Arch. Environ. Contam. Toxicol. 44, 30–35 (2003)
DOI: 10.1007/s00244-002-1213-4

ARCHIVES OF
Environmental
Contamination
and Toxicology

Bioavailability and Trophic Transfer of Sediment-Bound Ni and U in a Southeastern Wetland System

T. Punshon,[1,2] K. F. Gaines,[1] R. A. Jenkins, Jr.[3]

[1] University of Georgia, Savannah River Ecology Laboratory, Drawer E, Aiken, South Carolina 29802, USA
[2] Consortium for Risk Evaluation with Stakeholder Participation, Environmental and Occupational Health Sciences Institute, Division of Life Sciences, Rutgers University, 604 Allison Road, Piscataway, New Jersey 08854, USA
[3] Department of Entomology, Clemson University, Clemson, South Carolina 29634, USA

Received:18 October 2001/Accepted:12 June 2002

Abstract. Elemental composition of soil, herbaceous and woody plant species, and the muscle and liver tissue of two common small mammal species were determined in a wetland ecosystem contaminated with Ni and U from nuclear target processing activities at the Savannah River Site, Aiken, SC. Species studied were black willow (*Salix nigra* L.), rushes (*Juncus effusus* L.), marsh rice rat (*Oryzomys palustris*), and cotton rat (*Sigmodon hispidus*). Two mature trees were sampled around the perimeter of the former *de facto* settling basin, and transect lines sampling rushes and trapping small mammals were laid across the wetland area, close to a wooden spillway that previously enclosed the pond. Ni and U concentrations were elevated to contaminant levels; with a total concentration of 1,065 (± 54) mg kg^{-1} U and 526.7 (±18.3) mg kg^{-1} Ni within the soil. Transfer of contaminants into woody and herbaceous plant tissues was higher for Ni than for U, which appeared to remain bound to the outside of root tissues, with very little (0.03 ± 0.001 mg kg^{-1}) U detectable within the leaf tissues. This indicated a lower bioavailability of U than the cocontaminant Ni. Trees sampled from the drier margins of the pond area contained more Ni within their leaf tissues than the rushes sampled from the wetter floodplain area, with leaf tissues concentrations of Ni of approximately 75.5 (± 3.6) mg kg^{-1} Ni. Ni concentrations were also elevated in small mammal tissues. Transfer factors of contaminants indicated that U bioavailability is negligable in this wetland ecosystem.

The widespread contamination of soils and sediments with heavy metals at Department of Energy (DOE) facilities is a costly legacy of the U.S. defense mission. Priorities for restoration and management activities must be established that balance human and ecological risks. This can be accomplished using well-defined ecological endpoints that reflect contaminant impact to the entire ecosystem. When choosing an endpoint, the natural sources of variation in the ecosystem must be understood so they may be separated from stressor effects. This is very difficult to do in a laboratory setting. The potential for contaminant bioaccumulation and trophic transfer in the food web of an ecosystem must be established to understand the role of endpoints in the quantification of environmental risk. This work is an interdisciplinary investigation that examines the bioaccumulation of metals into the terrestrial food web in a riparian/wetland system impacted by four decades of uranium and metal discharges. Wetland ecosystems are effective at removing a wide range of inorganic contaminants from water, by converting them into insoluble forms (Sobolewski 1999). This amelioration arises at least in part from the characteristically low depth and large inputs of organic matter characteristic of wetland systems, which promotes biogeochemical metal removal processes. An examination of the bioavailability of metal contaminants in contaminated wetland systems can confirm the efficacy of this amelioration.

