January 2003

Bioavailability of uranium and nickel to vegetation in a contaminated riparian ecosystem

Tracy Punshon
University of Georgia

Karen F. Gaines
Eastern Illinois University, kfgaines@eiu.edu

Paul M. Bertsch
University of Georgia

Joanna Burger
Rutgers University

Recommended Citation

Punshon, Tracy; Gaines, Karen F.; Bertsch, Paul M.; and Burger, Joanna, "Bioavailability of uranium and nickel to vegetation in a contaminated riparian ecosystem" (2003). *Faculty Research & Creative Activity*. 57.
http://thekeep.eiu.edu/bio_fac/57

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 22, No. 5, pp. 1146–1154, 2003
© 2003 SETAC
Printed in the USA
0730-7268/03 $12.00 + .00

BIOAVAILABILITY OF URANIUM AND NICKEL TO VEGETATION IN A CONTAMINATED RIPARIAN ECOSYSTEM

TRACY PUNSHON,*†‡ KAREN F. GAINES,‡ PAUL M. BERTSCH,‡ and JOANNA BURGER†
†Environmental and Occupational Health Sciences Institute, Division of Life Sciences, Rutgers University, 604 Allison Road, Piscataway, New Jersey 08854, USA
‡University of Georgia Savannah River Ecology Laboratory, Drawer E, Aiken, South Carolina 29802, USA

(*Received* 3 *June* 2002; *Accepted* 31 *October* 2002)

Abstract—The lower portion of Tims Branch (TB), a second-order stream system on the Savannah River site (SC, USA), receives influx of mixed waste-contaminated sediments from Steed Pond, a former settling basin for target processing wastes for over three decades. The magnitude and distribution of U, Ni, and other metals and the potential for trophic movement were studied to facilitate risk assessment and determine potential remedial action. Total and sequential extraction of TB soils demonstrated contaminant heterogeneity both spatially and between operationally defined fractions. Metal concentrations were elevated within riparian zone soils in contrast to stream sediments, suggesting off-site transport. Leaf tissue from TB contained an order of magnitude more Ni than tissue from reference sites. Leaves from streamside trees contained no U but elevated Ni up to 75.4 (±25) mg/kg dry weight (dry wt). Understory flora (*Discanthelium* sp. and *Andropogon* sp.) contained high concentrations of U associated with leaves up to 518 (±7.5) mg/kg dry weight U. The contrast in contaminant content and ratio of streamside and understory vegetation may result from resuspension of particulate U and Ni onto leaf surfaces and represents a potential pathway for trophic movement. The findings of this study have important ramifications for remediation of the ecosystem, suggesting that a strategy based on contaminant immobilization may be the most appropriate.

Keywords—Bioavailability Trophic transfer Uranium Nickel Transport

INTRODUCTION

The majority of studies dealing with trophic transfer and bioaccumulation are performed and modeled on aquatic ecosystems [1], and comparatively fewer studies are available on terrestrial ecosystems. A need exists for additional site-specific studies of inorganic contaminant transfer in terrestrial ecosystems to support the development of accurate bioaccumulation models. The remediation and restoration of contaminated land and its return to an ecologically functional system requires a detailed understanding of how contaminants behave within a particular ecosystem before remedial activities are carried out. In addition, determining the efficacy of remediation and restoration activities requires baseline data from similar impacted and unimpacted ecosystems. Less attention has been devoted to the role of vegetation in trophic transfer, particularly for metals and mixed wastes in terrestrial systems, and many accepted models contain serious omissions [2]. The relationship between the concentration of inorganic contaminants in the soil and plants growing within it is complex [3]. That is, some species may accumulate extremely high metal concentrations, while others do not [4]. The transfer of inorganic contaminants from soil to vegetation is considered a significant step because plants represent the primary producers within an ecosystem and can give an early indication of potential food chain transfer risks to both wildlife and humans.

This study examines the role of vegetation in the movement of Ni and U in a riparian-wetland ecosystem on the Department of Energy's (DOE) Savannah River site (SRS), a former nuclear materials products facility near Aiken (SC, USA). The SRS currently has the second-largest volume of contaminated media among all sites within the DOE's stewardship [5]. This particular former waste unit is one of the most important contaminated sites within the SRS on which a risk assessment has not been carried out. The DOE owns tracts of land containing contaminated units in 34 states, representing one of the largest remediation and restoration challenges faced by the United States [5–7]. An understanding of which sites require remediation and the form these activities should take is crucial if this task is ever to be realistically approached. This study forms the basis of an ongoing multidisciplinary study that aims to improve understanding of bioavailability and trophic transfer of metals and radionuclides in a terrestrial system in order to aid the cost-effective risk assessment and remediation efforts of the DOE.

MATERIALS AND METHODS

Study site

The Steed Pond–Tims Branch (SP-TB) system is located in the northwest corner of the U.S. Department of Energy's SRS (Fig. 1A). The SRS is a 777-km² former nuclear weapons production facility near Aiken. The site began the manufacture of aluminum-clad U targets in the M area in 1954, and the discharge of process wastewater from these facilities led to extensive contamination of a nearby riparian ecosystem and underlying groundwater [8,9]. Large quantities of depleted and natural uranium, nickel, and aluminum entered the system as well as lesser amounts of copper, zinc, lead, and chromium. Associated wastes such as nitric, phosphoric, and sulfuric acids and sodium hydroxide were also discharged [10]. Waste materials such as aluminum-forming and metal-plating wastes were discharged primarily into TB, a second-order stream system draining a 16-km² watershed area. Effluent discharge di-

---

* To whom correspondence may be addressed (punshon@srel.edu).

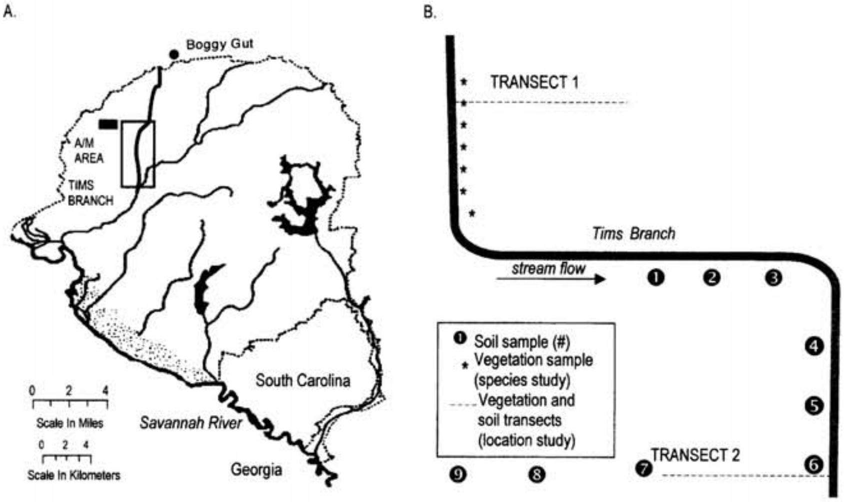

Fig. 1. The Savannah River Site (Aiken, SC, USA) and the location of the Tims Branch (SC, USA) riparian ecosystem (**A**), including a schematic of the study area (**B**) showing the location of separate soil and plant sampling points and two soil and plant transects from which final transfer factor data were derived.

