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SPATIAL AND TEMPORAL TRENDS IN CONTAMINANT CONCENTRATIONS IN *HEXAGENIA* NYMPHS FOLLOWING A COAL ASH SPILL AT THE TENNESSEE VALLEY AUTHORITY'S KINGSTON FOSSIL PLANT

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Abstract: A dike failure at the Tennessee Valley Authority Kingston Fossil Plant in East Tennessee, United States, in December 2008, released approximately 4.1 million m^3 of coal ash into the Emory River. From 2009 through 2012, samples of mayfly nymphs (*Hexagenia bilineata*) were collected each spring from sites in the Emory, Clinch, and Tennessee Rivers upstream and downstream of the spill. Samples were analyzed for 17 metals. Concentrations of metals were generally highest the first 2 miles downstream of the spill, and then decreased with increasing distance from the spill. Arsenic, B, Ba, Be, Mo, Sb, Se, Sr, and V appeared to have strong ash signatures, whereas Co, Cr, Cu, Ni, and Pb appeared to be associated with ash and other sources. However, the concentrations for most of these contaminants were modest and are unlikely to cause widespread negative ecological effects. Trends in Hg, Cd, and Zn suggested little (Hg) or no (Cd, Zn) association with ash. Temporal trends suggested that concentrations of ash-related contaminants began to subside after 2010, but because of the limited time period of that analysis (4 yr), further monitoring is needed to verify this trend. The present study provides important information on the magnitude of contaminant exposure to aquatic receptors from a major coal ash spill, as well as spatial and temporal trends for transport of the associated contaminants in a large open watershed. *Environ Toxicol Chem* 2016;35:1159–1171. Published 2015 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

Keywords: Coal ash Metals Contaminants Exposure Mayfly nymphs Ecotoxicology

INTRODUCTION

Coal remains a major fuel source for energy production in the United States. From 2005 through September 2013, coal accounted for an average of slightly more than 45% of the net power generated in the United States [1]. Although coal use for power generation in the United States has experienced a recent decline, it is expected to remain a major fuel source for energy production in the near future [1,2].

A major concern with continued use of coal for energy production is that the combustion process produces coal ash byproducts (fly ash and bottom ash) that are enriched with several potentially hazardous trace elements, such as arsenic, mercury, and selenium [3]. The specific elements and quantities present in coal ash depend on factors such as the coal source and combustion process [3,4]. If they are released into an aquatic environment, the mobilization and fate of these elements are affected by factors such as pH, redox potential, and the amount of organic matter present [5]. Given the many factors that can affect content, release, and fate of coal ash contaminants, each unintended release of coal ash into the environment can produce unique ecological consequences. Thus, each release must be evaluated to determine the most appropriate actions to take for minimizing negative effects.

On December 22, 2008, an earthen dike failed at the Tennessee Valley Authority's Kingston Fossil Plant in Kingston, Tennessee (USA), releasing approximately 4.1 million m^3 of coal ash into the Emory River [6]. The ash was deposited on the bottom and shoreline of an estimated 1.2 km^2 of the adjacent Emory River and adjoining embayments. The spill forced some ash as far as 3.5 miles upstream from the point of the spill in the Emory River, and trace ash deposits were found in the Tennessee River as far as 11.2 miles downstream. Since the spill occurred, the amount of ash present and its distribution have been potentially affected by several factors. From March 2009 through August 2010, an estimated 2.7 million m^3 of ash was removed from the Emory River. Not only did this significantly reduce the amount of the ash in the river, it also had the potential to resuspend contaminant-laden particles and facilitate their downstream movement [7]. The lower reaches of the Clinch and Emory Rivers are part of the Watts Bar Reservoir, created by a hydroelectric dam on the Tennessee River approximately 44 miles downstream of the spill site. Operation of Watts Bar Dam slows flow velocity in the lower Emory and Clinch Rivers substantially, which would enhance deposition of fine suspended particles, including coal ash. Finally, there were 4 significant high flow events between May 2009 and May 2010 that transported ash and contaminated sediments further downstream [8]. All of these factors could have affected both spatial and temporal trends in the movement of and exposure to contaminants, thereby confounding interpretation of trends.

Dissolved contaminants in aquatic environments from natural or anthropogenic sources generally adsorb to fine-grained sediment particles (i.e., clay and silt) that settle on the surface sediments in deposition zones [9–11], where the dissolved concentrations eventually reach some equilibrium value. After coal ash enters the water, trace elements that

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desorb from the particles follow the same process, eventually resorbing to available sediment particles until equilibrium is established. Some amounts of these elements remain with the ash particles, but both particulate phases ultimately settle onto the surface sediments. Thus, the surface sediments can become enriched with the contaminants at concentrations of potential ecological concern to the benthic invertebrates inhabiting the sediment [5,12].

The nymphs of the burrowing mayfly genus *Hexagenia* live in burrows they dig in soft depositional sediments. There they ingest detritus, algae, bacteria, and other organic matter that deposit on the substrate, as well as inorganic sediment particles [13,14]. Because of their close association with and ingestion of sediment, *Hexagenia* nymphs are exposed to and bioaccumulate contaminants that are present [15]. Furthermore, the nymphs and adults may be an important link in the transfer of these contaminants to higher trophic levels such as fish [16] and terrestrial consumers [17]. For these reasons, the status of populations of *Hexagenia* species has been widely used as an indicator of ecosystem health [18,19], and the nymphs have been used in a variety of laboratory and field studies addressing the toxicity and bioaccumulation of contaminants [16,20–22].

Concentrations of metals and metalloids (herein referred to collectively as metals or contaminants) that are common coal ash contaminants have been determined annually in nymphs of the mayfly *Hexagenia bilineata* inhabiting the affected watershed since the spill. In the present study we focus on exposure to potential ash-related contaminants from the spill at the Kingston Fossil Plant. We present results on spatial and temporal trends for concentrations of metals in nymphs collected from both ash-affected and unaffected reference sites to evaluate the magnitude of potential exposures of resident biota to ash-related contaminants and help distinguish coal ash contributions from other potential sources. The present study provides valuable information on spatial trends and early temporal trends of potential ash contaminants. Not many coal ash spills have been documented in the United States, and none of this magnitude. Furthermore, because the constituents of coal ash can be specific to each coal combustion facility, it is still not known if there are risks associated with coal ash spills that can be generalized across any spill. It is imperative to document the impacts on the affected biota and watershed so that future coal ash spill assessments can use and build on lessons learned in the present study to determine if there are generalizable risks to wildlife.

MATERIALS AND METHODS

Site description

The Tennessee Valley Authority's Kingston Fossil Plant is located in East Tennessee at the base of a peninsula between the confluence of the Emory and Clinch Rivers (Figure 1). The confluence of the 2 rivers is 4.4 miles upstream of the Clinch River's confluence with the Tennessee River (confluence at Tennessee River mile 567.6). Together these 3 rivers form the headwaters of Watts Bar Reservoir. The earthen dike that failed in December 2008 was located northeast of the main facility on the right descending shoreline of the Emory River at approximately river mile 2.5.