Vegetation is the gateway for contaminants to enter the food chain by either direct consumption or secondary consumption by organisms higher in the food web. Innate differences in the metal uptake and accumulation characteristics of different plant species and ecotypes (Baker 1981) are important determinants of bioavailability. Small mammals are a model group for examining contaminant uptake due to their high fecundity and omnivorous diets. Furthermore, their potential to accumulate contaminants and thus be vectors of contaminant transport to different groups of organisms makes them an integral part of quantifying the complete exposure pathway to species occupying higher trophic levels. Hunter *et al.* (1987a,1987b,1987c) quantified trophic transfer of Cu and Cd to small mammals in a terrestrial environment and showed that these metals were highly mobile through the soil-plant-invertebrate–small mammal pathway. Furthermore, this study showed that metal accumulation differed between organisms based on diet, with insectivores often accumulating more metals than herbivores. Although this study was very thorough, it was limited to the ecotoxicology of Cu and Cd in a grassland ecosystem. To date, there is a paucity of research that takes trophic transfer and bioavailability of metals into consideration in terrestrial ecosystems; the majority of studies are still carried out on marine and coastal environments. A study carried out by Torres

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Correspondence to: T. Punshon; *email:* punshon@srel.edu

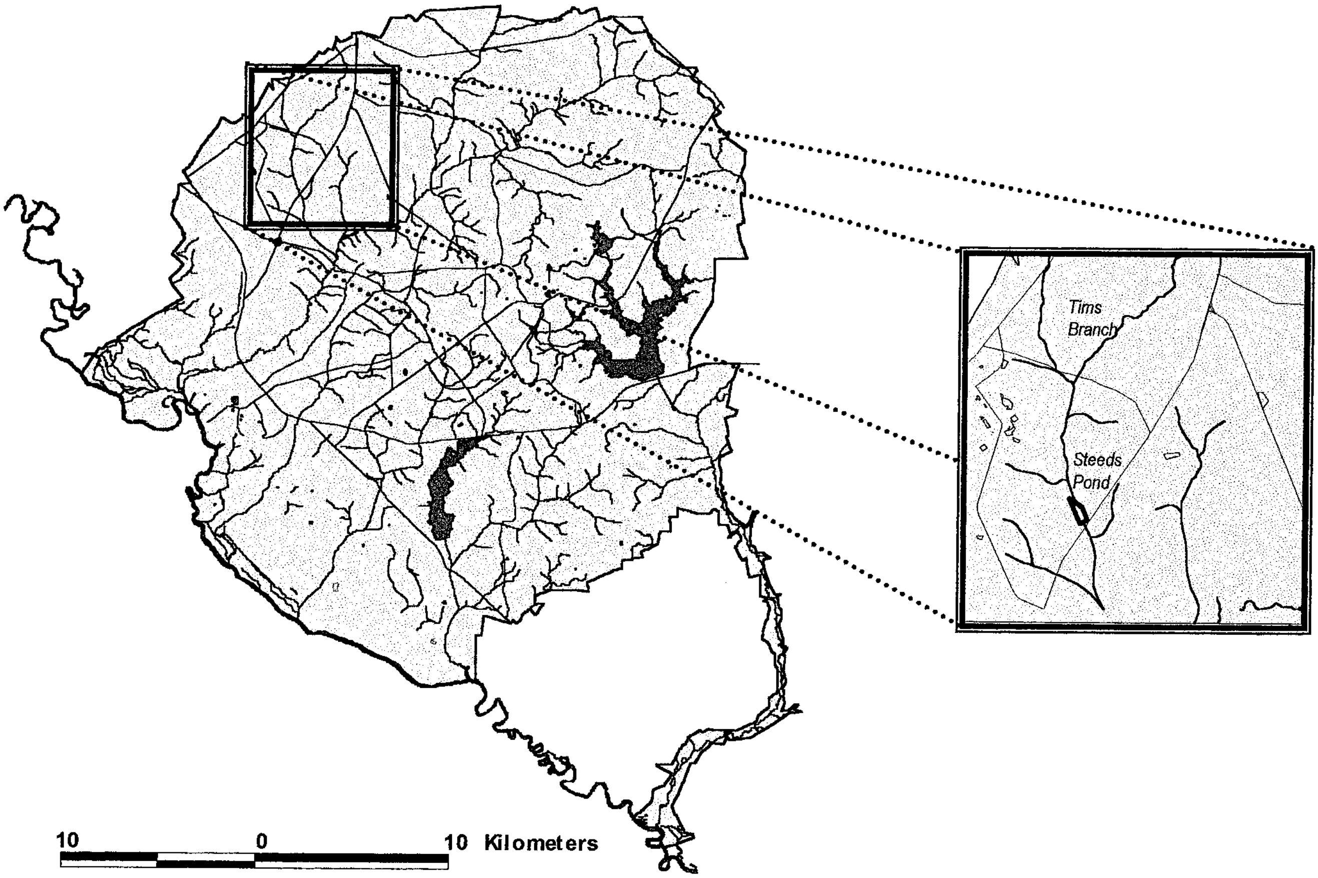

Fig. 1. Location of Steed Pond within the Savannah River Site (SRS), near Aiken, South Carolina

and Johnson (2001) measured Ni concentrations, as well as other metals in arthropods and mice at a seasonal wetland, finding a strong correlation between the concentration of Ni in the *Scirpus robustus* (alkali bulrush) with those of ambient soils. In this study there was no significant relationship noted between Ni within *Mus musculus* (house mouse) and the concentration within the wetland soils.

The objective of this study was to quantify metal concentrations within the main trophic compartments and thus determine if Ni and U were bioavailable to plants and small mammals inhabiting this riparian/wetland ecosystem.