rectly into TB ceased in 1982, and in 1989 the M-area settling basin was removed from service, stabilized, and capped [11]. Uranium contamination is the biggest regulatory concern in the SP-TB system, with an estimated U inventory estimated at about 43,500 kg. This constitutes 97% of the total gross alpha activity released by the SRS, the majority of which occurred between 1966 and 1968 [9]. Stream morphology had a strong impact on the deposition of metals and metal-laden sediments [9]; together with extensive erosion during peak discharges, contamination of the streambed nearest the initial point of discharge was limited, resulting from slopes as high as 3%, which then decrease abruptly at the main stream channel. Instead, deposition occurred primarily in natural and manmade impoundments, such as beaver ponds, wetlands, and former farm ponds (such as SP), that essentially functioned as settling basins. It is estimated that maintenance of SP as a wetland ecosystem effectively retained up to 70% of the radionuclide releases within the sediments [8,9]. Estimates from the M-area settling basin suggest that releases of Ni and U were similar in magnitude [12], although ongoing trophic transfer studies at SP indicate a shift in contaminant ratios, with a considerable loss of Ni from the system relative to U [13], which indicates higher Ni transport and bioavailability.

In 1984 the wooden spillway enclosing SP ruptured, allowing export of U-enriched sediments downstream through a series of erosion events and a drop in water level. It remains an unconfined wetland system prone to periodic erosion. Export of contaminated sediments from SP is approximately 1,500 to 2,800% of base flow during peak flow consistent with periodic storm events [14]. Detailed studies on SP have shown selective erosion of localized, unvegetated areas: hot spots characterized by elevated concentrations of metals and radionuclides, which precluded plant establishment in some areas following dam failure [14]. Additionally, sediment texture analysis has shown that it is the easily dispersible clay fraction of these southeastern coastal plain sediments that contain the highest concentration of metal and radionuclide contaminants [15,16]. This soil fraction is easily dislodged by raindrop impact and disturbance by animal activity [14].

Sediment sampling

Grab samples from surface sediments (0–15 cm) of the stream at TB were collected at 10-m intervals extending throughout the study area for a total of 23 sediment sampling locations with three replicate samples at each point. Samples were collected in acid-washed Nalgene® bottles (Rochester, NY, USA), packed on ice, and transported back to the laboratory. Samples were dried (105°C) for 48 h, and 0.3-g sediments were digested in triplicate with 10 ml 48% HF and HNO_3 via microwave digestion in Teflon® PFA vessels.

Soil sampling

Soil samples were collected from varying locations within the TB study area, using gamma activity readings of protactinium (^{234}Pa), a decay daughter of ^{238}U, to locate radiologically contaminated areas of varying magnitude (Model 12 count rate meter, series 10, Ludlum Measurements, Sweetwater, TX, USA) (Fig. 1B). Samples were collected from the upper 30 cm of soil using a Wildco stainless-steel soil corer (Wildco Instruments, Saginaw, MI, USA). Exact distance from the stream at TB was recorded for each sampling point. A second set of surface soil samples (0–30 cm) was collected from two transect lines extending 13.5 m from the stream, with samples taken at 1.5-m intervals to correspond to locations of vegetation collection. Transect 1 was located within an area of the site having lower gamma activity readings (Fig. 1B), whereas transect 2 was located in an area having higher gamma activity (soil sample TB6). Uncontaminated reference soils were collected from Boggy Gut (BG), a riparian zone, also formed from a former farm pond north of SP above the A/M area (Fig. 1A). The soils were sealed in Ziploc® bags (Dow, Midland, MI, USA) and stored on ice for transportation back to the laboratory. An additional reference site on TB (TB00), upstream of the A/M area outfall, characterized by Sowder et al. [12], was also included as a mineralogically similar background soil.

The pH and electrical conductivity of air-dried soil was determined using a slurry of soil with distilled deionized water

Table 1. Summary of sequential extraction procedure [16,20]

Reagent	Target fraction	pH	Extraction time
18 MΩ H_2O	Water soluble	5.5	16 h
0.5 M calcium nitrate	Exchangeable	5	16 h
0.44 M acetic acid + 0.1 M calcium nitrate	Acid soluble	2	8 h
0.01 M $HN_2OH{\cdot}HCl$ + 0.1 M HNO_3	Mn-oxides	1	0.5 h
0.1 M sodium pyrophosphate	Organically bound	10	24 h
0.175 M ammonium oxalate + 0.1 M oxalic acid	Amorphous Fe-oxides	3	4 h in dark
15 ml 0.15 M sodium citrate + 0.05 M citric acid buffer + 0.5 g fresh Na-dithionite	Crystalline Fe-oxides	5	0.5 h at 50°C
10 ml 48% HF + 1 ml Aqua regia	Residual	1	0.4 h (microwave)

at a ratio of 1:2 with a glass junction combination electrode with calomel reference (Acumet, Model 13-620-286, Fisher Scientific, Pittsburgh, PA, USA), maintained in a 10% HCl solution prior to use. Five separate subsamples were prepared for each soil, and for pH the geometric mean was determined via antilogarithms of the readings. The dissolved organic carbon concentration of soil extracts was determined using a Shimadzu TOC-5000 total organic carbon analyzer (Kyoto, Japan).

Soils were subject to a seven-step sequential extraction technique, modified from Miller et al. [17]. The major modification was eliminating the step associated with the removal of the occluded MnO_2 since previous studies have indicated that excessive U extraction occurs during this step, and since these sediments have low total Mn, the efficient removal of U has been shown to be an artifact of the extraction [12]. Extractants and experimental conditions used in the sequential extraction procedure are shown in Table 1. Extractions used 0.75 g of air-dried soil and 30 ml of each reagent in sequence, shaken at approximately 90 cycles/min for the prescribed duration (Table 1) and centrifuged at 10,000 *g* for 30 min, filtered (nylon, 0.1 μm), and preserved with 1% HNO_3 prior to analysis. A rinse step (20 ml 0.025-M $Ca[NO_3]_2$) was incorporated between each extractant. Total metal concentration and metals within the residual soil fraction were extracted using 0.3 g oven-dried (105°C) soil in 10 ml 48% HF and HNO_3 and microwave digestion in Teflon PFA vessels. Analyte concentrations of all extracted fractions were determined by inductively coupled plasma–optical emission spectrometry (ICP-OES) using an Optima 4300 DV optical emission spectrometer (Perkin-Elmer, Norwalk, CT, USA). National Institute of Standards ([NIST], Gaithersburg, MD, USA) standard reference materials used were San Joaquin soil (2709) and apple leaves (1515).

