

SELENIUM, ARSENIC, AND MERCURY IN FISH INHABITING A FLY ASH EXPOSURE GRADIENT: INTERSPECIFIC BIOACCUMULATION PATTERNS AND ELEMENTAL ASSOCIATIONS

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Abstract—Releases from coal ash impoundments can be a significant source of trace elements to the aquatic environment. In the present study, whole-body concentrations of As, Hg, and Se in various fish species inhabiting streams receiving a gradient of fly ash exposure are reported. High-exposure sites had elevated water concentrations of As, Mo, Se, and SO₄. Fish were collected during two seasons in 2007. Mercury concentrations in all fish samples were low (range = 1.2–99 ng/g wet wt); highest As concentrations occurred in fish from high-exposure sites. Whole-body Se concentrations were low at reference sites but increased as the magnitude of fly ash exposure increased. For all sites combined, a significant ($r^2 = 0.60$) correlation was observed between the geometric mean of each species' whole-body Se concentration and log-transformed water Se concentration. A significant inverse relationship was apparent with log-transformed whole-body Hg and Se concentrations ($r^2 = 0.56$ for all species and sites combined), suggesting that high tissue Se levels antagonistically regulated Hg bioaccumulation. Sunfish (*Lepomis* sp.) from high- and medium-exposure sites had significantly higher Se body residues, but significantly lower Hg, relative to fish from low-exposure and reference sites. Ninety percent of fish from high-exposure sites had a surplus of Se, whereas all fish from reference sites had Se/Hg molar ratios <1.0. These ratios increased as water Se increased. Where fish have moderate to high exposure to fly ash-influenced water, Se tissue levels can be expected to be elevated (as well as As, in some cases), but tissue Hg concentrations will likely be low. Environ. Toxicol. Chem. 2012;31:739–747. © 2012 SETAC

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INTRODUCTION

The release of contaminants from the disposal of coal combustion byproducts (CCBs) can, depending on exposure regimes, lead to deleterious effects to fish and wildlife. There have been a wide variety of observed effects on water quality, community structure and function, and trace element bioaccumulation when CCBs are released at levels (and durations) that result in long-term accumulation in abiotic media [1]. There are exposure settings, however, where deleterious or otherwise adverse effects caused by CCBs release are not observed [2], or water quality is improved by CCB impoundment water when background water quality is limiting [3]. Moreover, there are many reported cases where stable populations of seemingly Se-sensitive fish species occur despite relatively high concentrations of Se being measured in water and biota tissue [4,5].

Of all the CCBs produced by typical coal-fired power plants, fly ash is most often associated with contaminant accumulation and adverse effects to biota. According to the U.S. Environmental Protection Agency (U.S. EPA), the trace elements of most concern regarding exposure of ecological receptors to fly ash are Se, As, and Hg [6]. Fly ash is composed of fine-grain particles that have variable morphology and consist primarily of an amorphous glassy material. The elemental composition of fly ash is highly variable and is most affected by chemical properties and variations in parent coal and the operation of advanced air pollution control technologies. Like burned coal, fly ash has unique chemical signatures based on coal field origin [7].

When coal is combusted, much of the As, Hg, and Se is volatilized and (through condensation) collected onto electrostatic precipitators (ESPs). The mass of metals not removed by precipitators is removed by further downstream chemical removal technologies or is emitted at the stack in gaseous or aerosol fractions. Various factors control the partitioning of these trace elements between the vapor and solid phases such as elemental associations [8], overall efficiency of electrostatic precipitators [9], combustion temperature, and particle size. Most of the Se and As present in flue gas is the particulate form and thus a greater percentage (>60%) of these elements are captured in the ESP unit relative to Hg, which is largely in the vapor elemental form upstream of ESP devices [10].

Because a lower proportion of flue gas Hg is captured onto ESPs as ash particles, the enrichment factor (ratio of element concentration in fly ash to element concentration in parent coal) for Hg is relatively low. Moreover, the concentration of Hg in fly ash is typically lower than that of As and Se. For example, in an analysis of the distribution of fly ash trace element concentrations in nine Illinois Basin USA coal sources, the median concentrations of As, Hg, and Se were 59, 0.11, and 13 mg/kg, respectively [11].

The speciation of these trace elements on fly ash particles (as well as basic sluice water chemical variables such as pH and redox) influences the desorption rate from the particles and, hence, chemical composition of impoundment or leachate water [12]. For eastern bituminous coal fly ash, selenite (SeO₃-2) is the dominant form of Se and arsenate (AsO₄-3) is the principal inorganic form of As [13,14]. Moreover, selenite and arsenate are the dominant inorganic forms in fly ash impoundment water or landfill leachate where eastern bituminous coal is burned [15]. Total Hg concentrations in impoundment and leachate water are typically low (median concentration = 3.8 ng/L);

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monomethyl Hg is typically detected, but at lower levels (median concentration = 0.08 ng/L) [15]. The relatively low aqueous concentration of Hg in fly ash impoundment water (relative to As and Se) is due largely to a lower mass loading of this element onto fly ash particles.

The most severe, pervasive ecological impacts associated with the release of fly ash-derived Se have occurred in power plant reservoirs built exclusively for the discharge of heated water and treated fly ash wastewater (Belews Lake, NC; Hyc0 Reservoir, NC; Martin Lake, TX) [16,17]. In lotic receiving streams, there is some evidence that bioaccumulation and population-level impairments in fish may be less severe or less frequent [18–20]. One hypothesis is that the incorporation of dissolved Se into primary producers or particulate organic matter—a critical pathway to ultimate trophic transfer [21]—is less efficient in running waters relative to lentic habitats.