Materials and Methods

Study Site

The Steed Pond–Tims Branch depositional system is located in the northwest of the Savannah River Site (SRS), a 777 km^2 DOE reservation situated in the upper coastal plain of South Carolina, near Aiken (SC) (Pickett 1990) (Figure 1). Steed Pond is an abandoned farm pond that served as a *de facto* settling basin for contaminated sediments resulting from target processing facilities between the mid-1950s and 1985 (Evans *et al.* 1992). The pond was originally 5.7 ha, but was reduced to 4.5 ha after partial failure and repair of the enclosing dam in the 1960s. Contamination accumulated within the pond sediments as a result of fuel and target processing activities—predominantly U and Ni—were subsequently left exposed in the wetland environment. Vegetation quickly colonized the area, stabilizing much of the area from erosion, with the exception of several unvegetated areas.

It is estimated that approximately 44,000 kg depleted U were released into Steed Pond (Pickett 1990). Ninety-seven percent of the gross α-activity released by the SRS was to the Steed Pond–Tim's Branch stream system, with 61% of this activity released between 1966 and 1968 (Evans *et al.* 1992). Up until 1979, effluent discharge went to a drainage ditch that flowed into Tims Branch and then into Steed Pond. Although U deposition occurred in shallow beaver ponds upstream of Steed Pond, the depth, surface area, and longer retention time provided by Steed Pond allowed time for settling of suspended sediments. Following the breach of the wooden spillway in 1984, Steed Pond released sediment-bound contaminants into the Tims Branch depositional environment and continues to do so during episodic storm events (Batson *et al.* 1996). Site topography ranges from 3% slopes at the facility discharge point down to 0.4% at the entry to the former Steed Pond area, and as a result there was little or no accumulation of contaminants in the streambed.

Sample Collection

Direct determination of total contaminant concentrations in sediments collected from the floodplain was determined by ICP-MS analysis following microwave digestion in HF–aqua regia. Metals investigated were Al, As, Ba, Cr, Cu, Cd, Hg, Ni, Pb, Se, Zn, and U. Sediment pH was determined on air-dried samples in Milli-Q water using a 2:1 solution to sediment ratio.

