ECOLOGICAL IMPACTS OF THE TVA COAL ASH SPILL IN KINGSTON, TN: A TWO YEAR ASSESSMENT

A Thesis
by
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Foreword

The research described in this thesis will be submitted to the *Journal of Environmental Science and Technology*, which is a peer-reviewed journal published by the American Chemical Society. The material within this document has been prepared in accordance with the specifications outlined by the *Journal of Environmental Science and Technology*.

Abstract

ECOLOGICAL IMPACTS OF THE TVA COAL ASH SPILL IN KINGSTON, TN: A TWO YEAR ASSESSMENT (August 2011)

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A two year investigation into the environmental impacts of the largest industrial spill of coal combustion waste (CCW) in the history of the United States (U.S.) at the Tennessee Valley Authority (TVA) Kingston coal-fired power plant revealed several impacts. First, selenium concentrations were identified above criterion continuous concentration (CCC) set by the U.S. Environmental Protection Agency in total available water samples, and sediment samples were elevated up to 21 times background concentrations for arsenic following the spill. Second, fish body burdens for arsenic and selenium were statistically elevated in the months following the spill, particularly in redear sunfish. Third, body burdens were found to be statistically different between fish species for many elements including: arsenic (As), silver (Ag), selenium (Se), vanadium (V), and thallium (Tl), which can be attributed to diet and trophic level. Forth, concentration of selenium in fish tissues were found above proposed biological effects concentrations, particularly in redear sunfish which had hepatic selenium concentrations at levels above 20 mg/kg (Parts Per Million, ppm). Furthermore, fish were identified with pathological abnormalities such as exopthalmus, histopathological changes in the gills, reduced condition index, bacterial infections, and fin erosion. However, following an analysis of young bluegill sunfish demonstrating exopthalmus, no direct correlation could be made between these individuals and highly elevated contaminant concentrations. Fish populations are showing sustained elevation of toxic elements two years following the spill but are also likely benefitting from immigration/ emigration of individuals from healthy source populations creating sustained populations.

Dedication

To my loving parents, James and Teresa Jackson

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Introduction

On December 22, 2008, 4.1 million cubic meters of coal combustion waste (CCW) was released into the Emory River and Watts Bar Reservoir at the Tennessee Valley Authority (TVA) coal combustion plant in Kingston, Tennessee. [1] This event is the largest industrial spill in the history of the United States, releasing 5 times more volume than the BP gulf oil spill of 2010, and surpassing the Exxon Valdese spill of 1989 by over 100 times.[2, 3] The coal ash released during this event entered the environment with such force that it traveled 4 miles upstream and covered 300 acres of land and water with deposits up to 10 meters deep.[4] The majority of CCW released during this event flowed into the adjacent Emory River, which then joins the Clinch and Tennessee Rivers, 2 and 6 miles downstream, respectively (Figure 1).

Coal contains trace amounts of many toxic elements including arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), selenium (Se), and vanadium (V). However, during the combustion process, carbon is removed and these trace elements become concentrated.[5-7] According to the American Coal Ash Association annual production of CCW was over 136 million tons in 2008.[8] Storage of CCW is typically conducted by creating lagoons to hold the slurry of coal ash that has been mixed with water to minimize air transport; however, leaching of toxins from these lagoons has been well documented.[6] Previous studies of CCW releases have found that leachable trace elements have the potential to elicit histopathological and physiological effects on exposed organisms[6] and even cause the extirpation of entire aquatic populations.[9, 10]

Initial studies following the TVA spill found elevated concentrations of contaminants in sediments and water samples within the river system and in pore water where concentrations of arsenic reached up to 2000 μg/L.[1, 11] Despite these localized and highly elevated levels of contaminants within the environment, detrimental effects to aquatic organisms have not been previously reported.

The focus of this study was to provide a long-term assessment of the impacts of the TVA coal ash spill on the fish populations in the surrounding waters. This was performed by examining whether fish body burden levels reached reported toxic thresholds, documenting any pathologies commonly associated with CCW exposure,[12, 13] and determining tissue-level and species specific fates of specific CCW elements in fish populations.

Methods and Materials

Water, sediment, and fish were sampled from locations around the spill site (Figure 1) during seven collection dates from January 2009 to January 2011. Grab water samples were collected from mid-channel at all locations by dipping a plastic container one foot below the water's surface, and samples were fixed with concentrated nitric acid to a pH below 2 . Water samples were split into triplicates and prepared by microwave assisted acid digestion following U.S. Environmental Protection Agency (EPA) Protocol 3015A.[14] Sediment samples were collecting using an Ekman grab and stored in Whirl-paks® on ice for transport. Sediment samples were frozen at -20°C, dried by lyophilization, split into triplicates (0.5g), and prepared by microwave assisted acid digestion following EPA Protocol 3051B.[15] Fish samples were collected using an electrofishing boat, identified to species, any external abnormalities were noted, and total lengths were recorded. Fish selected for body burden analysis [mainly largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), gizzard shad (*Dorosoma cepedianum*), and redear sunfish (*Lepomis microlophus*)] were fish-tagged, with colors specific to collection location, and stored on ice for transport. In the lab, fish weight and total length were recorded and fish were dissected for muscle, liver, stomach, gastric caecum, spleen, and gonads. Liver and gonad weights were recorded. Tissues were individually wrapped in aluminum foil, frozen, lyophilized, homogenized, and digested following the Protocol EPA 3051B.[15] All samples were analyzed for elemental concentrations of silver (Ag), As, cobalt (Co), iron (Fe), manganese

(Mn), molybdenum (Mo), Se, thallium (Tl), and V using Inductively Coupled Plasma-Optical Emissions Spectrometry (ICP-OES) by EPA Protocol SW-846 Method 6010C.[16] National Institute of Standards and Technology (NIST) certified fish tissue samples were also microwave assisted acid digested following EPA protocol 3051B,[15] and quantified using ICP-OES by EPA Protocol SW-846 Method 6010C (Table S1).[16]

Gill Analysis

Gill analysis was conducted on largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), and redear sunfish (*Lepomis microlophus*) collected January 8 and 9, 2009. Gills were removed in the field and immediately fixed in 4% buffered paraformaldehyde. Gills were rinsed in phosphate buffered saline prior to paraffin processing. Paraffin blocks were sectioned at 7µm and adhered to microscope slides. Sections were stained with hematoxylin and eosin and examined using light microscopy Leica CME (Wetzlar, Germany).

Statistical Analysis

Means were highly heterogeneous among populations, so non-parametric Kruskal-Wallis tests were conducted using SAS version 9.2 (SAS Institute, Cary, NC). Rank means were then analyzed using general linear model analysis of variance (ANOVA) and differences among groups were determined using Tukey's Honest Significant Difference post-hoc test. Box plot construction was performed using Minitab 12 software (State College, PA); middle lines indicated median; bottom and top of boxes indicated 25^{th} and 75^{th} percent quartile range, respectively; vertical lines indicated range of data; and points outside of 1.5 times the inner-quartile range are indicated by stars.

