Are U, Ni, and Hg an Environmental Risk within a RCRA/CERCLA Unit on the U.S. Department of Energy's Savannah River Site?

Paul G. Edwards,[1] Karen F. Gaines,[1] A. Lawrence Bryan, Jr., [2] James M. Novak,[1] and Susan A. Blas[3]

[1]Department of Biological Sciences, Eastern Illinois University, Charleston, IL, USA;

[2]Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, USA;

[3]Savannah River Nuclear Solutions, Savannah River Site, Aiken, SC, USA

ABSTRACT

The U.S. Department of Energy's Savannah River Site (SRS) is a former nuclear weapon production facility. From 1954–1985, releases of Al, Cu, Cr, Hg, Ni, Pb, U, and Zn were discharged into the Tims Branch-Steed Pond water system. This study investigates whether metal concentrations in Tims Branch's sediment, biofilm and other biota exceed screening level risk calculations to determine if remedial actions should be pursued for the Contaminants of Potential Concern (U, Ni, Hg). Transfer factors (TFs) were calculated to determine metal concentration changes throughout lower trophic levels and results were compared with sediment benchmarks to create hazard quotients (HQs) to assess risk and a scientific-management decision point. Most TFs for Ni and U from lower to higher trophic level biota were < 1, suggesting no biomagnifications; however HQs > 1 and cumulative distributions showed the majority of the samples exceeded action levels. Elevated TFs and HQs > 1 in the upper trophic levels for Hg indicated a high degree of bioavailability and biomagnification. Monte Carlo resampling analyses supported these empirical results. This system should continue to be closely monitored to ensure that contamination does not move off the SRS.

Key Words: hazard quotient, mercury, nickel, RCRA/CERCLA, transfer factors, uranium.

INTRODUCTION

The purpose of this study was to determine if the current trace metal concentrations for Contaminants of Potential Concern (COPCs: uranium (U), nickel (Ni), mercury (Hg)) in a Superfund site located on the U.S. Department of Energy's (DOE) Savannah River Site (SRS) pose an environmental risk to the level where remedial actions should be pursued by the DOE. The SRS is a 777 km2 former nuclear weapons material production and current research facility located in west-central South Carolina along the Savannah River (Figure 1). In 1954, in the northwest region of the SRS (Figure 1), direct environmental releases were discharged through a drainage ditch into the Tims Branch-Steed Pond water system (Sowder et al. 1996) from a fuel and aluminum (Al)-clad U nuclear reactor target manufacturing facility called "M-Area". The wastewater discharges released into Tims Branch contained large volumes of inorganic wastes such as depleted and natural U, Al, and Ni along with smaller quantities of lead (Pb), copper (Cu), chromium (Cr), and zinc (Zn) (Pickett et al. 1987). An estimated 43,500 kg of U entered this system from 1954–1985, with most occurring from 1966-1968 (Evans et al. 1992).

Comparable amounts of Ni are thought to have been released, as Ni and U contamination was from the same waste, although the precise quantity is unknown (Sowder et al. 1996). Concomitant to the inputs into Tims Branch, Hg was discharged into the watershed from the 1950s–2000s via groundwater pumped into an outfall (A11) which discharges into an input tributary (all part of M-Area, Figure 1) leading into Tims Branch, including Hg-contaminated groundwater in 2005–2007. Starting in 2007, tin (II) chloride (SnCl2) was added to the groundwater prior to reaching a pre-existing air stripper leading into the A11 outfall to reduce and mobilize Hg with the purpose of volatizing the trace metal and forming tin oxides which are essentially non-toxic (Looney et al. 2012).

SRS was placed on the U.S. Environmental Protection Agency's (USEPA) National Priorities List (NPL) of contaminated sites in 1989. The NPL is used to determine sites that warrant cleanup under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as Superfund. The SRS is also required to meet RCRA1 requirements that govern the disposal of solid and hazardous waste. The SRS approach to environmental cleanup is guided by a Federal Facility Agreement (FFA) that integrates CERCLA response actions with corrective measures required by SRS's RCRA permit through an integrated Resource Conservation and Recovery Act (RCRA)/CERCLA process (SRS Index # 510.456, CERCLIS #:73, WSRC-OS-94-42; SRS FFA, 2012).

Due to the contamination from SRS activities, Tims Branch and Steed Pond are RCRA/CERCLA units falling under Superfund. Because this area is a Superfund site, the ecological risk assessment guidance for Superfund (ERGAS) was followed (USEPA, 1997b). In this 8-step process, a screening-level problem formation and toxicity evaluation (step 1) is performed, followed by a screening-level exposure estimate and risk calculation based on Hazard Quotients (step 2). Aluminum, Cr, Cu, Ni, Hg, Pb, and U have been classified as COPCs for this watershed with particular attention to U, Ni, and Hg due to bioavailability and high transfer factors (TFs) observed (e.g., Batson et al. 1996; Looney et al. 2012; Murray et al. 2010; Pickett et al. 1987; Pickett 1990; Punshon, et al. 2003a,b). Previous studies have shown that the contamination has not entered the Tims Branch terrestrial food web to the point of environmental risk (Reinhart 2003). However, other ecotoxicological studies have shown that the contamination is present in both the aquatic and transitional wetland communities (Murray et al. 2010; O'Quinn 2005; Punshon et al. 2003a,b;). Although these studies indicated, based on multiple endpoints, that there are risks to both wildlife communities from DNA strand-breakage, as well as humans who may consume aquatic organisms that move off the SRS from this Superfund site, the pathway of contamination movement through partitioning and availability in the contaminated riparian sediments, is not well understood (Sowder et al. 1996).

This study utilizes the most current data available to examine trophic transfer and how U, Ni, and Hg concentrations in water, Anuran and Anisopteran larvae, biofilms, and detritus samples compare to established action limits. We chose these taxa, because of unanswered questions left from previous studies examining higher trophic positions as well as sediment and plant tissue pathways in this system (Batson et al. 1996; Chow et al. 2005; Gaines et al. 2005; Murray et al. 2010, O'Quinn 2005; Punshon et al. 2003a,b; Sowder et al. 1996). Specifically, TFs were calculated as an index of change in U, Ni, and Hg trace metal concentration within and between lower trophic levels. Additionally, sample matrix metal concentrations were compared with benchmarks from multiple sources to create hazard quotients (HQs) as the environmental risk calculation. All established benchmarks that were applicable to environmental matrices of the COPCs in similar systems that could be found in the literature were used. As such, the evaluations presented below were based on the way the data were collected and the most appropriate benchmarks in both the government and peer-reviewed literature.

Rationale

Based on the Superfund ecological risk assessment process, after HQs are established (step 2), the "Baseline Ecological Risk Assessment" can be performed. This process includes: the problem formulation (step 3), study design (step 4), field site verification (step 5), data analysis (step 6), risk characterization (step 7), and risk management (step 8, not part of the assessment). This investigation performs the 7-step assessment process to provide insight for a risk management strategy concerning what future actions, if any, should be taken to remediate the Superfund site. To accomplish this, the U, Ni, and Hg concentrations and bioavailability in lower trophic level biota from seven beaver impounded ponds were quantified with a focus to determine their transport potential within the Tims Branch food web. These three COPC metals are used because previous studies in this system have shown they pose the most risk to both plant and animal species inhabiting or potentially consuming biota from this system (Batson et al. 1996; Chow et al. 2005; Gaines et al. 2005; Murray et al. 2010; O'Quinn 2005; Punshon et al. 2003a,b; Sowder et al. 1996, SRNS 2011). Specifically, studies have shown an increase of 1500– 2800% of U transported through SRS tributaries during storm events when compared to base flow measurements indicating that U is still present in the aquatic systems even though it may not be as mobile (Batson et al. 1996; Buettner et al. 2011). Moreover, exposure models (Chow et al. 2005; Gaines et al. 2005; Murray et al. 2010) show risk to human consumers as well as DNA damage to fauna that reside in the system from both U and Ni exposure.

