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Uranium Absorption Ability of Sunflower, Vetiver and Purple Guinea Grass

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ABSTRACT

The ability of sunflower, vetiver and purple guinea grass to absorb uranium from yellowcake solution was compared. Using an image plate technique, beta and gamma rays from uranium daughter nuclides were used to stimulate image plate phosphor to determine the distribution of uranium in plants. The results showed that all three plants could accumulate uranium in their roots better than in shoots, while sunflower showed the best uranium absorption in general. In both solution and sand culture systems, sunflower absorbed uranium better than purple guinea, while vetiver had the least absorption. Moreover, sunflower absorbed higher quantities of uranium as the growing time increased. Although yellowcake, at pH 4, facilitated better uranium absorption than at pH 7, growth was found to be better at pH 7.

Key words: phytoremediation, radionuclide, uranium, sunflower, vetiver, purple guinea

INTRODUCTION

Many human activities cause radionuclide contamination to soil and water, for example, nuclear weapon testing, disposal of nuclear waste, nuclear accident and nuclear industries. Uranium mining, milling and processing have resulted in uranium-contaminated soils in large areas of the world (Huang *et al.*, 1998). Moreover, metallic mining activities also produce large amounts of radioactive waste, which has been found in tin mining areas in Malaysia and Nigeria (Ibeanu, 2003), and in monazite activities in India, USA, Australia, Brazil, China and Thailand (Paschoa and Filho, 1995). Radionuclide contaminants pose serious problems to biological systems, due to chemical toxicity and radiological effects. Hence, it is essential to achieve effective reclamation of contaminated sites.

At present, phytoremediation has been applied to remove pollutants, such as heavy metals, herbicides, pesticides, organic matter and radioactive elements from soil, sediment and water. This green technology is gaining in importance and attractiveness due to its simplicity and cost-effectiveness. Plant species used to decontaminate radioactive wastes (radiophytoremediation) must be resistant to radiation, fast growing and have a high capacity for uptake of radionuclides (Gulati *et al.*, 1980; Sheppard *et al.*, 1989; Saric *et al.*, 1995). The ability of different plants to absorb radionuclides also depends on the environment and the soil properties (Entry *et al.*, 1996; Broadley and Wiley, 1997; Entry *et al.*, 1999). Radioactive waste in Thailand mainly comes from the application of radioisotopes in medicine, industry and agriculture (FNCA, 2007). However, tin mining and monazite mining enhance natural radioactivity in the

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environment. Therefore, research is needed to identify appropriate plant varieties that can be used effectively for radiophytoremediation.

The current study compared the uranium absorption ability of different plant species. Three types of plant with promising potential for radiophytoremediation were selected. Sunflower plants have exhibited potential for treating radionuclide contamination in soil and water (Cooney, 1996; Dushenkov *et al*., 1997; Prasad, 2007). Vetiver is a fast growing grass with the ability to grow well under adverse conditions and has been used for soil conservation and decontamination of sites containing heavy metals (Roongtanakiat, 2009), as well as having potential for radiophytoremediation (Singh *et al*., 2007). Purple guinea grass is one of the best grazing grasses, with a high leaf production that is able to adapt to a wide range of soil types (Tudsri, 2005). Imaging plate technology was applied to investigate the distribution of uranium in shoot and root parts. The results should be beneficial for the development of nuclear technology to be used more broadly in an environmentally sustainable way.

MATERIALS AND METHODS

Plant and uranium source

Plant samples of sunflower (*Helianthus annuus,* Valentine) and purple guinea grass (*Panicum mazimum*, TD58) were germinated from seeds. Plantlets of vetiver grass (*Chrysopogon nemoralis*), Ratchaburi ecotype, were prepared from tissue culture.

Yellowcake, a product from monazite processing, was the radionuclide material used as a uranium source in this study. It was obtained from the Rare Earth Research and Development Center, Office of Atoms for Peace. The analysis of yellowcake samples was carried out with an X-ray fluorescence spectrometer (Oxford ED2000). The concentrations of yellowcake solution were 880 and 440 mg L^{-1} prepared for plant culture using nitric acid and adjusted to pH 4 and pH 7 with potassium hydroxide. The total radionuclide activity in the solution was measured by a liquid scintillation counter (Wallac1220 Quantulus).

