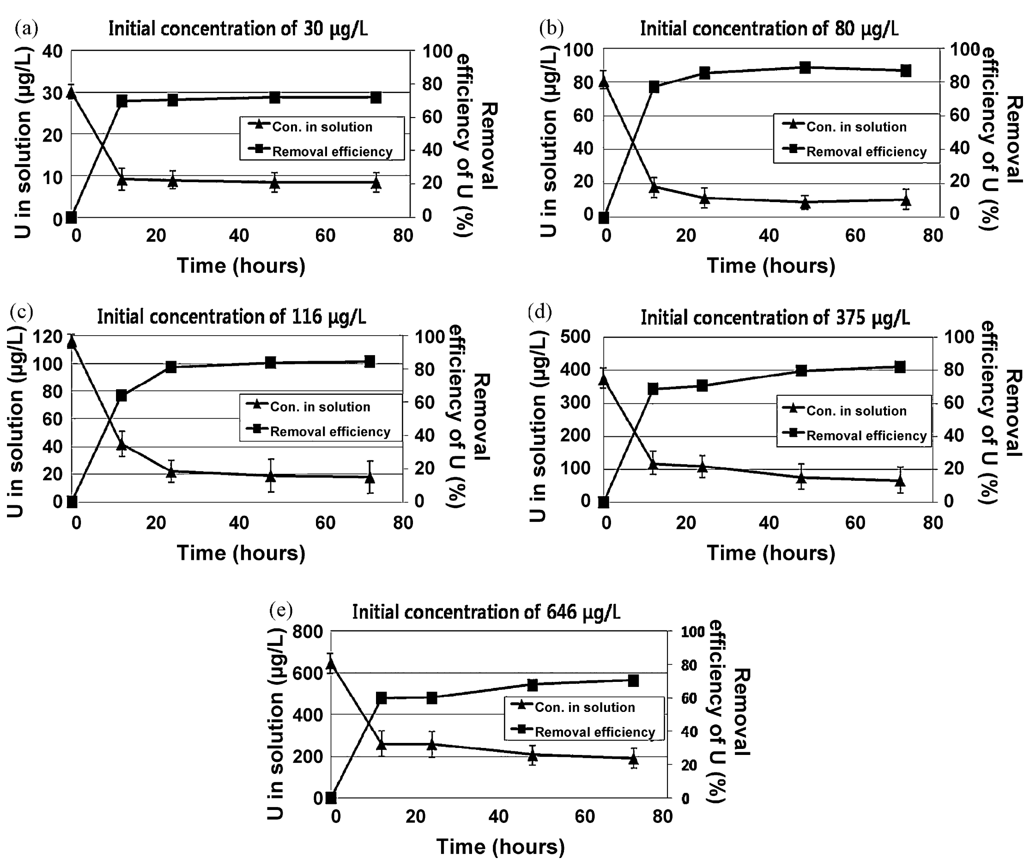

Fig. 3. Results of rhizofiltration batch experiments using bean with five different uranium concentrations of artificial groundwater.

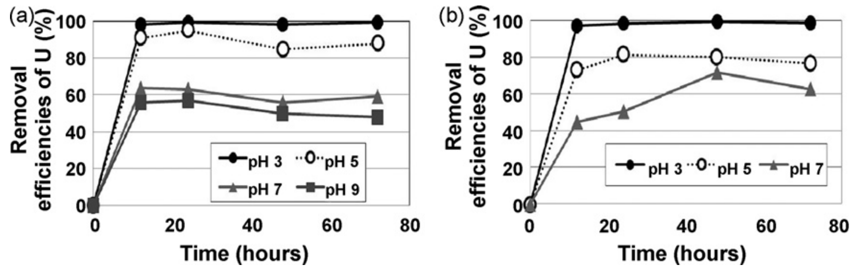

Fig. 4. Results of uranium removal efficiencies at different pH of solution ((a) sunflower and (b) bean).