Mercury contamination on the SRS is a result of site processing (coal combustion and waste disposal), atmospheric deposition as well as from Savannah River water, contaminated from upstream industry, which was used in SRS activities such as the construction and maintenance of reactor cooling reservoirs (Kvartek et al. 1994; Sugg et al. 1995). Availability of Hg to the food web, particularly in wetland habitat types, is associated with episodes of flooding (Rudd 1995) as Hg binds to sediments in combination with an increase in microbial processes during these periods, forming highly bioavailable methylmercury (MeHg; Jackson 1988; Snodgrass et al. 2000). Beaver ponds and wetland habitats have also been known to increase Hg methylation (Brigham et al.

2009; Driscoll 1998; Krabbenhoft 1999; Mason et al. 2000; Roy et al. 2009a,b), raising concerns specifically for Tims Branch due to the dynamic habitat types and periods of drought that occur in this system.

Based on these studies, it is hypothesized that the COPCs are bioavailable, could be biomagnifying, and are high enough in concentration to be considered harmful (e.g., above action limits). Therefore, to better understand how trophic relationships affect Ni, U, and Hg concentrations in baseline organisms and to determine the risks associated with those concentrations, the specific objectives of this study were: 1) to quantify trophic transfer of contaminants from biofilm and detritus to purged Anuran and Anisopteran larvae in each of the seven pond impoundments of the Tims Branch system on the SRS; 2) compare sample matrix concentrations with soil toxicity action levels to identify potential risks to human and non-human environments; 3) compare appropriate sample matrix concentrations with water quality numeric criteria for the protection of aquatic life and human health.

METHODS

Study Site

The point source contamination into the Tims Branch-Steed Pond water system from "MArea" settled into Steed Pond, a farm pond prior to construction of the SRS, located roughly 1 km downstream of the drainage ditch (Figure 1). The pond was dammed and converted to the main settling basin for the M-Area effluent between 1954 and 1985 (Pickett et al. 1987). In 1979, significant releases into Tims Branch ended and all subsequent inputs were redirected into the M-Area settling basin. However, Tims Branch still received small releases until 1982 when all untreated effluent was redirected to the M-Area basin. The wooden spillway within Steed Pond ruptured in 1984, releasing sediment-bound metals downstream through Tims Branch (Evans et al. 1992) and contamination transport still occurs during episodic storm events (Batson et al. 1996). "Pond 25" (labeled Pond 5a/b in the sequential sampling regime outlined below, Figure 1) acted as the settling basin for the contaminant release before it was also breached releasing the sediment-bound contaminants into Upper Three Runs (Arnett and Spitzer 1994). The M-Area settling basin was stabilized and capped in 1989.

Overall, the Tims Branch watershed undergoes durations of drought and has high beaver activity resulting in ponding, which makes summarizing and predicting the ecotoxicological components of this system a challenge. In fact, Steed Pond has dried and ponded numerous times during the past decade. While some of the pollution has washed downstream toward the Savannah River, much still remains due to sorption to sediment-bound particles. Due to the residual contamination and the ambiguity of how these toxicants may progress through the food web, the Tims Branch watershed remains closely monitored.

Tims Branch is a black water second order stream system currently consisting of small stream channels and beaver ponds that flow into Upper Three Runs Creek, which then flows into the Savannah River. The portions of Tims Branch included in this study were seven beaver impounded ponds in the northwest SRS (Figure 1). Preceding the M-Area input are two ponds labeled Ponds 1A and 1 with Pond 1 considered the control as it occurs directly before the MArea input. Pond 1A was treated as impacted due to receiving wastewater discharges and storm water runoff. Due to drought conditions, Pond 1A is mainly disconnected from Pond 1. All other ponds were considered impacted as a result of contaminant releases into these systems. Following the M-Area input were five consecutive beaver ponds labeled 2, 3, 4, 5A and 5B (5A and 5B are also referred to as Pond 25 in previous Tims Branch studies). In order to observe the current state of the lower trophic levels within Tims Branch, biofilm, detritus and Anuran and Anisopteran larvae were collected based on availability from all ponds.

Sample Collection

Polycarbonate plates (1 ft x 1 ft) were utilized as a surface for biofilm growth (Kröpfl et al. 2006). These plates were inserted into previously slit untreated (2 in x4 in) Standard Yellow Pine stakes. Each stake contained four horizontal plates with two on each side with a vertical distance of 3 in between plates (N = 4 plates/stake). Three stakes were placed in sun lit areas and allowed a 2-week growth period in the summer and fall months of 2010. Following each growth period, samples were collected and freeze dried. In the spring of 2011, plates were collected after 2, 4, and 8 weeks, however, no differences were observed between time periods so all samples were pooled for further analyses. Sample sizes for biofilm were: Pond 1A (n = 5), Pond 1 (n = 34), Pond 2 (n = 58), Pond 3 (n = 23), Pond 4 (n = 24), Pond 5A (n = 9) and Pond 5B (n = 49).

In the summer months of 2010, Anuran (tadpole) larvae were collected from the littoral region of all ponds using minnow traps and dip nets. Tadpoles were purged for 24 hours in deionized water and each individual was identified to species, weighed (g), measured (snout/vent length [mm]), and Gosner staged (Gosner 1960). Only Ponds 1A, 1, 2, 5A and 5B were available for collection in 2011. Sample sizes for tadpoles were: Pond 1A (n = 77), Pond 1 (n = 60), Pond 2 (n = 72), Pond 3 (n = 10), Pond 4 (n = 1), Pond 5A (n = 39) and Pond 5B (n = 36).

Dragonfly larvae were collected from the littoral regions of each pond in the summer months of 2010 and allowed a 24-hour purge time prior to weighing and identification to infraorder Anisoptera. Sample sizes for dragonfly larvae were three for each pond. Detritus(n = 2 for each pond, except Pond 5B where n = 7) was also collected from each pond in the summer months of 2010 with the exception of Pond 1A which was unavailable due to drought. Samples collected for 2010 and 2011 were combined by taxa/sample matrix due to the inability to distinguish between the effect of year and natural variation. All samples were collected, handled, and processed in accordance with the University of Georgia Animal Care Use Protocols.

Trace Metal Analyses

In order to achieve a homogeneous sample matrix, all samples were freeze dried and ground prior to processing for trace metal analysis. Sample preparation followed USEPA method 3052 (USEPA 1996c) utilizing microwave assisted acid digestion. All samples were analyzed for Mn, V, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Hg, Pb, and U using a Perkin Elmer NexION 300X inductively coupled plasma-mass spectrometer (ICP-MS) operating in Kinetic Energy Discrimination (KED) mode following USEPA method 6020A (USEPA 2007) at the Savannah River Ecology Laboratory, Aiken, South Carolina, USA (Bryan et al. 2011; Metts et al. 2012; O'Quinn 2005; Punshon et al. 2003a,b; Reinhart 2003). Manganese and Cd were removed from further analyses due to concentrations outside the standard curves. All concentrations were reported on a dry-weight basis with an allowable spike recovery range of 80–120%.