In the present study, whole-body concentrations of As, Hg, and Se in nine species of fish were measured. The fish were collected from stream or large river sites having a gradient of fly ash exposure. At the power plant sites studied, fly ash is sluiced to a large impoundment, and the final treated discharge is released directly to receiving streams of various sizes. The objective of the present study was to evaluate patterns of trace element bioaccumulation—and assess potential elemental associations—as a function of fly ash exposure.

MATERIALS AND METHODS

Study sites

Fish and water samples were collected twice during 2007 at fly ash pond discharge receiving streams located proximal to coal-fired power plants in several U.S. states, including West Virginia (Conner Run, Fish Creek, Little Scary Creek), Kentucky (Blaine Creek), and Indiana (Ohio River; Fig. 1). All of the facilities burn bituminous coal from the Northern and Central Appalachian Coal Regions, and all of the facilities are equipped with coal-side ESPs for removal of fly ash in the flue gas. Most of the facilities are also equipped with selective catalytic reduction and wet flue gas desulfurization air pollution control systems. Conner Run (CR) and Little Scary Creek (LSC) were considered high-exposure sites as the fly ash discharge comprised >95% of total stream flow. Fish Creek (FC) is the receiving stream for CR, and collections were made in the immediate mixing zone (confluence). This site was considered a medium-exposure site. Blaine Creek (BC) was considered a medium-exposure site and the Ohio River (OR) site near Tanners Creek Plant was considered low exposure. At the OR site, samples were collected in the fly ash pond discharge mixing zone. Samples were also collected at two reference locations: the Kanawha River upstream of LSC confluence and the OR near the low-exposure location (upstream of fly ash discharge input). A suitable reference site on BC was not available due to stream access difficulties.

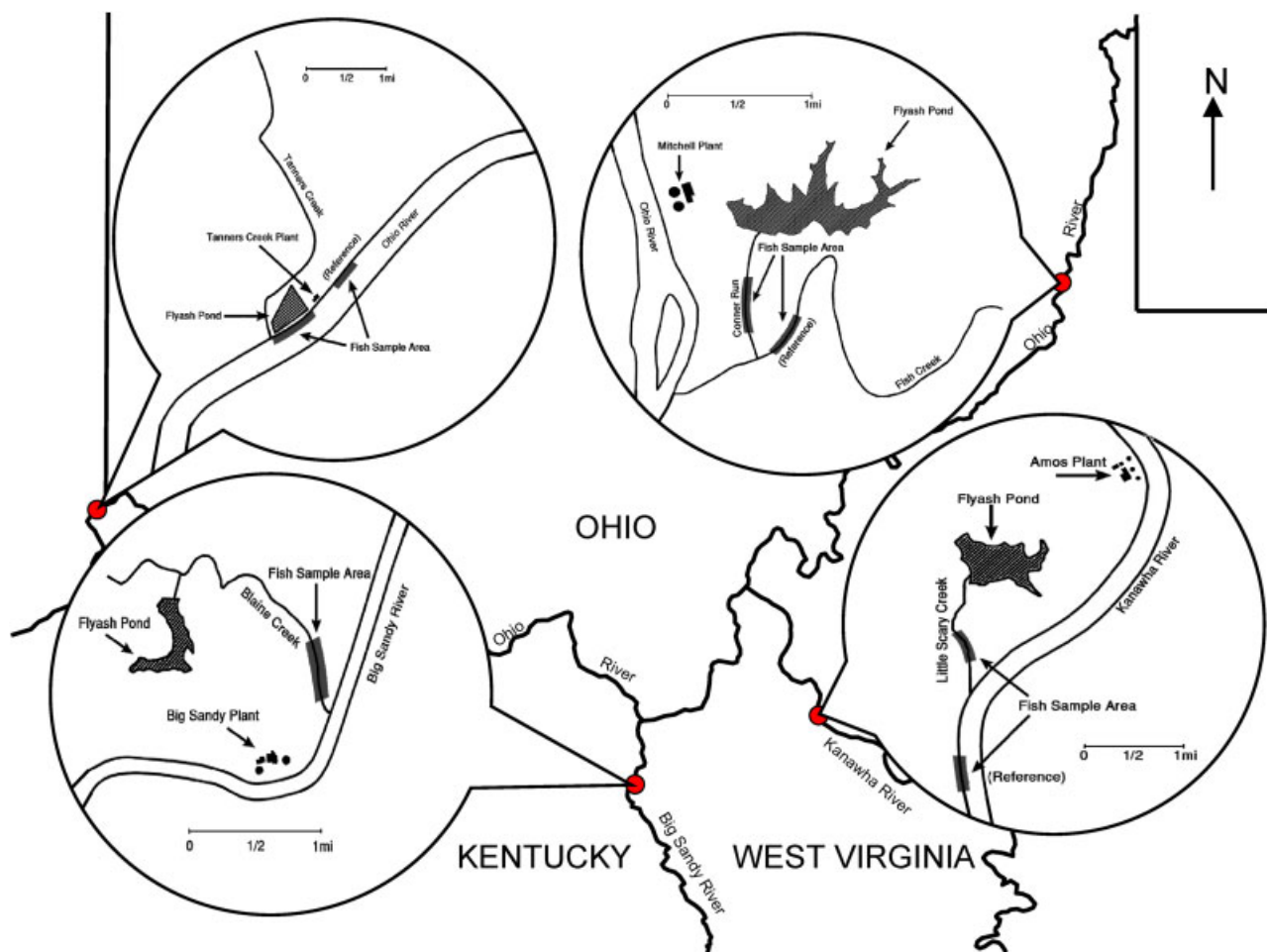


Fig. 1. Location of water quality and fish collection sites near four United States Midwestern coal-fired power plants. The magnitude of fly ash exposure, per stream, was as follows: Conner Run and Little Scary Creek (High); Blaine Creek and Fish Creek (Medium); Ohio River near Tanners Creek Plant (Low). The Kanawha River and Ohio River upstream of Tanners Creek Plant were water sample and fish collection reference sites.