3. Results and discussion

3.1. Results for batch experiment with artificially uranium contaminated solution

The results of the uranium removal efficiencies via rhizofiltration using two plant cultivars with five different uranium contaminated solutions are shown in Figs. 2 and 3. Sunflower removed more than 80% of the uranium from groundwater (30 μg/L of uranium) and the uranium concentration of the residual solution was maintained at about 6–7 μg/L, which is much lower than the USEPA drinking water limit of 30 μg/L (Fig. 2). The uranium removal ability of sunflower abruptly increased within 12 h and was stably maintained after 24 h. For the solution having 80 μg/L of uranium, the uranium removal efficiency reached more than 89% within 24 h of rhizofiltration. Even when the initial uranium concentration of groundwater was 543 μg/L (Fig. 2(e)), sunflower removed more than 97% of the initial uranium by rhizofiltration within 24 h and the uranium concentration of the treated groundwater was reduced to 18 μg/L, suggesting that rhizofiltration using sunflower has powerful capability to remediate groundwater having very high uranium concentrations.

For bean, more than 70% of the uranium was removed from the solution (30 μg/L of uranium) and the uranium concentration in the groundwater was reduced to 10 μg/L in 24 h (Fig. 3(a)). As the initial concentrations of uranium in groundwater were 80 and 116 μg/L, the uranium removal efficiencies exceeded 80%. However, with high uranium concentrations of groundwater (375 and 646 μg/L), the removal efficiency of rhizofiltration was reduced to approximately 60% and the uranium concentrations in treated water were 120 and 256 μg/L, respectively (Fig. 3(d) and (e)). These results suggest that rhizofiltration using bean also has strong capability to remove uranium in groundwater; however, it might be limited when the concentration of uranium in the groundwater is greater than 350 μg/L.

3.2. Results for batch experiment with different pH of solution

The effect of the pH of the solution on the uranium removal efficiency for two plant cultivars during rhizofiltration is shown in Fig. 4. The uranium removal efficiency of sunflower decreased from 99% to 50% as the pH of the solution was increased from pH 3 to 9. This suggests that the ability of sunflower to uptake uranium was influenced by the pH of water, due to the differences in uranium speciation in solution (Fig. 4(a)). At pH 3–5, uranium was present predominantly as oxidated free uranyl cations, the most amenable form for rhizofiltration, and was readily adsorbed to the plant roots, resulting in a significant increase of uranium bioaccumulation on the root [25,26,29]. For bean, like sunflower, the greatest uranium accumulation in the plant root occurred at a solution pH 3–5 and the uranium removal efficiency was over 80% (Fig. 4(b)). Results suggested that a solution having low pH (pH 3–5) yields very high uranium accumulation for both plants in rhizofiltration and the uranium removal efficiencies were significantly dependent on the pH of the groundwater.

3.3. Results for batch experiment with genuine groundwater

Results of rhizofiltration using sunflower to remediate uranium contaminated groundwater from three different sites are shown in Fig. 5. The uranium removal efficiency for Deogpyeongri groundwater (initial concentration: 22 μg/L) was more than 90% within 48 h of rhizofiltration while for Wadong groundwater (initial concentration: 81 μg/L) the removal efficiency of sunflower was 87%. Despite the high uranium concentration of groundwater at Oesamdong (initial concentration: 242 μg/L), the uranium removal efficiency reached more than 87% within 48 h and the uranium concentration in treated groundwater was 25 μg/L. This is lower than the