Risk Evaluation

Transfer factors (TFs) were calculated based on a modified equation given by Shaw et al. (1989; Equation 1) in order to determine transfer of trace metals to higher trophic levels

$TF = [M_{plant}] / [M_{soil}]$) (1)

where $[M_{plant}]$ is the metal concentration of the plant tissue and $[M_{soil}]$ is the metal concentration within the soil. Transfer factors were calculated for contaminant transfer and bioavailability from biofilm and detritus to tadpoles and dragonfly larvae (Equation 2).

$TF = [M_{htl}] / [M_{bl}]$ (2)

where $[M_{htl}]$ represents higher trophic level biota metal concentrations (tadpoles and dragonfly larvae) and $[M_{bl}]$ represents baseline metal concentrations (biofilm and detritus). Transfer factors were calculated from biofilm to tadpoles across all combined ponds and each pond individually for the maximum (Max), 75_{th} percentile, 50_{th} percentile, 25_{th} percentile, and the minimum (Min) concentration for Ni, U, and Hg. This follows the USEPA approach for HQ values to be calculated for the central tendency as well as the reasonable maximum exposure (RME). Frequency histograms were calculated (see below) so any threshold value could be used for the HQ.

Hazard Quotients (HQs) were calculated based on the USEPA's (1989) ecological risk characterization procedures (Equation 3)

$HQ = [E] / [TRV]$ (3)

where [E] is the Site Exposure Level and [TRV] is the Toxicity Reference Value. HQs were based on sediment quality benchmarks for Ni, Hg, and U trace metal concentrations (Table 3, Friday 1998).

Empirical cumulative distributions of Ni, U, and Hg were calculated for all combined ponds based on the converted wet weight metal concentrations displaying the maximum, 75^{th} percentile, 50_{th} percentile, 25_{th} percentile, and minimum for each trace metal for each sample matrix. Percentiles of observations were used based on their utility in biological assessment (Suter 2006), in particularly because the 25th percentile has been used as reference values to define impairment in some states (Yoder and Rankin 1995). Biofilm and detritus were converted to wet weight metal concentrations based on a 90% wet weight (Gangadhara et al. 2008; Wright et al. 2008), dragonfly larvae were converted using an 84% wet weight (Muscatello et al. 2008; Muscatello et al. 2009), and tadpoles were converted based on a 90% wet weight (Loumbourdis et al. 1999; Bradford 1984). Added to these distributions were wet weight water quality numeric criteria as established by the South Carolina Department of Health and Environmental Control (S.C. DHEC) for the protection of aquatic life and human health. Criterion maximum concentrations (CMC) and criterion continuous concentrations (CCC) were given for acute and chronic exposure limits, respectively, for concentrations of Ni and Hg and maximum concentration levels (MCLs) for U and Hg (S.C. DHEC, 2008).

Empirical cumulative distributions were also plotted using the aforementioned parameters for biofilm and tadpoles for each pond sampled in the Tims Branch system. As defined by the S.C. DHEC, the CCC is the highest in-stream concentration of a toxicant or an effluent to which the organisms can be exposed to protect against effects. The USEPA develops chronic criteria from longer term (often greater than 28 days) tests that quantify survival, growth, reproduction, and in some cases bioconcentration. Similarly, the CMC is the highest in-stream concentration of a toxicant or an effluent to which the organisms can be exposed to for a short duration without causing an acute effect. The USEPA derives acute criteria from 48- to 96-hour tests of lethality or immobilization.

Monte Carlo simulations based on fitted distributions were performed in Crystal Ball 11.1 (2013) for TF and HQ estimates to provide probabilistic estimates of those measures being greater than 1 based on the threshold levels for all combined ponds. Each simulation consisted of 10,000 iterations, resulting in a representative picture of the variability of the distribution of the corresponding measures. Probabilistic models were based on fitted distributions to each of the datasets for the respective taxa.

RESULTS

Transfer Factors

Transfer factors greater than 1 signify amplification in concentration as trophic levels increase and thus, by definition, are indicative of biomagnification. Transfer factors less than 1 indicate a reduction in contaminant concentrations as trophic levels increase, and therefore show a reduction in bioavailability. Although, TFs from biofilm to tadpoles for U were slightly higher than Ni, almost all fell below 1 (Table 1). Probabilities generated through Monte Carlo simulations support the empirical findings. However, when investigating each pond separately, TFs in Pond 1 (control) were all greater than 1 for U (except for the Min interval). Mercury TFs were above 1 for all intervals for all ponds. Pond 1A was the only pond with intervals resulting in TFs less than 1 due to biofilm Hg concentrations generally being higher than those observed in tadpoles. The highest values for the Hg TFs were those observed in the 25th percentile and Min intervals for most ponds, demonstrating a greater variability in biofilm Hg concentrations compared to Hg concentrations in tadpoles. A similar pattern of TFs below 1 for Ni and U were observed from detritus and biofilm to tadpoles whereas TFs were greater than 1 for Hg (Table 2). Furthermore, TF values from biofilm and detritus to dragonfly larvae for all metals were generally less than 1 with the exception of the Max and 75th percentile intervals for Hg.

Hazard Quotients

According to the USEPA guidelines, a HQ greater than 1 indicates a potential concern for ecological health (US EPA 1989). HQs greater than 1 indicate risk to organisms within the area as their concentrations are greater than those suggested to cause adverse effects. Hazard quotients for threshold effect concentrations (TECs – see Table 3 for abbreviations) over all combined ponds for the Max intervals for both biofilm and detritus were above 1 for Ni (Table 3). Threshold effect concentrations HQs (TEC-HQs) for tadpoles were above 1 for the majority of the Max intervals. Dragonfly larvae HQs were mainly below 1 for TECs. Most Hg TEC HQs were greater than 1 for all biota at the Max interval. Most TEC HQ were >1 for both Ni and Hg for tadpoles. All U sample matrices were above a HQ of 1 (Table 3). Probabilities generated through Monte Carlo simulations using the entire distribution (not just maximum values) support the empirical findings (Table 3).

Pond 1 (control) HQs for Ni for all sample matrices were below 1 (accept for Biofilm CLP-PQL that had an HQ of 1.42), although biofilm and tadpole Max HQs were greater than 1 for U. The TEC Hg Max HQs for all matrices were above 1 for USEPA Region IV, as well as the TEL and TV. In contrast to the control pond, all Ni HQs for biofilm and detritus in the impacted ponds were above 1 for TECs; however TEC HQs for tadpoles were below 1 for IV. Dragonfly larvae intervals did not exceed 1 except for CLP-PQL. All sample matrix HQs were greater than 1 for U with notably high indices of 83 for biofilm, 137 for detritus, 11 for tadpoles, and 15 for dragonfly larvae. TEC HQs for Hg exceeded 1 for all sample matrices accept IV.