Plant culture

Plantlets of the three plant species were cultured in test tubes with roots immersed in 20 ml of the yellowcake solutions at pH 4 and pH 7 using both concentrations (880 and 440 mg L^{-1}) for 3, 6 and 9 d. Each treatment was replicated three times. Besides culturing in the yellowcake solution, the plants were also grown in pots containing 1.5 kg sand and were treated with the yellowcake solutions at pH 4 and pH 7 at the rate of 20 ml per pot each day. All plants were harvested 20 d after planting.

Plant analysis

At the end of each experiment, plants were harvested with their root system and thoroughly washed with distilled water. Plant samples from the first experiment were well flattened with pressing paper until the plants were dry. PE (Polyethylene) film was used to wrap the dry plants in order to protect the imaging plate from contamination by radionuclides. The wrapped plants were placed against the imaging plate (BAS-MS, Fuji film Co.) for 48 h and then read with an imaging analyzer (Fujifilm Bas-2500).

For the radioactivity determination, dry plant samples from both experiments were incinerated at 400°C for 6 h and then the ash was digested in a mixture of hydrochloric and nitric acid (1:3, v:v). Hydrofluoric acid was added to complete the digestion until clear solutions were obtained. A Wallac1220 Quantulus liquid scintillation counter was used to detect the activity of the aliquots in term of count rate (count per minute, cpm) using Optiphas HiSafe 3 as the scintillation cocktail.

Data analysis

The means of the three replications, as well as associated standard errors (SE) were calculated. The range of alpha particles in the PE was calculated with the SRIM program of Ziegler *et al*. (2008).

RESULTS AND DISCUSSION

Radionuclide and radioactivity of yellowcake solution

The major constituent of yellowcake used in the culture solution was uranium (86.067%). Thorium, rubidium, niobium, iron, silicon, sodium and rhodium were found as impurities (Table 1), with minor traces of manganese, sulfur, calcium, copper and zinc. Yellowcake typically contains 70-90% triuranium octoxide (U_3O_8) by weight. Hence, the most common radionuclide in the culture solution was uranium (^{235}U, ^{238}U) and its daughter nuclides (^{231}Th, ^{234}Th, ^{231}Pa, ^{234}Pa etc.).

At pH 4, the radiation count rate obtained from the yellowcake solution concentration of 880 mg L^{-1} was higher than that at the concentration of 440 mg L^{-1}, due to the higher quantity of yellowcake. In contrast, at pH 7, both concentrations showed no difference in the count rate, which was very much lower than for the pH 4 solution (Table 2). This was most likely due to precipitation of uranium under the neutral or alkaline conditions.

Radiographic image of radionuclide accumulation in plant parts

The image plate, a radiation image sensor, contained a sensitive layer of BaFBr.Eu^{2+} phosphors that trapped and stored radiation energy (Ohuchi and Yamadera, 2002). The stored energy was released as luminescence by stimulation with a laser beam on the image plate reader. Then, the photostimulated luminescence was transformed to obtain a highly sensitive image of the original pattern of radiation.

The image plate was sensitive to all alpha, beta and gamma rays. Uranium, the major radionuclide in yellowcake, emits alpha particles (from ^{235}U) with maximum energy of 4.679 MeV (U.S. Department of Health, Education and Welfare, 1970). The range of an alpha particle was calculated by the SRIM program and showed that a 4.679 MeV alpha particle would interact and deposit its entire energy in a PE film that was 31.33 µm thick. In this experiment, the plant samples were wrapped with 60-µm-thick PE film and the image plate (BAS-MS) was coated with 9-µm-

Table 1 Element constituents of the yellowcake used in the study.

Element	Content (%) + SE	Element	Content (%) + SE
U	86.067 ± 0.233	Nb	1.760 ± 0.137
Th	3.240 ± 0.165	Rh	5.530 ± 0.181
Rb	2.120 ± 0.023	Si	0.257 ± 0.039
Na	0.796 ± 0.052	Fe	0.092 ± 0.008

Table 2 Average count rate of yellowcake solution used for plant culture.