Results and Discussion

Water analysis for the total available metals indicated higher levels of several elements within the water column near the spill in the first months following the event with a subsequent decrease in concentration over time (Table 1). Total available Se concentrations in the water consistently exceeded the US EPA criterion continuous concentration (CCC) for chronic exposure to freshwater life of 5.0 µg/L[17] during the 25 month study period, with the peak concentrations found during May 2010, which was a period of active dredging in the Emory River where Se averaged 11 µg/L. All sampling locations were identified with exceedances of Se above EPA critical concentration criteria at some point during this study. However, as indicated in Table 1, the ICP-OES instrument detection limit (IDL) for Se was 6µg/L, which is above the EPA regulated CCC. Other than the first sampling at 17 days, no waterborne contaminants tested besides Se exceeded EPA criteria during the course of the analysis.

Sediment analysis indicated elevated concentrations of many contaminants following the spill (Table 2), including As, Co, Mo, Se, and V. Concentrations of sediment contaminants found in January 2009 are consistent with a recent study characterizing the trace elements released during this event, which are up to 21 times background levels as demonstrated by As.[1] As a result of dredging, resuspension and downstream re-entrainment, and transport of sediments from upstream reaches during flooding events, contaminant levels in sediment at the site of the spill decreased over the two year period but still remained elevated over reported background levels for the area for some elements (Table 2).[1] As, which has been identified as a good indicator of the presence of coal ash within this system,[1, 11] decreased at the site of the spill over time, while gradually increasing at collection sites up to 6 miles downstream(Table 3). V was also recognized during this study as a good indicator of coal ash presence within sediment samples, as it was greatly elevated along with arsenic in areas where coal ash was deposited (Tables 2, 4).

Kruskal-Wallis analysis of fish body burden indicated a significant statistical difference between contaminant concentration and fish species for all elements tested ($p < 0.001$; Table 5). Redear sunfish (*Lepomis microlophus*) exhibited the highest concentrations of selenium, with an average concentration for all tissues of 8 mg/kg dry weight basis (Table 5). These results support previous findings that centrarchids often accumulate the highest concentrations of selenium.[18] As (5 mg/kg) and Tl (0.05 mg/kg) concentrations were the highest within another centrarchid species, the white crappie (*Pomoxic annularis*); however, sampling size for this species was small (N = 15 for all tissues; Table 5). Gizzard Shad (*Dorosoma cepedianum)* average body burden contained the highest levels for Co (1 mg/kg), Fe (85 mg/kg), Mn (69 mg/kg), and V (2 mg/kg). The elevated levels of these elements within shad samples could be attributed to the gut content contained in whole body samples. Whole body sampling was only conducted for shad samples as a result of small size and difficulty of dissection of internal organs within this species.

As body burdens were found highest in white crappie, black crappie, and redear sunfish; however, as indicated in the previous paragraph, crappie had incomplete collections (Figure 2A). A comparison of redear sunfish tissues showed that liver samples had higher concentrations of As than other tissues (Liver > Testes > Gastric Caecum > Stomach > Spleen > Ovary > Muscle), with a mean concentration of 11 mg/kg for all sites over two years (Figure 2B). Liver concentrations in redear sunfish have also shown a significant difference between the first collection date and the subsequent collections, with the peak liver concentrations in redear sunfish occurring during the January 2010 collection and averaging 15 mg/kg for all sites (Figure 2C). Complete redear sunfish collections (N = 35) were only gathered at one location over the two year period, Clinch River mile 5.5 (CRM 5.5, Figure 1). Liver samples collected from CRM 5.5 over this period are shown in Figure 2D, where concentrations of As peaked in January 2010 with a mean concentration of 24 mg/kg.

There was a significant difference between species and Se concentration, with redear sunfish having the highest concentrations (Figure 3A). Differences in Se concentrations by tissue were also found to be statistically significant (Figure 3B), and like As, the greatest concentrations of Se were found in liver samples. Body burden of Se in both muscle (Figure 3C) and liver (Figure 3D) indicate an increase due to the spill, with the greatest concentrations being found at the site of the spill and sites just downstream when compared to upstream and collections further downstream. Mean Se liver burden was also found to be elevated during all collection dates following the first collection taken 17 days after the spill, indicating that this was a result of this event (Figure 3E). There was no significant difference between collection date and Se concentration in redear sunfish liver samples at CRM 5.5; however, all samples from this site were greatly elevated compared to other sites and peaked during the January 6, 2010 collection (day 379) at about 20 mg/kg (Figure 3F). While there were not any statistically significant differences in collection days at CRM 5.5, liver Se values of 20 mg/kg were toxicologically significant. A proposed biological effect concentration for fish liver tissue is 12 mg/kg, which results in reproductive failure.[19] Selenium levels of 12 mg/kg in liver samples have been shown to result in changes in red blood cell counts and blood iron concentrations in Rainbow Trout (*Oncorhynchus mykiss*).[20] It has been shown that reproductive failure occurs at Se concentrations of 22 mg/kg in bluegill sunfish liver,[21] and mortality can occur at 32 mg/kg.[22] Whole body gizzard shad samples contained a mean Se concentration of 4 mg/kg (dry weight basis; Table 5), which is also above the suggested threshold of 3 mg/kg for dietary toxicity from prey organisms.[19]

Both As and Se body burdens were highest in redear sunfish livers, where tissue levels were documented at over 20 mg/kg (ppm) for both contaminants at CRM 5.5 (Figures 2F, 3F). Similar concentrations of both As and Se were found in redear sunfish liver samples at other locations (Figure S1 and S2) but the sample sizes were smaller. The gut contents of redear sunfish most likely explain why As and Se accumulated in the livers. Dissection of redear sunfish stomachs indicated a diet primarily of Sphaeriidae fingernail mussels, which are filter feeders that inhabit the contaminant rich benthos. Diet has been identified as the dominant mode of uptake for Se due to its propensity to bioaccumulate,[23] and the discrepancy between the accumulation of contaminants in redear sunfish and other fish species occupying the same trophic level from the same location indicates differences in diets as the likely cause. Accumulation of toxins in the liver may also be attributed to the high metabolic activity and detoxification pathways occurring in the liver as opposed to other tissues.[24, 25]

V concentrations showed a similar trend as As and Se, with the highest concentrations found in redear sunfish liver tissue, which averaged 6 mg/kg. V was also significantly elevated in all collections following the initial collection taken 17 days after the spill. There were also differences between sites and vanadium concentrations, with Emory River Mile (ERM) 2.0 averaging the highest concentration for all tissues at 1 mg/kg; however, vanadium concentrations remained well below reported toxic thresholds (Figure S3).[26, 27] Mn is another element that has shown significant differences between samples, but concentrations of Mn (mean 14 mg/kg) remained well below toxicity thresholds throughout the study period, and are not further discussed here (Figure S4). No statistical differences were found between date and/or collection sites for cobalt, silver, or thallium, which had means of 0.5, 0.04, and 0.04 mg/kg, respectively, for all fish tissues collected during this study period. All of these concentrations are below toxicity thresholds and are not discussed further (Figure S5-S7).