Sampling sites for mayflies included locations on the Emory, Clinch, and Tennessee Rivers adjacent to and downstream of the spill, as well as locations that received no ash from the spill (i.e., reference sites). In 2009, the ash-affected sites assessed included Emory River mile 2.5 (ERM 2.5) immediately adjacent to the spill, Emory River mile 1.0 (ERM 1.0) near the confluence with the Clinch River, and Clinch River mile 1.5 (CRM 1.5) near the confluence with the Tennessee River. Reference sites (designated by an R at the end of the site name) included an upstream site on the Emory River at mile 6.0 (ERM 6.0-R); a site on the Clinch River upstream of its confluence with the Emory River at mile 6.0 (CRM 6.0-R); and a site on the Little Emory River, a tributary to the Emory River, 1.0 mile (LERM 1.0-R) upstream of its confluence with the Emory River at mile 5.5. After 2009 the spatial coverage of the area affected by the ash spill was expanded. A site at ERM 4.0 was added to assess the upstream presence of ash-related contaminants, and a site was added at CRM 3.5 approximately 1.0 mile downstream of the confluence with the Emory River. Three sites on the Tennessee River were added including 2 downstream of the confluence with the Clinch River at river miles 566.3 (TRM 566.3) and 560.8 (TRM 560.8), and a reference site upstream of the Clinch River at mile 572.5 (TRM 572.5-R). In the present study, reference sites represent conditions under which biota have not been exposed to coal ash from the spill.

Target species

Burrowing mayflies in the genus *Hexagenia* (Ephemeridae), including *H. bilineata*, are found throughout much of the United States from the central plains to the east coast, where they generally prefer larger rivers and impoundments [13,14]. *Hexagenia* nymphs live in shallow U-shaped burrows they excavate in highly adhesive, soft mud (clay and silt) that is common in depositional areas. The nymphs create a current of water through their burrows that carries in nearby deposited and suspended organic and inorganic particles, helps remove wastes, and is thought to help supplement oxygen needs [23]. They feed on detritus, algae, bacteria, and other organic matter that deposit around their burrows, and they also ingest large quantities of inorganic sediment particles either as an aid in grinding food in their foregut or incidentally as aggregates with detritus [24,25].

Field procedures

Mayfly nymphs were collected once annually between early May and mid-June. Four (2009) or 3 (2010–2012) samples were collected from each site with a Peterson dredge. Each sample was a composite of nondepurated nymphs collected in $\geq$10 drops of the dredge across transects perpendicular to the river channel; sampled transects at each site were approximately 100 m apart. The dredged material was washed with water from the site in a large nylon-mesh sieve, and the nymphs were removed with stainless steel forceps and placed in small glass bottles (60–120 mL). Samples were held on ice for transport back to the laboratory. Sample bottles were either new I-Chem Certified or reused bottles that had been acid washed [26].

Laboratory procedures

After return to the laboratory, the nymph samples were triple rinsed with distilled water, returned to the sample bottle, which was triple rinsed, and stored in a freezer (at $<-20\,^{\circ}$C) until further processing could be completed. Samples were later thawed, and the nymphs were lightly blotted on a clean Kimwipe tissue to remove excess external moisture. Individuals were added to new 40-mL glass I-Chem vials until the desired mass was reached ($>$1.5 g). Samples were refrozen (at

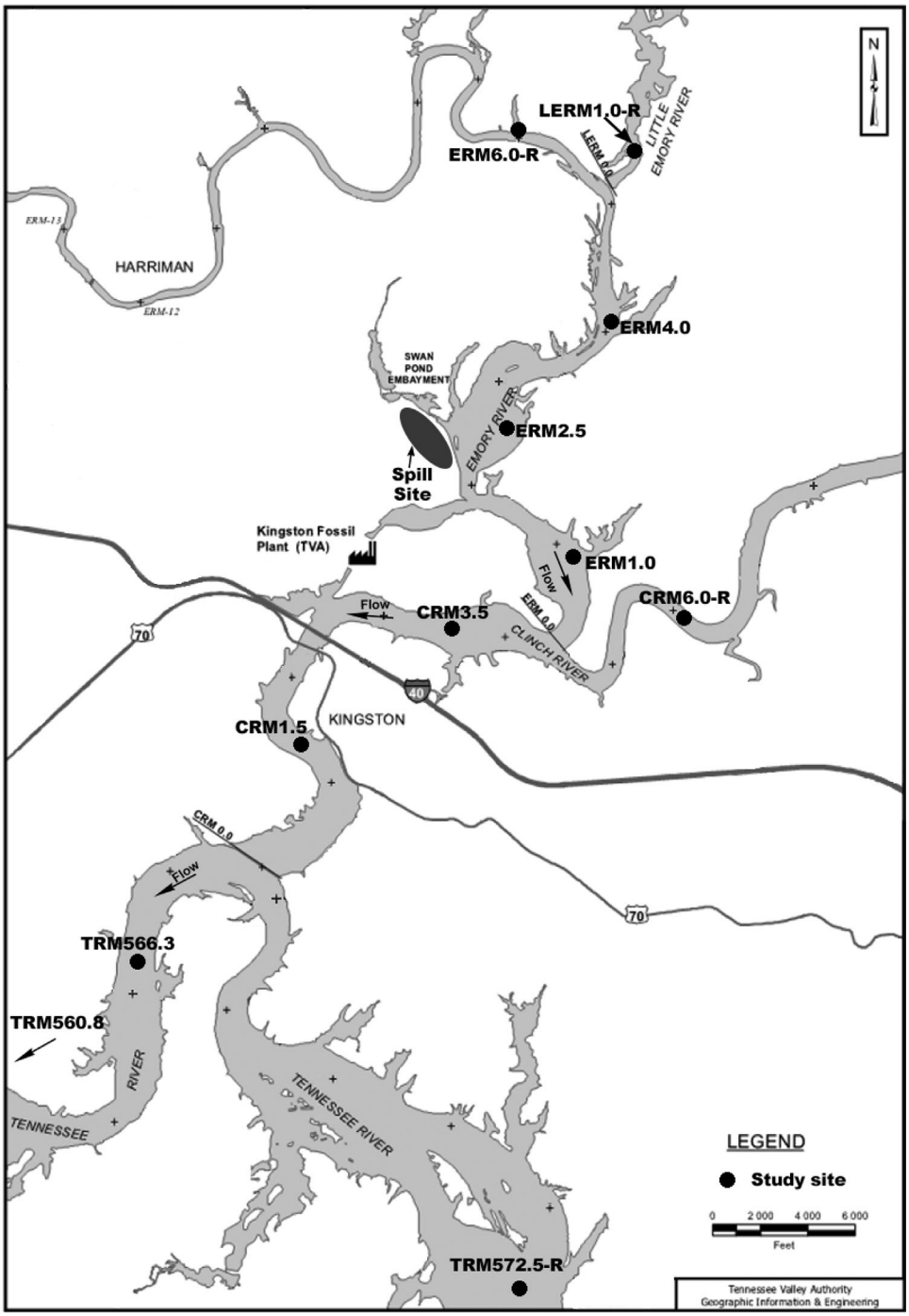

Figure 1. Map of mayfly nymph sampling sites on the Emory (ER), Clinch (CR), Tennessee (TR), and Little Emory Rivers (LER). Sampling sites are labeled with miles upstream from mouth, for example, ERM 2.5 refers to the sampling site located 2.5 miles upstream from the Emory River's confluence with the Clinch River. Tennessee River site TRM 560.8, which is downstream from TRM 566.3, is not shown on the map. The present study area shown on the map encompasses much of the upper Watts Bar Reservoir watershed.