Vegetation analysis

The effect of species. Following a vegetation survey of the area, leaf samples ($n = 10$) of dominant vegetation were collected from TB, with corresponding species collected from BG. The samples were analyzed separately to give an indication of contaminant variability within each site. Both the species name and the relative location (Fig. 1B) of the plant material were noted. Material for the species study was collected close to the stream edge.

Plant material was dried (60°C for 48 h), ground (<2 mm), and digested in triplicate aliquots of 0.25 g with 10 ml 5-*M* HNO_3 (trace metal grade) in pure Teflon PFA vessels using a microwave digestion apparatus (MDS-2000, CEM, Matthews, NC, USA), after which several drops of H_2O_2 were added to each sample. A HNO_3 blank and a NIST standard reference material (1515—apple leaves) were included with each carousel of microwave extractions, after which samples were analyzed by ICP-OES.

The effect of location. Actively growing herbaceous understory vegetation was collected from two distinct 13.5-m transects at 1.5-m intervals, moving away from the stream (Fig. 1B). Species consisted of *Andropogon* sp. and *Discanthelium* sp. leaf and root material were placed in separate polythene bags and stored on ice until being transported back to the laboratory. Leaf and root tissue were separated, and extraneous debris was washed from root systems using DI water. Plant material was prepared and analyzed in triplicate as described previously.

Statistical analysis

Statistical analysis of soil data used individual one-way analysis-of-variance (ANOVA) tests to investigate differences between the total analyte concentrations of samples collected from various locations. Investigation of significant differences in analyte partitioning between soil fractions was carried out by arcsine transformation of the percentage of total analyte within each fraction, then subjecting the data to individual one-way ANOVA tests with interaction effects. Nonsignificant interaction effects were omitted from the final models presented. Bonferroni correction (elimination of errors due to the likelihood of false-positive results) was used where appropriate; a critical *p* value was derived from the standard value of 0.05 divided by the number of tests giving a final critical *p* value of 0.00714. Coassociation of metals with one another within individual soil samples was determined by performing a Pearson's correlation between total analyte concentrations of all sampling locations. For remaining analyses, data were log transformed where appropriate and subjected to ANOVA statistical tests to determine significant differences where $p < 0.05$. Throughout the study, statistical annotations are as follows: *** indicates a *p* value of < 0.001, ** indicates $p < 0.01$, * indicates $p < 0.05$, and NS indicates that no significant differences were observed. The *F* values, where expressed, are followed by the degrees of freedom and the error degrees of freedom.

RESULTS

Sediment analysis

Data indicated significant variation in the concentration of Al, Cu, Fe, Mn, and U within stream sediments collected from varying locations along the streambed ($p < 0.0001$). The concentration of Ni did not vary with location ($F_{23,48} = 1.16$NS), and the majority of samples fell below detection limits (0.838 μg/kg). Average sediment U concentration was 4.18 mg/kg (±9.58). Pearson's correlation tests showed a strong correlation between Al and Cr, Cu, Fe, Mn, and Zn ($r^2 > 0.8$) within

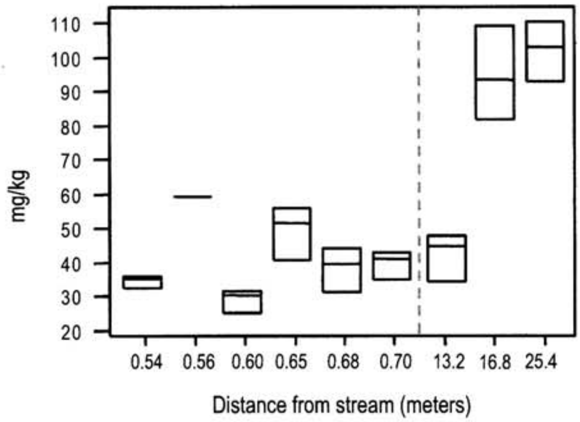

Fig. 2. Concentration of dissolved organic carbon (mg/kg) in Tims Branch soils (SC, USA). Boxes represent 25th and 75th percentile bisected by the median; whiskers represent minimum and maximum values ($n = 3$).

sediments but weak correlations for U: Al and U ($r^2 = 0.332$) and Fe and U ($r^2 = 0.183$). Nickel was strongly correlated with Fe only ($r^2 = 0.802$) within stream sediments.

Soil analysis

The TB soils were acidic, with average pH 4.6 (±0.1) with an electrical conductivity of 0.42 (±0.1) dS/m, typical of riparian sediments in the southeastern coastal plain. Soils at BG had a pH of 4.4 (±0.03) and an electrical conductivity of 0.54 (±0.01) dS/m. In all cases, no significant differences were observed between analysis replicates, and no significant variation was observed with sample location. Dissolved organic carbon concentration varied significantly with location ($F_{8,16} = 37.05^{***}$), where organic carbon concentration increased at greater distances from the stream (Fig. 2).

Total metal concentration

Total and sequential metal analysis of soils considered Al, Cr, Fe, Ni, Cu, Zn, Pb, and U as target analytes. Mean concentrations and standard deviation (where $n = 3$) for each analyte (expressed as mg/kg dry wt) are shown in Table 2, compared with two uncontaminated soil sources. Data indicate that Ni and U are the only analytes present at concentrations high enough to qualify them as contaminants, using both uncontaminated reference sites as a basis for comparison and average SRS soil concentrations reported by Pickett [8]. Concentrations of Ni at TB were four times higher than TB00 and approximately nine times higher than the BG control site (130 ± 90 mg/kg dry wt). The average U concentration in contaminated TB soils (289 ± 166 mg/kg dry wt) was about 35 times higher than both TB00 and BG. The elevated standard deviation of data collected from the various locations at TB indicated significant spatial heterogeneity in analyte concentration for Ni, Cr, and U, confirmed by one-way ANOVA tests between locations (Fig. 3A–C), with particularly elevated concentrations at approximately 0.6 m from the stream. Given the relatively small spatial scale over which this enrichment occurred (in comparison with the Ni, Cr, and U concentrations at 0.65, 0.68, and 0.70 m, respectively), it is likely that this represents an anomaly or a hot spot, an area characterized by

Table 2. Mean analyte concentration of contaminated Tims Branch ([TB] SC, USA) soils collected from nine discrete sampling points (TB 1–9), TB00, and Boggy Gut ([BG] SC, USA) soils (reference uncontaminated areas on- and off-site, respectively) expressed as mg/kg dry weight (±standard deviation where $n = 3$), showing F values from analysis of variance tests between TB 1–9 analyte concentration and distance from the stream edge (m)