Yellowcake solution concentration (mg L^{-1})	pH	Average count rate (cpm mL^{-1}) ± SE
440	4	708 ± 1.7
	7	13 ± 0.2
880	4	1,228 ± 2.2
	7	14 ± 0.2

thick PE film. The total thickness of the PE films was greater than the maximum required to completely capture the range of alpha energy in the PE film; therefore, the alpha particles were blocked completely from reaching the sensitive layer of the image plate. Only beta and gamma rays emitted from uranium daughter nuclides could pass through the PE films and stimulate the phosphor layer to produce a radiographic image.

No radiographic image was received from the plant samples incubated in the yellowcake solution at pH 7. This implied that the plants had absorbed a very low amount of uranium from the solution to produce no image. In contrast, with the pH 4 solution, all plant species (sunflower, vetiver and purple guinea) absorbed high amounts of uranium and accumulated more in the roots compared to the shoots (Figure 1), as has been reported by many researchers (Entry *et al*., 1996; Petrescu and Bilal, 2003; Chen *et al*., 2007; Prasad, 2007). The accumulation of uranium in the plants increased as the growing period increased (Figure 2). Sunflower was the most effective of the three species in the absorption of uranium. The results were similar to those reported by Entry *et al*. (1996) and Huhle *et al*. (2008). Dushenkov *et al*. (1997) revealed that all uranium was concentrated in the sunflower roots and the amount of uranium transported to the shoots was negligible. However, the radiation image of the sunflower samples indicated the distribution of uranium to shoots; this might have been due to translocation of the uranium daughter nuclides to the shoots. The absorption of other radionuclides in sunflower plants has been studied. Autoradiography, using X-ray monitoring Film T2, by Soudek *et al*. (2004), showed that the strongest exposure to ^{137}Cs occurred in the youngest parts of the sunflower plant, as well as in the internode segments, while ^{90}Sr nuclides were relatively evenly distributed within the plant. This could be explained by assuming that the absorption and distribution of radionuclides in plants are nuclide specific.

Effect of exposure period on plant growth and uranium absorption

Whilst the three plant species absorbed radionuclides, they did not grow well when the plants were left longer in the test solution at pH 4,

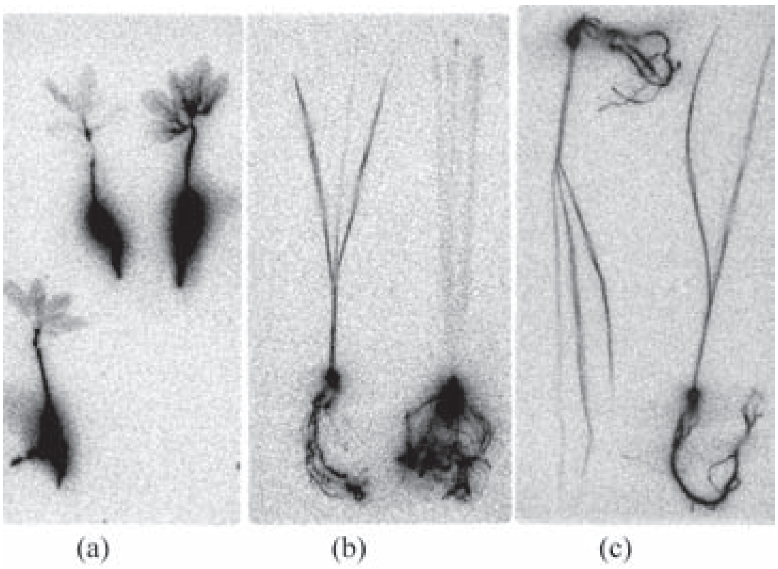

Figure 1 Radiographic images of plants cultured in 440 mg L^{-1} yellowcake solution at pH 4 for 9 d for: (a) sunflower; (b) vetiver and (c) purple guinea.

as shown in Table 3 by the reduction in plant biomass (ash weight). This may have been due to the acidic conditions, which were detrimental to plant growth. Also, it may have been due to the non-addition of plant nutrients to the culture solution, in order to prevent competition between uranium and nutrient absorption. Yatim (1991) reported that the absorption of uranium was reduced as the nutrient concentration of the solution increased. However, the amount of uranium in the plants increased with the length of the exposure period. Similar results were reported by Dushenkov *et al*. (1997), where the longer plants were grown in a radioactive solution, the more radionuclides were absorbed.