$<-20\,^{\circ}$C) before drying to a constant weight in a freeze dryer ($\geq$90 h). Mean sample wet weights were 2.1 g ($\sim$427 mg dry wt/sample) in 2010 and $\geq$2.8 g ($\geq$600 mg dry wt/sample) in all other years. Dried samples were stored in a secure freezer (at $<-20\,^{\circ}$C) until submission for chemical analysis. Sample handling procedures followed those recommended by the US Environmental Protection Agency (USEPA) [27]. As samples were processed, distilled water rinsates of equipment potentially coming into contact with the nymphs were collected in new 1-L I-Chem bottles (i.e., Kimwipes [$n=3$], forceps [$n=2$], and Petri plates [$n=4$]). After collection, enough nitric acid (trace metal grade) was added to each sample to lower the pH to $\leq$ 2.0, and then stored in a refrigerator ($\leq 4\,^{\circ}$C) until analysis.

Analytical procedures and quality assurance

Samples (tissue and rinsates) were analyzed for 17 metals: antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr), vanadium (V), and zinc (Zn). One

aliquot from each sample was analyzed for mercury with USEPA method 7473 in a DMA-80 automatic mercury analyzer (Milestone) [26], and a second aliquot was first digested in nitric acid per USEPA method 3052 [28] and analyzed for the remaining 16 metals with USEPA method 6020A and inductively coupled plasma–mass spectrometry (ICP–MS) [29]. All quality assurance procedures (e.g., blanks, matrix spikes, etc.) were as specified for each analytical method [28,29]. The sample mass analyzed varied among samples; therefore, method detection limits were calculated separately for each sample; mean method detection limits for the metals for the nymph samples and rinsates are presented in Supplemental Data, Table S1. Concentrations of all metals in the rinsates were below the method detection limits.

Data analysis

For statistical analyses, an amount equal to half the method detection limit was used as a replacement value when concentrations were below the method detection limit (i.e., boron = 11% of samples, chromium = 2%, mercury = 6%, and molybdenum = 3%). Descriptive statistics (e.g., mean and standard error of the mean) were calculated using Statistical Analysis System (SAS) software procedures (SAS for Windows, Ver 9.2). A principal components analysis was used to visualize spatial and temporal patterns in metal concentrations, and to help identify the metals contributing to those trends. Principal components analysis is a widely used and accepted data reduction method that helps visualize and summarize large datasets with many variables [30]. Principal components were computed from a correlation matrix of $\log_{10}$ transformed metal concentrations from the individual replicate samples. Use of a correlation matrix in principal components analysis centers and standardizes the data [30], so no further adjustments were made. Scree plots and percentage of variance explained were used to help determine the number of meaningful axes to retain for follow-up analyses, and a varimax rotation was used to facilitate interpretation of the results [31]. The SAS procedure PROC FACTOR was used for principal components analysis (SAS for Windows, Ver 9.2).

A linear regression analysis was used to examine the relationship between scores (dependent variable) for the first 2 principal components (i.e., principal components analysis axis 1 or PC1, and axis 2 or PC2) and time (year; independent variable) for each site; only those sites sampled in all 4 yr were included in this analysis (ash-affected sites ERM 2.5, ERM 1.0, and CRM 1.5; reference sites ERM 6.0-R, CRM 6.0-R, and LERM 1.0-R). A slope significantly different from zero ($p \leq 0.05$) was considered an indication that overall metal concentrations changed with time. The SAS procedure PROC REG was used for the regression analyses (SAS for Windows, Ver 9.2).

RESULTS

The first 2 principal components (i.e., axes) accounted for 75.9% of the total variation, and were determined to be the most informative (Table 1 and Supplemental Data, Figure S1). Spatial and temporal differences were clearly evident on both axes. Reference sites were separated along PC1 from all ash-affected sites except ERM 4.0 and Tennessee River sites. Ten of the 17 metals (Sb, As, Ba, Be, B, Cu, Mo, Se, Sr, and V) had positive loadings >0.6 on PC1, whereas none of the metals had strong negative loadings (Table 1). The reference sites also showed some separation from ash-affected sites on PC2; samples from the ash-affected sites except ERM 4.0 and CRM 1.5 generally had positive scores, whereas scores for all reference sites except TRM 572.5-R had negative scores (Figure 2 and Supplemental Data, Figure S1). Chromium and Pb had positive loadings >0.6 on PC2, and Ba and V had high positive loadings on both PC1 and PC2. Loadings for Cd, Co, Hg, and Zn, in contrast, were relatively low on the first 2 axes.

Table 1. Loading for metals on the first 2 varimax rotated principal components from the principal components analysis[a]

Metal	PC1 (64.1%)	PC2 (11.8%)
Sb	0.8066[b]	0.4174
As	0.8284[b]	0.4200
Ba	0.6567[b]	0.6196[b]
Be	0.7086[b]	0.5676[c]
B	0.6919[b]	0.2990
Cd	0.0454	0.1967
Cr	0.4199	0.770[b]
Co	0.4136	0.3584
Cu	0.6227[b]	0.4840
Pb	0.2509	0.8500[b]
Hg	0.0790	0.1928
Mo	0.9429[b]	0.0414
Ni	0.4983	0.5011[c]
Se	0.6950[b]	0.3337
Sr	0.8165[b]	0.4842
V	0.6374[b]	0.6847[b]
Zn	−0.3335	−0.0621

[a]The percentage variance explained by each axis is given in parentheses.
[b]Loadings ≥ 0.6 and ≤ –0.6
[c]Loadings from 0.5 to 0.59 and –0.59 to –0.5.
PC1 and PC2 = principal components 1 and 2, respectively.

Plots of mean site scores for the first 2 principal components further highlighted spatial trends (Figure 2). Mean scores for PC1 were highest for ERM 1.0, and the site scores decreased with distance from the spill site. The mean PC1 score for ERM 2.5 was similar to the mean scores for CRM 1.5 and CRM 3.5, whereas the mean for ERM 4.0 was most similar to the reference site scores. Mean PC2 scores for the ash-affected sites were generally higher than those for the reference sites except for TRM 572.5. Also, the reference sites showed their greatest variation on PC2 and less variation was evident among ash-affected sites on that axis. Mean PC2 scores for the ash-affected sites were generally higher than those for the reference sites except for TRM 572.5.

Temporal changes in overall metal concentrations at sites downstream of the spill were most evident on PC1, but some temporal change was evident along PC2 as well (Figure 3 and Supplemental Data, Figure S1). The regression slopes for PC1 versus time were significantly different from 0 for all 3 ash-affected sites tested (Table 2 and Figure 3). ERM 6.0-R was the only reference site with a significant slope, but unlike the ash-affected sites, the slope for ERM 6.0-R was positive. Only PC2 scores for ERM 1.0 and CRM 1.5 had significant slopes, and the r^2 values were lower than those for PC1. These results suggest that temporal trends in overall metal concentrations were generally similar at ash-affected sites whereas the reference sites showed no consistent trends over the 4-yr period.