Gill analysis of fish collected at ERM 2.0, seventeen days after the spill revealed edema in the filamental epithelium, lamellar vasodilation, lifting of the lamellar epithelium, and lamellar fusion due to cell proliferation, compared to reference fish collected 6 miles downstream in the Tennessee River (Figure 4). These pathological changes have been hypothesized to be defense mechanisms whereby separation of the epithelia and lamellae results in a greater distance that pollutants must diffuse across before reaching the bloodstream.[28]

Exopthalmus, or protruding eyeball, was found in many fish collected during this study, including the January 2010 collection where 33 individuals (9%) from Tennessee River Mile (TRM) 567 were identified with this anomaly (Figure 5). The vast majority of individuals displaying exopthalmus were either bluegill or redear sunfish; however, several largemouth bass were also identified with this deformity. These findings are supported by other studies which found this condition most often in centrarchids as well.[13, 29] In a review,[12] exopthalmus was identified as the result of incorporation of selenoproteins into internal organs, which produced “leaky” membranes and increased internal blood pressure. We hypothesize that another potential cause of exopthalmus could be the result of the decreased osmoregulatory ability within the gills, leading to edema and increased internal pressure. Although we have documented elevated levels of Se within centrarchids following this event, a comparison of bluegill sunfish identified with exopthalmus and an equal number of individuals collected from the same location during the same period without this abnormality, showed no correlation between selenium concentration and exopthalmus occurrence.

Several individuals collected during the study period were emaciated and had a severe decrease in condition index [weight (g) / length (cm)], an indication of poor health. Some individuals collected from the spill site had significantly lower condition indices than comparable fish at downstream reaches; however, there was not a statistical difference between condition index in fish from the spill site and downstream sites ($p = 0.56$). Our findings are also supported by other studies which showed fish populations exposed to CCW had much lower condition indices than non-exposed, control populations.[30, 31]

Fish collected during our investigation were also identified with fin and operculum deformities; however, the deformities found here were not consistent with those associated with teratogenic defects described in previous studies due to Se exposure[12] and were more consistent with fungal infection or fin erosion, which can be a result of depressed immune function.[32]

Other abnormalities observed in individual fish were lesions on the body, parasitic worms, and bacterial infections on the fins and operculum, all of which occurred at very low frequency. None of these symptoms have been identified by previous studies as a direct result of exposure to CCW but may be general indications of poor health or compromised immune function in fish populations.[32]

During this study, Se body burdens were found at or exceeding proposed thresholds for toxicity, were at or above documented levels where reproduction was adversely affected, and abnormalities commonly associated with contaminant exposure were identified. However, no correlation was found between fish containing abnormalities and increased contaminant body burden. Gill samples were the only tissues examined for histopathological changes; however, other studies have commonly shown alterations in other tissues.[9, 31] Reproductive ability of individuals also could have been characterized, which when combined with histopathological studies, may have shown an effect resulting from high body burden concentrations. Although we have documented elevated levels of contaminants and abnormalities commonly associated with elevated contaminants levels, fish populations do not seem to be severely affected, which is probably a result of immigration and emigration of healthy source populations from/to unaffected areas within the large (40,000 acre) reservoir. Due to the lack of correlation between elevated levels of contaminants and observed effects, individual fish in the area of the TVA coal ash spill can only be described as being at risk of experiencing toxic effects from CCW exposure.

Table 1. Total Available Metals in Water Samples Collected by Date from all Locations Surrounding the Tennessee Valley Authority (TVA) Power Plant in Kingston, TN.

Total Available Metals																				
	Jan 09			July 09			Jan 10			May 10			Sep 10			Jan 11				
Element	Mean	*SD*	*N*	Mean	SD	N	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	IDL	CCC
Ag	NA	NA	NA	2	2	21	BDL		15	BDL		18	BDL		15	BDL		18	0.06	3.2*
As	4	4	18	7	6	21	BDL		15	BDL		18	BDL		15	BDL		18	4	150
Co	NA	NA	NA	2	1	21	0.8	0.3	15	0.9	0.4	18	0.6	0.4	14	1.2	0.3	18	0.3	
Fe	547	314	18	223	102	21	285	68	15	11	4	18	263	38	15	193	49	18	3	1000
Mn	NA	NA	NA	58	12	21	62	13	15	66	8	18	42	10	15	56	21	18	0.07	
Mo	2	1	18	7	9	19	0.1	0.2	2	11	11	4	BDL		15	BDL		18		
Se	6	4	16	7	4	21	7	5	12	11	6	18	BDL		15	BDL		12	6	5
Tl	NA	NA	NA	BDL		21	BDL		15	BDL		18	BDL		15	BDL		18	4	
V	NA	NA	NA	6	4	21	BDL		15	BDL		18	BDL		15	2	1	18	0.9	

Concentrations are reported in µg/L (ppb) and are reported as averages over all sampling locations during each collection date. Instrument detection limit (IDL) is also reported for each element, and EPA critical concentration criteria (CCC) for chronic exposure is reported. * Indicates acute CCC. BDL indicates below detection limit for the instrument. N/A indicates that samples were not analyzed for a particular element. Standard Deviation is indicated by *SD*. Total number of samples analyzed is indicated by *N*. Dates are indicated by Jan 09 (January 2009), July 09 (July 2009), Jan 10 (January 2010), May 10 (May 2010), Sept 10 (September 2010), and Jan 11 (January 2011). Elements are indicated by silver (Ag), arsenic (As), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), thallium (Tl), and vanadium (V).

Table 2. Element Concentrations in Sediment Samples by Collection Date in all Locations Surrounding the Tennessee Valley Authority (TVA) Power Plant in Kingston, TN.

Sediments																						
	Jan 09			March 09			July 09			Jan 10			May 10			Sept 10			Jan 11			Backgroud Levels	
Element	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*	*N*	Mean	*SD*
Ag	0.01	0.01	3	0.01		1	0.01	0.01	3	6	1	3	0.092	0.002	3	BDL		3	BDL		3		
As	76	2	3	62	4	3	76.1	0.2	3	2.5	0.3	3	14	2	3	21.9	0.8	3	18	4	3	4	2
Co	18	1	3	18	2	3	17	2	3	BDL		3	4.3	0.4	3	3.1	0.4	3	8	5	3	7	8
Mn	98	4	3	193	6	3	84	3	3	BDL		3	98	16	3	BDL		3	BDL		3	1056	1007
Mo	3.06	0.03	3	2.8	0.3	3	2.5	0.03	3	0.6	0.5	3	0.6	0.2	3	BDL		3	BDL		3	0.2	0.4
Se	6.4	0.3	3	2	1	3	2.9	0.3	3	0.3	0.3	3	0.8	0.5	3	1.1	0.6	3	1.0	0.5	3	0.9	2
Tl	0.9	0.2	3	0.6	0.2	3	0.4	0.3	3	BDL		3	BDL		3	0.34	0.06	3	0.4	0.2	3		
V	78.6	0.9	3	51	3	3	84	5	3	10.6	0.2	3	9	1	3	30	1	3	25	4	3	20	4

Concentrations are reported in mg/kg (ppm) and are reported as averages over all sampling locations during each collection date. BDL indicates below detection limit. Background levels are reported from a previous study of sediment on concentrations in this area following the spill.[1] N/A indicates that samples were not analyzed for a particular element. Standard Deviation is indicated by *SD*. Total number of samples analyzed is indicated by *N*. Dates are indicated by Jan 09 (January 2009), March 09 (March 2009), July 09 (July 2009), Jan 10 (January 2010), May 10 (May 2010), Sept 10 (September 2010), and Jan 11 (January 2011). Elements are indicated by silver (Ag), arsenic (As), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), thallium (Tl), and vanadium (V).