	Reference sites				
Analyte	BG		TB00[a]	TB 1–9		ANOVA[b] by location (*F* values)
Al	5,829	(856)	18,000	7,170	(3,012)	3.37**
Cr	8.6	(1.0)	89.6	27.5	(12.2)	11.47***
Fe	1,705	(176)	34,285	7,576	(2,236)	4.65**
Ni	14.8	(0.2)	26.8	130	(90)	39.94***
Cu	2.35	(0.23)	24.6	15.3	(5.6)	83.50***
Zn	2.52	(1.3)	34.7	43.2	(14.8)	6.09**
Cd	6.28	(1.2)	0.03	0.18	(0.06)	16.77***
Pb	27.4	(2.5)	43.4	26.7	(6.8)	20.70***
U	8.46	(0.23)	8.2	289	(166)	35.81***

[a] Sowder et al. [12]; *** $p < 0.0001$; ** $p < 0.001$.
[b] ANOVA = analysis of variance.

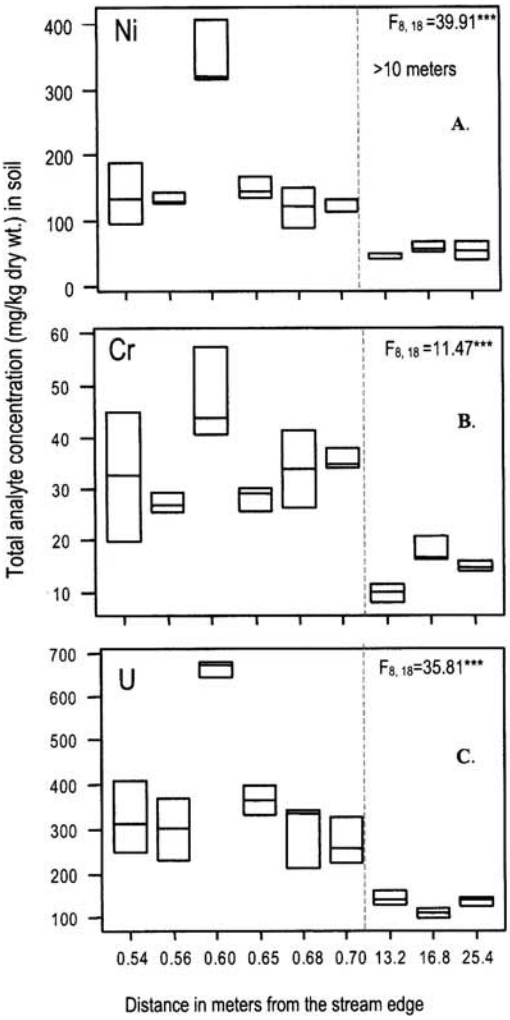

Fig. 3. Total soil concentration (mg/kg dry wt) of Ni (**A**), Cr (**B**), and U (**C**) at Tims Branch (SC, USA) with respect to distance from the stream. Boxes represent 25th and 75th percentile bisected by the median; whiskers represent minimum and maximum values ($n = 3$). F values from one-way analysis-of-variance statistical tests between concentration and distance are shown in the upper-right-hand corner.

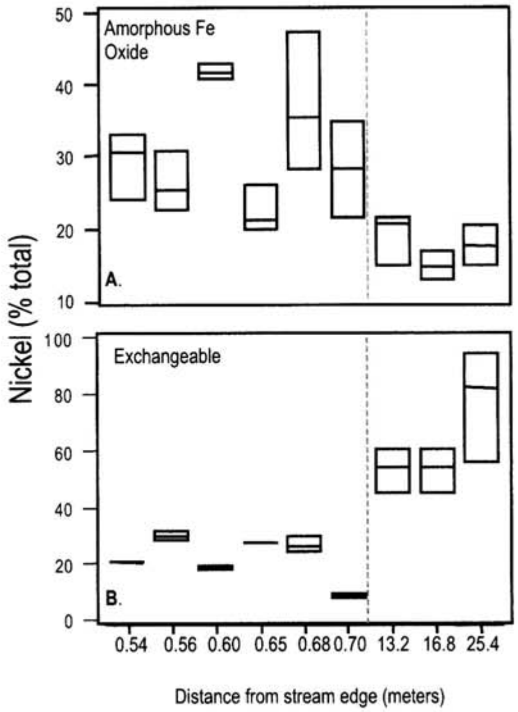

Fig. 4. Percentage of nickel within acid-soluble (**A**) and exchangeable (**B**) Tims Branch (SC, USA) soil fractions with respect to distance from the stream. Boxes represent 25th and 75th percentile bisected by the median; whiskers represent minimum and maximum values (n = 3).

a significantly higher concentration of contamination. These areas may have arisen by a variety (and combination) of factors, not least the localized topographic features where deposited sediments have tended to accumulate during episodes of increased stream flow. The fact that these anomalies generally occurred closer to the steam may simply be a product of how far the contaminated sediments were deposited during individual flooding incidents. Correlations were particularly strong between Fe and Ni, Ni and Cu, and Ni and U (r^2 = 0.845) and appeared to be a distinct signature of the contamination.

Sequential extraction of metals

Nickel was detected primarily within the amorphous Fe oxide fraction of the soil (Fig. 4A), an association that may explain the high correlation between these two elements in both stream sediments and soil. Lower but significant concentrations of Ni were present in both the exchangeable and the residual fraction of TB soils. A significant separation in trends was observed in soils sampled >10 m from the stream edge as opposed to those collected within 10 m from the stream. In samples TB7, TB8, and TB9, over 50% of the total Ni burden was extracted from the exchangeable fraction of the soils (Fig. 4B). For TB9, this amounted to 77% (±19) of the Ni burden, whereas in samples closer to the stream, Ni distribution between fractions followed the general order amorphous Fe oxide ≈ residual > exchangeable, suggesting that Ni is bound to more geochemically labile fractions in soils farther away from the stream (Fig. 4B). A stable pH of 4.5 throughout the TB study area ruled out a pH effect, but dissolved organic carbon concentration increased from 34.4 (±1.6) mg/kg at 0.54 m to 102 (±7.1) mg/kg at 25 m away from the stream edge [18]. Dissolved organic matter exerts a strong influence on metal solubility, and in general the majority of the dissolved metal is found in metal-organic complexes [19]. For this particular contaminated system, previous studies have found that complexation with organic matter increases metal solubility [14,20] because of weaker sorption of metals to native humic substances in comparison with soil minerals [21].