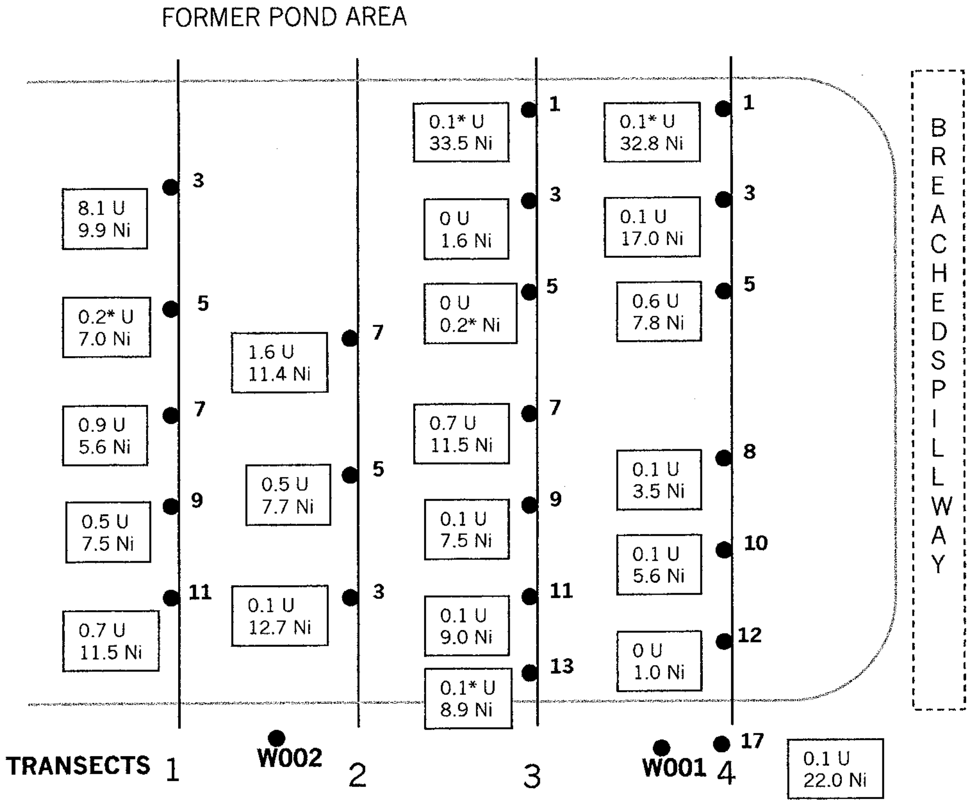

Fig. 2. Schematic diagram of the Steed Pond former settling pond area, showing the four transect lines and locations along each transect from which *Juncus effusus* vegetation samples were collected. Numbered points correspond to the small mammal trap numbers, which were placed every 10 m along each transect. Boxed numbers refer to the U and Ni concentrations found in plant tissues expressed as mg kg^{-1} DW and are means of triplicate samples (* indicates SD $>$ 15% mean). W001 and W002 are the locations of mature *Salix nigra* trees also sampled

Dominant vegetation from the Steed Pond floodplain area was sampled between June and August 1999, consisting of *Salix nigra* L. (black willow) at the margins of the floodplain close to the breached wooden spillway, and in transects of *Juncus effusus* (common rush) across the floodplain area. Due to classification of the floodplain as a soil contamination area, vegetation samples from it were restricted to leaf material only. Locations of trees sampled and the reed transects are shown schematically in Figure 2. Samples were collected in bulk, stored on ice during transportation, and dried to a constant weight (60°C, air assisted). Prior to drying, all root material was washed thoroughly in distilled water to remove extraneous soil material. Plant tissue was homogenized and ground to a fine power in a sample grinder, and digested in 5 M HNO_3 + 30% H_2O_2 using a microwave dissolution technique in pure Teflon PFA vessels (CEM Corporation MDS-2000).

Adult *Sigmodon hispidus* ($>$ 80 g; cotton rat) and *Oryzomys palustris* ($>$ 50 g; marsh rice rat) were collected by live-trapping from 30 June–15 August 1999. Four trap lines were placed across the length of Steed Pond, with each trap being placed 10 m apart along the same transect lines as the *J. effusus* samples (Figure 2). Trapping activities gave sample sizes of 19 *O. palustris* and 21 *S. hispidus* adult individuals. Individuals were sacrificed within 24 h of capture, and the liver and muscle tissue were removed and frozen until metals analysis. Samples were analyzed in triplicate by ICP-MS using a Perkin Elmer ELAN 6100 DRC in standard operating mode. Samples were analyzed using an external calibration and by the method of standard additions.