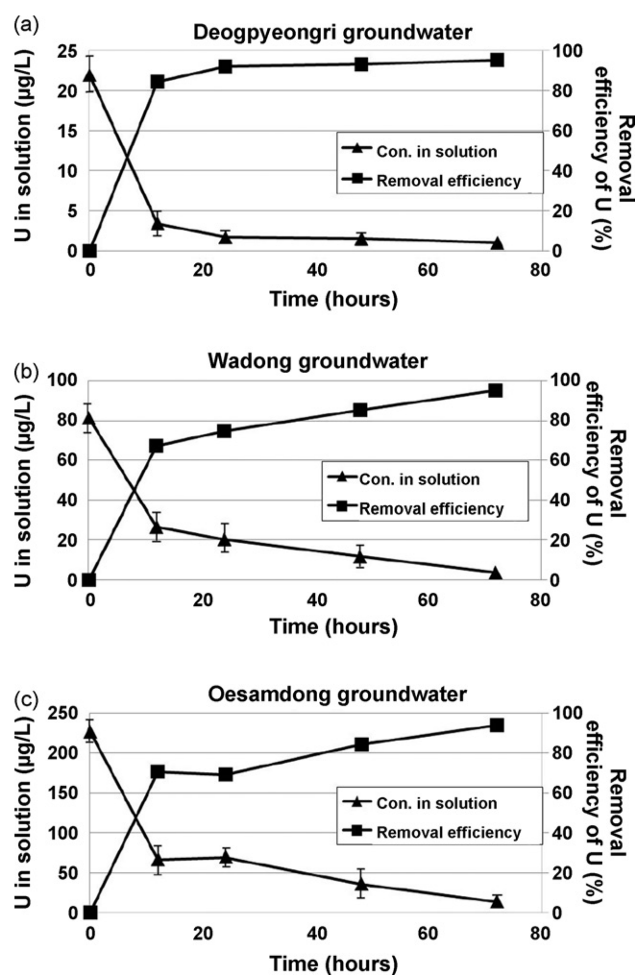

Fig. 5. Results of uranium removal efficiencies for genuine groundwater.

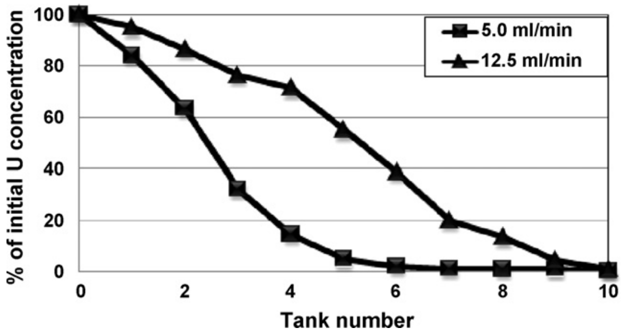

Fig. 6. Results of uranium concentration ratio for each rhizofiltration box in the continuous clean-up system for 1 pore volume treatment (18 L) at two flow rates.

USEPA Water Quality Standard limit (30 μg/L), suggesting that the use of sunflower for rhizofiltration is an extremely viable means of removing uranium from genuine groundwater.

3.4. Results for a lab-scale continuous rhizofiltration clean-up system

Fig. 6 shows the ratio of the uranium concentration of treated groundwater to the initial concentration of groundwater in each box of the clean-up system. The initial uranium concentration of groundwater was 270 μg/L and the one pore volume of the clean-up system for groundwater was 18 L. For a flow rate of 5.0 mL/min, the uranium removal efficiency of the final tank with sunflower was maintained at over 99% and the uranium concentration of treated groundwater from the final tank was below 5 μg/L for treatment of 18 L of groundwater (Fig. 6). As the amount of injected groundwater was increased to 36 L (two pore volumes of the clean-up system),

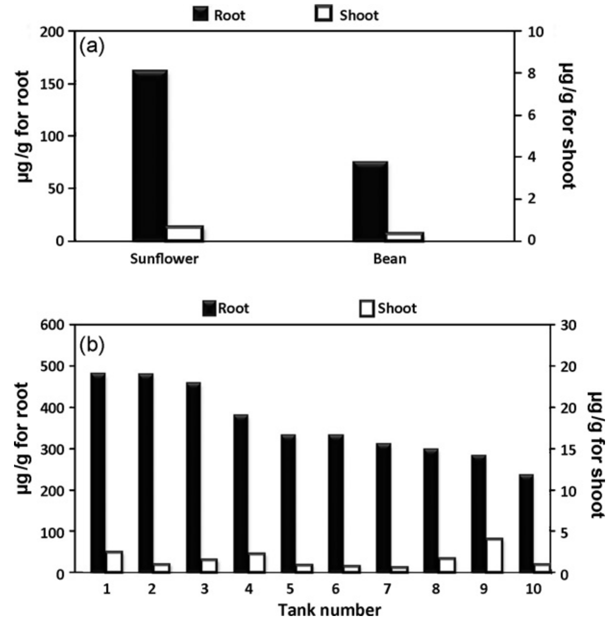

Fig. 7. Results of uranium accumulation in root and shoot part for (a) batch experiment at initial U concentration of 80 μg/L and (b) continuous clean-up system at initial U concentration of 270 μg/L.