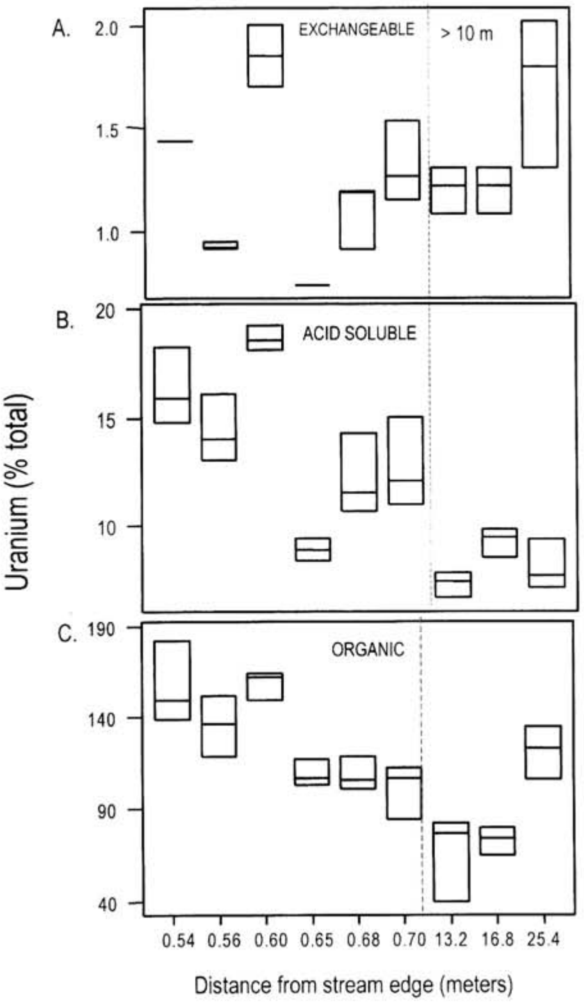

Fig. 5. Percentage of uranium within exchangeable (**A**), acid-soluble (**B**), and organic (**C**) fractions of Tims Branch (SC, USA) soils with respect to distance from the stream. Boxes represent 25th and 75th percentile bisected by the median; whiskers represent minimum and maximum values (n = 3).

Data from all sampling points showed that the majority of U was extracted from the organic fraction. Artifacts arising from the overly aggressive extractant used to define this fraction (0.1 M sodium pyrophosphate, pH 10) are suspected to be responsible for a total U recovery of above 100% [12]. In common with Ni, U distribution between fractions also differed in soils collected greater than 10 m away from the stream edge, with significantly less U detected within the acid soluble fraction of the soil (Fig. 5). Significant differences were observed between the concentrations of all contaminants within operationally defined soil fractions from the various sampling locations, indicating that bioavailability is also spatially heterogeneous. In general, this heterogeneity took the form of an

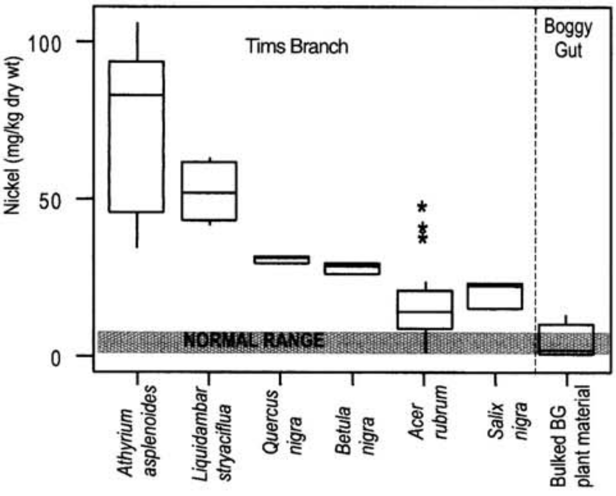

Fig. 6. Concentration of Ni (mg/kg dry wt) within the leaf tissues of dominant vegetation at Tims Branch (SC, USA) and composite samples of vegetation from the reference site, Boggy Gut (SC, USA). Boxes represent 25th and 75th percentile bisected by the median; whiskers represent minimum and maximum values ($n = 3$) and asterisks are outliers.

increase in the percentage of metal sorbed to soluble phases at sites that were farther away from the stream and therefore significantly less to more recalcitrant phases, such as the acid soluble and organic phases. Figures 4 and 5 give examples of the metal distribution trends with respect to distance.

Vegetation analysis

Survey. The TB overstory is dominated by *Acer rubrum* (red maple), *Betula nigra* (red birch), and *Salix nigra* (black willow), with scattered individuals of *Liquidambar stryaciflua* (sweet gum). Throughout the site, the Southern Lady fern *Athyrium asplenoides* was found to be abundant, especially closer to the stream edge. The canopy structure (≈9 m height) is typical of secondary regeneration, with older mixed pine and hardwood (predominantly *Pinus taeda*, *L. stryaciflua*, and *Quercus nigra*) on the outskirts of the site. Vegetation structure suggested that the water level at TB was formerly several meters higher than the current time. The understory is made up of blackberry (*Rubus* spp.) and *Vitis rotundifolia*, with widespread *Andropogon* sp. and *Discanthelium* sp. and scattered *Juncus* sp. in moister areas. The overstory becomes sparser farther away from the stream, at approximately 6 m high, and is composed mainly of *A. rubrum* and *S. nigra* with scattered individuals of *Myrica cerifera* (wax myrtle). The understory becomes denser farther away from the stream, dominated by *Rubus* sp. and some *Juncus* sp., again suggesting earlier flooding, as well as *Cyperus* sp., *Andropogon* sp., *Poa*, and *Discanthelium* sp. Samples (10 per species per site) of dominant species *A. asplenoides*, *A. rubrum*, *L. stryaciflua*, *B. nigra*, *S. nigra*, and *Q. nigra* were collected from TB study area with corresponding species collected from the reference site at BG.

Influence of plant species

Aluminum, Co, Cr, Cu, Fe, Mn, Ni, Pb, U, and Zn were considered as analytes, with the addition of Ca, K, and Mg considered to be cations that may influence metal uptake and binding within plant tissues. Analyte concentrations within leaf tissue collected from BG did not differ significantly between species (e.g., for Ni, $F_{5,47} = 2.84$NS), with an average Ni concentration of 4.75 mg/kg dry weight (±5.81), considered to be within the normal range for plants, and U concentrations at or below detection limits (≥0.01 mg/kg dry wt). Therefore, for comparison with species collected from TB, BG data were grouped together. The ANOVA tests indicated that plant species collected from TB contained significantly higher concentrations of Ni than those from BG ($F_{5,57} = 30.49$***; Fig. 6) by approximately one order of magnitude. The highest Ni concentration was detected within the fern *A. asplenoides* (75.4 ± 25 mg/kg dry wt). Uranium concentrations were at or below detection limits within all leaf material collected from streamside sediments at TB, with no significant difference in the U concentrations in plant material collected from TB and BG ($F_{6,57} = 1.02$NS). All other analytes showed no significant differences between plants from TB and BG. When individual species data within TB were considered, analysis showed significant species differences in Al, Cu, and K only, with all other analytes having highly consistent concentrations between species.