Plots of mean metal concentrations provided further evidence of an association with ash for some metals downstream of the spill, even as far downstream as TRM 560 (Figure 4; e.g., see plot for Sr). With few exceptions (e.g., Cu) the highest concentrations for the metals with high loadings on PC1 (Table 1) were at ERM 1.0 and not ERM 2.5, the site closest to the spill. Concentrations of several metals generally decreased with distance from ERM 1.0. Likewise, concentrations of

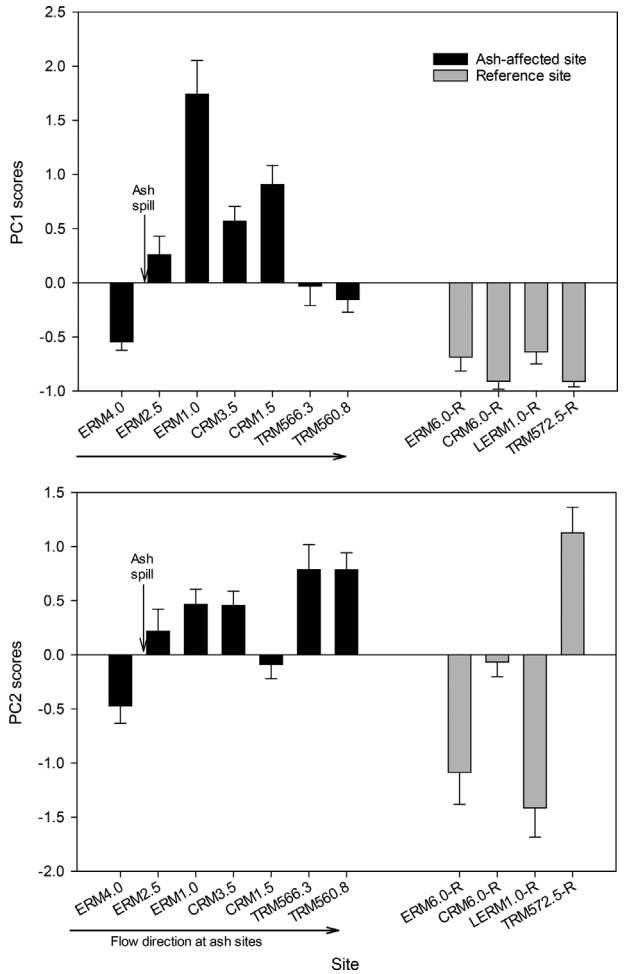

Figure 2. Mean (± standard error) principal components analysis site scores for PC1 and PC2. Means were calculated from principal components analysis axis scores across all years. ERM = Emory River Mile; CRM = Clinch River Mile; TRM = Tennessee River Mile.

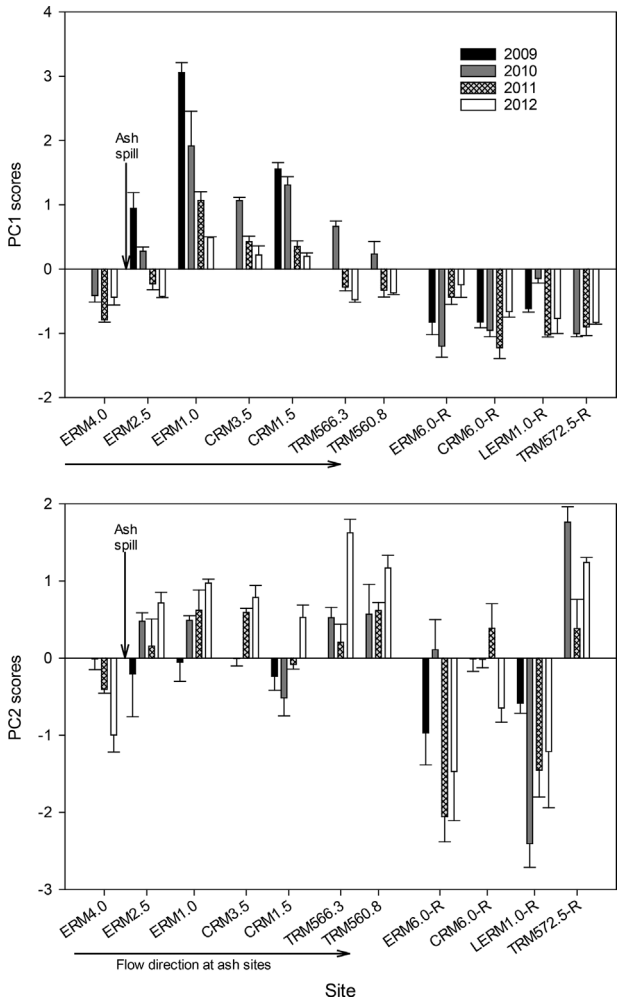

Figure 3. Spatial and temporal trends in annual means (± standard error) for principal components analysis site scores for PC1 and PC2. ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile; TRM = Tennessee River Mile.

several metals appeared to drop after 2010 at most of the ash-affected sites. A few metals such as Cr and Pb appeared to be elevated at most ash-affected sites, but their concentrations also appeared to be comparatively high at 1 or more reference sites. The highest concentrations of Cd, Hg, and Zn were found at 1 or more reference sites. Mercury was clearly much higher in the Clinch River, with nymphs from CRM 6.0-R having the highest concentrations. Similarly, Zn concentrations were highest at TRM 572.5-R, followed by the 2 Tennessee River sites

Table 2. Regression statistics for test that the slope = 0 in the relationships between PC1 and PC2 scores from the principal components analysis versus collection year[a]

		PC1				PC2			
Site	N[b]	Slope	Intercept	r^2	p value	Slope	Intercept	r^2	p value
Ash-affected sites									
ERM 2.5	14	–0.476	957.2	0.776	<0.001[c]	0.261	–523.6	0.166	0.148
ERM 1.0	13	–0.865	1740.4	0.841	<0.001[c]	0.327	–656.7	0.585	0.002[c]
CRM 1.5	13	–0.498	1002.9	0.870	<0.001[c]	0.252	–507.2	0.408	0.019[c]
Reference sites									
ERM 6.0-R	13	0.232	–467.4	0.354	0.032[c]	–0.333	668.2	0.138	0.211
CRM 6.0-R	13	0.0105	–22.1	0.002	0.881	–0.137	275.1	0.113	0.262
LERM 1.0-R	12	–0.135	269.9	0.168	0.186	–0.0926	184.8	0.013	0.722

[a]Only sites sampled in all 4 yr were included.
[b]N = Total number of samples in the analysis. From 2010 to 2012, 3 samples from each site were analyzed; in 2009, 4 samples were analyzed for all sites except Emory River Mile 2.5 (5 samples) and Little Emory River Mile 1.0-R (3 samples).
[c]Relationships for which the slope ≠ 0.
PC1 and PC2 = principal components 1 and 2, respectively; ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile; R = reference.