Empirical Cumulative Distributions

The empirical cumulative distribution above the 25th percentile for both the food source (in this case biofilm) and higher trophic sample matrices of all combined ponds exceeded the action levels set by the South Carolina Department of Health and Environmental Control water quality numeric criteria for the protection of aquatic life and human health for CMC and CCC for Ni and the MCL for U (Figure 2(a-c); S.C. DHEC 2008). The biofilm cumulative distribution above the 50th percentile for Hg were beyond all limits (CMC, CCC, and MCL) for all combined ponds, however for detritus, only the portion of the cumulative distribution above the 75th percentile exceeded these criteria. The tadpole Hg empirical cumulative distribution above the 25th percentile exceeded all three limits (CMC, CCC, and MCL). The Hg cumulative distribution exceeded the effects criteria for dragonfly larvae only above the 75th percentile. Biofilm cumulative distributions for Pond

1 above the 25^{th} percentile exceeded limits for both Ni and U however, sample matrices were below these criteria for Hg (Figure 3). For all impacted ponds, cumulative distributions were beyond the limits for both Ni and U for all measured samples (Figure 4(a-b)). Biofilm, detritus, and dragonfly larvae cumulative distributions exceeded all limits only above the 50^{th} percentile, however tadpole distributions were above these limits above the 25^{th} percentile (Figure 4(c)).

DISCUSSION

Risk Analysis

Transfer factors (TFs) indicate the degree to which a contaminant is transferred from one trophic level to the next; however additional indices are necessary as TFs alone are not sufficient to predict risk. TFs are useful when they are considered in context with other indices such as the HQ. Remediation activities conducted by the SRS are based on a comparison of ecological risk associated with COPC screening values to actual contaminant concentrations that may possibly result in adverse effects (Friday 1998). Typically, these benchmark concentrations are provided for a number of sample matrices such as water, soil, or sediment. The screening values are referenced from multiple sources and are the recommended values for decision-making in the SRS remediation program. We chose these same benchmarks for this study based on their applicability to the environmental conditions at the study site as well as to facilitate the identification of specific hazards for the DOE-SRS to address. Sediment benchmarks were used not only due to the propensity for exposure through ingestion by upper trophic level biota, but also due to particulates being trapped in biofilm samples. Moreover, we used these benchmarks to determine if these risk measures were consistent to findings showing risk in this system based on physiological, morphological, and chemical speciation endpoints (Batson et al. 1996; Chow et al. 2005; Gaines et al. 2005; Murray et al. 2010, O'Quinn 2005; Punshon et al. 2003a,b; Sowder et al. 1996). Therefore, the HQs generated from these screening values will help to evaluate potential risk at both the organismal and environmental system level.

Nickel

TFs for Ni from food source sample matrices to higher trophic level biota (e.g., dragonfly larvae and tadpoles) for all intervals indicated that Ni is not biomagnifying in this system; however, bioaccumulation and trophic transfer are occurring (Tables 1–2). In contrast, Ni concentrations were extremely elevated in Tims Branch (Figure 2) and exceeded action levels, showing the risk for adverse effects as established by local, state, and federal agencies, as these particular receptors were selected for their potential to assess population-level impacts. These multiple action levels, derived from toxicity tests and field survey data, were chosen specifically from a variety of sources to best assess the associated risk as different toxicity tests may show adverse effects at different concentrations. These results show that TFs must be taken in context with the system being measured as well as the particular COPC. That is, trophic transfer is relative to the bioavailability of the chemical species as well as how the organism uses the environment

(Chow et al. 2005; Gaines et al. 2005; Murray et al. 2010).

Although the TFs were less than 1 for tadpoles with low Monte Carlo simulation probabilities (Table 2), trophic movement of Ni was enough to exceed all action levels for the highest interval (Table 3). Both TFs and HQs for dragonfly larvae were most often lower than those observed for tadpoles, suggesting that dragonfly larvae diet does not solely consist of biofilm and detritus. Comparing the upper trophic level biota Ni concentrations to sediment toxicity benchmarks indicate a possible risk at the highest concentrations. However, Ni concentrations in both food source items and upper trophic level biota exceed both the Ni CCC (chronic) and CMC (acute) limits set by the S.C. DHEC, which are established in order to protect aquatic life and human health (Figures 2–4). Exceeding both of these limits suggests possible adverse effects for long-term and single exposure indicating Ni as a risk in this system. Previous Tims Branch studies have also shown Ni as a risk (Punshon et al. 2003 a,b) with TFs for Ni greater than 1. TFs greater than 1 for these past studies are likely due to the elevated mobility for Ni within the Tims Branch terrestrial ecosystem. Another recent Tims Branch study has revealed DNA double strand breakage in water snakes as a result of low level exposure to Ni in the presence of the other COPCs (Murray et al. 2010) showing that TFs are not the most important factor in quantifying risk from Ni exposure in this system. Although the direct effects of Ni have not been widely studied for Anuran larvae, one study has suggested population sinks as a result of Ni contamination in Hyla crucifer and Bufo americanus (Glooschenko et al. 1992).

Uranium

As expected, TFs were below 1 for U indicating no biomagnification of this metal in Tims Branch. However, TFs were greater than those found in Ni. The Monte Carlo simulations support this finding. This coupled with the fact that the action level is much lower for U than Ni, shows that U is very much a hazard in the system with HQs showing risk. It should be noted that TFs for U in Pond 1 were likely exaggerated due to the extremely low food source and consumer U concentrations. This is another example of why TFs must be considered in context when evaluating risk. As with Ni, trophic transfer of U is occurring from food source matrices to the upper trophic level biota (Tables 1-2). Sowder et al. (1996) indicated that U is tightly bound to the sediment, as such, this metal's trophic mobility is most probably from the gut of the organism from ancillary sediment/soil ingestion. That is, the U is still mostly associated with the sediment as seen in other studies versus being assimilated by the organism (Batson et al. 1996; O'Quinn 2005; Punshon et al. 2003 a,b; Sowder et al. 1996; Edwards 2012). This is supported by the fact that Edwards (2012) showed U to be 3 times lower in tadpoles with the digestive tract removed compared to those with the digestive tract intact, indicating that U stays mostly in the gut and is not absorbed into the blood. U bioavailability is directly associated with the chemical speciation of the metal which in turn is affected by the characteristics of the system including pH, water hardness, and alkalinity. Although data for speciation and bioavailability of U in sediments in limited, OU_2^{2+} and UO_2OH^+ are suggested to be the common forms bioavailable to organisms (Markich 2002). Other studies indicate that U attaches at the cell surface in place of Ca (Chao and Lin-Shiau

1995), making it available for absorption as there is no known use for U within organisms.

Aside from the control pond (Pond 1), U concentrations in food source samples and upper trophic level biota all exceeded action limits (Tables 3–5) and in some cases (Pond 2), concentrations of U were extremely elevated for food source samples (>500 μg kg^{-1}). Due to an extremely limited number of available sediment TRVs, only one action level was available for calculating the HQs, therefore, additional reference values are needed to better determine if concentrations that exceed limits may result in adverse effects. Due to this limitation, sample matrix concentrations were also compared to S.C. DHEC's MCL of U for the protection of aquatic life and human health. Uranium exceeded all action levels given by S.C. DHEC for both control and impacted ponds suggesting possible adverse effects of U throughout the entire Tims Branch system (Figures 2–4). Effects of U contamination as a result of mining have been documented to include histopathological abnormalities (liver, spleen, lungs, and testes) in Rana perezi (Marques et al. 2009). Laboratory tests have also shown morphological effects such as decreased snout-vent length and tail deformities with behavioral effects such as lowered stimulus reactions for this species (Marques et al. 2008). As U concentrations were above all provided limits and threshold values regardless of the TF, this metal continues to pose a threat to higher trophic level organisms in Tims Branch due to the extremely elevated concentrations observed.

