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Elevated Uptake of Th and U by Netted Chain Fern (*Woodwardia areloata*)

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We assessed the ability of netted chain fern (*Woodwardia areloata*) to uptake U and Th from wetland soils on the U.S. Department of Energy's Savannah River Site in South Carolina. Netted chain fern had the highest Th and U concentrations of all plants collected from the wetland. Ferns grown in contaminated soil (329 mg kg^{-1} Th, 44 mg kg^{-1} U) in a greenhouse contained 6.4 mg kg^{-1} Th and 5.3 mg kg^{-1} U compared with 0.13 mg kg^{-1} Th and 0.035 mg kg^{-1} U in Bermuda grass (*Cynnodon dactylon*). Netted chain fern has potential for the phytoremediation of soils contaminated with Th and U.

Introduction

The use of plants to remove contaminants from soils is emerging as a potential strategy for the cost effective and environmentally sound remediation of contaminated sites. Plants must produce sufficient biomass while accumulating high concentration of contaminants for phytoremediation to be successful. The degree to which contaminants are sorbed to soils is commonly the key factor determining how easily plants take up contaminants. There are several reports on the phytoremediation of soils contaminated with trace elements[1]. Recently, phytoremediation has also been considered for remediation of environments contaminated by radionuclides such as ^{137}Cs, ^{90}Sr, U and

^{99}Tc[2,3], but there is little on the phytoremediation of soil/water contaminated with U or Th.

The objective of this paper is to describe the extraction of Th and U from contaminated soils by netted chain fern (*Woodwardia areloata*) in field screening and greenhouse studies. The chain fern is a native plant in the ecologically sensitive TNX wetland located at the U.S. Department of Energy's Savannah River Site (SRS) near Aiken, South Carolina. The wetland sediment is contaminated with Th, U and other radionuclides and heavy metals[4].

EXPERIMENTAL

Field screening study

The objective of the field screening study was to identify plants that effectively remove U and Th from contaminated soils in the TNX wetland. This paper presents the results on netted chain fern and other selected species; more detailed results on additional species are presented in Kaplan et al[5]. Three factors were considered when deciding where to collect plant samples: 1) soil contaminant concentration, 2) number of soil contaminants present, and 3) the plant species present. Fern samples were collected on Nov. 11, 1999, March 22, 2000, and April 18, 2000 to observe seasonal changes in contaminant uptake. About 200 g of fresh plant material was collected on each date by cutting the plants 5-cm above ground with garden shears. Plant samples were washed, dried, and microwave digested for contaminant analyses via ICP-MS, using 0.5-g dry weight plant material in 5 mL HNO_3 and 3 mL H_2O_2. On Nov. 11, 1999 a soil sample (contaminated soil A) was collected from the root zone of the fern samples by hand auguring from 0 to 0.15 m below the leaf–litter level.

Fallen leaves were collected to compare Th and U uptake between netted chain ferns and other plants in the study area. The collected leaves were separated by species, and analyzed for radionuclide and metal content in the same manner as the fern samples.

Greenhouse study

The objective of the greenhouse study was to determine the proportion of Th and U that netted chain fern and Bermuda grass (*Cynnodon dactylon*) would extract from two TNX soils. The soils were collected from 0 to 0.15 m below the leaf-litter level in the contaminated area (contaminated soils A and B) and a nearby uncontaminated area (uncontaminated soil) at TNX. The netted chain fern was selected for this test because the field screening study showed that it had relatively high Th and U concentrations. Bermuda grass was selected as an example of a monocotyledon, which generally do not translocate metals from roots to the aboveground portion of the plant. There were 6 treatments (3 soils and 2 plants), and each treatment had 4 replicates. The experiment was conducted in 5- kg containers. Netted chain ferns collected April 18, 2000 from a non-contaminated area were transplanted on the same day to the pots in a greenhouse (two plants per pot). Bermuda grass was planted from seeds (0.5 g of seeds per pot) on the same day. Both plants were harvested after 6 weeks. Plant samples were washed, dried, and microwave digested via ICP-MS, using 0.5- g dry weight plant material in 5 mL HNO_3 and 3 mL H_2O_2, and analyzed for Th and U.

Soil analysis

Contaminated soils A and B and the uncontaminated soil were characterized by standard methods[6]. Soil pH was determined on 1:1 soil/water equilibrations. Soil organic C was determined by a modification of the unheated potassium dichromate method.