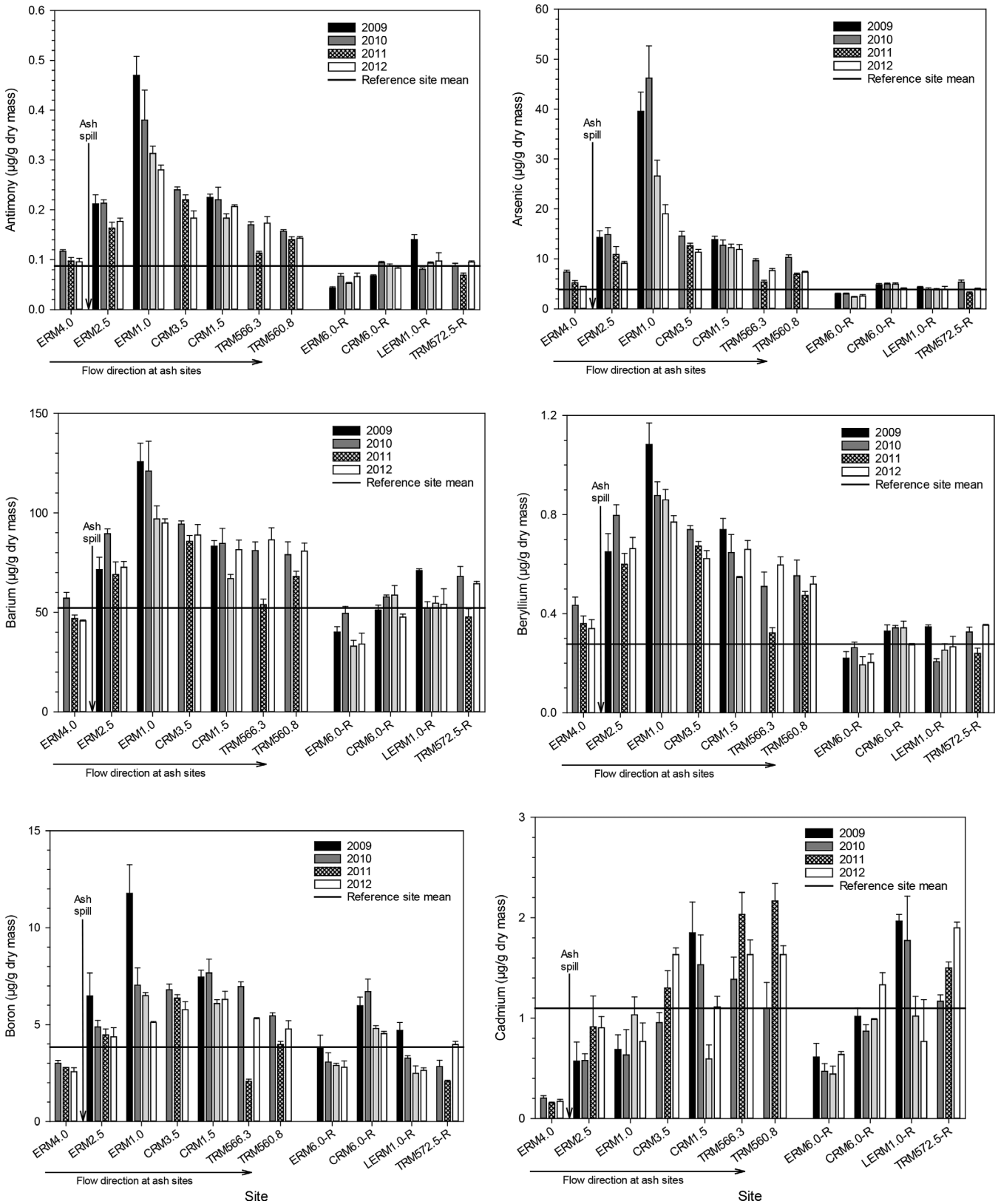

Figure 4. Spatial and temporal trends for mean (± standard error) metal concentrations (µg/g dry mass) in *Hexagenia* nymphs. The horizontal black line on each plot is the mean concentration for the reference sites across all years; the reference-site mean for Hg excludes Clinch River Mile 6.0-R. Horizontal gray lines on the selenium plot indicate the toxic effect thresholds suggested by Deforest et al. [56] and Lemly [55] for selenium in fish food items. ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile; TRM = Tennessee River Mile.

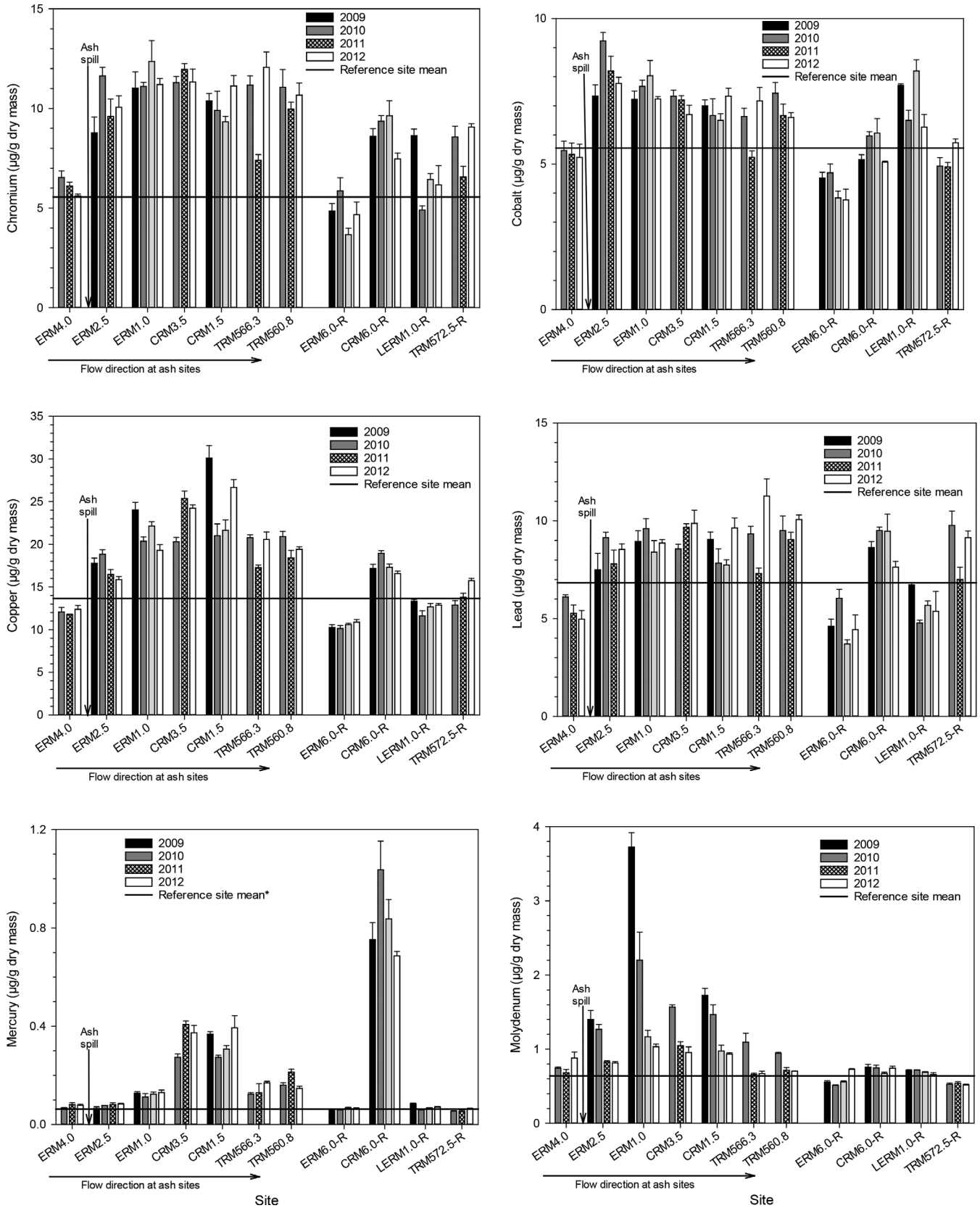

Figure 4. Continued.

