Environmental Toxicology and Chemistry, Vol. 32, No. 9, pp. 2065–2068, 2013

Printed in the USA

Short Communication

THE USE OF TETRAGNATHID SPIDERS AS BIOINDICATORS OF METAL EXPOSURE AT A COAL ASH SPILL SITE

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(Submitted 8 January 2013; Returned for Revision 3 February 2013; Accepted 6 May 2013)

Abstract: On 22 December 2008, a dike containing coal fly ash from the Tennessee Valley Authority Kingston Fossil Fuel Plant (TN, USA) failed, resulting in the largest coal ash spill in US history. The present study was designed to determine sediment metal concentrations at multiple site locations and to determine whether site-specific bioaccumulation of metals existed in tetragnathid spiders. Selenium and nickel were the only 2 metals to exceed the US Environmental Protection Agency sediment screening levels. Selenium concentrations in spiders were significantly higher at ash-affected sites than in those from reference sites. The ratio of methylmercury to total mercury in spiders was found to be similar to that in other organisms (65–75%), which highlights the potential use of tetragnathid spiders as an indicator species for tracing contaminant transfer between the aquatic and terrestrial ecosystems. *Environ Toxicol Chem* 2013;32:2065–2068. © 2013 SETAC

Keywords: Spiders Tetragnathid Selenium Mercury Coal ash

INTRODUCTION

On 22 December 2008, the largest coal ash spill in US history occurred when a dike containing coal fly ash from the Tennessee Valley Authority Kingston Fossil Fuel Plant near Kingston, Tennessee, USA, failed and released over 3.7 million cubic meters of wet coal ash into adjacent waterways and over land [1–3] (Figure 1). Approximately 2.3 million cubic meters of ash was deposited in the Emory River, filling the channel with up to 9 m of fly ash [3].

Beyond the immediate biotic and abiotic effects of the spill, long-term toxicological concerns exist due to the potential exposure to constituents associated with coal ash [4,5]. Many of these constituents, including selenium, mercury, arsenic, and zinc, have been shown to negatively impact aquatic and terrestrial ecosystems [4,6–8].

Two constituents of particular concern are mercury and selenium. In the aquatic environment, inorganic mercury can be methylated to methylmercury, which has been shown to bioaccumulate and biomagnify, potentially resulting in high concentrations in biota from low aquatic doses [9–11]. Selenium also has the propensity to bioaccumulate within the food chain [12] at field sites impacted by coal-fired power plants [7,8,13,14], including the Kingston fly ash spill site [15].

Ecological subsidies, which describe the transfer of materials from one habitat to another, can play a vital role in contaminant export from the aquatic to the terrestrial ecosystem [16–22]. Emergent aquatic insects have been shown to be important vectors between these 2 ecosystems, with their consumption being directly linked to contaminant exposure in riparian predators, such as birds [9,11,18,19]. Recently, the use of riparian spiders has gained attention as a potential bioindicator for contaminant flux [20–22]. Of particular interest are tetragnathid spiders due to their global distribution in riparian zones [23], specialized consumption of aquatic insects [21], and usefulness in tracking contaminant transport from aquatic to terrestrial ecosystems [20,21].

Given the short time since this spill occurred, only a limited number of studies have addressed its potential impacts [1,3,4,15]. The present study is the first to investigate the potential transfer of ash-associated metals from the aquatic to the terrestrial ecosystem using tetragnathid spiders. The specific objectives were to determine: 1) whether site-specific bioaccumulation of metals exists in tetragnathid spiders and whether spider metal concentration relates to sediment metal concentrations; 2) the relative ratio of methylmercury to total mercury in tetragnathid spiders; and 3) the usefulness of tetragnathid spiders as bioindicators for metal transfer between the aquatic and terrestrial ecosystems.

MATERIALS AND METHODS

Site locations and spider collection

Tetragnathid spiders (Araneae, Tetragnathidae) were collected from 2 sites affected by the ash spill: Emory River mile (ERM) 1 and ERM 3 (Figure 1). Spiders were also collected from 2 reference sites upstream of the spill site: ERM 6 and Little Emory River mile (LERM) 2. Spiders were collected by hand from riparian vegetation (primarily hanging tree branches over the water) in July 2012 (sample sizes: ERM 1, $n = 130$; ERM 3, $n = 173$; ERM 6, $n = 129$; LERM 2, $n = 128$). All spiders were stored on ice after collection, weighed to the nearest 0.1 mg, and stored at –20 °C until metal analysis.