Cation and anion exchange capacity were determined by exchange with potassium and nitrate ions on sediments that were not pH adjusted. Iron oxide concentrations were determined by the dithionite reduction procedure. Particle size distribution (sand, silt, and clay concentrations) was determined by the pipette method. The bioavailability of Th and U was determined by the diethylenetriaminepentaacetic acid (DTPA) chelation method[7].

RESULTS AND DISCUSSION

Field study - uptake of contaminants by the netted-chain fern

Ferns as a group are very adaptable and can grow in environments where they would not be found naturally and under conditions that would seem unsuitable. They are shallow–rooted plants, with a large percentage of the roots near the soil surface. Netted chain fern is typically found in bogs and swamps. It has fronds with unpaired leaflets, broad, dark-green sterile fronds, and fertile fronds that are narrow and more erect. Two chain-like rows of spore cases may be seen on the back of the leaf.

The field screening study showed that netted chain fern had the highest Th and U concentrations of all plants tested (Table 1). Cobalt, Cr and Hg concentrations were also highest in netted chain fern (Table 1). Concentration ratios (CR) were calculated as the contaminant concentration in dry plant (mg/kg) divided by the contaminant concentration in dry soil (mg/kg) in which the plant was growing. A low CR value indicates low contaminant bioavailability to plants or low contaminant translocation within plants. The CR values for Th and U in netted chain fern were the highest among all native plants collected at the TNX wetland (Table 1) indicating greater uptake and translocation of these elements to the leaves by ferns than by the other plants in Table 1.

Plant concentrations of Th and U increased ten-fold during the growing season (Figure 1). A possible mechanism was vigorous spring growth that resulted in high uptake of water and dissolved constituents, followed by the concentration of contaminants in the plant tissue as water was transpired from the leaves over the course of the growing season. Previous work has shown that some ferns can take up high concentrations of metals[8,9], and fern species have been intensively screened for hyper-accumulation of trace elements by different laboratories. Ozeki et al[10] analyzed the accumulation of trace elements in 96 species of ferns by instrumental neutron activation analysis and found that trace element accumulation was highly variable and species dependent. *Dryopteris erythrosora* took up large amounts of the lanthanides and rare earth elements under natural conditions[10]. Recently, Ma et al[11] found that the Brake fern (*Pteris vitatta*) hyperaccumulates arsenic, with concentration ratios as high as 200. They theorized that the presence of arbuscular mycorrhizal fungi contributed to this ability. Arbuscular mycorrhizal fungi grow within plant roots and into the soil and greatly increase the ability of the plant to extract soil nutrients and solutes.

Greenhouse study – uptake of Th and U by the netted chain fern and Bermuda grass

For the greenhouse study, two soils (A and B) were collected from contaminated areas and one was collected from a nearby uncontaminated area (i.e., background soil). Selected properties of the three tested soils are presented in Table 2. Thorium concentrations were high in both contaminated soils (201 and 329 mg/kg, respectively in soil A and B) and low in the background area (mean of 8.9 mg/kg, Table 2).

The TNX Facility utilized primarily depleted uranium; i.e., U in which ^{235}U was removed, leaving ^{238}U in relatively high abundance. The mean ^{238}U concentration in the contaminated area was almost 450 times greater than in the non-contaminated area. The U concentrations in the non-contaminated soils were similar to the average U concentration reported in non-contaminated soils world wide (1.3 mg/kg)[12].

Mobility is the capacity for elements to move from contaminated materials to any compartment of the soil or groundwater, and bioavailability is the fraction of a contaminant that can be taken up by plants. Both processes play an important role in phytoremediation. The bioavailability of Th and U, as estimated by the concentrations of these elements in DTPA extracts, was low for Th and especially U (10.5 and 0.17 mg/kg, respectively, Figure 2). Kaplan and Serkiz[13] reported similarly that Th in TNX soil associated mostly with the organic and the amorphous Fe-oxide fractions, and only 1% of the total Th was found in the exchangeable form.