Rat tissues were freeze-dried prior to microwave digestion. Approximately 25 mg of homogenized sample was placed in a Teflon microwave digestion vessel to which 5 ml distilled HNO_3 was added. The vessel was capped and microwave-digested using a variable powered program with increasing microwave power applied over 1 h. After cooling, the vessels were uncapped and 1 ml H_2O_2 was added; the vessels were then recapped and subject to an identical microwave heating procedure. After the vessels had cooled, the digest was brought to a final volume of 25 ml using volumetric flasks. Two duplicate samples, one blank, and two SRMs were included per digestion set. SRMs used for soil, plant, and animal tissue were NIST-SRM 2709 (San Joaquin soil), 1515 (apple leaves), and DORM-2 and DOLT-2 (NRC-CNRC, Ottowa, Canada), respectively. Analysis data with less that 95% SRM recovery were rejected. The digested tissue samples were analyzed for Al, Cr, Ni, Cu, Zn, Cd, Ba, Hg, and Pb following the methodology outlined in EPA method 6020. Quality control procedures were based on EPA SW-846. Where possible, material collected from Steed Pond was compared to an off-site, uncontaminated reference site, outside the influence of radiological discharges.

Student's *t*-tests were used on metal concentration data to determine significant differences in metal concentrations between *S. hispidus* and *O. palustris.* All statistical tests were considered significant at $p \leq 0.05$.

Results

Total Concentration of Contaminants of Concern

Elemental analysis of soil, root and leaf tissue of *S. nigra,* and leaf tissue from *J. effusus* from the floodplain area at Steed Pond confirm the presence of U and Ni within soils and the

Table 1. Concentration of contaminants of concern in soil and apportioned *Salix nigra* leaf and root material

	Uranium (mg kg^{-1})	Nickel
Soil	1,065 (54)	526.7 (18.3)
S. nigra roots	139 (4.2)	156 (7.6)
S. nigra leaf tissue	0.03 (0.002)	75.5 (3.6)
SRS reference soil	8.13 (0.2)	14.8 (0.2)
SRS reference leaf	< BDL	4.7 (5.8)

Data are means ± SD (n = 5).
< BDL: Below detection limits.

Table 2. Metal concentration (mg kg^{-1}) in liver and muscle tissues of *Oryzomys palustris* and *Sigmodon hispidus* collected from the contaminated Steed Pond wetland habitat.

	Oryzomys palustris		Sigmodon hispidus	
	Liver	Muscle	Liver	Muscle
Ni	1.89 (1.86)	2.11 (2.43)	1.84 (1.31)	2.60 (2.52)
	0.2–8.1	*0.2–10.4*	*0.1–4.5*	*0.2–10.4*
	1.19 (0.32)[a]		0.56 (0.15)[a]	
U	0.03 (0.02)	0.04 (0.07)	0.02 (0.01)	0.4 (0.4)
	0.004–0.08	*0.006–0.32*	*−0.0006–0.05*	*−0.0003–0.1*

Data show means (± SD) and range (in italics), where n = 19 and 21, respectively.
[a] Background data from Peles and Barrett (1997).

presence of contaminants of concern for the site within plant tissues (Table 1). All other metals analyzed showed no enrichment over background levels and are therefore not shown. Concentrations of U within root tissues of *S. nigra* were much higher than leaf tissues: 139.5 (± 4.2) and 0.03 (± 0.002) mg U kg^{-1} respectively.

Collation of transect-orientated data from the floodplain itself shows elevation of Ni within *J. effusus* (Figure 2), with low U concentrations, similar to the *S. nigra* samples (Table 1). Due to the abundance of *J. effusus* on the floodplain itself, and the lower number of mature *S. nigra* trees, relatively more tissue concentration data was collected.