Analytical methods

Three composite replicates of approximately 20 to 25 spiders from each site were weighed and freeze-dried (average spider moisture ± standard deviation [SD] = 65.8 ± 1.8%) prior to

* Address correspondence to Ryan.Otter@mtsu.edu.
Published online 18 May 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2277

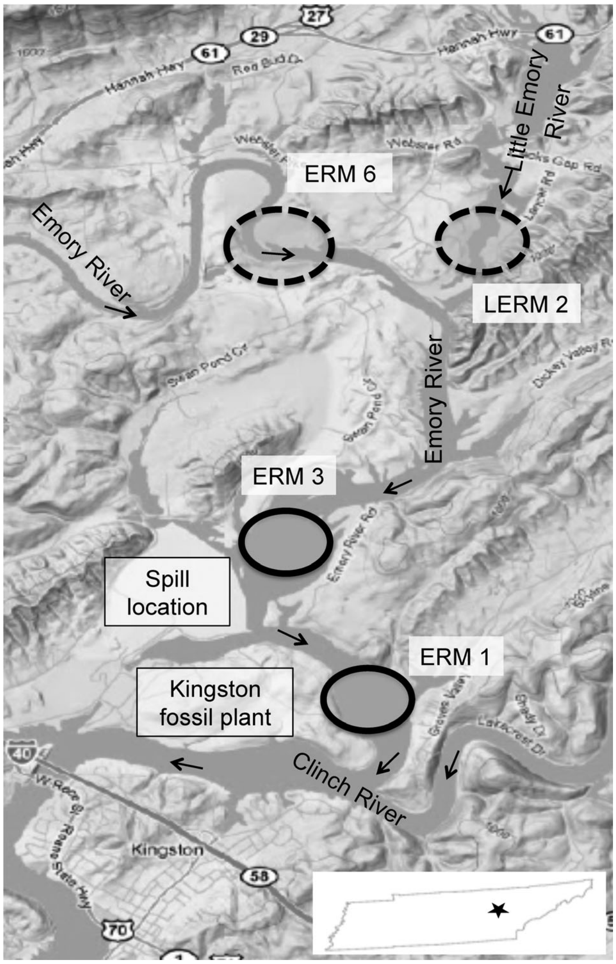

Figure 1. Map of spill area and sampling locations. Sampling locations are labeled by river mile marker on the Emory River (ERM) and Little Emory River (LERM 2). Dashed circles represent spider collection areas for reference sites (ERM 6 and LERM 2), and complete circles represent spider collection areas for ash-impacted sites (ERM 1 and ERM 3). Arrows indicate water flow direction. Base map provided by Google maps.

analysis. Superficial (>15 cm) sediment samples from each site ($n = 3$–7) were collected between 23 May 2011 and 12 December 2012. Spiders and sediment were analyzed for aluminum, lead, molybdenum, nickel, silver, strontium, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, vanadium, zinc, and selenium (dry wt) using inductively coupled plasma mass spectroscopy (US Environmental Protection Agency [USEPA] method 6020). Quality control measures consisted of blanks, duplicates, spikes, and analysis of reference standard material every 20 samples. Likewise, 3 composite replicates (~20–25 spiders) and sediment from each site were also analyzed for total mercury and methylmercury concentrations via USEPA method 1631 and method 1630, respectively.

Statistical analysis

Site differences in metal concentrations of spiders and sediment were investigated using one-way analyses of variance with Tukey's post hoc tests. All analyses were performed using JMP Pro 9 software. Significant differences were defined by p values < 0.05.

RESULTS

Significant differences in spider bioaccumulation of silver ($F_{(3,11)} = 144.5$; $p < 0.001$), strontium ($F_{(3,11)} = 4.85$; $p = 0.032$), cobalt ($F_{(3,11)} = 7.20$; $p = 0.012$), zinc ($F_{(3,11)} = 12.52$; $p = 0.002$), and selenium ($F_{(3,11)} = 54.91$; $p < 0.001$) were observed between sites (Table 1). Silver and zinc concentrations in spiders were significantly higher at reference sites compared with ash sites. In contrast, cobalt was found to be significantly elevated at ERM 1 compared with ERM 6 ($p = 0.0253$) and LERM 2 ($p = 0.0179$). Selenium concentrations were significantly higher at ash-associated sites than at reference sites ($p < 0.001$ for each comparison) (Figure 2). Analysis of total mercury ($F_{(3,11)} = 26.31$; $p < 0.001$) and methylmercury ($F_{(3,11)} = 14.99$; $p = 0.001$) in spiders showed that site was a significant factor, with concentrations at ERM 6 being significantly higher than at all other sites (Figure 3). Methylmercury was on average 70.5% of the total mercury