Influence of location

The concentrations of the previously mentioned analytes and major plant nutrients were analyzed in leaf and root tissue samples of herbaceous ground flora at increasing distances away from the stream at TB in two distinct transect locations (Fig. 1B). The concentrations of Ni within leaf tissue from transect 1 were in the range of 34.6 (±2) to 272 (±3) mg/kg dry weight and from transect 2 were in the range of 36.0 (±9) to 326 (±100) mg/kg dry weight. Uranium concentrations within the leaf tissues of understory vegetation were within the range of 13.6 (±1.6) to 518 (±7.5) mg/kg dry weight in transect 1 and 35.9 (±3.1) to 119 (±20) in transect 2, far higher than was detected in trees and fern species (showing mainly nondetects) growing close to the stream at TB (Table 3). The ANOVA analysis showed significant differences in tissue concentration between the two transects for Al, Cr, K, Mg, Pb, and U (Table 3), and because of their distinct locations at the site, the transects were considered separately in terms

Table 3. Concentration of contaminants of concern within leaf and root tissue (mg/kg dry wt) of herbaceous understory flora collected from Tims Branch (SC, USA); data are means ± standard deviation where $n = 30$

Factor	Al	Ca	Cr	Cu	Fe	K	Mg	Ni	Pb	U
Transect 1										
Leaves	3,637 (3,251)	6,941 (4,022)	7.0 (5.5)	9.2 (2.8)	2,698 (1,640)	5,072 (4,189)	1,830 (385)	95.1 (71)	3.9 (3.2)	150 (151)
Roots	1,782 (1,956)	5,434 (1,677)	2.4 (2.5)	6.1 (2.1)	1,920 (2,182)	5,560 (2,126)	1,491 (560)	82.2 (51.2)	2.2 (2.2)	62.8 (70)
Transect 2										
Leaves	1,493 (341)	4,303 (1,453)	2.68 (0.7)	9.1 (4.2)	768 (327)	1,608 (2,673)	1,100 (587)	118 (80)	3.38 (1.6)	102 (36)
Roots	1,677 (642)	4,235 (2,528)	2.43 (0.9)	10.7 (5)	921 (280)	1,359 (688)	812 (323)	160 (47)	4.1 (2.6)	126 (71)

Table 4. Transfer factors (TFs) for Al, Cr, Cu, Ni, Pb, and U derived from total soil and leaf tissue concentrations at Tims Branch (SC, USA) and the reference site, Boggy Gut (SC, USA). Data are mean TF ± standard deviation where $n = 3$; figures in brackets represent the range of TFs between 0 and 12 m from the stream. ND = no data

	Tims Branch		
Analyte	Transect 1	Transect 2	Boggy Gut
Al	0.66 (0.7) [0.2–2.2]	0.19 (0.07) [0.1–0.3]	0.022
Cr	0.31 (0.3) [0.05–1.1]	0.06 (0.01) [0.03–0.08]	0.08
Cu	1.42 (0.9) [0.3–2.98]	0.48 (0.3) [0.1–1.1]	2.46
Ni	1.09 (0.9) [2.7–9.5]	0.59 (0.4) [0.1–1.5]	0.319
Pb	0.34 (0.4) [0.07–1.2]	0.1 (9.06) [0.03–0.2]	ND
U	0.86 (0.8) [0.09–3.06]	0.18 (0.07) [0.09–0.3]	0.006

of statistical analysis. Concentrations of all analytes, with the exception of Mn, were higher in transect 1 (roots and shoots considered together) than transect 2. Significant difference in the concentration of U between transects is in contrast with Ni, and no significant differences were observed in either Ni or U with distance from the stream.

Total contaminant concentration in samples of surface soil collected from transects showed that transect 2 soils contained higher concentrations of both contaminants than transect 1. The ANOVA showed no significant differences in Ni or U concentration with increasing distance from the stream (p = 0.933 and 0.996, respectively); therefore, mean values (± standard deviation) for the separate transects were 107 (±60) and 205 (±45) mg/kg Ni for transects 1 and 2 and 217 (±154) and 577 (±190) mg/kg U for transects 1 and 2. Correlation (Pearson's) between Ni and U concentrations was $r^2 = 0.924$, giving U:Ni ratios of 2 and 2.8 for transects 1 and 2.

Transfer factors

Traditionally, transfer factors are described by the equation

$$[M_{\text{plant}}]/[M_{\text{soil}}]$$

with $[M_{\text{plant}}]$ representing the metal concentration within the plant, which can be tissue specific, and $[M_{\text{soil}}]$ representing the total metal concentration within the soil [22]. We assume that a more accurate expression of transfer can be obtained using the concentration of bioavailable metal within the soil rather than total, although in a practical sense this can be estimated only using chemical extractions such as those used previously. Further, the use of a soil bioassay may present confounding information on bioavailability because digestive enzymes can enhance metal lability and give a false-positive result. In addition, the metal tolerance or susceptibilities of the test organism will influence its response to the contaminants, and this is infrequently determined during bioassays. Given this, we tested for the most significant correlation between the leaf tissue concentrations (the portion of the plant most frequently utilized as a food source) with those from a truncated sequential extraction series (roughly equivalent to the plant-accessible fraction), consisting of soluble, exchangeable, organic, and the logical combination of those extracts (soluble + exchangeable, soluble + exchangeable + organic) carried out on the transect soil samples. This showed that no individual extractant or progressive combinations of extractions correlated significantly with the concentration of analyte within leaf, root, or a combination of the two tissues. Traditional transfer factors were derived from the mean total soil and leaf tissue analyte concentrations (Table 4) in comparison with transfer factors (TFs) arising from the BG reference site to give an approximation of contaminant movement. In general, TFs at TB were far higher than BG, with the exception of Cu, which was similar at both locations. Transfer of Al, Cr, Ni, Pb, and U was approximately one order of magnitude higher at TB, suggesting that an increased soil burden leads to greater transfer into plant tissue. Differences in TFs between transects suggest considerable contaminant heterogeneity. Transfer factors were higher in transect 1, especially for Ni and U, with U transfer an order of magnitude higher at this point than at transect 2.