The radiation count rate per ash mass of plants (Table 3) indicates that that sunflower accumulated the highest amount of uranium from the yellowcake solution, followed by purple guinea and vetiver. This confirmed the results obtained

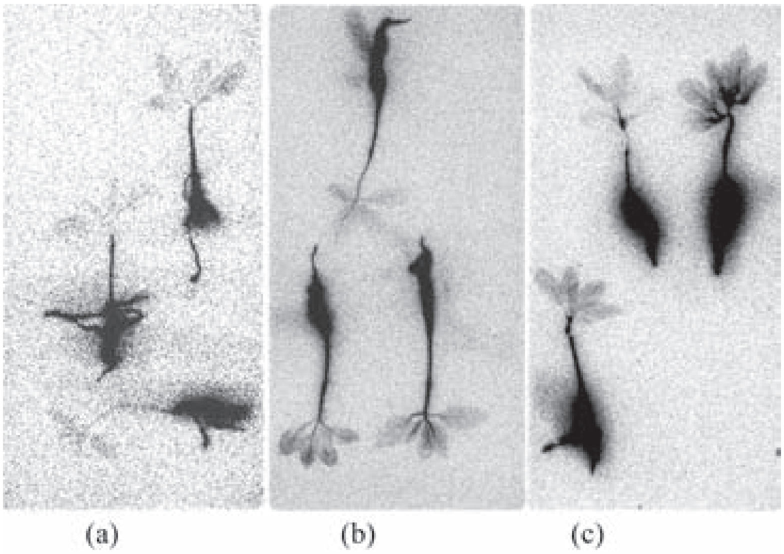

Figure 2 Radiographic image of sunflower plants cultured in 440 mg L^{-1} yellowcake solution at pH 4 for: (a) 3 d; (b) 6 d; and (c) 9 d.

Table 3 Mean ash weight per plant and radioactivity (counts per min) of three plant species, cultured in yellowcake solution at pH 4 for 3, 6 and 9 d.

Plant species	Period of culture (day)	Mean plant ash weight in milligrams (mg) ± SE	Counts per minute per milligram (cpm mg^{-1}) ± SE
Sunflower	3	31.3 ± 6.4	88.1 ± 0.1
	6	65.3 ± 15.8	144.7 ± 0.7
	9	50.8 ± 8.8	176.2 ± 3.6
Vetiver	3	119.5 ± 17.7	34.3 ± 2.4
	6	167.6 ± 42.8	43.4 ± 1.2
	9	109.7 ± 1.6	73.0 ± 1.1
Purple guinea	3	64.0 ± 5.7	40.3 ± 1.9
	6	46.9 ± 14.2	83.2 ± 7.4
	9	33.5 ± 8.1	120.9 ± 4.7

from the radiographic images. However, the most desirable property of plants for radiophytoremediation is a high growth rate and thus high biomass production in a given environment (Soudek *et al.*, 2004). Greger (2004) also reported that a high root biomass often resulted in numerous roots exploring the soil, thus producing a higher uptake than in plants with low root biomass. Vetiver absorbed the least uranium among the three plant species; however, it had potential for radiophytoremediation as reported by Singh *et al.* (2007) because of its long, dense root system. Therefore, comparisons of biomass production and uranium uptake by these three plant species on contaminated sites should be further investigated.

Effect of pH on uranium absorption by plants

The three plant species were grown in two different plant growth media (solution and sand), to investigate the effect of pH solution on uranium absorption. In the solution culture, the radiation count rate from sunflower plants after incubation in a solution concentration of 440 mg L^{-1} at pH 4 for 3, 6 and 9 d was 88.4, 144.7 and 176.2 cpm mg^{-1}, respectively. Purple guinea plants produced a count rate of 40.3, 83.2 and 120.9 cpm mg^{-1}, respectively, while the count rate for vetiver was 34.3, 43.5 and 73.0 cpm mg^{-1}, respectively, over the same periods. However, at pH 7, the count rates were in the range of 5.2-6.1 cpm mg^{-1}, which were much lower than those at pH 4, and there were no differences among treatments, plant species and exposure periods (Figure 3).