Table 1. Bioaccumulation of metals (mg/kg dry wt) in tetranathid spiders and sediment

	ERM 1		ERM 3		ERM 6		LERM 2	
Metal	Spider	Sediment	Spider	Sediment	Spider	Sediment	Spider	Sediment
Al	13.43 ± 0.58	13933 ± 950[c,d]	17.30 ± 3.60	10315 ± 979[c,d]	17.03 ± 5.69	2810 ± 708	15.30 ± 3.19	6167 ± 1326
Pb	0.09 ± 0.01	16.5 ± 1.57[c]	0.09 ± 0.01	12.4 ± 0.49[c]	0.14 ± 0.02	5.9 ± 1.3	0.09 ± 0.01	21.2 ± 5.8[c]
Mo	0.16 ± 0.03	− ± -	0.14 ± 0.01	− ± -	0.16 ± 0.02	5.94 ± 0.43	0.13 ± 0.01	3.81 ± 0.01
Ni	0.21 ± 0.03	25.1 ± 1.5[c,d]	0.19 ± 0.01	16.5 ± 1.3[c,d]	0.22 ± 0.02	8.2 ± 1.8	0.23 ± 0.03	8.5 ± 1.4
Ag	0.06 ± 0.01[a,b]	0.92 ± 0.02	0.15 ± 0.02[a,b]	0.75 ± 0.01	0.31 ± 0.01[b]	0.74 ± 0.05	0.73 ± 0.04[a]	0.48 ± 0.01
Sr	1.43 ± 0.12	124.3 ± 3.2[c,d]	2.23 ± 0.19	83.3 ± 46.5[c,d]	2.90 ± 0.60[b]	5.9 ± 0.4	1.33 ± 0.18[a]	4.0 ± 0.2
As	0.49 ± .07	− ± -	0.43 ± 0.04	− ± -	0.35 ± 0.03	2.3 ± 0.4	0.38 ± 0.03	9.9 ± 3.1
Ba	0.87 ± 0.09	229.3 ± 5.4[c,d]	1.10 ± 0.06	151.7 ± 42.6[c,d]	1.57 ± 0.26	38.1 ± 9.8	1.21 ± 0.14	62.1 ± 12.7
Be	0.07 ± 0.01	1.96 ± 0.06[c,d]	0.07 ± 0.01	1.56 ± 0.57[c,d]	0.07 ± 0.01	0.61 ± 0.05	0.07 ± 0.01	0.41 ± 0.03
Cd	1.63 ± 0.18	0.18 ± 0.01	1.73 ± 0.09	0.15 ± 0.01	2.33 ± 0.59	0.21 ± 0.06	1.27 ± 0.12	0.41 ± 0.13
Cr	0.44 ± 0.06	21.4 ± 1.1[c,d]	0.41 ± 0.03	14.8 ± 3.6[c]	0.42 ± 0.02	3.9 ± 0.7[d]	0.53 ± 0.10	12.9 ± 4.3[c]
Co	0.37 ± 0.10[a,b]	16.4 ± 0.9[c,d]	0.07 ± 0.03	10.8 ± 0.3[c]	0.08 ± 0.02	6.0 ± 1.4	0.05 ± 0.00	9.4 ± 2.6
Cu	61.93 ± 2.72	27.9 ± 0.8[c,d]	61.03 ± 6.85	19.4 ± 6.9[c,d]	53.10 ± 5.00	4.2 ± 1.0	48.67 ± 2.10	5.7 ± 0.7
V	0.17 ± 0.02	41.7 ± 3.0[c,d]	0.14 ± 0.01	27.3 ± 9.2[c,d]	0.14 ± 0.02	5.5 ± 1.2	0.16 ± 0.03	13.2 ± 3.9
Zn	175.33 ± 7.31[a,b]	64.7 ± 3.1[c,d]	190.33 ± 3.28[a]	46.0 ± 3.1[d]	226.00 ± 5.86	27.5 ± 6.2	206.67 ± 7.26	26.8 ± 3.2

[a]Significantly different spider concentration from ERM 6 (ANOVA; post hoc Tukey's test; $\alpha = 0.05$)
[b]Significantly different spider concentration from LERM 2 (ANOVA; post hoc Tukey's test; $\alpha = 0.05$)
[c]Significantly different sediment concentration from ERM 6 (ANOVA; post hoc Tukey's test; $\alpha = 0.05$)
[d]Significantly different sediment concentration from LERM 2 (ANOVA; post hoc Tukey's test; $\alpha = 0.05$)
ERM = Emory River mile; LERM = Little Emory River mile; ANOVA = analysis of variance.