Small Mammal Metal Concentrations

Small mammal metal concentrations are presented in Table 2. *t*-test results showed that muscle and liver metal concentration were not significantly different between *S. hispidus* and *O. palustris* for the majority of the metals analyzed. For U, liver metal concentration was significantly higher in *O. palustris* than in *S. hispidus* ($p = 0.002$). *t*-tests established no significant differences in the concentration of Ni in liver tissue. ($p = 0.45$) or muscle tissue ($p = 0.27$) between the two rodent species, although liver data collected from Steed Pond were higher than published values for the same rodent species collected from a reference uncontaminated site at Ellenton Bay on the SRS (Peles and Barrett 1997). *t*-tests also showed no difference in U concentration of muscle between species ($p = 0.43$).

Table 3. Calculated transfer factors in leaf tissue of *Juncus* spp. (Herbs), *S. nigra* (Trees), and muscle tissue of small mammal species, *O. palustris* (*O.P*) and *S. hispidus* (*S.H*) as a function of the total extractable concentration of contaminant in Steed Pond soil

Metal	Leaf Tissue		Muscle Tissue	
	Herbs	Trees	*O.P.*	*S.H.*
Ni	0.01796	0.14488	0.00400	0.00493
U	0.00055	0.00009	0.00003	0.00003

Discussion

Analysis of Steed Pond sediments showed significant enrichment of soils with U and Ni (Table 1). Average total concentrations of Ni and U within sediments on the SRS are 10 and 2 mg kg^{-1}, respectively (Pickett 1990), and concentrations of Ni and U immediately outside the influence of target processing wastes were 14 and 8 mg kg^{-1}, respectively. The concentration of Ni within the leaf tissues of *S. nigra* are also above published background concentrations of approximately 0.05–5.0 ppm (Adriano 2001) by a factor of approximately eight. Because Ni is a contaminant of concern, this confirms significant entry of this contaminant into the primary-producer compartment of the wetland food web. Furthermore, the low concentrations of plant tissue–associated U compared to the total concentration of the soil and sediments suggest that it is currently predominantly in a nonavailable form. Although root tissues were thoroughly washed prior to analysis, there were high concentrations of U found associated with these tissues; it is likely a more aggressive desorption step (*e.g.*, using $PbNO_3$) may be required for a complete removal of the U bound to exterior surfaces. Considering the low U content in leaf tissues, it is likely that U does not enter the symplasm.

Nickel levels in liver tissues for both *O. palustris* and *S. hispidus* were higher than those levels found in liver from animals collected from areas on the SRS not directly impacted from activities associated with releases of those trace elements (Peles and Barrett 1997), although U was not analyzed in that study. Small mammals are an important intermediate for the transfer of toxic metals to higher trophic levels (Laurinolli and Bendell-Young 1996). This is especially important because the two species used in this study have different foraging strategies, which maximizes the potential to show metal bioaccumulation and subsequent trophic transfer in this system. Furthermore, both small mammal species were particularly good indicators of trophic transfer within Steed Pond due to the confinement of their home ranges, provided by the dense stand of conifers surrounding the pond. Marsh rice rats and cotton rats typically avoid heavily forested habitats and prefer instead to feed within the marshy areas provided by Steed Pond. *S. hispidus* is a more terrestrial omnivore that favors herbaceous food items, whereas *O. palustris* prefers a more aquatic environment and will often feed on invertebrates on the SRS (Cotheran *et al.* 1991).

To quantify the magnitude of contaminant transfer between the source of the contamination and the receptors, transfer factors (TFs) are usually employed. Although a relatively crude indicator of bioavailability and valuable only in a broad context, they allow comparison between contaminants within a specific ecosystem, and can allow estimation of risk. Table 3