The plants cultured in sand irrigated with 880 mg L^{-1} yellowcake solution at pH 4 and pH 7 gave similar results to those from the plants cultured in the solution. At pH 4, plants absorbed more uranium than plants at pH 7 (Figure 4), which was similar to the results of Dushenkov *et al.* (1997), who reported that sunflower roots absorbed more uranium at pH 5 compared to pH 7. The current study result may have been because at pH 4, most of the uranium in the solution was in the form of uranyl ions, which are available for plant uptake as reported by Edds *et al.* (1998), Yang and Volesky (1999) and Edds *et al.* (2001). At low pH, uranium is present in the solution, mainly in the form of uranyl ions (UO_2^{2+}) and was more readily taken up and translocated by the plants. Precipitation of uranium under neutral conditions to an unavailable form for plant uptake, as shown by the low count rate of the solution at pH 7 (Table 2), could explain the lower amount of uranium accumulated in plants grown in a medium under neutral conditions. Hence, in order to promote uranium uptake for the purpose of radiophytoremediation, the planting medium should have a low pH. Edds *et al.* (1998) suggested that a soil pH below 5.5 was required to provide uranium absorption in the most available form for plants.

CONCLUSION

Yellowcake, composed of 86% uranium, was used as the uranium source. Beta and gamma rays from uranium daughter nuclides transferred energy to the image plate, creating a radiation image, which showed the uranium distribution in the plants. Sunflower, vetiver and purple guinea differed in uranium accumulation, which occurred in roots more than in shoots. The solution and sand culture tests indicated that sunflower exhibited the best absorption ability, followed by purple guinea and vetiver. The amount of uranium in the plants increased as the growing period increased. The pH of the solution had a dominant effect on plant growth and uranium absorption; at pH 4, plants could absorb more uranium compared with pH 7. For further study, an experiment with contaminated soil should be carried out, in order to get information on soil-plant relationships and uranium uptake under actual growing conditions in the field.

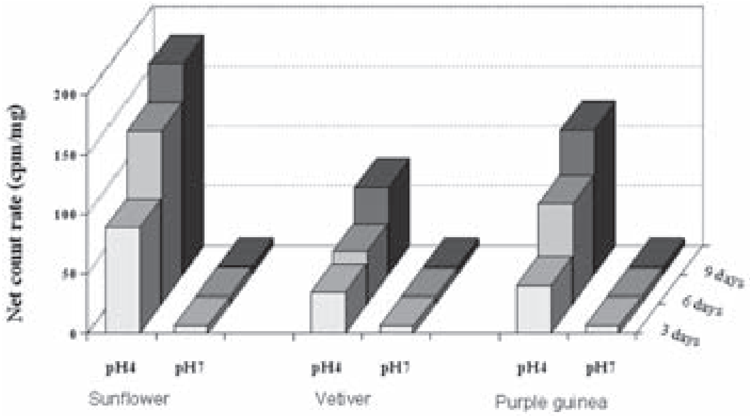

Figure 3 Radiation count rate per ash weight of sunflower, vetiver and purple guinea plants grown in 440 mg L^{-1} yellowcake solution at pH 4 and pH 7 for 3, 6 and 9 d.

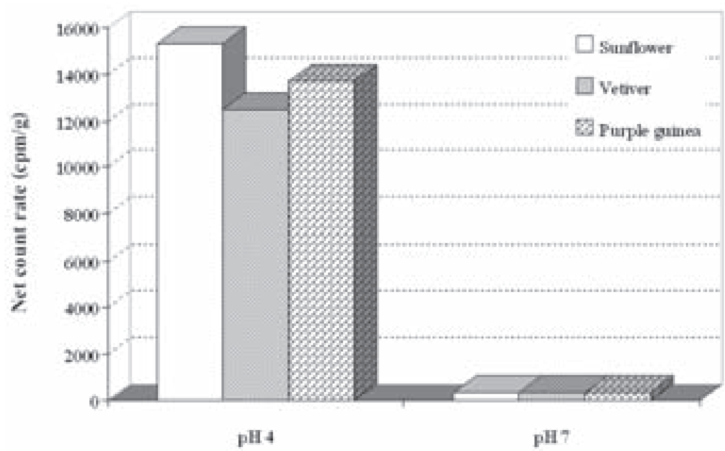

Figure 4 Radiation count rate per ash weight of sunflower, vetiver and purple guinea plants grown in sand irrigated with yellowcake solution at pH 4 and pH 7 for 20 d.

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