Water sample analysis

Water samples were collected at the time of fish collection, and in previous months at some locations. At FC, access to a suitable water collection point was difficult due to the presence of a thick bed of fly ash particles at the confluence delta. Thus, mixing zone water quality was simulated by taking a 1:1 mixture of CR and FC (outside of CR influence) water and analyzing the composites. Parameters measured at all sites were total recoverable elements (Al, Ag, As, Be, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Tl, and Zn using U.S. EPA Method 200.7 or 200.8), as well as specific conductance (25°C), total dissolved solids (TDS), dissolved salts (Ca, Cl, Mg, SO₄), pH, total hardness, and alkalinity. Samples for the analysis of low-level Hg were collected and analyzed according to U.S. EPA methods [22]. Samples for Hg analysis were collected at the fly ash discharge monitoring location (BC, CR, LSC). Ohio River ambient Hg data were obtained from the Markland Lock and Dam monitoring location downstream of Tanners Creek Plant, provided by the Ohio River Valley Water Sanitation Commission (www.orsanco.org/clean-metals/196-clean-metals-data). Kanawha River ambient Hg data were obtained from samples collected at American Electric Power's John M. Amos generating station (56 km upstream from the OR). Inorganic Se species (selenite, selenate) were determined by hydride generation ion chromatography. Quality assurance and quality control measures included the analysis of lab reagent and fortified blanks, calibration verifications, matrix spikes, and matrix spike duplicates.

Fish collections and analysis

Fish were collected in CR, LSC, and BC using DC-pulse electrofishing, following standardized Ohio EPA protocols for sampling in wading sites [23]. Fish were collected in the OR and in FC using DC-pulse boat electrofishing. The target species for obtaining metals tissue data were sunfish, as at least two of these species (bluegill and largemouth bass) have been shown to be sensitive to Se exposure in both laboratory and field studies [24,25]. Other fish species were collected for analysis if no sunfish, or few sunfish, were obtained. A total of nine fish species were collected for tissue analyses (all sites combined), and these samples represented a mixture of composite (three to five fish) and individual fish samples. All fish were measured for total length and wet weight and preserved in dry ice in the field. Fish were collected during July and October to November 2007. At the CR site fish were also collected during October 2008.

Fish tissue analysis

All analyses for determination of trace elements in fish whole-body samples were conducted by Brooks Rand Laboratories (Seattle, WA). All composite and individual samples were homogenized and percent solids was determined. After digestion with HNO₃, the samples were analyzed for total Hg (U.S. EPA Method 1631), and total As and Se (U.S. EPA Method 1638; modified using inductively coupled plasma-mass spectrometry with Dynamic Reaction Cell to minimize matrix interferences). Quality assurance and quality control measures (with appropriate recovery or acceptability criteria) included certified reference materials (dogfish muscle and liver), laboratory fortified blanks, matrix spike and matrix spike duplicate analysis, method blanks, and (where appropriate) post-preparation spike analysis. All sample results were blank-corrected. Results are provided as mg/kg dry weight for As and Se and ng/g wet

weight for Hg. Whole-body tissue molar concentrations of Se and Hg are provided as $\mu\text{mol/g}$.

Statistical analysis

Because only a few species were collected at all exposure settings, statistical analyses of metal concentrations (spatial comparisons) were conducted with this subset. Sunfish of the genus *Lepomis* were chosen for these comparisons. These species included bluegill (*L. macrochirus*), green sunfish (*L. cyanellus*), and longear sunfish (*L. megalotis*). Trace element concentrations in these sunfish species, collected from Se-enriched fly ash impoundments, have been shown to be comparable (within an order of magnitude) [26,27]. Concentrations of As, Hg, and Se were rank-transformed and compared between exposure settings using the Kruskal–Wallis test. When this statistic was significant ($p \leq 0.05$), levels among two exposure settings were compared using the Mann–Whitney *U* test. An analysis of the ranks of tissue Se/Hg molar ratios between high exposure (combined medium and high exposure settings) and low exposure (combined reference and low exposure sites) sites was conducted using the Mann–Whitney *U* test. Elemental associations in tissue samples were evaluated (Pearson Correlation analysis) using log₁₀-transformed metal data.

Linear regression was used to elucidate relationships between tissue Se and water Se (geometric mean concentrations at each of the six exposure and reference locations); this analysis was conducted first for all species combined, and second for *Lepomis* species only. Stepwise multiple regression analysis was conducted using mean location-specific whole-body Se concentrations for the three *Lepomis* species as the dependent variable and mean concentrations of water quality variables (metals, routine parameters) as independent variables. All of these data were log₁₀-transformed prior to analysis. All statistical analyses were conducted using Numerical Algorithms Group statistical add-ins for Microsoft Excel.