Table 3. Arsenic Concentrations in Sediment Samples by Collection Date and Proximity to the Spill Site.

Arsenic								
River Miles From Spill Site	Jan 09	Mar 09	July 09	Jan 10	May 10	Sept 10	Jan 11	
	1.6	82	1.2	N/A	5.3	4.8	1.6	Mean
-2	0.4	3	0.4	N/A	0.3	0.2	0.3	*SD*
	76	62	76.1	2.5	14	21.9	18	Mean
0	2	4	0.2	0.3	2	0.8	4	*SD*
	40	16	31	22.9	19.0	19	16	Mean
2.5	2	3	5	0.2	0.4	1	1	*SD*
	21.8	20	20	22.8	31	18	22.8	Mean
6	0.7	8	2	0.5	2	2	0.5	*SD*

All concentrations are in mg/kg (ppm) dry weight. Negative values indicate upstream and positive values indicate downstream. Standard deviation is indicated by *SD*. N/A indicates samples were not taken. Dates are indicated by Jan 09 (January 2009), March 09 (March 2009), July 09 (July 2009), Jan 10 (January 2010), May 10 (May 2010), Sept 10 (September 2010), and Jan 11 (January 2011). Collection locations are indicated by -2 (Emory River Mile 4), 0 (Emory River Mile 2), 2.5 (Clinch River Mile 3.5), 6 (Tennessee River Mile 567).

Table 4. Vanadium Concentrations in Sediment Samples by Collection Date and Proximity to the Spill Site.

Vanadium								
River Miles From Spill Site	Jan 09	Mar 09	July 09	Jan 10	May 10	Sept 10	Jan 11	
-2	5	78	2.56	N/A	9.1	10.0	3.5	Mean
	1	2	0.07	N/A	0.5	0.8	0.2	*SD*
0	78.6	51	84	10.6	9	31	25	Mean
	0.9	3	5	0.2	1	1	4	*SD*
2.5	56	34	42	33	33.0	35	28.3	Mean
	1	4	3	2	0.8	2	0.5	*SD*
6	35	35	32	31	40	33	31	Mean
	1	8	2	1	5	3	1	*SD*

All concentrations are in mg/kg (ppm) dry weight. Negative values indicate upstream and positive values indicate downstream. Standard deviation is indicated by *SD*. Dates are indicated by Jan 09 (January 2009), March 09 (March 2009), July 09 (July 2009), Jan 10 (January 2010), May 10 (May 2010), Sept 10 (September 2010), and Jan 11 (January 2011). Collection locations are indicated by -2 (Emory River Mile 4), 0 (Emory River Mile 2), 2.5 (Clinch River Mile 3.5), 6 (Tennessee River Mile 567).

Table 5. Fish Body Burden by Species from all Dates and Locations Surrounding the Tennessee Valley Authority (TVA) Power Plant in Kingston, TN.

	Species																							
	BG			BLKC			CC			LMB			RES			SHAD			SMB			WTC		
Element	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***	**Mean**	***SD***	***N***
Ag	0.005	0.014	8	0.002	0.004	14	0.06	0.44	75	0.04	0.65	539	0.04	0.33	272	0.007	0.022	92	0.01	0.03	48	0.02	0.02	15
As	0.4	0.2	8	3	1	14	1	1	75	2	0	539	4	4	272	2	2	92	2	1	48	5	2	15
Co	1	1	8	0.2	0.2	14	0.7	0.7	75	0.4	0.4	539	0.7	0.9	271	1	1	92	0.4	0.4	48	0.2	0.2	15
Fe	54	63	6	45	57	10	79	106	35	66	70	407	82	125	199	85	80	53	75	73	40	56	81	12
Mn	19	21	8	5	5	14	13	23	74	7	6	539	15	28	270	69	62	84	8	10	48	7	5	15
Mo	0.3	0.3	7	0.3	0.2	14	1	1	45	0.4	0.6	348	0.5	0.6	159	0.1	0.2	53	0.3	0.3	23	0.4	0.4	15
Se	3.4	2.4	8	5	3	14	5	3	75	6	2	539	8	4	271	4	2	92	6	2	48	5	2	15
Tl	0.02	0.05	7	0.02	0.05	14	0.03	0.08	75	0.04	0.12	539	0.04	0.14	271	0.04	0.10	91	0.04	0.11	43	0.05	0.08	15
V	0.2	0.2	8	0.07	0.06	14	1	2	75	0.1	0.2	539	1	3	271	2	3	92	0.2	0.3	48	2	2	15

Concentrations are reported in mg/kg (ppm) and are reported as averages over all sampling dates, locations, and tissues. Standard Deviation is indicated by *SD*. Total number of samples analyzed is indicated by *N*. Fish species are indicated by; BG (*Lepomis macrochirus*, bluegill sunfish), BLKC (*Pomoxis nigromaculatus*, black crappie), CC (*Ictalurus punctatus*, channel catfish), LMB (*Micropterus salmoides*, largemouth bass), RES (*Lepomis microlophus*, redear sunfish), SHAD (*Dorosoma cepedianum*, gizzard shad), SMB (*Micropterus dolomieu*, smallmouth bass), and WTC (*Pomoxis annularis*, white crappie). Elements are indicated by silver (Ag), arsenic (As), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), thallium (Tl), and vanadium (V).