DISCUSSION

Data showed significant elevation of Ni and U within TB soils up to a distance of 25 m away from the stream, with Ni concentrations nine times higher and U approximately 35 times higher than the reference site upstream of the A/M area. Sequential chemical extraction indicated that a greater percentage of Ni was associated with the more chemically labile fractions than U (soluble, exchangeable, and acid soluble), 12% for Ni and 0.3% for U, suggesting comparatively greater bioavailability of Ni than U. Further, the association of both Ni and U with the most chemically labile fractions of the soil increased with increasing distance from the stream. Previous investigations involving soils collected from this contaminated stream system strongly suggest that dissolved organic matter is a significant factor in controlling metal solubility, particularly Ni and U [12,14,20,21]. In a recent review, Sauvé [19] developed a semimechanistic model of metal partitioning, compiling data from over 70 individual studies, and showed that predicting partitioning coefficients could be achieved via a knowledge of total metal concentration, soil solution pH, and organic matter content. Organic carbon is particularly significant in this system, especially in the control of U solubility. Sowder et al. [12] showed that the solubility of U and Ni in extracts from the sediments at SP increased with dissolved organic carbon.

Data from TB indicated that the majority of U was extracted from the organic fraction. However, recoveries of U from the organic fraction of the soil amounting to greater than 100% strongly suggest artifacts associated with the extractant used to define this fraction (0.1 M sodium pyrophosphate, pH 10). Previous studies of SRS soils suggest that it is overly aggressive for U [12,14,20,21,23]. Consistent overestimation of U recovery from this phase, despite some modifications tailored for SRS soils in particular [14], strongly indicate the technique's nonspecificity for U. As an actinide, U has a markedly different aqueous geochemistry to the transition elements for which this method was originally developed [17,24] and in particular the high solubility of U (VI) under alkaline conditions such as those provided by the sodium pyrophosphate step. The nonspecificity for U should not be extrapolated to include the other analytes studied.

Analysis of native flora at TB showed differences in Ni uptake between species and a complete lack of U uptake by streamside plants. This confirmed the data provided by the weakest sequential extraction steps for U. The most significant finding was the highly elevated U and Ni concentration of ground vegetation collected away from the stream. Contaminant ratios detected within the leaf and root tissue of ground flora were markedly different to those within woody species at the stream edges but very similar to the soil. The correlation (r^2) between Ni and U within the soil was about 0.9. Nickel and U associated with leaf and root tissue of ground flora also gave r^2 values of 0.985 and 0.952 for transects 1 and 2, respectively, although U concentrations were below detection limits in the leaves of woody plants. This strongly suggests soil contamination of plant tissue whose leaves are closer to the ground. In addition, no relationship was observed between the plant tissue and soil concentrations within transects; concentrations of Ni and U in plants collected from transect 1 were higher than transect 2, although total Ni and U concentrations within the soil were far higher at transect 2. Analysis of SP-TB soils using x-ray adsorption near edge structure extractions has shown that U speciation is dominated by the insoluble U (IV) form [25], with very little soluble hexavalent U(VI), supporting evidence against the uptake of high concentrations of U by plants. Previous studies have indicated that the fraction of the soil bearing the majority of U and Ni contamination is the easily dispersible clay fraction [26] and that disturbance of the surface soil through either rainfall or animal activity could dislodge this fraction, coating the surface of the smaller plants. In support of this, fern species collected from the stream edge contained the highest levels of contamination, and although Pteridophytes have been found to contain elevated metal concentrations in the past [4], the alternative explanation in this case is more likely to be that it is closer to the soil and that its leaves are more likely to intercept contaminants dislodged from the soil than mature woody plants. Leaf tissue from the trees (Fig. 6) had lower concentrations of Ni within tissues, possibly because their leaves were out of range of potential resuspension.

The concentrations of Ni and U detected within herbaceous ground flora remains a concern, however, as it represents the contaminant load that can be transferred to the next trophic level. The relationship between soil and plant metal burden is obscured by mass loading of metals onto plant surfaces; however, previous studies have shown that this pathway of contaminant transfer is frequently overlooked [2]. It is unclear how the uptake transfer factors compare to resuspension transfer factors, and further studies are required to clarify the importance of resuspension. This TF therefore represents the concentration of metal and radionuclide available to the food web and is several orders of magnitude higher than published TFs from herbaceous plant material collected from SP [13]. The Ni levels in the potential food items are unlikely to pose any direct toxic effects, although U concentrations are a concern in this system. The median lethal dose (LD50) of U and Ni in small mammals is 400 mg/kg U and 2,000 mg/kg Ni, respectively [27]. However, the LD50 concept is a more applicable tool for human risk assessment than it is a measure of potential ecosystem or trophic transfer risk. Elevated input of metals and radionuclides on the scale observed in this study presumably have a deleterious ecological effect prior to the observance of significant mortality in small mammals, which are more difficult to detect—such as exclusion of species through habitat degradation. Most laboratory studies dealing with animal exposure utilize LD50s; however, in the field this is an inappropriate endpoint. Assessment of risks to populations using more sophisticated techniques better quantify damage and potential risk to ecosystem structure. Measuring bioavailability of contaminants in an impacted site should be the first step in determination of ecological risk because a logical estimate of exposure to multiple contaminants that can cause both acute and chronic effects must be made. For example, U is an alpha-emitting radionuclide with daughter gamma products that can cause DNA damage [28]. Further, Ni has been shown to contribute to the cytotoxicity and genotoxicity of mammalian cells in combination with gamma/x-ray/ultraviolet radiation and/or benzo[*a*]pyrene [29,30], and the history of this particular former waste unit shows that an additional risk exists from volatile organic compounds [12]. Risk assessment models must be adequately refined to be predictive of exposure. Only then, through examination of ecological endpoints, such as DNA damage, will we be able to gain a more realistic understanding of how complex mixtures of contaminants behave as they move through trophic levels. This study forms the basis of a much wider consideration of ecological risk from a mixture of metals and radionuclides for the purpose of linking contaminant levels to their true ecological endpoints.

It is likely that contaminants were transported into TB during periods of elevated stream flow, after which the water level subsided, leaving fine particulate stream sediments deposited on the surface of riparian soils along the TB corridor. In addition to a comparatively greater Ni bioavailability, this deposition has allowed resuspension of both Ni and U onto plant surfaces via similar processes as those resulting from aerial deposition with which studies of mass loading are traditionally associated [31]. These findings indicate that resuspension processes may pose an increased risk to humans and wildlife, and that even this vegetated site requires some form of stabilization.

Remediation techniques that can immobilize metals and radionuclides, preferably before they are eroded from the source term and enter TB, would potentially reduce ecological risks. This stabilization would ideally take first a chemical form involving binding contaminants to hydroxyapatite [32] in combination with a physical stabilization of the eroding former waste unit. Apatite minerals ($Ca_5[PO_4]_3V$ (X = halide, hydroxyl) react with transition and heavy metals as well as metalloids and radionuclides forming secondary phosphate precipitates that are stable over a wide range of geochemical conditions [20] and could be combined with unvegetated, eroding material at the source term to render the metals insoluble before they are transported into the riparian depositional environments downstream. Combined with a rebuilding of the enclosing spillway, these measures would protect the SP-TB system from further ecological damage and potential bioaccumulation.