RESULTS

Water sample analysis

Table 1 indicates the generating capacity of each facility, source of water for fly ash sluicing (to a treatment impoundment), Se and As levels in the fly ash pond effluent during the study period, and the Instream Waste Concentration (proportion of waste water in receiving stream at critical low flow conditions) at each location. Mean total Se concentrations among the fly ash pond discharges ranged from 18.2 to 73 $\mu\text{g/L}$. The predominant inorganic form of Se was selenite (Se + 4). Total As concentrations were elevated relative to expected ambient background concentrations; however, the mean concentrations did not approach acute or chronic thresholds for protection of freshwater aquatic life [28].

Table 2 indicates mean concentrations of trace elements and routine variables at reference and exposure sites at the stream reach where fish were collected. Analysis results for all water quality variables and measurements are provided (Supplemental Data, Table S1). Most of the fly ash exposure sites were characterized by comparatively high levels of Mo, Se, hardness, SO₄, and TDS. The CR site (high exposure) had the highest concentrations of all trace metals analyzed, as well as hardness, SO₄, and TDS. Mean total Hg concentrations at the high- and medium-exposure sites were low and varied between 1.3 to 1.8 ng/L. These values were markedly lower than concentrations measured at reference locations.

Table 1. Power plant generating capacity, source of fly ash sluice water, flow rate of fly ash discharge and selected trace element concentrations in the fly ash discharge (mean \pm Standard Deviation), January to October 2007

Facility	Net generating capacity (MW) ^a	Fly ash sluice water source ^b	Fly ash discharge characteristics				Instream waste concn (%) ^d
			Average flow rate (MGD) ^c	Total selenium ($\mu\text{g/L}$)	Selenite (Se^{+4}) ($\mu\text{g/L}$)	Total arsenic ($\mu\text{g/L}$)	
Amos	2,900	Kanawha River	8.3	31.9 \pm 10.5	22.2 \pm 7.3	17.6 \pm 12	> 95
Big Sandy	1,060	Big Sandy River	6.6	18.2 \pm 11.4	20.3 \pm 6.9	55.1 \pm 4.3	40
Mitchell	1,560	Ohio River	8.2	73 \pm 64.5	49.2 \pm 2.1	22.9 \pm 8.4	> 95
Tanners Creek	1,000	Ohio River	20.9	51.7 \pm 16.8	49.9 \pm 11.9	102 \pm 164	< 5

^a MW = Megawatts.^b Source water used to sluice fly ash to fly ash impoundment. All stream sites are located in the USA.^c MGD = Million gallons per day.^d Ratio of effluent flow to receiving stream flow during critical (low flow) conditions.Table 2. Water chemistry analysis results for fly ash pond receiving streams and reference sites, July to October 2007^a

Receiving stream site ^b	Mean concentration ($\mu\text{g/L}$); mercury (ng/L)							Mean concentration (mg/L)				pH (s.u.) ^d
	As	Cu	Hg	Mo	Se	Se^{+4}	Se^{+6}	Alkalinity	Total Hardness	Sulfate	TDS ^c	
Little Scary Creek (EXP-H)	9.0	14	1.7	298	20	16.5	2.9	15	197	225	445	6.7
Kanawha River (REF)	0.4	< 5	5.2	< 2	< 0.5	< 0.5	< 0.5	43	66	37	106	7.5
Conner Run (EXP-H)	84	< 5	1.8	332	143	129	27	137	365	811	1,720	8.0
Fish Creek (EXP-M)	22.1	< 5	ND ^e	71	51.3	16.3	10.1	93	249	327	786	7.6
Blaine Creek (EXP-M)	7	6	1.3	131	6	5	< 0.5	39	340	375	710	6.7
Ohio River (EXP-L)	4.1	< 5	ND	10	4	5.2	< 0.5	83	165	94	366	7.4
Ohio River (REF)	1.6	< 5	4.6	8	1	< 0.5	0.7	82	169	88	378	7.4

^a Site designations: EXP (exposure): high (H); medium (M); low (L), and reference (REF). $n = 2$ to 5 for all sites. At some sites, mercury results reflect samples collected from 2007 to 2009.^b All stream sites are located in the USA.^c TDS = Total dissolved solids.^d Geometric mean values indicated; s.u. = standard units.^e ND = no samples collected.

Fish tissue analysis

A total of nine species, and one hybrid, were represented among all fish analyzed for trace element content (Table 3). Twenty-nine discrete samples were analyzed, with 21 of these being composite samples. Nine of these composites represented replicate seasonal samples. At one location (CR), bluegill and longear sunfish samples were obtained in two seasons and in two years.

Relationships with metal levels and fish size were assessed on a species-specific basis for species with the greatest number of discrete tissue samples (bluegill, longear sunfish, and black basses, *Micropterus* sp.). There was no association between length (and wt) and As concentration for all sunfish species ($p > 0.05$). For bluegill, Hg content increased with fish length ($r^2 = 0.70$; $p < 0.01$) while Se content was negatively correlated with fish length ($r^2 = 0.72$; $p < 0.01$) and fish weight ($r^2 = 0.68$; $p < 0.01$). For pooled spotted bass and largemouth bass samples, there was a marginal positive correlation between fish length and Hg content ($r^2 = 0.34$). There were no significant correlations between trace metal content and fish size for longear sunfish.