Mercury

Unlike Ni and U, all Hg TFs were > 1 with high Monte Carlo generated probabilities, providing evidence of biomagnification for this metal (Tables 1–2). With a known high absorption rate, MeHg (methyl mercury) is the likely chemical species responsible for trophic migration of Hg in Tims Branch (Berglund et al. 1971; Charbonneau et al. 1976; Miettinen 1973). Mercury methylation is a concern in Tims Branch due to the dynamic conditions of this system undergoing periods of flooding and drought as these processes are known to increase the methylation process (Brigham et al. 2009; Driscoll 1998; Krabbenhoft 1999; Mason et al. 2000; Roy et al. 2009 a,b). Edwards (2012) found similar concentrations of Hg in Tims Branch between whole tadpoles and those with the digestive tract removed, supporting the high absorption rate for Hg in this system. Burger et al. (1998) has also shown consistent Hg concentrations on the SRS in tadpoles purged for 24, 48, and 72 hours. Evidence supporting Hg biomagnification can also be seen as tadpoles' concentrations exceed the action limits resulting in higher HQs when compared to the food sources.

With the exception of Pond 1A, tadpoles consistently resulted in higher HQs when compared to food sources; however, dragonfly larvae HQs were variable for each pond suggesting alternative food sources contributing to their diet in addition to biofilm and detritus. It is suggested that dragonfly larvae sequester certain metals into the exoskeleton and also have a tolerance to certain metals such as Cd, Cu, and Pb (Lukan 2009; Tollet et al. 2009), which may explain some of the variability observed between ponds as metal concentrations were dependent on pond. Interestingly, the TFs were mostly below 1 for

Pond 1A (Table 1), most likely due to its hydrological separation from the other Ponds in the system. That is, Pond 1A never received Hg input from M-Area (Looney et al. 2012). Therefore, the majority of Hg contamination in this pond is most likely from atmospheric deposition versus SRS activities.

Although the remediation using tin (II) chloride ($SnCl_2$) to the groundwater to reduce and mobilize Hg resulted in a reduction of Hg concentrations in redfin pickerel (Esox americanus) of up to 72% in 2010 when compared to fish sampled in 2006 before the addition of $SnCl_2$ (Looney et al. 2012), Mercury concentrations exceeded action limits and increase with trophic level posing a risk to higher trophic level organisms. A study using treatments of methylmercuric chloride contaminated water resulted in mortality of Rana pipiens tadpoles (≥ 0.05 mg kg^{-1}) and inhibited development (0.001–0.01 mg kg^{-1}) over a 48-hour time period (Chang et al. 1974). Aside from Pond 3 (<0.045 mg kg^{-1}), tadpoles consistently exceeded these concentrations in all ponds collected suggesting risk for these organisms (Figures 2–4). While biofilm cumulative distributions were variable for each pond when compared to S.C. DHEC CCC and MCL limits, tadpole concentrations surpassed these limits for all ponds from which they were sampled, including the control (Figures 2–4). Although remediation activities for Hg have already begun with additions of $SnCl_2$ into Tims Branch (Looney 2012), Hg still appears to be bioavailable and is biomagnifying while exceeding action limits, demonstrating a higher mobility than the other metals analyzed. With elevated Hg TFs and HQs for upper trophic level organisms, it is likely that Hg is moving in the system and accumulating at higher trophic levels, posing a risk to these organisms.

CONCLUSION

The best practice for deriving reliable inferences when risk indices are used, is to consider the findings of all appropriate measures, taking the relative strengths and weaknesses of each method into account and identify discrepancies to give way to the correct conclusion. This study yielded similar trends across measures indicating confidence in the risk measures. These measures supported previous findings (Batson et al. 1996; Chow et al. 2005; Gaines et al. 2005; Murray et al. 2010, O'Quinn 2005; Punshon et al. 2003a,b; Sowder et al. 1996, SRNS 2011) showing risk based on human and environmental endpoints. That is, high HQs and large areas of the cumulative distributions exceeding action levels support the conclusions of these studies. However, how these action limits and endpoint measures fit into the Tims Branch system management plan must be considered, since there is no access to Tims Branch on the SRS by the public. Given that the focus on this system as a RCRA/CERCLA unit is how these COPCs may adversely affect environmental health, the data suggest that tadpoles are the best receptor organism for monitoring over the long-term, as they are vulnerable based on their life history, are relatively stationary within the system, are food for higher organisms, are easy to observe, and tend to show adverse effects genotypically and phenotypically. During this study, tadpoles were identified to have Ranavirus (Hoverman et al. 2012; Robert et al. 2012), which is indicative of stress. It is unclear as to whether entire populations are at risk within the system, however, it is clear that the COPCs are still of concern and that the system should be closely monitored.

Since this site is unique in terms of contamination events, COPC mixtures, and ecosystem dynamics it is extremely important to evaluate these finding in context as its own RCRA/CERCLA site and therefore the empirical data is extremely important. With that, Monte Carlo simulations based on a best fitting distribution are valuable as a probabilistic measure to evaluate risk. However, caution should be taken because these analyses can bias interpretations in terms of the precision of the estimate due to the fact that the analysis calculates the probabilities based upon a fitted distribution, not the empirical data. The actual fit of the distribution to the data is not used in the calculation of the probabilities and thus introduces an additional uncertainty into the analysis.

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1 Editor's note: The Resource Conservation and Recovery Act (RCRA) gives USEPA the authority to control hazardous waste from the "cradle-to-grave." This includes the generation, transportation, treatment, storage, and disposal of hazardous waste. USEPA has authority to grant permits to facilities capable of hazardous waste management.

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Table 1. Transfer factors (TFs, Eq. 2) for Ni, U, and Hg calculated from biofilm to tadpole from each sampled pond within the Tims Branch tributary. TFs are calculated for the minimum (Min), 25%, 50%, 75%, and maximum (Max) values of each trace metal concentration.

Value	Metal	Ponds						
		All	1A	1	2	3	5A	5B
Max	Ni	0.148	0.219	0.421	0.235	0.196	0.090	0.116
	U	0.186	0.254	4.038	0.200	0.152	0.171	0.243
	Hg	1.953	1.222	1.758	1.953	2.032	8.00	2.327
75%	Ni	0.106	0.188	0.350	0.056	0.171	0.064	0.109
	U	0.140	0.201	2.695	0.070	0.135	0.113	0.186
	Hg	1.899	0.297	3406.871	1.630	4.675	6.13	3.624
50%	Ni	0.106	0.189	0.262	0.050	0.159	0.055	0.099
	U	0.150	0.185	1.842	0.059	0.190	0.088	0.192
	Hg	4.032	0.256	3937.854	1.842	1876.056	6.22	3.656
25%	Ni	0.113	0.143	0.226	0.046	0.154	0.063	0.097
	U	0.523	0.156	1.397	0.048	0.156	0.081	0.185
	Hg	2133.779	0.000	3154.302	1.710	4134.800	5.03	2358.051
Min	Ni	0.000	0.087	0.000	0.005	0.244	0.043	0.113
	U	0.001	0.090	0.001	0.022	0.242	0.067	0.271
	Hg	1.460	0.000	2211.976	3412.019	4115.376	4.54	2233.792

Table 2. Transfer factors (TFs, Eq. 2) for Ni, U, and Hg calculated from biofilm (n = 202) and/or detritus (n = 17), to tadpole (n = 295) and/or dragonfly larvae (n = 21) sample matrices from all combined ponds located within the Tims Branch tributary. TFs are calculated for the minimum (Min), 25%, 50%, 75%, and maximum (Max) values of

each trace metal and tissue type. Values in parentheses ( ) show the probabilities of TFs ≥ 1 based on the variability of the concentrations as quantified via Monte Carlo resampling.