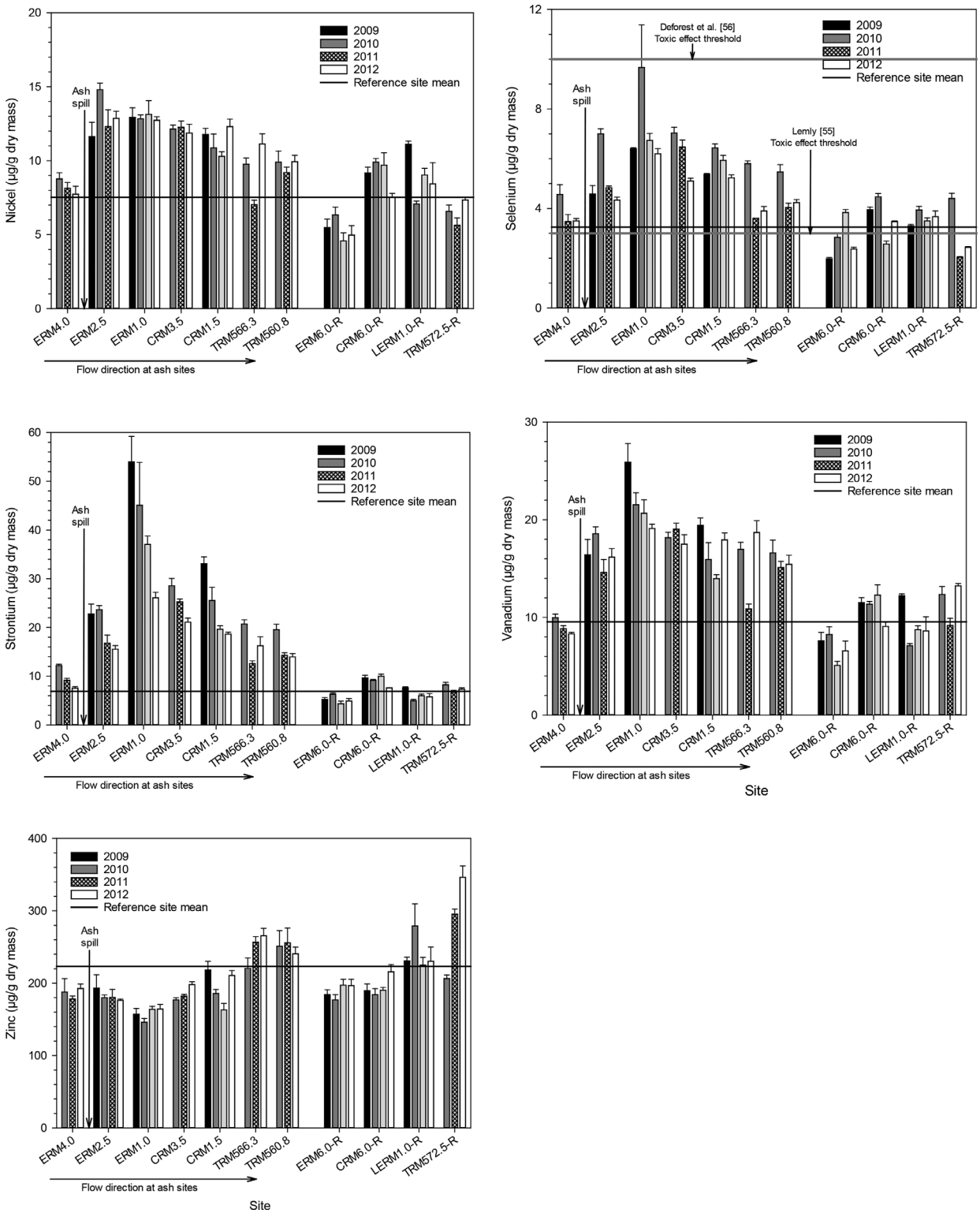

Figure 4. Continued.

downstream of the Clinch River confluence. Cadmium showed no clear spatial or temporal trends.

DISCUSSION

The presence of metals in coal ash and the negative consequences to biota from some of these metals when coal ash is present in aquatic environments have been well documented (see Rowe et al. [3] for a review). Metal content and concentrations in coal ash vary considerably because of factors such as the source of the coal and the combustion process [3,4]. Furthermore, the mobilization and fate of metals associated with coal ash released into aquatic environments are affected by a range of environmental factors such as pH, redox potential, and quantity of organic matter present [5]. Even though the release of potentially large quantities of metals would be expected from a

coal ash spill of the magnitude experienced at the Tennessee Valley Authority's Kingston Fossil Plant site, no prerelease data were available for comparison with post-spill conditions. Given the lack of historical data, the inherent variability that characterizes the content, release, and concentrations of metals in coal ash, and a documented history of pollutants present in the affected watershed [7], establishing a link between contaminant concentrations in resident organisms and the ash spill is challenging. This uncertainty not only affects estimates of risk to wildlife, it also potentially affects decisions identifying and selecting the correct remedial actions to reduce or eliminate risks.

Spatial trends in metals

Soon after the ash spill, the Tennessee Valley Authority completed a trace constituent analysis on samples of pond ash that remained in the storage cell that ruptured, as well as ash that spilled from the cell [32]. Many of the metals with elevated concentrations in mayflies were also elevated in the pond ash, although concentrations of most metals in the nymphs were 20% to 90% lower than those in the ash. Concentrations of As, Ba, Be, Mo, Sb, Se, Sr, and V were elevated in the pond ash, and the principal components analysis results and trends in concentrations for nymphs provided strong evidence that they were likely enriched in nymphs because of the ash spill as well [32]. These metals exhibited similar spatial and temporal trends in nymphs downstream from spill; their concentrations were higher at ash-affected sites than at reference sites; they all had high loadings on PC1 of the principal components analysis; and concentrations of all but Se were lower in nymphs than the reported concentration in the pond ash. Selenium concentrations in nymphs from ash-affected sites were either comparable to or even higher than the reported concentration in pond ash. The highest concentrations of these metals were generally at ERM 1.0, and then concentrations decreased with distance downstream. Enrichment of some of these metals in nymphs, particularly Sr and Be, was detected at TRM 560.8, which is approximately 14 miles downstream of the spill site. The spatial gradient found in nymph concentrations was generally consistent with reported distributions of ash and contaminant concentrations in sediments and fish downstream of the spill [5,33–35].