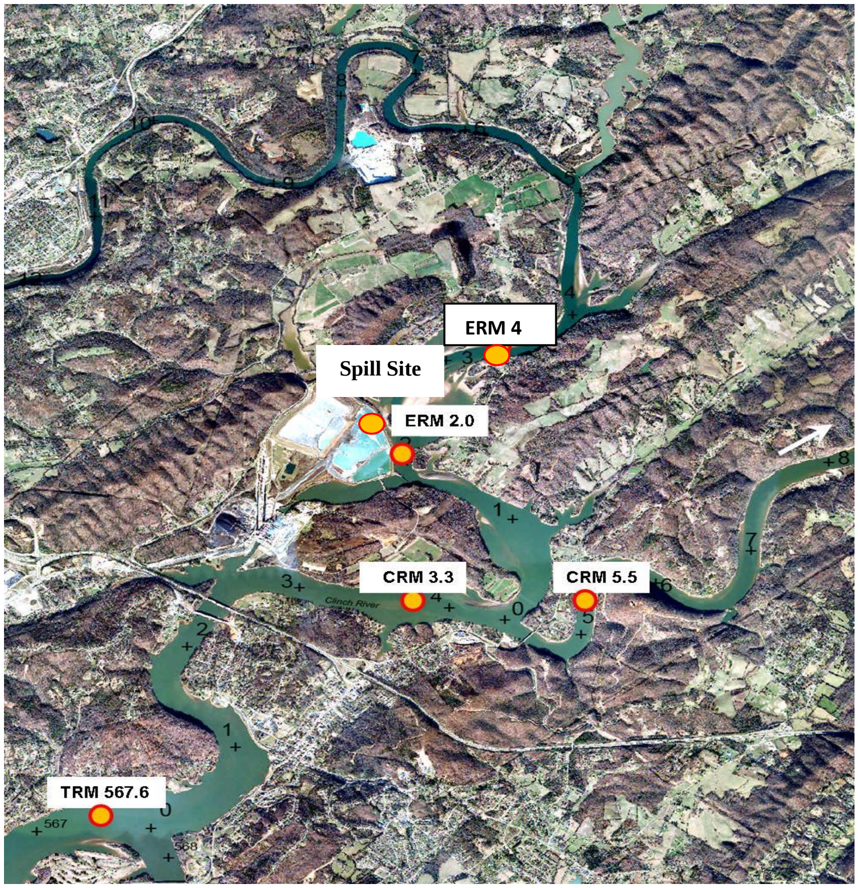

Figure 1. Satellite Image Identifying Locations of Collection Sites Around the Ash Spill Site. Two collection sites are located on the Emory River (ERM 2 and 4), two collection sites are located on the Clinch River (CRM 3.5 and 5.5), and one site is located on the Tennessee River (TRM 567.6). Image created by Marshall Adams, Oakridge National Laboratory.

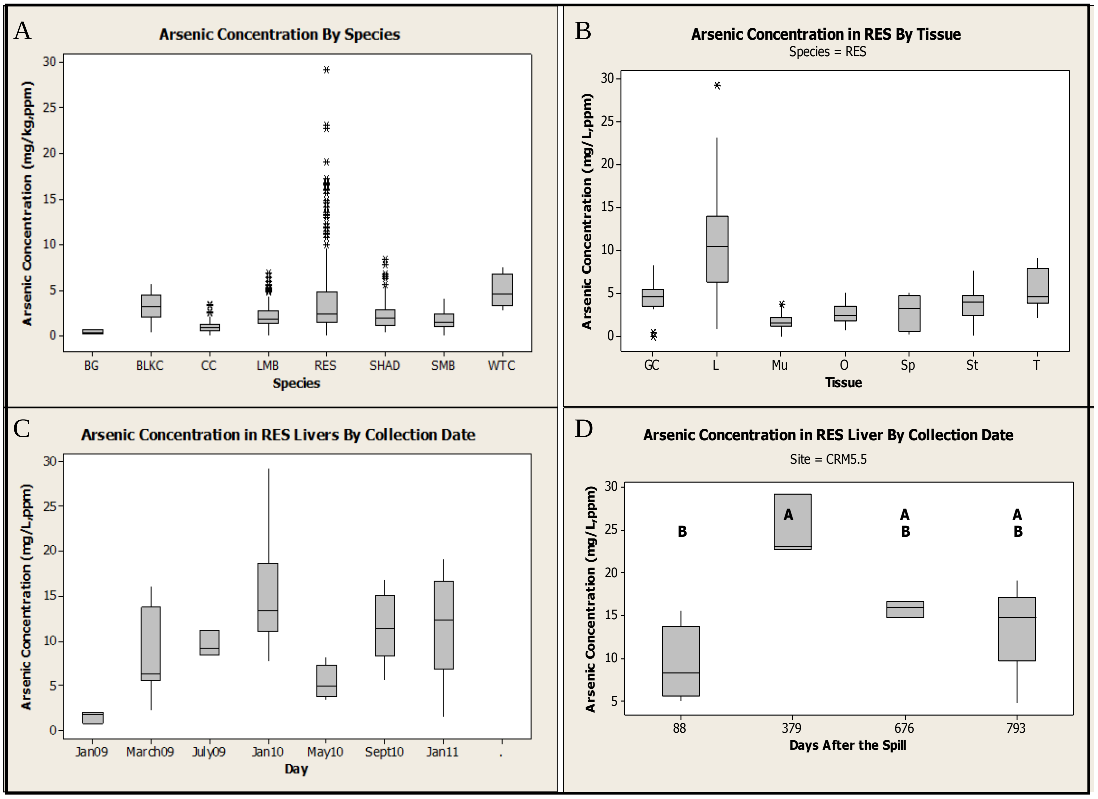

Figure 2. Arsenic Body Burdens in Fish Collected over the Two Year Period following the Tennessee Valley Authority (TVA) Coal Ash Spill. A) Arsenic body burden by fish species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad) SMB (Smallmouth Bass), and WTC (White Crappie). B) Arsenic concentration by redear sunfish tissue; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), and T (testes). C) Arsenic concentration in redear sunfish livers by collection date; Jan 09 (January 2009), March 09 (March 2009), July 09 (July 2009), Jan 10 (January 2010), May 10 (May 2010), Sept 10 (September 2010), and Jan 11 (January 2011). D) Arsenic concentration in redear sunfish livers at Clinch River mile (CRM) 5.5 by days after spill; 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011). Populations with different letters (A, B) indicate a statistical difference among groups.