the uranium removal efficiency decreased to 80% with a flow rate of 5.0 mL/min and the final uranium concentration of treated water was below 30 μg/L. These results suggest that the uranium removal capability of the two plants used in the clean-up system exceeded 20 mg/kg of wet plant. For a flow rate of 12.5 mL/min, the removal efficiency of the final tank was about 98% for treatment of 18 L of

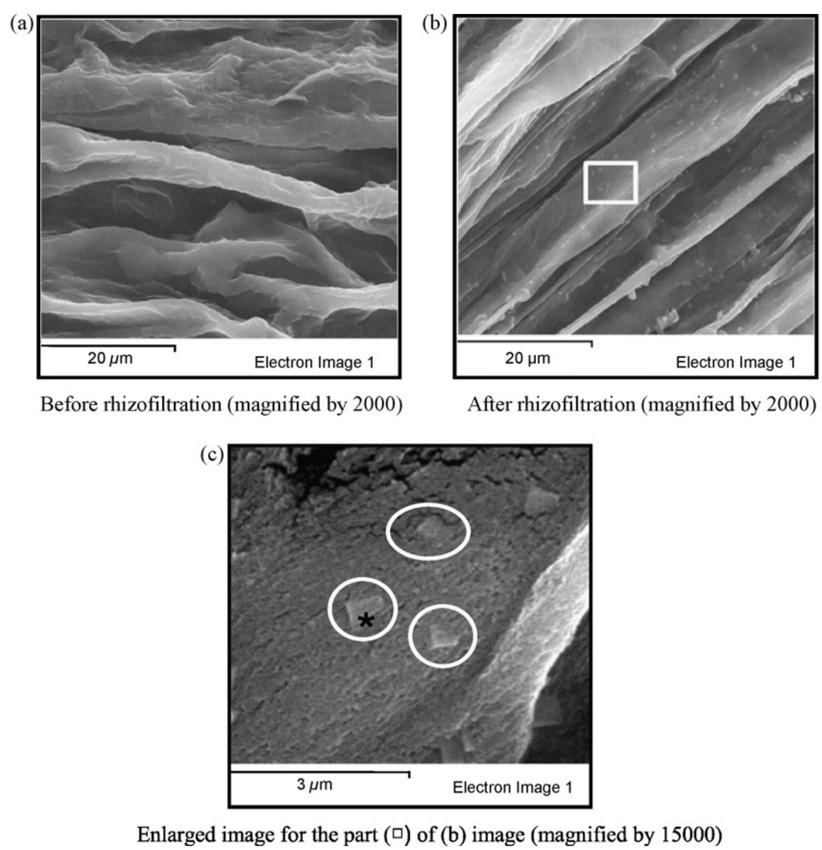

Fig. 8. SEM images of the surface of the sunflower root before and after rhizofiltration ((★) the spot for EDS analysis).