Boron, Cr, Co, Cu, Ni, and Pb also appeared to have some association with the coal ash. Except for Pb, concentrations of metals in this group were lower in nymphs than in pond ash [32]; concentrations of Pb in nymphs and pond ash were similar. Maximum concentrations of these metals were generally at 1 or more of the ash-affected sites, but concentrations at 1 or more reference sites were generally comparable to those at most ash-affected sites. Furthermore, there was little or no indication of a concentration gradient with distance from the spill site. These spatial patterns suggested possible contributions from other sources as well. For example, the highest concentrations of Cu were at Clinch River sites downstream of the Emory River. Concentrations of Cu were higher at ERM 1.0 and ERM 2.5 than at ERM 6.0-R and LERM 1.0-R, but were similar to those at CRM 6.0-R; thus, some of the Cu in nymphs from Clinch River sites downstream of the spill could have come from a source in the Clinch River, such as legacy contaminants from US Department of Energy facilities in Oak Ridge (TN, USA) [36]. The streams that received these contaminants drain into the Clinch River between river miles 11 and 21. Similarly, Cr concentrations were higher downstream of the spill site compared with ERM 6.0-R and LERM 1.0-R, but were similar to concentrations found at CRM 6.0-R and TRM 572.5-R. Concentrations of Co at ERM 2.5 and ERM 1.0 were higher than those at all reference sites except for LERM 1.0-R, but Co concentrations at ERM 4.0 were similar to those found at ERM 6.0-R, CRM 6.0-R, and TRM 572.5-R. The slightly higher concentrations of Co in nymphs at a reference site upstream of the spill (LERM 1.0-R) suggested the possibility of a source unrelated to the ash spill, but the values were not substantially higher and showed considerable interannual variability, making it difficult to infer that LERM 1.0-R was a source of Co at sites downstream of the spill.

Mercury appeared to have a weak association with the ash spill at most. Concentrations of Hg reported in pond ash [32] were more than 2-fold higher than in nymphs from ERM 1.0. However, concentrations in nymphs from Clinch River sites, including the reference site, were nearly 2 to 3.5 times higher than in the pond ash. It is well documented that significant quantities of Hg were released into East Fork Poplar Creek from the Department of Energy's Y-12 National Security Complex (Y-12 Plant) in Oak Ridge (TN) [36]. East Fork Poplar Creek is in the Clinch River drainage upstream of CRM 6.0-R, and releases of Hg from the Y-12 Plant have been a significant source of Hg in the lower Clinch River.

In contrast to the other metals, spatial trends for Cd and Zn in nymphs showed no clear association with the coal ash spill, and their concentrations in pond ash [32] were at least 70% lower than in nymphs from any ash-affected site. Furthermore, unlike other metals, Cd and Zn concentrations increased with distance from the spill site, and concentrations in nymphs at LERM 1.0-R and TRM 572.5-R were generally comparable to (Cd) or higher than (Zn) the highest concentrations found at ash-affected sites. Zinc is a widespread and common pollutant that is often a significant contaminant in urban runoff [37]. A relatively large metropolitan area (i.e., Knoxville, TN, USA) is located upstream of TRM 572.5-R, and thus may have been a source of some of the Zn at the Tennessee River sites.

Even though there was little or no clear association between the ash spill and concentrations of Cd, Hg, and Zn in mayflies, it cannot be concluded from the present study that the spill was not a source. Coal ash is typically enriched with these contaminants [3], and all were found in pond ash from the spill site [32]. Bartov et al. [38] used Hg stable isotopic signatures as a means of identifying sources of Hg in river sediments downstream of the Kingston Fossil Plant spill site. From their study they concluded that coal ash was the dominant source of Hg in Emory River sediments downstream from the spill, whereas historical releases into the Clinch River from US Department of Energy facilities were the primary source of Hg in lower Clinch River sediments. Thus, definitively distinguishing sources of these metals may not be possible with the analytical methods used in the present study.

Maximum concentrations of most of the metals were found not at the site closest to the spill (ERM 2.5), but further downstream at ERM 1.0. Several factors may have contributed to this trend. One factor may have been related to the removal of an estimated 2.7 million m^3 of ash by hydraulic dredge from the Emory River between river miles 1.75 and 3.2 from March 2009 through August 2010 [7]. No dredging was performed downstream of ERM 1.75 or in the Clinch River to avoid disturbing sediments with legacy radiological contaminants released from the US Department of Energy's facilities in Oak Ridge. Thus, although remediation efforts removed much of the coal ash upstream of ERM 1.75, not only did ash remain in the lower Emory River, but some of the contaminants from upstream dredged areas may have been resuspended and deposited further downstream during dredging operations [39].

Another factor contributing to higher concentrations at ERM 1.0 could have been the existence of complex hydrological conditions in the affected watershed [7]. The lower reaches of the Clinch and Emory Rivers are part of the Watts Bar Reservoir headwaters. Watts Bar Dam is a hydroelectric dam on the Tennessee River at river mile 530. Reservoir levels are maintained to provide navigation, not only in the Tennessee River, but also in portions of the Clinch and Emory Rivers. When releases from Watts Bar dam are minimized, flow velocity in the lower Emory and Clinch Rivers decreases. Melton Hill Dam is a second hydroelectric dam in the watershed located at CRM 23. The reservoir created by this dam (Melton Hill Reservoir) is a run-of-river reservoir; thus releases of water are frequent. The channel of the Emory River widens considerably near river mile 1.3, and the channel of the Clinch River widens considerably just downstream of the confluence with the Emory River. The wider channels slow flow velocity, which would increase the settling rate of suspended particles. This can be exacerbated in the Emory River when minimum water release from Watts Bar Dam coincides with a release from Melton Hill Dam. Not only can this slow flow velocity in the Emory River, but some of the water from the Clinch River can be forced upstream in the Emory River. Sediments are a sink for metals, especially the clay and silt fractions (i.e., particles $< 64.0\ \mu m$) [11,40]. Characterization of fly ash from Kingston Fossil Plant has shown that approximately 75% of the ash particles are the size of silt (i.e., 4.0–64.0 μm), and another 22% of the particles are the size of clay (i.e., $<4.0\ \mu m$) [32]; thus, the factors that slow the flow velocity in the lower Emory River create more favorable conditions for the smaller and potentially most contaminant-enriched particles to settle and accumulate.

Finally, 4 significant flow events occurred between May 2009 and May 2010 that not only redistributed some ash further downstream [8], but also could have added to the ash deposits already present at ERM 1.0. The most significant of these events occurred on 4 May 2009. Discharge in the Emory River during this event peaked at approximately 1982 m^3/s (3-yr return flood) and transported an estimated 90 982 m^3 of coal ash downstream of the spill site at Kingston Fossil Plant. On 24 January 2010, a high-flow event with an estimated peak flow of 1699 m^3/s (also a 3-yr return flood) transported an estimated 6743 m^3 of coal ash downstream of the spill site. Somewhat smaller flow events in December 2009 and May 2010 (estimated peak flow for both was 1416 m^3/s) transported an estimated 3181 m^3 and 1430 m^3 of ash, respectively [8].