In the greenhouse study, U concentrations in netted-chain fern increased from 0.112 mg/kg in the control treatments to 1.36 and 5.25, respectively in soils B and A over six weeks (Figure 3). Uranium is not considered essential for plant growth[12]. Plant uptake is limited to mobile U species and is affected by soil pH. Typical concentration factors are usually from 10^{-2} to 10^{4}[4,12]. Recently, there have been reports on the high bioavailability of U to agricultural plants. Some legumes from the *Brassicaceae* family accumulate more U than grasses, and sunflower, and leafy vegetables accumulate more U than other plants[2,14]. Mobilizing agents that increase U uptake by plants are chelating agents and inorganic and organic acids[15]. For example, Huang et al[15] observed that organic acids increased U accumulation in *Brassica juncea* shoots more than 1000-fold.

Thorium concentrations in netted chain fern increased from 0.273 mg/kg (control treatment) to 6.4 and 9.1 mg/kg, over six weeks in the treatments with soil A and B, respectively (Figure 3). The Th concentration in Bermuda grass in soil A remained the same as in the control treatment and increased to 0.95 mg/kg in soil B (Figure 3). Thorium is not considered essential for plant growth[12]. Its mobility may be restricted in plants by adsorption on cell wall material[16].

Thorium and U CR values for netted chain fern grown in soil B in the greenhouse study were 80 and 270 times higher, respectively, than the geometric mean CR values presented by Sheppared and Evenden[17] for Th and U. Their review of about 100 CRs for U and Th showed an overall geometric mean CR of 0.0045 for U and 0.0036 for Th[17]. Similarly, Zararsiz et al[16] reported that Th plant concentrations were typically several orders of magnitude lower than soil concentrations (e.g., CR values for annual grass species range from 0.001-0.05). The much higher values that we observed indicate that U and Th uptake at the TNX wetland is greater than expected.

CONCLUSIONS

Thorium and U concentrations in fern were at least an order of magnitude greater than in Bermuda grass. The netted chain fern may have potential for phytoremediation of Th and U, two contaminants that strongly bind to wetland soils. The use of an indigenous species to passively remediate the ecologically sensitive wetland environment may provide a viable alternative to less environmentally-friendly remediation approaches.

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LIST OF FIGURES

Table 1. Selected element concentrations and concentration ratio (CR) values for some plants from the field screening study.

Plant species	Co		Cr		Hg		Pb		U		Th	
	mg/kg	CR	mg/kg	CR	mg/kg	CR	mg/kg	CR	mg/kg	CR	mg/kg	CR
Netted chain fern	2.4	0.65	4.7	0.11	0.8	0.12	0.7	0.17	20.7	0.11	21.5	0.107
Switchcane	0.8	0.10	1.7	0.04	BDL	---	0.6	0.15	0.6	0.003	0.8	0.004
Red maple	0.3	0.08	1.0	0.02	0.1	0.01	0.3	0.07	0.3	0.002	0.3	0.002
Bald-cypress	0.4	0.11	0.8	0.02	0.0	0.00	0.2	0.05	0.2	0.001	0.3	0.002
Sweetgum	0.5	0.14	1.0	0.02	0.1	0.01	0.2	0.05	4.0	0.021	0.3	0.002

B.D.L. = below detection limit, which is ~0.01 mg/L Hg.

Table 2. Characterization of three tested soils

Parameter/ Element	Unit	Uncontaminated Soil	Contaminated Soil A	Contaminated Soil B
pH		4.16 (0.01)	4.00 (0.08)	4.53 (0.04)
Org. C	mg/kg	1395	1493	1427
Sand	%, wt	79.4 (2.10	48.8 (6.8)	82.0 (3.2)
Silt	%, wt	13.6 (0.3)	23.6 (1.6)	13.0 (1.5)
Clay	%, wt	6.3 (0.8)	6.4 (1.1)	5.0 (0.6)
CEC*	cmol (+)/kg	4.8 (0.08)	9.0 (0.09)	7.3 (0.14)
AEC**	cmol (-)/kg	1.6 (0.17)	2.4 (0.05)	1.1 (0.06)
Al	mg/kg	1915	6252	2620
Ba	mg/kg	22	78.7	147.7
Cd	mg/kg	<0.0002	0.3	1.4
Cr	mg/kg	2.8	44.6	33.4
Co	mg/kg	1.2	3.7	0.9
Cu	mg/kg	30	88.2	na
Fe	mg/kg	16470	7533	19954
Hg	mg/kg	0.08	6.8	3.7
Mn	mg/kg	127	114.4	36
Ni	mg/kg	1.9	18.8	na
Pb	mg/kg	4.5	17.6	10.9
Th	mg/kg	8.9	201	329
U	mg/kg	0.4	188.9	44