CONCLUSION

Physical mobilization of contaminated wetland sediments from SP has contributed a significant amount of Ni and U to the soil and vegetation at TB. These studies indicate that Ni bioavailability is a concern at TB and that although U bioavailability is negligible, transfer to soil invertebrates and herbivores through mass loading onto plant tissue may still result in bioaccumulation.

Acknowledgement—This research was supported by Financial Assistance Award Number DEFG2600NT-40938 from the U.S. Department of Energy (DOE) to the Consortium for Risk Evaluation and Stakeholder Participation and DE0FC09-96SR18546 from the U.S. DOE to the University of Georgia Research Foundation and National Institute of Environmental Health Sciences (ESO 5022). The authors gratefully acknowledge the technical assistance of Christina Zabinski and Gregg O'Quinn with laboratory analysis and Virginia Jin for advice on the vegetation survey.

REFERENCES

1. Sharpe S, Mackay D. 2000. A framework for evaluating bioaccumulation in food webs. *Environ Sci Technol* 34:2373–2379.
2. Hinton TG. 1992. Contamination of plants by resuspension: A review, with critique of measurement methods. *Sci Total Environ* 121:177–193.
3. Baker AJM. 1981. Accumulators and excluders—Strategies in the response of plants to heavy metals. *J Plant Nutr* 3:643.
4. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED. 2001. A fern that hyperaccumulates arsenic. *Nature* 409:579.
5. U.S. Department of Energy. 1997. Linking legacies: Connecting the cold war nuclear weapons production processes to their environmental consequences. DOE/EM-0319. Washington, DC.
6. U.S. Environmental Protection Agency. 1997. Promoting watershed stewardship. *Proceedings,* 5th National Volunteer Monitoring Conference. EPA 841-R-97-007. Washington, DC.
7. U.S. Department of Energy. 1996. Charting the course: The future use report. DOE/EM-0283. Washington, DC.
8. Pickett JB. 1990. Heavy metal contamination in Tim's Branch sediments. OPS-RMT-90-900200. Westinghouse Savannah River, Aiken, SC, USA.
9. Evans AG, Bauer LR, Haslow JS, Hayes DW, Martin HL, McDowell WL, Pickett JB. 1992. Uranium in the Savannah River site environment. WSRC-RP-92-315. Westinghouse Savannah River, Aiken, SC, USA.
10. Pickett JB, Colven WP, Bledsoe HW. 1987. M-area settling basin and vicinity. Report DPST-85-703. E.I. Du Pont, Nemours & Company, Savannah River Laboratory, Aiken, SC, USA.
11. Arnett MW, Mametey AR, Spitzer D. 1995. Savannah River Site Report for 1994. WSRC-TR-95-075. Westinghouse Savannah River, Aiken, SC, USA.
12. Sowder AG, Bertsch PM, Morris PJ. 2003. Uranium and nickel speciation and bioavailability in riparian sediments: Impact of ageing, source term and geochemical controls. *J Environ Qual* 32:(in press).
13. Punshon T, Gaines KF, Jenkins RA Jr. 2003. Bioavailability and trophic transfer of sediment bound Ni and U in a southeastern wetland system. *Arch Environ Contam Toxicol* 44:30–35.
14. Batson VL, Bertsch PM, Herbert BE. 1996. Transport of anthropogenic uranium from sediments to surface waters during episodic storm events. *J Environ Qual* 25:1129–1137.
15. Chisholm-Brause CJ, Morris DE. 1992. Speciation of U (VI) sorption complexes on montmorillonite. *Proceedings,* 7th International Symposium on Water-Rock Interaction, Park City, UT, USA, pp 137–140.
16. Borovec Z. 1981. The adsorption of uranyl species on fine clay. *Chemical Geology* 42:45–58.
17. Miller WP, Martens DC, Zelany LW. 1986. Effect of sequence in extraction of trace metals from soils. *Soil Sci Soc Am J* 50:598–601.
18. Punshon T, Burger J, Bertsch PM. 2002. Bioavailability and trophic transfer of metals and radionuclides in a riparian ecosystem: First stage trophic transfer. SREL-65 UC-66e. CRESP, Rutgers University, University of Georgia Savannah River Ecology Laboratory, Aiken, SC, USA.
19. Sauve S, Hendershot W, Allen HE. 2000. Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden, and organic matter. *Environ Sci Technol* 34:1125–1131.
20. Arey JS, Seaman JC, Bertsch PM. 1999. Immobilization of uranium in contaminated sediments by hydroxyapatite addition. *Environ Sci Technol* 33:337–342.
21. Seaman JC, Arey JS, Bertsch PM. 2001. Immobilization of nickel and other metals in contaminated sediments by hydroxylapatite addition. *J Environ Qual* 30:460–469.
22. Shaw G, Bell JNB. 1989. The kinetics of cesium absorption by roots of winter wheat and the possible consequences of the derivation of soil to plant transfer factors for radiocesium. *J Environ Radioact* 10:213–231.
23. Clark SB, Johnson WH, Malek MA, Serkiz SM, Hinton TG. 1996. A comparison of sequential extraction techniques to estimate geochemical controls on the mobility of fission product, actinide and heavy metal contaminants in soils. *Radiochim Acta* 74:173–179.
24. Langmuir D. 1997. *Aqueous Environmental Geochemistry.* Prentice Hall, Upper Saddle River, NJ, USA.
25. Hunter DB, Bertsch PM. 1998. In situ examination of uranium contaminated soil particles by micro-X-ray and micro-fluorescence spectroscopics. *J Radioanal Nucl Chem* 234:237–242.
26. Batson VL. 1994. Surface water transport and distribution of uranium in contaminated sediments near a nuclear weapons processing facility. MS thesis. Texas A&M University, College Station, TX, USA.
27. Koranda JJ, Cohen JJ, Smith CF, Ciminesi FJ. 1981. Genotoxic materials in the surface environment. Ucrl-53215. Lawrence Livermore National Laboratory, Hayward, CA, USA.
28. Gimore G, Hemmingway JD. 1995. *Practical Gamma-Ray Spectrometry.* John Wiley, Chichester, UK.
29. Hartwig A. 1995. Current aspects in metal genotoxicity. *BioMetals* 8:3–11.
30. Denkhaus E, Salnikow K. 2002. Nickel essentiality, toxicity and carcinogenicity. *Crit Rev Oncol-Hematol* 42:35–56.
31. Pinder JEI, McLeod KW. 1989. Mass loading of soil particles on plant surfaces. *Health Phys* 57:935–942.
32. Arey JS, Seaman JC, Bertsch PM. 1990. Immobilization of uranium in contaminated sediments by hydroxyapatite addition. *Environ Sci Technol* 33:337–342.