Temporal trends in metals

There was strong evidence that concentrations of ash-associated metals in nymphs began to decline after 2010. The principal components analysis indicated that overall contaminant concentrations in nymphs downstream of the spill had dropped considerably by 2012, and concentrations of some metals such as As, Se, Sr, Sb, Mo, and V were at or near their lowest level of the present study in 2012, not only at ERM 1.0, but generally at other sites downstream of the spill as well. Even though concentrations of some ash-associated metals had begun to decrease, these metals were still elevated at some sites in 2012 relative to reference sites (e.g., As, Se, and Sr).

The release of coal ash from Kingston Fossil Plant was a single large event with a clear beginning and end (i.e., pulse disturbance) [41]. Although contaminant concentrations typically decrease over time after a source is significantly reduced or removed [42,43], the temporal trajectory and magnitude of decline in concentrations varies among contaminants and species [44,45]. These and other chemical, physical, and biological attributes contribute to variation in body burdens [45] that can be considerable over long periods of time [4,46,47]. Although a large proportion of the coal ash was removed from the Emory River via dredging, some coal ash remains in the Emory, Clinch, and Tennessee Rivers. Natural hydrological processes will cause further mixing of some of the ash with native sediments, and deposition of native sediments will cover some ash in place [48]; both of these processes will likely add variation to concentrations in biota. Given the relatively large size of the water bodies affected by the ash spill, the nature of the spill (i.e., a single large pulse release), the estimated amount of ash dredged from the Emory River, and the downstream movement of ash that has occurred during floods, it is likely that localized concentrations of most or possibly all ash-related contaminants in sediments and surface waters may have already peaked. Even so, given the ongoing dynamics of the remaining ash and the relatively short length of study to date, additional temporal data are needed to confirm the trends observed thus far.

Ecological relevance of contaminant exposures

The release of such a large volume of coal ash into the Emory River undoubtedly caused mortality of benthic invertebrates in those areas where ash deposits were significant. However, a study of invertebrate community structure over a 2-yr period after the spill found no differences between ash-affected and reference sites that could be definitively associated with the spill [49]. Although there were no prespill data on the invertebrate community from the affected area, differences in the invertebrate community at downstream and reference sites appeared to be related primarily to differences in physical habitat unrelated to the ash spill. For example, ERM 6.0-R and CRM 6.0-R were more riverine-like, with narrower channels and a greater proportion of coarse substrate (i.e., sand and gravel). Invertebrate communities at these sites were generally dominated by chironomids and oligochaetes. Ash-affected sites, in contrast, were more lake-like, with wider, deeper channels, and a greater proportion of silt and clay in the substrate. These sites typically had higher proportions of mayflies (*Hexagenia*) and bivalves (Sphaeriidae and *Corbicula*). The taxa present at all sites were typical inhabitants of reservoirs that are capable of adapting to change in habitat from lotic to lentic conditions caused by construction of dams, or species that normally live in lentic conditions [50,51]. Furthermore, even though *Hexagenia* nymphs are generally indicative of mesotrophic conditions, they exhibit enough sensitivity to changes in the environment that they have been used extensively as indicators of the environmental health of water bodies for many years, especially the Great Lakes and Mississippi River in the United States [18,20,52,53]. The near disappearance of *Hexagenia* from the Great Lakes and portions of the Mississippi River in the mid-20th century provided clear evidence of the poor ecological conditions caused by years of high inputs of pollutants (e.g., nutrients, organic compounds, metals) from various sources [18]. Thus the persistence of *Hexagenia* adjacent to and downstream of the Kingston Fossil Plant ash spill, the lack of distinct effects on the invertebrate community, and the modest enrichment of potentially toxic metals would suggest that overall, any negative effects (either physical or toxicological) from the ash spill to invertebrates were probably short-lived (i.e., confined to the initial months after the spill) and relatively localized.

Finally, although results from the present study and the study on benthic macroinvertebrate community structure [49] seem to

suggest that any negative effects to macroinvertebrates from the ash spill have been relatively short-lived, the concentrations of some metals (e.g., Se) found in the mayfly nymphs at some sites have been high enough at times to still have posed some potential risk to invertebrates and some of the animals that consume them [54]. *Hexagenia* and other insects with an aquatic stage in their life cycle are important trophic links that can facilitate contaminant transport from the sediment to higher trophic levels in aquatic and terrestrial habitats. One metal associated with coal ash that is a major concern is Se. The recommended dietary thresholds for Se in fish range from 3.0 μg/g dry weight [55] to 10 μg/g dry weight in warm water species [56]. Mean Se concentrations in the present study exceeded the most conservative of these recommended dietary Se thresholds in all years at all ash-affected sites, and during at least 1 yr at all reference sites except ERM 6.0-R. However, mean concentrations at no site exceeded the most liberal recommended threshold of 10 μg/g dry weight for warm water species [56].

Studies on vertebrate consumers inhabiting areas affected by the Kingston Fossil Plant spill have found evidence of bioaccumulation of some metals from the ash in insectivorous birds and fish. Dietary studies of nestling and adult tree swallows (*Tachycineta bicolor*) around the spill site suggest some trophic transfer through insects that have an aquatic stage (mostly chironomids, but mayflies also were found in the diet). However, the concentrations of contaminants were not associated with any lethal or sublethal physiological effects [57,58]. Downstream of the ash spill redear sunfish (*Lepomis microlophus*), a species likely to have a diet predominantly of aquatic organisms, were found to have elevated levels of Se [59] and evidence of possible sublethal physiological changes [60]. In contrast to the aforementioned studies, Sousa et al. [61] found no evidence of metal bioaccumulation or negative health effects in raccoons (*Procyon lotor*) from the spill site that could be attributed to the ash. With much uncertainty remaining in our current and future understanding of the overall ecological consequences of the ash spill at Kingston Fossil Plant, and the general uncertainty that remains in overall scientific understanding of the environmental fate and effects of the associated contaminants, further study is needed to ensure there are no delayed effects at higher trophic levels.

CONCLUSIONS AND IMPLICATIONS

The present study provides an essential and important contribution to the overall effort to understand the ecological consequences from the coal ash spill at the Kingston Fossil Plant, because it clearly delineates the patterns of contamination. Trends for several metals showed a strong ash signature that was distinguishable from other potential contaminant sources (e.g., As, Se), whereas concentrations of a few metals, such as Hg, showed little or no association with the ash. The chemical signatures provided information on the downstream movement of ash-associated contaminants, as well as documentation of an apparent decline in concentrations within the first 4 yr after the spill. Although declines in concentrations over a relatively short time period might be expected after a source of a contaminant is eliminated or significantly reduced, there currently is not enough available information from which the rate of decline in the present study could have been predicted given the magnitude of the spill experienced at the Kingston Fossil Plant site. The peak contaminant concentrations in *Hexagenia* seemed to be modestly elevated but were below levels that would be expected to cause widespread negative ecological effects. However, taxa at other trophic levels may be differentially affected, which is a topic that will be explored in future studies. Finally, because several ash-associated contaminants released with the spill were clearly elevated in *Hexagenia* nymphs, ongoing monitoring will help verify recovery trends, and identify any potential delayed effects at higher trophic levels.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3253.

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Data availability—Data may be obtained from the senior author upon request.

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