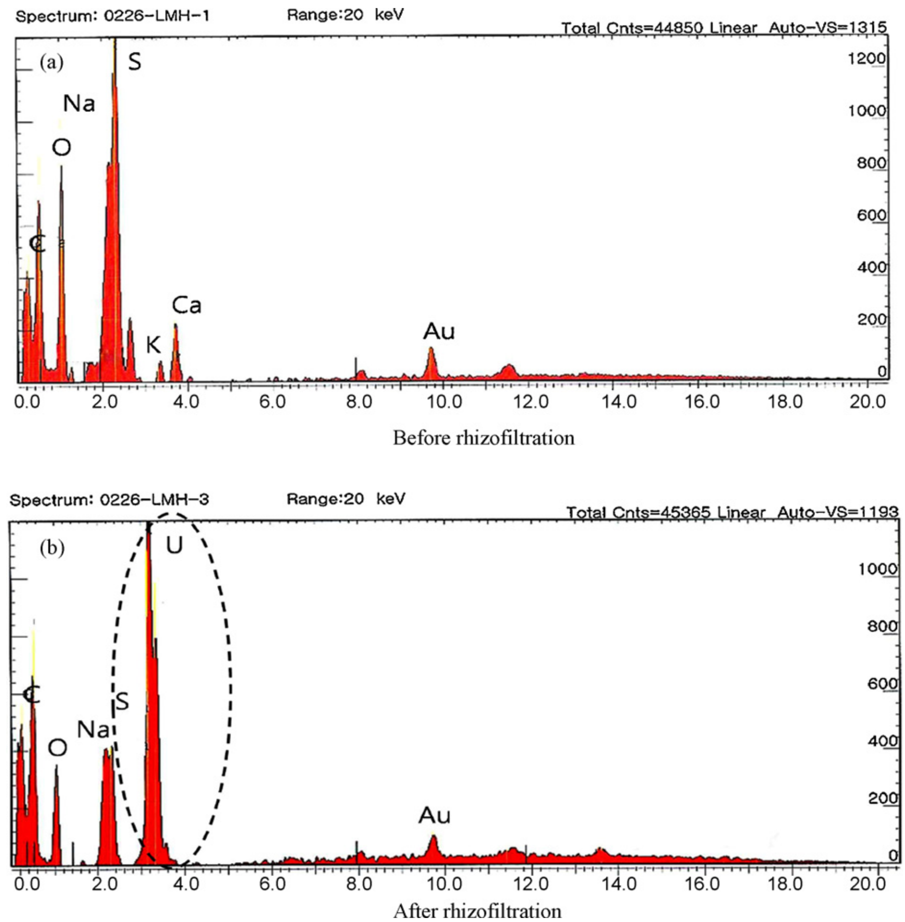

Fig. 9. Results of EDS analysis before and after rhizofiltration (the spectra result of (b) is from an analysis of the 'EDS spot' in Fig. 8(c)).

groundwater, whereas the efficiencies of other tanks were lower than those at 5.0 mL/min, suggesting the residence time of groundwater in tank control the removal efficiency.

3.5. Results for analysis of uranium accumulation in plant parts

The results from the measurement of uranium content in each part (the root or the shoot) for batch experiments (initial uranium concentration of 80 μg/L in groundwater) are shown in Fig. 7(a). For sunflower and bean, despite the small volume of root compared with that of the shoot, more than 90% of uranium accumulated in the root and the amount transported to the shoot was negligible (less than 10%). The content of uranium accumulated in each part of sunflower during operation of the continuous clean-up system was also measured and the results showed that most of the uranium accumulated in the plant roots (Fig. 7(b)). It was found that the uranium removal capability of the two plants via rhizofiltration was approximately 25 mg/kg of plant, but the capability of the root part in isolation was more than 500 mg/kg. Finally, the results suggest that only the roots of the fully grown plant used for rhizofiltration should be disposed or post-treated. The cost and time to treat massive amounts of grown plants after rhizofiltration could thereby be dramatically reduced when sunflower and bean are used for rhizofiltration in the real field.

SEM micrographs and EDS (Energy X-ray Dispersion Spectroscopy) spectra of sunflower roots were taken before and after rhizofiltration (Figs. 8 and 9). SEM micrographs show that the root surface was covered with many clusters resembling small uranium crystal formations (Fig. 8(c)) and these observations were confirmed by EDS analytical results, comparing uranium spectrums before/after rhizofiltration (Fig. 9). In Fig. 9(b), the distinct uranium peaks in the EDS spectra of the cluster on the root surface can be observed after rhizofiltration and the uranium mass ratio of the cluster spot was measured to be 0.5% of the total component weight. These findings suggest that sorption or precipitation on the root surface might be the major mechanisms to remove uranium from groundwater by rhizofiltration.

4. Conclusion

For the rhizofiltration with artificially uranium contaminated solution, more than 70% of the initial uranium was removed by using sunflower and bean. The root ability to uptake uranium was influenced by the pH of the water, due to differences in uranium speciation. For both plants, the highest uranium removal occurred at pH 3–5, at which the removal efficiency exceeded 90%. The uranium removal efficiency of the continuous clean-up system for rhizofiltration using sunflower was over 99% at a 5.0 mL/min flow rate and the uranium removal capability of sunflower roots used in the clean-up system exceeded 500 mg/kg of plant. From the SEM and EDS spectra analyses on the sunflower root, it is speculated that the main mechanism of the rhizofiltration to remove uranium might be strong adsorption including precipitation and exchangeable sorption on the root surface.

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