*Cation exchange capacity
**Anion exchange capacity

Figure 1

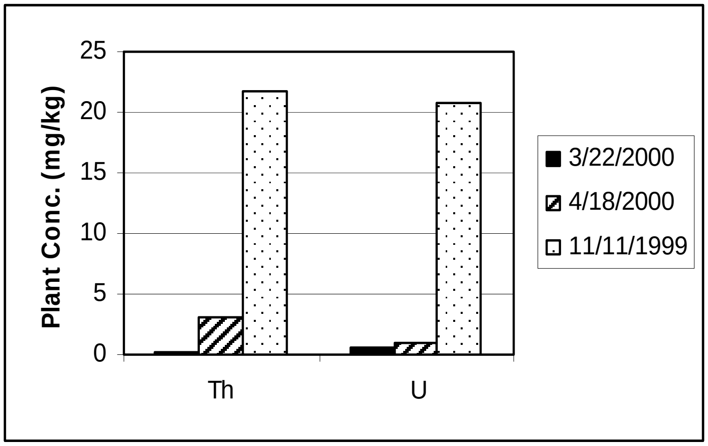


Figure 2

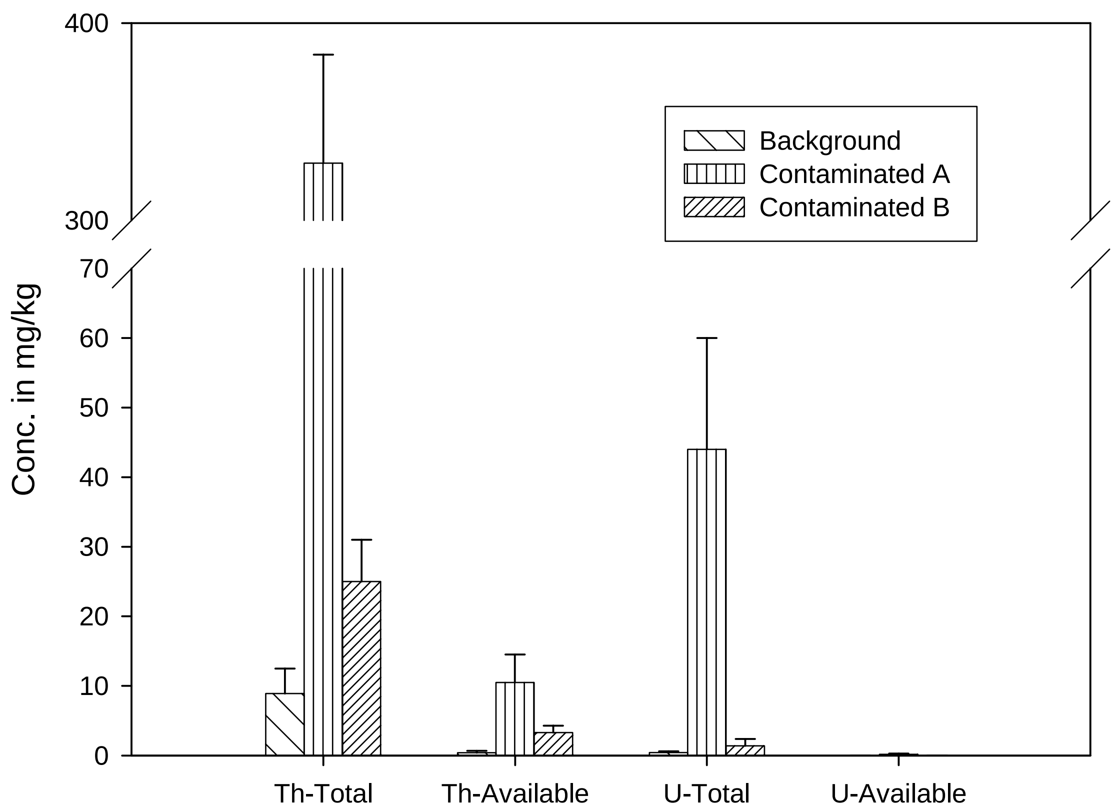
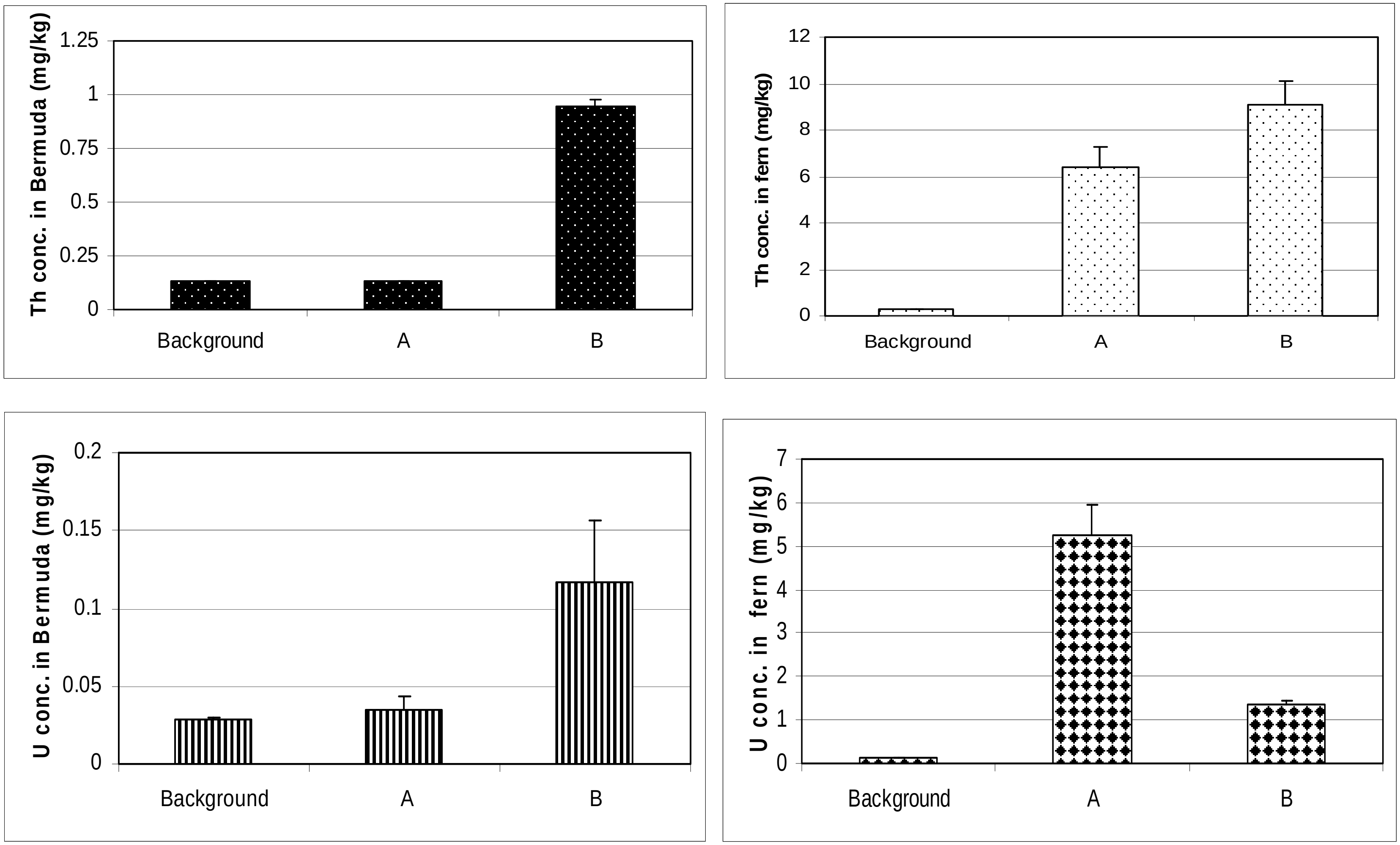

400
300
70
60
50
40
30
20
10
0
Conc. in mg/kg
Background
Contaminated A
Contaminated B
Th-Total
Th-Available
U-Total
U-Available

Figure 3


Th conc. in Bermuda (mg/kg)
1.25
1
0.75
0.5
0.25
0
Background
A
B
Th conc. in fern (mg/kg)
12
10
8
6
4
2
0
Background
A
B
U conc. in Bermuda (mg/kg)
0.2
0.15
0.1
0.05
0
Background
A
B
U conc. in fern (mg/kg)
7
6
5
4
3
2
1
0
Background
A
B