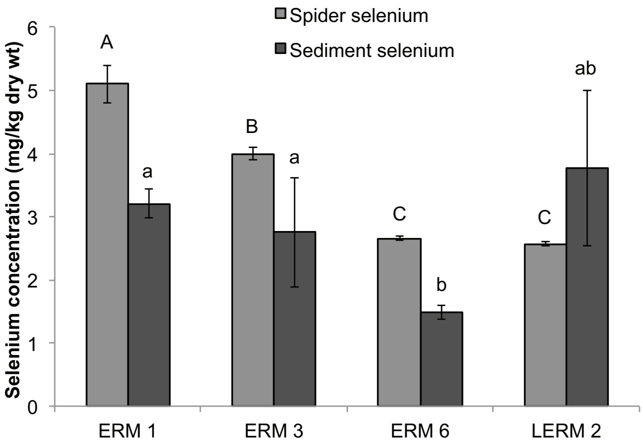

Figure 2. Total selenium concentration in tetragnathid spiders and sediment. Significant differences between sites are indicated by different upper case letters (spiders) and lower case letters (sediment) ($p < 0.05$, analysis of variance). Data presented as mean ± SE.

found in spiders across all sites, with little variation between sites (ERM 1 = 72%, ERM 3 = 67%, ERM 6 = 66%, and LERM 2 = 76%).

Analysis of sediment metal concentrations revealed significantly higher concentrations of aluminum ($F_{(3,20)} = 33.25$; $p < 0.001$), nickel ($F_{(3,20)} = 26.93$; $p < 0.001$), strontium ($F_{(3,20)} = 11.87$; $p < 0.001$), barium ($F_{(3,20)} = 43.68$; $p < 0.001$), beryllium ($F_{(3,20)} = 28.81$; $p < 0.001$), copper ($F_{(3,20)} = 33.92$; $p < 0.001$), and vanadium ($F_{(3,20)} = 28.01$; $p < 0.001$) at both of the ash-impacted sites compared with both of the reference sites (Table 1). Sediment concentrations of chromium ($F_{(3,20)} = 25.20$; $p < 0.001$), cobalt ($F_{(3,20)} = 11.61$; $p < 0.001$), selenium ($F_{(3,20)} = 6.56$; $p = 0.003$), and zinc ($F_{(3,20)} = 13.72$; $p < 0.001$) were significantly higher at impacted sites compared with at least 1 reference site.

DISCUSSION

The large-scale spill at the Kingston Fossil Fuel Plant provides a unique opportunity to better understand the bioavailability of coal-ash–related metals. To date, the vast majority of research investigating the potential environmental impacts of coal ash in the environment has focused on chronic effects in engineered impoundments or small receiving streams [14,24,25], but little information exists on the transfer of ash-associated contaminants from aquatic to terrestrial consumers. In the present study, sediment concentrations revealed that no metal exceeded USEPA sediment screening levels, with the exception of selenium and nickel. Selenium exceeded the threshold of 2.0 mg/kg at each site, except at ERM 6, and nickel exceeded its threshold of 22.7 mg/kg only at ERM 1.

Spiders have previously been used as bioindicators at metal-contaminated field sites [26,27] due to their propensity to accumulate metals and their status as a valuable prey item to higher trophic–level organisms (e.g., birds) [9,28]. In the present study, the majority of metals analyzed showed no significant site differences in spiders (Table 1). In contrast, sediment metal concentrations were higher at ash sites compared with reference sites, showing an increased presence of metals but indicating a lack of bioavailability. Bioavailability of metals in sediment is complex and been shown to be influenced by many factors, including organic carbon [29,30]. Because fly ash from this spill is known to have a considerable amount of organic carbon (~4.5%) [3], it is possible that this could, at least partially, explain the differences observed between metal concentrations in sediment and spiders.