Value	Metal	Matrix Type			
		Biofilm/ Tadpoles	Detritus/ Tadpoles	Biofilm/ DF Larvae	Detritus/ DF Larvae
Max	Ni	0.148	0.108	0.058	0.043
	U	0.186	0.112	0.139	0.084
	Hg	1.953	2.855	1.025	1.499
75%	Ni	0.106	0.142	0.095	0.127
	U	0.140	0.132	0.112	0.105
	Hg	1.899	2.777	0.735	1.075
50%	Ni	0.106	0.122	0.129	0.149
	U	0.150	0.195	0.138	0.180
	Hg	4.032	2752.888	0.001	0.954
25%	Ni	0.113	0.087	0.137	0.106
	U	0.523	0.400	0.182	0.139
	Hg	2133.779	1753.525	0.856	0.703
Min	Ni	0.000	0.000	0.622	0.302
	U	0.001	0.000	0.657	0.273
	Hg	1.460	0.755	1.460	0.755
Prob	*Ni*	*(0.040)*	*(0.079)*	*(0.048)*	*(0.089)*
TF ≥	*U*	*(0.065)*	*(0.211)*	*(0.086)*	*(0.226)*
1	*Hg*	*(0.793)*	*(0.655)*	*(0.468)*	*(0.177)*

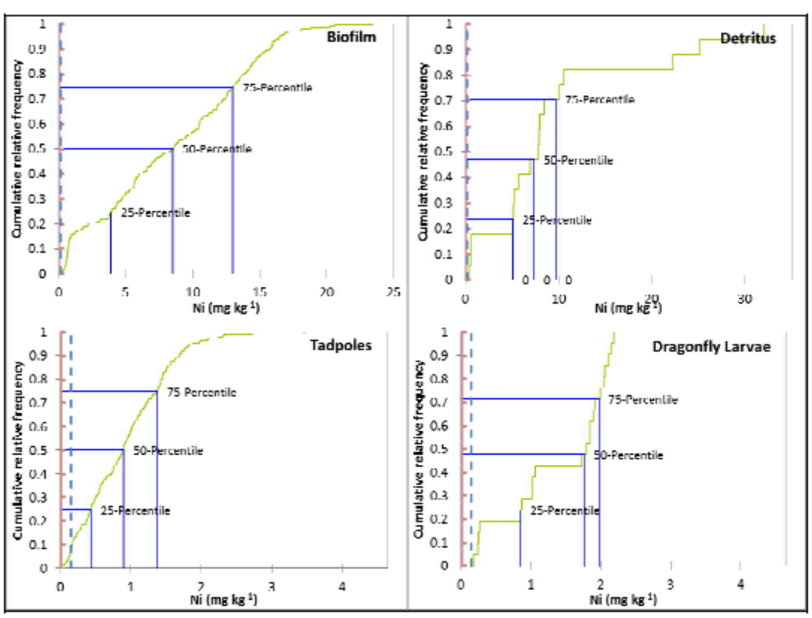
Table 3. Hazard Quotients (HQs) for Ni, U, and Hg (maximum concentrations) calculated for biofilm (n = 202), detritus (n = 17), dragonfly larvae (n = 21), and tadpole (n = 295) sample matrices from all combined ponds (Eq. 3) using the ecological screening (threshold effect concentration) values (mg kg^{-1}) for soil and sediment that are used by the SRS (Friday, 1998). To determine raw concentrations, the HQs can be multiplied by the action levels. Values in parentheses ( ) show the probabilities of HQs ≥ 1 based on the variability of the concentrations as quantified via Monte Carlo resampling.

Metal	Sample Matrix		Threshold Effect Concentrations								
			EPA Region IV			Ecotox Thresholds	Environment Canada		Dutch Ministry Standards		ORNL
			EV	CLP-PQL	SV	ERL	TEL	PEL	TV	IV	SP*
Ni		Action Level	15.9	8	15.9	21	18	35.9	35	210	-
	Biofilm	HQ	14.77	29.35	14.77	11.18	13.04	6.54	6.71	1.12	-
		Prob HQ ≥ 1	*(0.867)*	*(0.909)*	*(0.867)*	*(0.837)*	*(0.856)*	*(0.752)*	*(0.757)*	*(0.000)*	
	Detritus	HQ	20.15	40.05	20.15	15.26	17.80	8.92	9.15	1.53	-
		Prob HQ ≥ 1	*(0.844)*	*(0.916)*	*(0.844)*	*(0.799)*	*(0.824)*	*(0.682)*	*(0.689)*	*(0.111)*	-
	Tadpoles	HQ	2.18	4.33	2.18	1.65	1.93	0.97	0.99	0.17	-
		Prob HQ ≥ 1	*(0.090)*	*(0.376)*	*(0.090)*	*(0.032)*	*(0.060)*	*(0.001)*	*(0.001)*	*(0.000)*	-
	Dragonfly Larvae	HQ	0.86	1.71	0.86	0.65	0.76	0.38	0.39	0.07	-
		Prob HQ ≥ 1	*(0.107)*	*(0.499)*	*(0.107)*	*(0.036)*	*(0.069)*	*(0.001)*	*(0.002)*	*(0.000)*	-
Hg		Action Level	**0.13**	**0.02**	**0.13**	**0.15**	**0.174**	**0.486**	**0.3**	**10**	-
	Biofilm	HQ	8.32	54.07	8.32	7.21	6.21	2.23	3.60	0.11	-
		Prob HQ ≥ 1	*(0.271)*	*(0.444)*	*(0.271)*	*(0.255)*	*(0.239)*	*(0.131)*	*(0.180)*	*(0.000)*	
	Detritus	HQ	5.69	36.98	5.69	4.93	4.25	1.52	2.47	0.07	-
		Prob HQ ≥ 1	*(0.730)*	*(0.938)*	*(0.730)*	*(0.694)*	*(0.654)*	*(0.231)*	*(0.459)*		
	Tadpoles	HQ	16.24	105.57	16.24	14.08	12.13	4.34	7.04	0.21	-
			(0.837)	*(0.915)*	*(0.837)*	*(0.821)*	*(0.802)*	*(0.495)*	*(0.679)*	*(0.000)*	
	Dragonfly Larvae	HQ	8.53	55.43	8.53	7.39	6.37	2.28	3.70	0.11	-
		Prob HQ ≥ 1	*(0.224)*	*(0.349)*	*(0.224)*	*(0.214)*	*(0.203)*	*(0.125)*	*(0.161)*	*(0.000)*	-
U		Action Level	-	-	-	-	-	-	-	-	**5**
	Biofilm	HQ	-	-	-	-	-	-	-	-	82.45
		Prob HQ ≥ 1									*(0.859)*
	Detritus	HQ	-	-	-	-	-	-	-	-	136.98
		Prob HQ ≥ 1									*(0.917)*
	Tadpoles	HQ	-	-	-	-	-	-	-	-	15.29
		Prob HQ ≥ 1									*(0.827)*
	Dragonfly Larvae	HQ	-	-	-	-	-	-	-	-	11.46
		Prob HQ ≥ 1	-	-	-	-	-	-	-	-	*(0.745)*