The highest whole-body As concentrations were measured in two minnow species from LSC (creek chub and blacknose dace; mean concentrations 3.01 and 3.03 mg/kg dry wt, respectively); there was, however, no consistent trend of higher As concentrations in fish from high- and medium-exposure sites. The highest mean Hg concentrations were measured in samples of

spotted bass from the OR reference site (70.4 ng/g wet wt) and BC, a medium-exposure site (67.7 ng/g). Regarding Se, the highest mean species whole-body concentration was for two green sunfish composites collected from CR (57.36 mg/kg). A single composite sample of blacknose dace collected from LSC had a Se content of 55.44 mg/kg. In general, fish from the two high-exposure sites, and fish from one medium-exposure site, had the highest mean species' whole-body Se levels. All but one fish sample collected from high- and medium-exposure locations had whole-body Se concentrations that exceeded the U.S. EPA's proposed body burden criterion (protective of freshwater fish) of 7.91 mg/kg dry weight (Table 3) [29].

To evaluate further distinctions in metal tissue levels between exposure settings, concentrations of metals for the three *Lepomis* species (bluegill, green sunfish, longear sunfish) were pooled in each exposure category. Results of a nonparametric analysis of variance test using ranks indicated that the distribution of As levels in sunfish among the four exposure settings was not significantly different (Kruskal-Wallis Test; $p > 0.05$). For Hg, differences in tissue level distributions among the exposure settings were significant ($H = 9.42$; $p < 0.05$). In addition, Hg levels in high- and medium-exposure setting fish were statistically lower than levels measured in fish from low-exposure and reference site locations ($p < 0.01$; Mann-Whitney U test). Similarly, there was evidence that the distribution of Se levels in *Lepomis* samples was significantly different among the four exposure types ($H = 17.99$; $p < 0.001$). Subsequent analyses indicated that Se levels in

Table 3. Concentrations of arsenic, mercury, and selenium, and molar Se/Hg ratios in whole body fish samples collected at five fly ash exposure and two reference locations in the Ohio River basin, July to October 2007. For each species at a particular location, concentration values represent the geometric mean of seasonal samples (where these were available)

Location	Fly ash exposure	Species	Number of samples ^a	Mean total length (mm)	Geometric mean whole body concentration: As, Se as mg/kg dry weight; Hg as ng/g wet weight			Mean molar ratio, $\mu\text{mol Se}/\mu\text{mol Hg}$
					As	Hg	Se	
Little Scary Creek	High	Creek Chub <i>Semotilus atromaculatus</i>	2 COMP	121	3.01 ± 1.8	13.0 ± 1.8	27.68 ± 4.0	5.41
		Blacknose Dace <i>Rhinichthys atratulus</i>	1 COMP	72	3.03	7.3	55.44	19.29
		Sunfish Hybrid ^b	2 IND	86	0.31 ± 0.01	64.8 ± 71	19.76 ± 14.4	1.84
		Green Sunfish <i>Lepomis cyanellus</i>	2 COMP	126	0.68 ± 0.29	15.5 ± 1.9	26.62 ± 11.8	4.3
		White Crappie <i>Pomoxis annularis</i>	1 IND	133	0.30	26.9	13.47	1.27
		Bluegill <i>Lepomis macrochirus</i>	1 IND	103	0.99 ± 0.47	12.4 ± 12.3	25.75 ± 20.2	11.92
Conner Run	High	Green Sunfish	3 COMP					
		Longear Sunfish	2 COMP	123	1.31 ± 0.52	4.3 ± 4.3	57.36 ± 4.24	68.03
		Spotted Bass <i>Lepomis megalotis</i>	2 COMP	98	1.59 ± 0.81	38.0 ± 48.5	40.72 ± 35.52	15.25
		Spotted Bass	1 IND	124	0.57	46.7	12.06	0.56
		White Bass <i>Micropterus punctulatus</i>	1 IND	226	0.98 ± 0.47	29.3 ± 19.4	18.69 ± 11.98	2.52
		Bluegill <i>Morone chrysops</i>	1 COMP	73	1.95	4.1	44.47	27.59
Fish Creek	Medium	Longear Sunfish	1 COMP	92	2.16	3.78	65.13	43.39
		Spotted Bass	1 IND	124	0.57	46.7	12.06	0.65
Blaine Creek	Medium	Bluegill	1 IND	110	0.85 ± 0.70	23.0 ± 2.3	9.53 ± 7.24	1.1
		Longear Sunfish	2 COMP	108	0.47 ± 0.08	26.8 ± 3.3	10.13 ± 3.27	0.95
		Spotted Bass	1 IND	231	0.55 ± 0.27	67.7 ± 44.3	5.38 ± 0.65	0.25
Ohio River	Low	Bluegill	1 COMP					
		Longear Sunfish	2 COMP	151	0.80 ± 0.04	64.8 ± 8.2	2.91 ± 0.01	0.12
		Spotted Bass	2 COMP	122	0.61 ± 0.30	54.3 ± 10.0	3.86 ± 0.64	0.19
		Largemouth Bass <i>Micropterus salmoides</i>	1 COMP	150	1.04	61.6	3.95	0.31
Kanawha River	Reference	Largemouth Bass	2 COMP	209	0.81 ± 0.03	50.5 ± 11.0	2.27 ± 0.64	0.12
		Bluegill	1 COMP	161	0.33	36.5	2.81	0.2
		Green Sunfish	1 COMP	119	0.27	49.0	3.09	0.16
Ohio River	Reference	Longear Sunfish	1 COMP	85	0.43	28.3	3.65	0.33
		Bluegill	1 COMP	121	1.98	33.6	3.19	0.24
		Longear Sunfish	1 COMP	120	0.75	46.4	2.81	0.15
		Spotted Bass	1 COMP	159	0.80	70.4	3.20	0.12
Largemouth Bass	1 COMP	208	0.90	56.6	2.59	0.12		

^a Composite sample designated as COMP; single fish individual sample designated as IND.