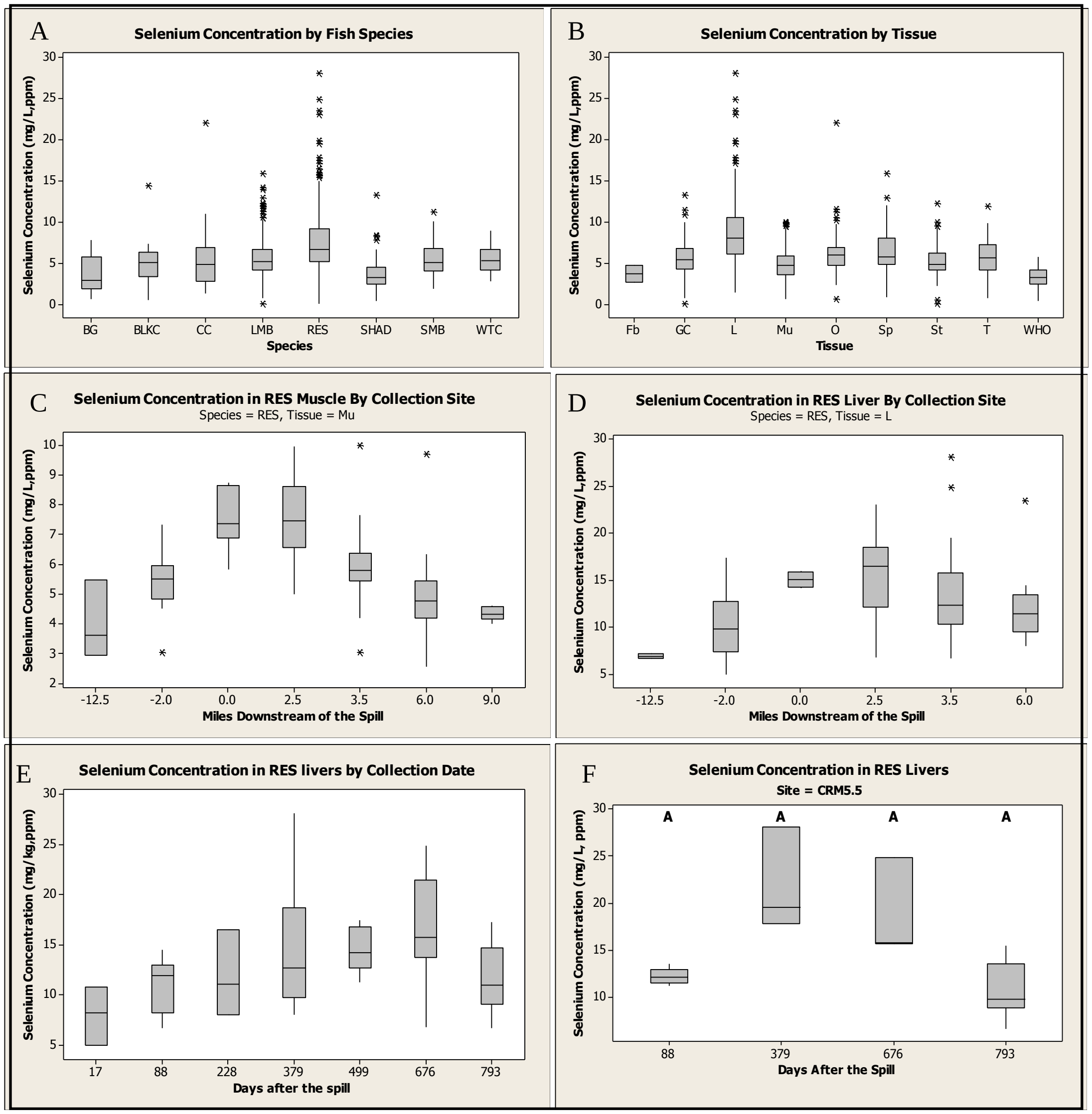

Figure 3. Selenium Body Burden in Fish Collected over the Two Year Period following the Tennessee Valley Authority (TVA) Coal Ash Spill. A) Selenium body burden by fish species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad) SMB (Smallmouth Bass), and WTC (White Crappie). B) Selenium concentration in fish tissues; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), and T (testes). C) Selenium concentration in redear sunfish muscle by collection site; Jan 09 (January 2009, day 17), March 09 (March 2009, day 88), July 09 (July 2009, day 228), Jan 10 (January 2010, day 379), May 10 (May 2010, day 499), Sept 10 (September 2010, day 676), and Jan 11 (January 201, day 793). D) Selenium concentration in redear sunfish livers by collection site; 12.5 (Emory River Mile 14), -2 (Emory River Mile 4), 0 (Emory River Mile 2), 2.5 (Clinch River Mile 3.5), 3.5 (Clinch River Mile 5.5), 6 (Tennessee River Mile 567), and 9 (Tennessee River Mile 564). E) Selenium concentration in redear sunfish livers by collection date. F) Selenium concentration in redear sunfish livers at CRM 5.5 by days after spill.

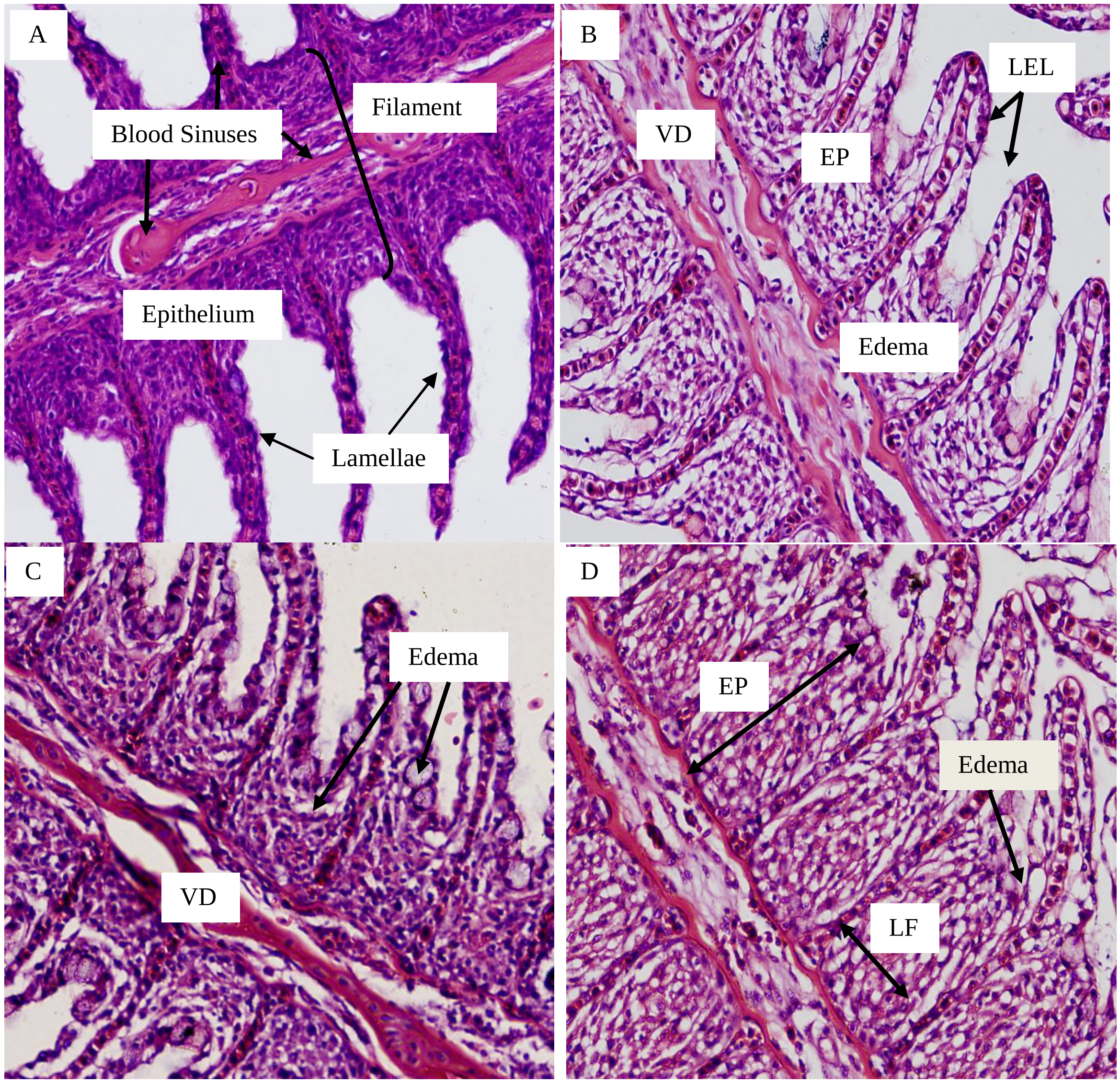

Figure 4. Histological Sections of Catfish Gills (10x magnification) from the reference site at Tennessee River Mile (TRM) 567 (A) and the ash spill site at Emory River Mile (ERM) 2.0 (B-D). The reference gill (A) shows normal tissue arrangement, while the gills from the 3 ash exposed catfish (B-D) all express pathology consistent with toxic element exposure including edema (B-D), vasodilation (VD; B, C), epithelial proliferation (EP; B, D), lamellar epithelium lifting (LEL; B), and lamellar fusion (LF; D).