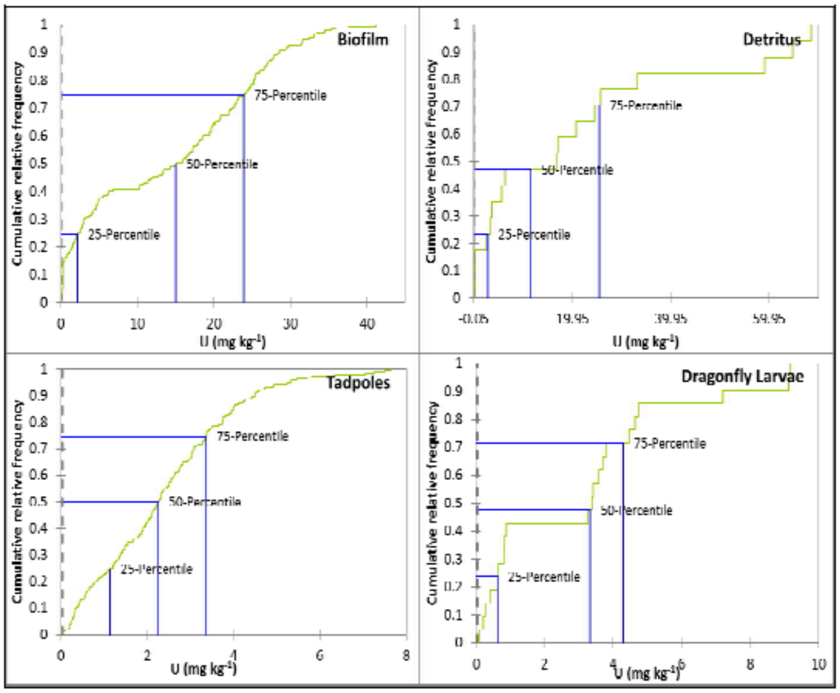
EV = Effects value (EPA, 1995), CLP-PQL = Practical Quantification Limit (EPA, 1995), SV = Screening Value (EPA, 1995), ERL = Effects Range-Low (EPA, 1996b), TEL = Threshold Effects Level (Environment Canada, 1995), PEL = Probable Effects Level (Environment Canada, 1995), TV = Target Value (Ministry of Housing, Spatial Planning and Environment, 1994), IV = Intervention Value (Ministry of Housing, Spatial Planning and Environment, 1994) ORNL = Oak Ridge National Laboratory, SP = Soil Phytotoxicity (Efroymson, et al., 1997), - = No guideline

Figure 1. Map of the U.S. Department of Energy's (DOE) Savannah River Site (SRS) located in western South Carolina, USA highlighting the Tims Branch study area. The schematic of the Tims Branch study area, a second order stream system consisting of impounded ponds and braided streams, shows the location of the A & M area, the M-Area drainage ditch contaminant input, all ponds sampled (1A, 1, 2, 3, 4, 5A, and 5B) as well as Steed Pond (currently unimpounded).

2(a)



2(b)



2(c)

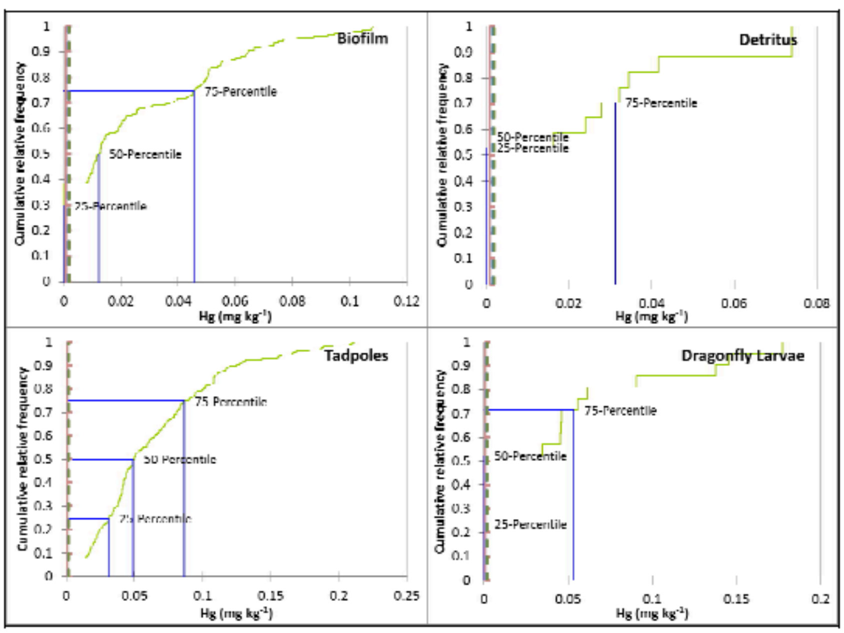


Figure 2 (a-c). Empirical cumulative distributions of Ni(a), U(b), and Hg(c) for biofilm, detritus, tadpole, and dragonfly larvae sample matrices from all combined ponds within the Tims Branch tributary. Included in these graphs are the South Carolina Department of Health and Environmental Control water quality numeric criteria for the protection of aquatic life and human health utilizing criterion continuous concentrations (CCC, ¬¬) and criterion maximum concentration (CMC, ---) for Ni and Hg, and the maximum concentration level for U and Hg (---, MCL, S.C.DHEC, 2008).

Pond 1 (control)