^b Bluegill X green sunfish.

high- and medium-exposure fish were significantly greater than respective levels from low-exposure and reference locations (z statistic = 4.19; $p < 0.0001$). Thus, at least for *Lepomis* sp. sunfish, whole-body levels of As did not differ among the four setting types, whereas fish from high- and medium-exposure locations had significantly greater Se concentrations and significantly lower Hg concentrations.

To evaluate the importance of water column Se levels as a determinant of whole-body tissue levels, least squares regression analysis was conducted using log-transformed water and tissue levels. An analysis was conducted using tissue levels measured in all fish species ($n = 9$), while a separate analysis was performed using tissue levels for the three sunfish species. For all species combined, the relationship between water Se and tissue Se was significant ($p < 0.001$; $r^2 = 0.60$). A stronger association ($p < 0.0001$; $r^2 = 0.83$) resulted when the analysis was limited to tissue levels for the three *Lepomis* species (Fig. 2).

To evaluate potential elemental associations in analyzed tissues, linear regression was used to compare the relationship between trace metals for all locations and species pooled. Log-transformed As levels were weakly correlated (positively) with log-transformed Se concentrations ($r^2 = 0.21$; $p < 0.01$), but were less correlated with Hg levels ($r^2 = 0.11$; $p < 0.05$). For all samples combined, there was a significant negative relationship between Se and Hg concentrations ($r^2 = 0.56$; $p < 0.001$; Fig. 3). Results of regression analysis using results for high- and medium-exposure locations only, and low-exposure and reference site locations only, indicated that the inverse relationship was considerably more pronounced in the former group ($r^2 = 0.38$; $p < 0.01$) compared with the latter ($r^2 = 0.03$; $p > 0.05$).

Selenium to Hg molar ratios for all species collected (ratio of $\mu\text{mol Se/g}$ to $\mu\text{mol Hg/g}$) are also provided in Table 3. Overall, there was a strong demarcation in molar ratio values between fish from the higher fly ash exposure sites relative to fish from

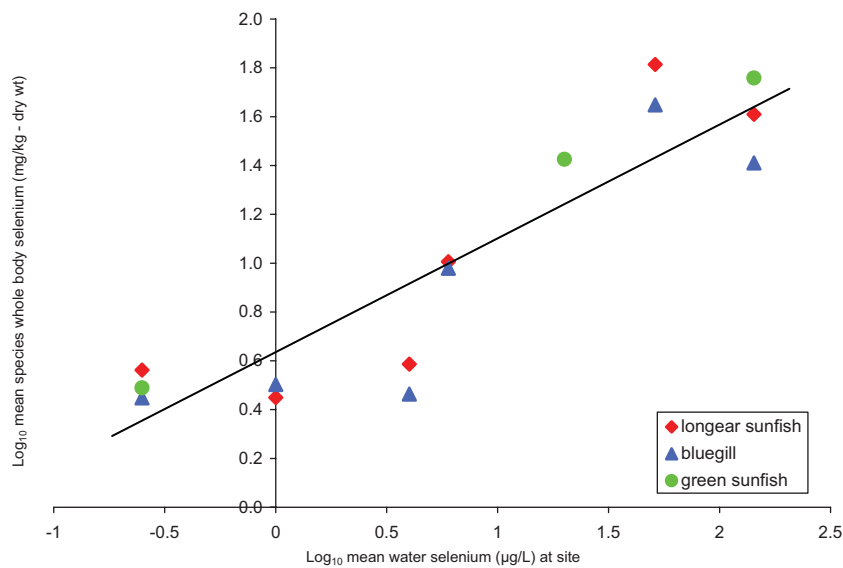


Fig. 2. Plot of mean whole-body Se concentration in three sunfish species versus mean water column Se concentration at five fly ash exposure and two reference stream locations. All concentration data shown are log₁₀-transformed; $r^2 = 0.83$.

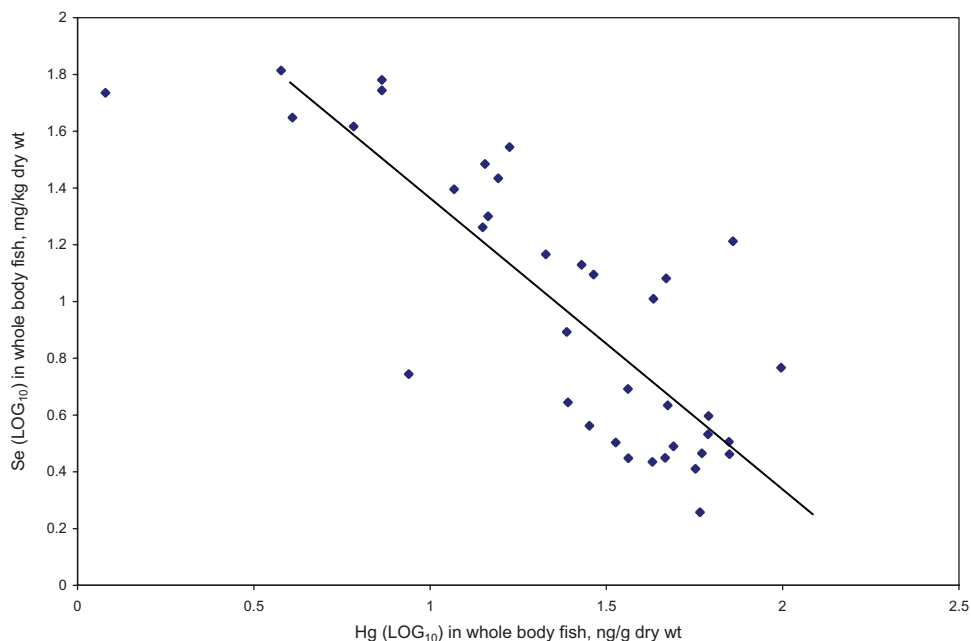


Fig. 3. Plot of whole-body Hg concentration versus whole-body Se concentration for all fish samples analyzed from both reference and fly ash exposure locations. All concentration data shown are log₁₀-transformed; $r^2 = 0.56$.