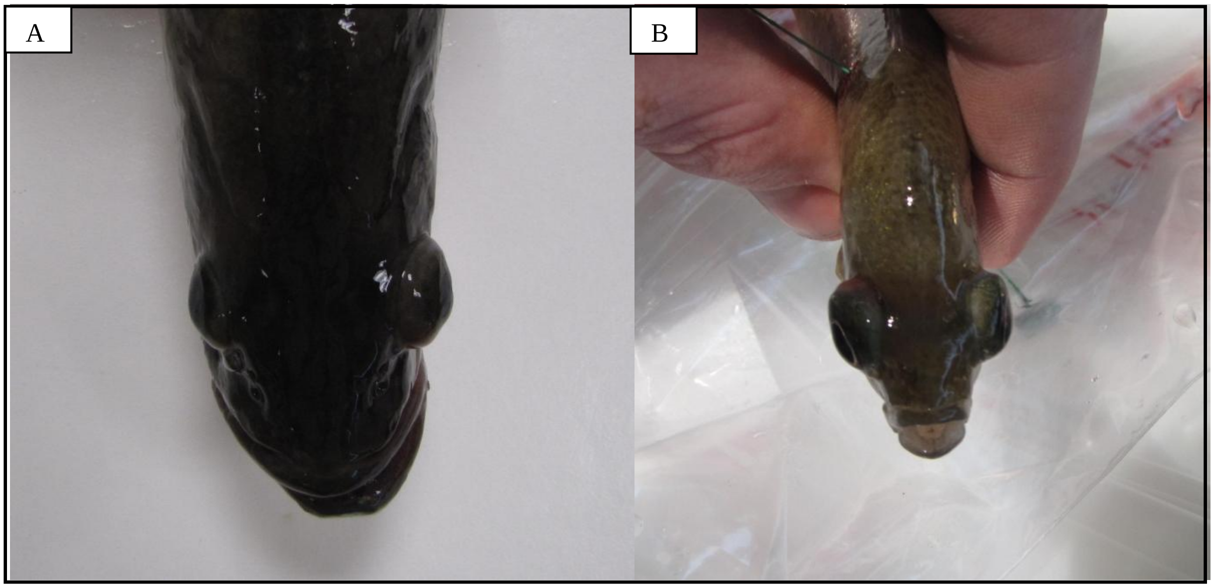

Figure 5. Fish Identified with Exopthalmus. A) Largemouth Bass collected at Emory River Mile (ERM) 2.0 in January 2010 displaying exopthalmus (popeye) in both eyes, and B) Bluegill sunfish collected in September 2010 displaying exopthalmus in both eyes.

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Supporting Information

Table S1. Elemental Concentrations in National Institute of Standards and Technology (NIST) Certified Fish Tissue Samples.

NIST Samples			
Elements	Mean	*SD*	*N*
Ag	0.01	0.01	10
As	3	0.4	10
Co	0.03	0.02	10
Mn	0.7	0.3	10
Se	3	1	10
Tl	BDL		10
V	5	10	10

Concentrations are averages of all samples processed during this study and are in mg/kg (ppm) dry weight. Elements are indicated by silver (Ag), arsenic (As), cobalt (Co), manganese (Mn), molybdenum (Mo), selenium (Se), thallium (Tl), and vanadium (V).Standard deviation is indicated by *SD*. Total number of samples analyzed is indicated by *N*. BDL indicates below detection limit for the instrument.

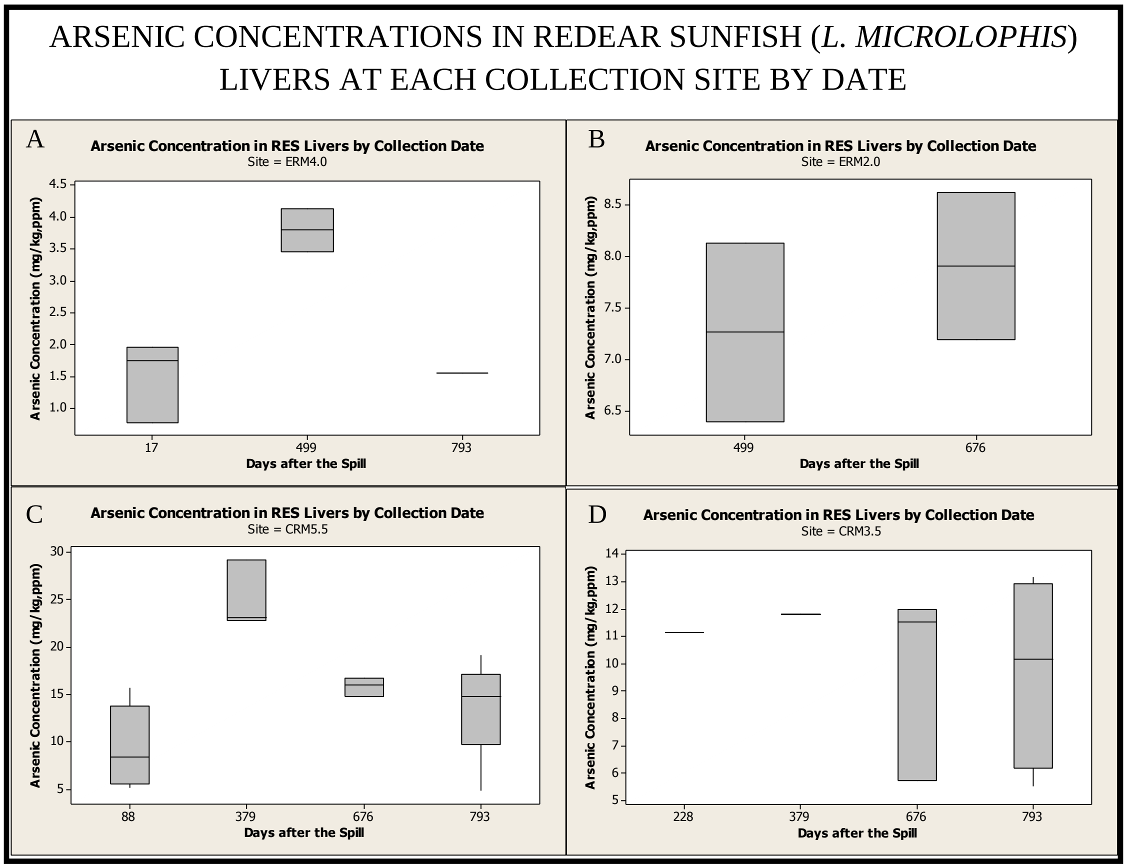

Figure S1: Arsenic Liver Burden in Redear Sunfish (*L. microlophis*) at Collection Sites over the Two Year Period Following the Spill A) Emory River Mile (ERM) 4.0, B) Emory River Mile (ERM) 2.0, C) Clinch River Mile (CRM) 5.5, and D) Clinch River Mile (CRM) 3.5. Days following the spill correspond to 17 (January 2009), 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011).