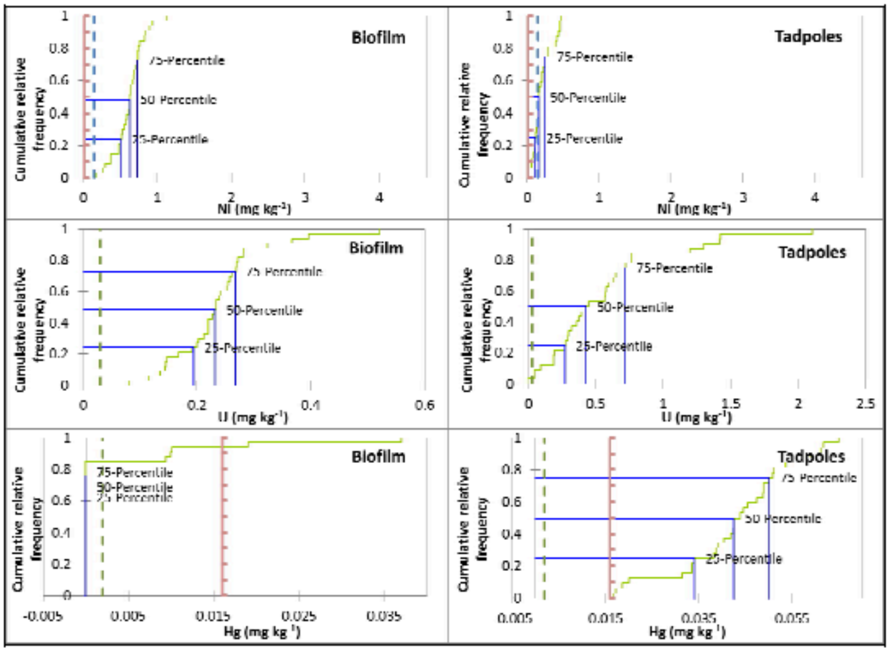
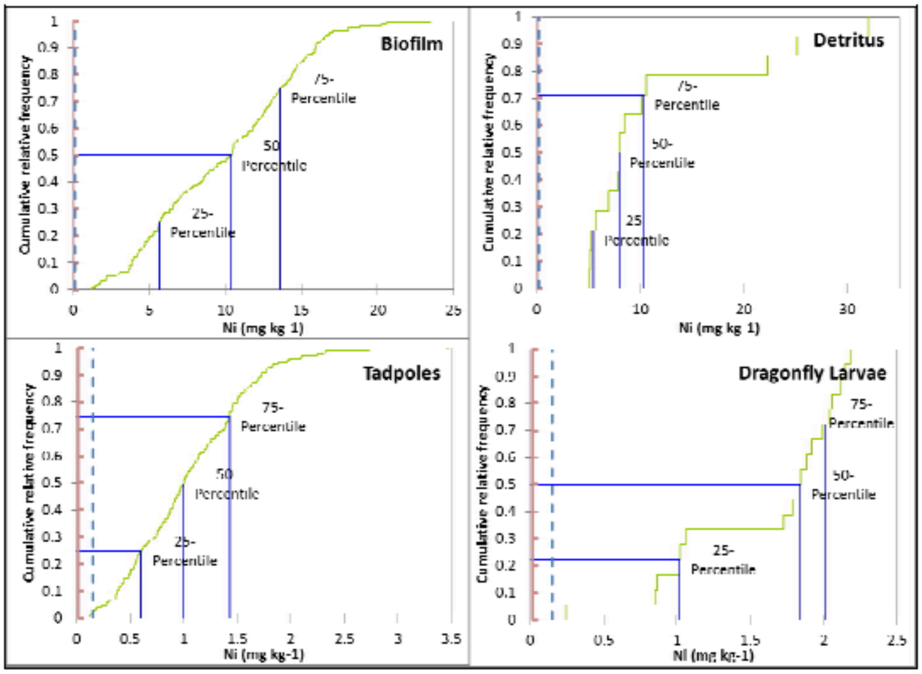
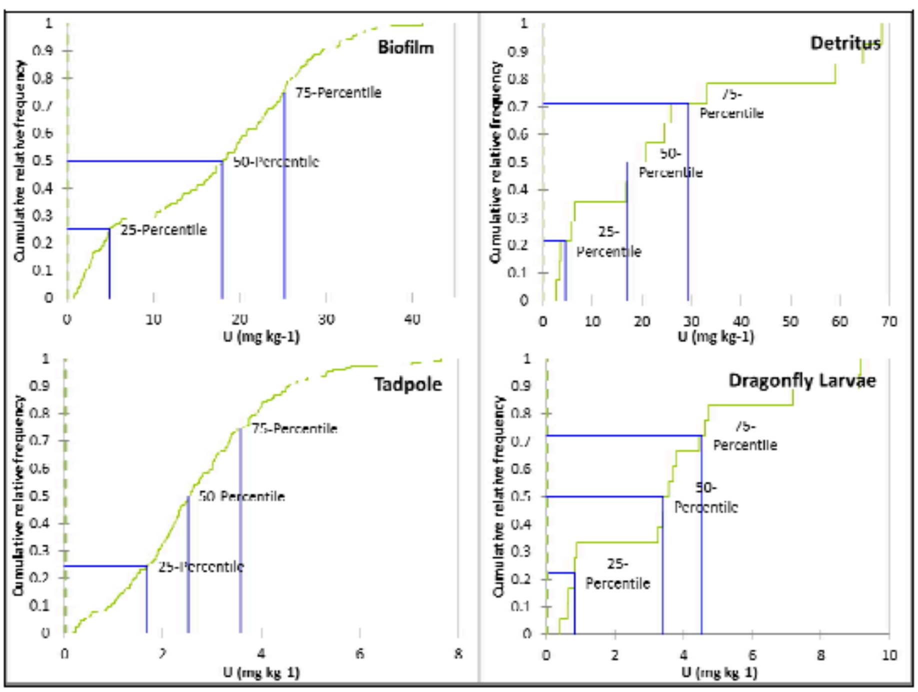


Figure 3. Empirical cumulative distributions of Ni, U, and Hg for biofilm (N = 34) and tadpole (N = 60) sample matrices from Pond 1 (control) within the Tims Branch tributary. Included in these graphs are the South Carolina Department of Health and Environmental Control water quality numeric criteria for the protection of aquatic life and human health utilizing criterion continuous concentration (CCC, ¬¬) and criterion maximum concentrations for Ni and Hg (CCC, ---), and the maximum concentration level for U and Hg (---, MCL, S.C.DHEC, 2008).

4(a)


Biofilm
Detritus
Tadpoles
Dragonfly Larvae
25-Percentile
50-Percentile
75-Percentile
Cumulative relative frequency
Ni (mg kg-1)

4(b)


Biofilm
Detritus
Tadpole
Dragonfly Larvae
25-Percentile
50-Percentile
75-Percentile
Cumulative relative frequency
U (mg kg-1)

4(c)

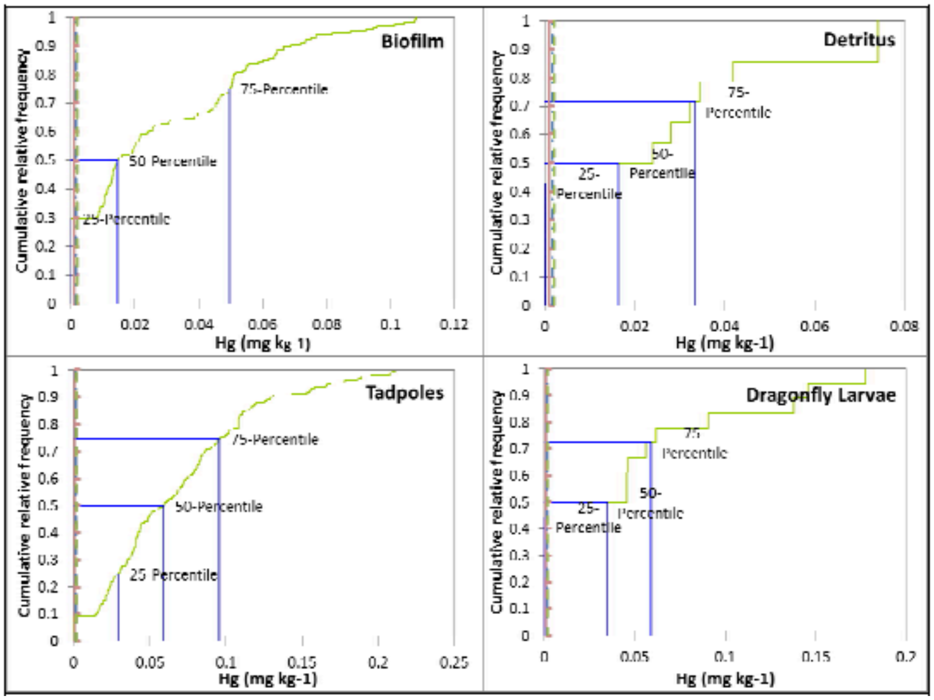


Figure 4 (a-c). Empirical cumulative distributions of Ni(a), U(b), and Hg(c) for biofilm, detritus, tadpole, and dragonfly larvae sample matrices from all impacted ponds within the Tims Branch tributary. Included in these graphs are the South Carolina Department of Health and Environmental Control water quality numeric criteria for the protection of aquatic life and human health utilizing criterion continuous concentrations (CCC, ¬¬) and criterion maximum concentration (CMC, ---) for Ni and Hg, and the maximum concentration level for U and Hg (---, MCL, S.C.DHEC, 2008).