low-exposure or reference sites. Using combined molar ratio data for the three *Lepomis* species and comparing these between two exposure classifications (combined high- and medium-exposure; combined low-exposure and reference), results using the Mann–Whitney U test indicated that ratio values in the higher exposure group were significantly higher compared with values in the low-exposure/reference grouping ($p < 0.001$).

In assessing what factors were associated with elevated Se to Hg tissue molar ratios, only water Se concentrations were influential. When plotting mean species molar ratio values for all fish species from each exposure category against mean log₁₀-transformed Se concentration in water, a significant positive relationship was observed ($r^2 = 0.61$; $p < 0.001$;

Fig. 4). However, if this same analysis was restricted to only *Lepomis* sunfish species, the resulting r -square value increased somewhat ($r^2 = 0.79$; $p < 0.001$). The response of Se to Hg molar ratios to water Se concentrations suggested that a minimum Se concentration ($\sim 4 \mu\text{g/L}$) is required before ratios increase markedly with increasing water concentration (Fig. 4).

Stepwise multiple regression analysis (whole-body Se as dependent variable) indicated that tissue Se in the three *Lepomis* species was best explained (i.e., highest r^2 value) when six water quality parameters were combined as independent variables (total As, Mo, and Se; total hardness, SO_4 , and TDS). This relationship was highly significant ($p < 0.0001$;

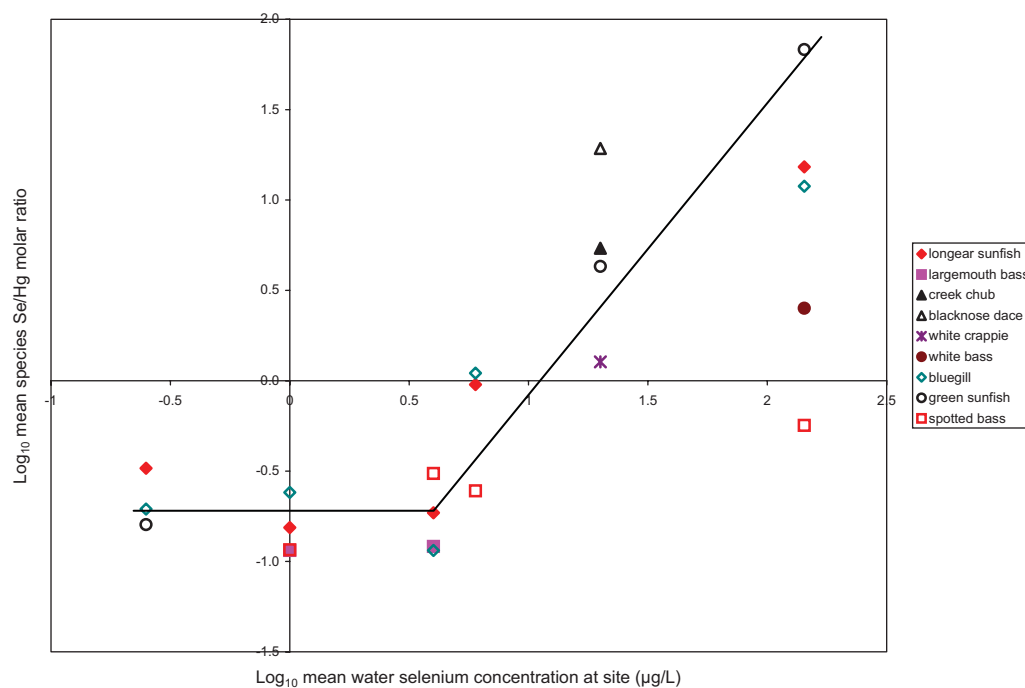


Fig. 4. Plot of species mean Se to Hg molar ratio versus mean water Se concentration at five fly ash exposure and two reference stream locations, all species collected; $r^2 = 0.61$.

$r^2 = 0.96$). The best two-, three-, and four-variable model explaining variation in tissue Se levels were As and Se ($f = 45.6$; $r^2 = 0.86$); As, Se, and SO_4 ($f = 48.8$; $r^2 = 0.91$); and As, Se, SO_4 , and TDS ($f = 45.8$; $r^2 = 0.91$). Clearly, Se and As water concentrations were the first and second most important variables explaining variability in whole-body Se concentrations.

DISCUSSION

The results of the present study indicate distinct tissue trace element profiles in freshwater fish that are exposed in situ to a chemical matrix originating from leached fly ash particles. In general, the magnitude of trace metal tissue departure from reference stream fish was exposure-dependent. There did appear to be, however, a certain threshold of fly ash exposure magnitude that, if not exceeded, did not manifest in unique Hg and Se tissue level signatures compared with reference fish not exposed to fly ash. In extrapolating an Se water concentration that is associated with potentially harmful elevated whole-body concentrations, results from the present study indicate that a water Se concentration of approximately $4.9 \mu\text{g/L}$ was associated with a whole-body Se concentration of 7.91 mg/kg , a value proposed to be protective of freshwater fish [29].