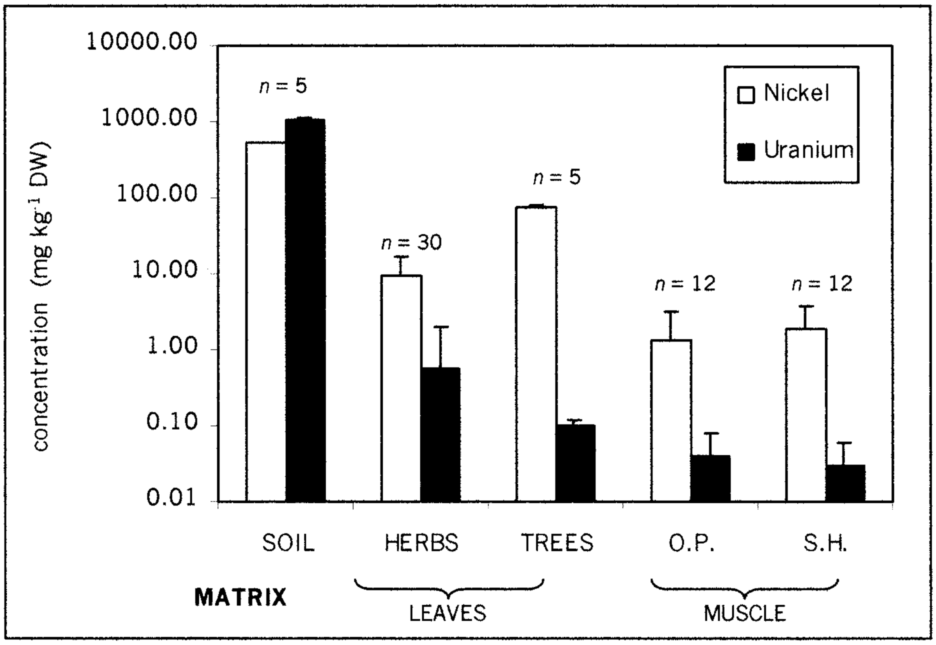

Fig. 3. Concentration of U and Ni within soil, *S. nigra* (TREES) and *J. effusus* (HERBS) leaf tissues, and muscle tissue of *O. palustris* and *S. hispidus.* Data are means ± SD; n is indicated on each column

shows the TFs calculated as a function of the total extractable concentration of contaminants from the soil at Steed Pond and showed that Ni was far more bioavailable than U. TFs indicate that the most significant route of Ni transfer in the species studied was between the soil and the leaf tissues of the *S. nigra* trees occurring at the edge of the floodplain area. *Salix* plays a significant ecological role within wetland ecosystems due to the large number of invertebrate and wildlife species they typically support (Sommerville 1992). Therefore, Ni enrichment of a primary producer that supports such a large diversity and abundance of biota becomes a significant vector for further transfer.

It is generally accepted that U is more bioavailable to biota from a soil matrix in oxidizing conditions as U (VI), where it exists as the uranyl dioxo cation (UO_2^{2+}) (Bender *et al.* 2000). In the typically reducing sediments of wetland ecosystems, however, the sparingly soluble U (IV) predominates, and in addition to high organic matter associated with wetlands, U may effectively be trapped as long as it retained under these conditions. Ni was bioavailable and accumulated in both *S. hispidus* and *O. palustris* muscle and liver tissues, whereas U was not. The relative trophic movement of Ni and U from soil to small mammals is summarized in Figure 3, showing the steep reduction in U concentrations in U compared to the relatively shallow drop in Ni concentrations. This amounts to a reduction in U concentration by six orders of magnitude between the soil and small mammals living in the former pond area and a drop of four orders of magnitude for Ni.

Ni is carcinogenic to both humans and animals (Hartwig 1995). Furthermore, Ni (II) has been shown to preferentially damage distinct chromosome regions and has been shown to interfere with the repair of UV- and X-ray-induced DNA damage through the damage recognition/incision and ligation stages. Although U was shown not to be bioavailable, its daughter gamma products— ^{214}Bi, ^{226}Ra, and ^{214}Pb (Gilmore and Hemmingway 1995) (which can cause DNA damage), are present in the Steeds Pond–Tim's Branch ecosystem above background levels. Simply measuring the bioavailability of contaminants should not be the final determination of ecological risk. From these measurements, one could conclude that U does not pose an ecological risk to the environment. However, the secondary by-products of U in the presence of Ni may very well pose further risks. Only through examination of ecological endpoints, such as DNA strand-breakage, will give a more realistic understanding of risk from complex mixtures of contaminants than measurement of their concentration and solubility alone.

Acknowledgments. This research was supported by Financial Assistance Award Number DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation.

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