Selenium is a contaminant of concern at the Kingston spill site due to risks to aquatic and terrestrial ecosystems [4,6]. The bioavailability of selenium from coal ash can be affected by many environmental factors [31]. Previous studies at coal-ash–influenced sites have shown significant bioaccumulation of selenium in various organisms, including redear sunfish [7,15], turtles [25], snakes [32], tadpoles [32], crayfish [25], caddisflies [14], bluegill [14,24], and green sunfish [24]. In the present study, concentrations of selenium in tetragnathid spiders were found to be significantly elevated at ash-associated sites compared with references sites (Figure 2). Unfortunately, limited data exist to predict whether the concentrations of selenium found in spiders was high enough to cause concern for potential predators. The USEPA lists a draft tissue concentration of 7.9 mg/kg as the chronic aquatic life criteria for selenium [33]; however, this value is based on whole-body fish concentrations (juvenile bluegill), and it is unclear whether this value is relevant for spiders. Plausible explanations for the selenium differences observed could be a simple increase of selenium in typical spider prey items or a possible shift in spider food availability to more selenium-rich prey species, resulting from the ash spill. These data gaps highlight the need for continued investigations into selenium food web dynamics, including those at the Emory River spill site, and tetragnathid spiders as potential vectors of contaminant transfer. The results of the present study are similar to those found for redear sunfish at the same sites in the Emory River, with ovary and liver concentrations exceeding 7.9 mg/kg [15]. Results from the same study [15], however, showed no significant bioaccumulation of selenium in other typically sampled fish (largemouth bass and white crappie) compared with reference sites, indicating the need for careful selection of indicator species to fully understand the movement and fate of ash-associated contaminants.

The proportion of methylmercury found in spiders ranged from 66% to 76% of total mercury (Figure 3). Such high proportions of methylmercury have been reported in other invertebrates such as crayfish (70–80%), dobsonflies (79%), dragonflies (77–94%), and caddisflies (48%) [9–11]. Cristol et al. [17] showed that 49% of the mercury found in spiders (grouping all spider taxa together) collected from a contaminated

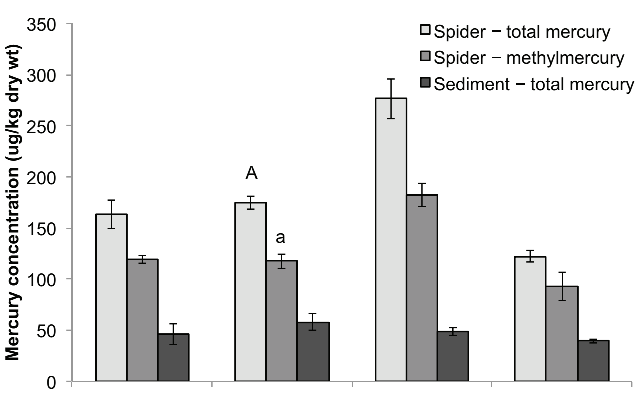

Figure 3. Total mercury and methylmercury in tetragnathid spiders and sediment. Significant differences between sites are indicated by different upper-case letters (total mercury) and lower-case letters (methylmercury) ($p < 0.05$, analysis of variance). No significant differences between sites were observed for sediment ($p < 0.05$, analysis of variance). Data presented as mean ± standard error.

field site was methylmercury. Using a similar approach of grouping spider taxa, Edmonds et al. [9] reported that spiders from forested wetlands across the boreal and Acadian ecoregions of Canada and the United States have approximately 73% of their total mercury as methylmercury. The present study is the first to report methylmercury proportions in tetragnathid spiders specifically. This focus on tetragnathids instead of sampling all spiders (Araneae), is important because previous work has shown that tetragnathids fill a unique riparian niche, with a specialized feeding preference for emergent aquatic insects.

Tetragnathid spiders are specialized riparian predators that feed largely on emergent aquatic insects. Previous research has suggested their use as key indicator species in the transfer of persistent organic contaminants from aquatic to terrestrial ecosystems [20,21], and results from the present study suggest they may be useful for studying the transfer of metals as well. The present study provides evidence for the continued use of tetragnathid spiders as a bioindicator species in studies on the environmental fate of contaminants. In addition, the present study shows that ash-associated selenium is still in flux in the system and highlights the continued need for the monitoring of selenium in response to the Kingston coal ash spill.

Acknowledgment—The present study was funded by the Tennessee Valley Authority as part of a larger project investigating the environmental consequences of the coal ash spill at the Kingston Tennessee fossil fuel plant. Additional support was provided by Middle Tennessee State University's Undergraduate Research Experience and Creative Activity Program. The Oak Ridge National Laboratory is managed by UT-Battelle for the US Department of Energy under contract number DE-ACO5-000R22725. Special thanks to B. Gendron for assistance with the collection of spiders and to T. Jeff for final laboratory preparation of samples.

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