Due to the nature of field studies, a relevant question to address is the demographic fidelity of fish collected at the various exposure and reference locations. While fish collected from high-exposure settings (CR and LSC) are likely permanent residents of the fly ash impoundment receiving streams (previous studies of these streams have documented unique biochemical and hematological parameters in resident fish, e.g., [30]), there was some uncertainty in the actual long-term fly ash exposure for fish collected from BC and FC (medium-exposure sites) and the OR (low exposure). Depending on the movement patterns of fish in these streams, their actual exposure to fly ash-influenced water may be less than was assumed or anticipated.

Despite this uncertainty, levels of Se in fish from medium-exposure and low-exposure settings were intermediate between levels in fish from high-exposure settings and at reference sites.

Previous studies of fish inhabiting Se-enriched fly ash impoundments (or fly ash impoundment receiving streams, as in the present study) have documented an extensive array of individual or population-level aberrations. Commonly, fish exposed to fly ash-enriched water have elevated levels of trace elements relative to reference fish. Elevated Se tissue levels, due partly to the relatively high enrichment factor of this trace element, is a typical indicator of fly ash exposure in fish and invertebrates [1].

What seems to be highly variable, in aquatic life exposed to fly ash, is how other trace elements covary with Se concentrations. In a field study, both Se and As concentrations in largemouth bass collected from a fly ash basin were elevated in four distinct tissue types, whereas elevated levels of other trace elements were not nearly as consistent [31]. At Belews Lake, NC, Se was the only trace metal elevated in fish collected from the fly ash-influenced portion of the reservoir [32]. In contrast, several metals (including Se) were elevated in sunfish and minnow species collected from fly ash pond receiving streams in Ohio and West Virginia [30,33]. It may be that the propensity for one (or more) trace elements to become elevated in fish and invertebrates is largely dependent on the relative mass, and bioavailability, of contaminants desorbed from fly ash particles.

There have been comparatively few reports of Se–Hg tissue interactions in fish inhabiting water bodies affected by fly ash exposure. In a fly ash treatment quarry, whole-body tissue levels of Hg increased when fly ash input into a treatment quarry ceased [34]. In a study concerning two sunfish species collected proximal to (and distant from) coal-fired power plants, fish collected near the facilities had lower tissue Hg and higher tissue Se relative to fish collected at lakes more distant ($> 14 \text{ km}$

distance) [35]. While the authors suggested that proximity to coal-fired power plants may have resulted in higher external loadings of Se due to atmospheric deposition or wastewater inputs, no actual exposure data were presented (Se and Hg levels in water or sediment).

The present study provides evidence demonstrating the relationship between fish tissue Se and Hg concentrations along a spectrum from reference (no fly ash exposure) to high fly ash exposure. While the highly significant inverse relationship between fish tissue Se and Hg concentrations observed cannot conclusively prove a direct *in vivo* antagonistic effect of Hg accumulation caused by Se, statistical associations between Se and Hg levels in aquatic biota are not uncommon in the literature. Significant relationships between Se and Hg concentrations in young walleye (*Sander vitreum*) collected from a series of boreal lakes downwind of a major air pollution source (nickel smelter) were reported [36]. Like the present study, the authors found that the Se content in tissues was closely related to water Se concentration. Likewise, tissue Hg and methyl Hg levels were negatively correlated with water column Se. Last, the authors observed that levels of methyl Hg in various tissue types decreased only after a threshold level of Se had been reached. This pattern may be analogous to the observation in the present study that Se to Hg molar ratios increased sharply only when water Se concentrations (a variable, itself, which explained significant variation in whole-body Se concentrations) exceeded an apparent threshold concentration. In a laboratory study, the rate of methyl Hg bioaccumulation in a freshwater oligochaete decreased when either organic matter or moderately high levels of Se were added in sediments [37]. More comprehensive reviews of Se's affect on Hg accumulation and toxicity in aquatic biota are available [38,39].

As indicated by results of multiple regression analysis, water column levels of Se and As were the most important variables in predicting tissue Se levels. Other chemical variables, however, were also associated with whole-body Se levels but to a lesser degree: total Mo, total hardness, TDS, and SO₄. Leachate water samples collected at fly ash impoundments (at facilities where bituminous coal is burned) had median concentrations of Mo and SO₄ of 214 g/L and 171 mg/L, respectively [15]. Levels of these constituents in water samples collected at the two high-exposure sites, and one medium-exposure site, were higher than these median values. SO₄ is known to mitigate the acute toxicity of selenate (SeO₄-2) [40]. Moreover, concentrations of total Hg in muscle tissue were negatively correlated with water SO₄ concentrations for some, but not all, species of freshwater fish collected from a variety of watersheds in the U.S. [41]. Whether comparatively high SO₄ concentrations in the high fly ash exposure streams contributed to the accumulation patterns of trace elements analyzed in the present study is unknown, as the relatively low sample sizes precluded a robust statistical evaluation.

Due to upcoming regulatory initiatives that are expected to promulgate more stringent controls on the disposal of CCBs, the release of coal ash impoundment water to surface waters will likely decrease and, eventually, this form of disposal may be phased out [42]. Thus, loadings of Se and other trace elements to water bodies from these treatment facilities will decrease. This development will provide opportunities to study the response of fish and other aquatic life (e.g., changes in bioaccumulation, population health, and community structure and function) to this cessation of external metals loading.

SUPPLEMENTAL DATA

The Supplemental Data includes one table (16 kb).

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