SELENIUM CONCENTRATIONS IN REDEAR SUNFISH (*L. MICROLOPHIS*) LIVERS AT EACH COLLECTION SITE BY DATE

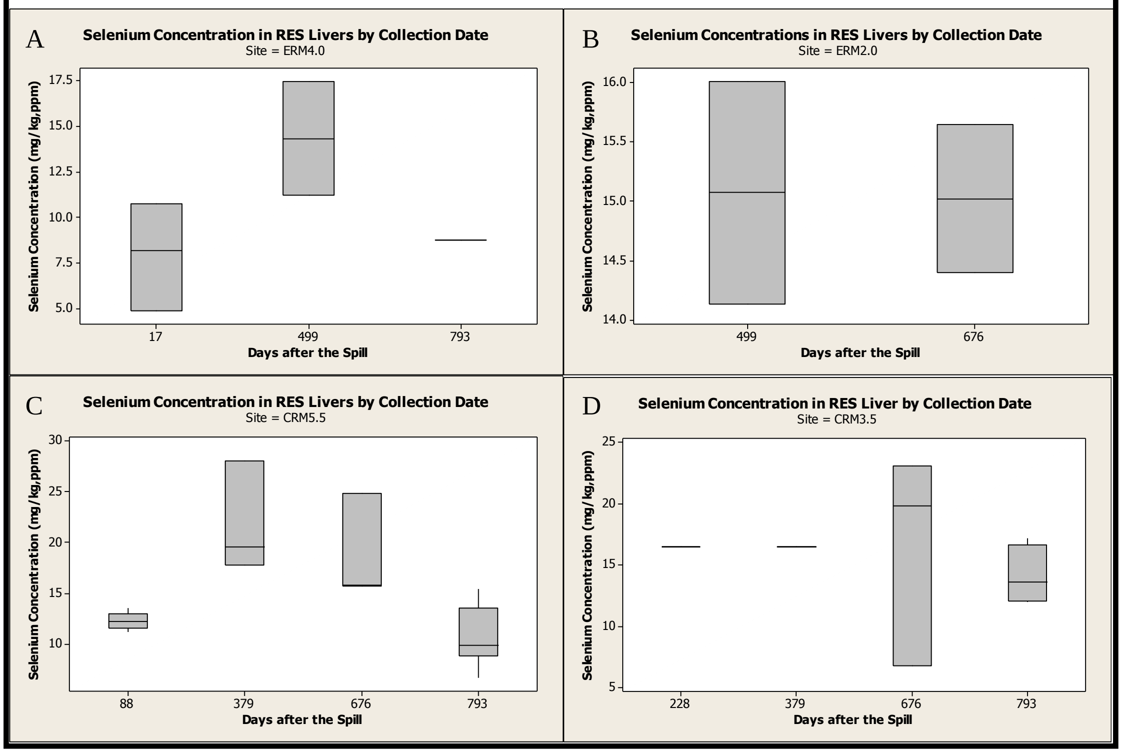

Figure S2: Selenium Liver Burden in Redear Sunfish (*L. microlophis*) at Collection Sites over the Two Year Period Following the Spill A) Emory River Mile (ERM) 4.0, B) Emory River Mile (ERM) 2.0, C) Clinch River Mile (CRM) 5.5, and D) Clinch River Mile (CRM) 3.5. Days following the spill correspond to 17 (January 2009), 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011).

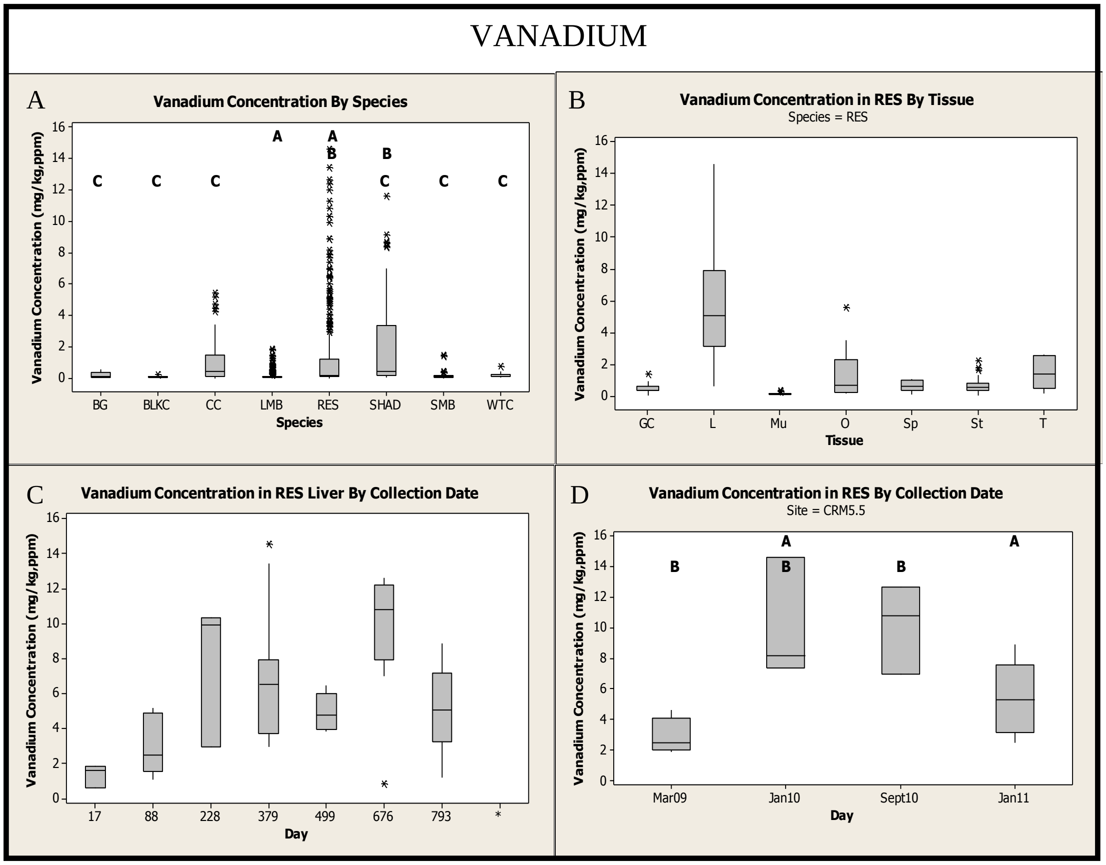

Figure S3. Vanadium concentration in fish collected over the two year period at collection sites surrounding the TVA coal ash spill. A) Vanadium concentrations by species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad), SMB (Smallmouth Bass), and WTC *(*White Crappie). B) Vanadium concentration in redear sunfish by tissue; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), and T (testes). C) Vanadium concentration in redear sunfish liver samples by days following the spill; 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011). D) Vanadium concentration in redear sunfish livers at CRM 5.5 by collection date; Jan 09 (January 2009), March 09 (March 2009), July 09 (July 2009), Jan 10 (January 2010), May 10 (May 2010), Sept 10 (September 2010), and Jan 11 (January 2011). Populations with different letters (A, B) indicate a statistical difference among groups.

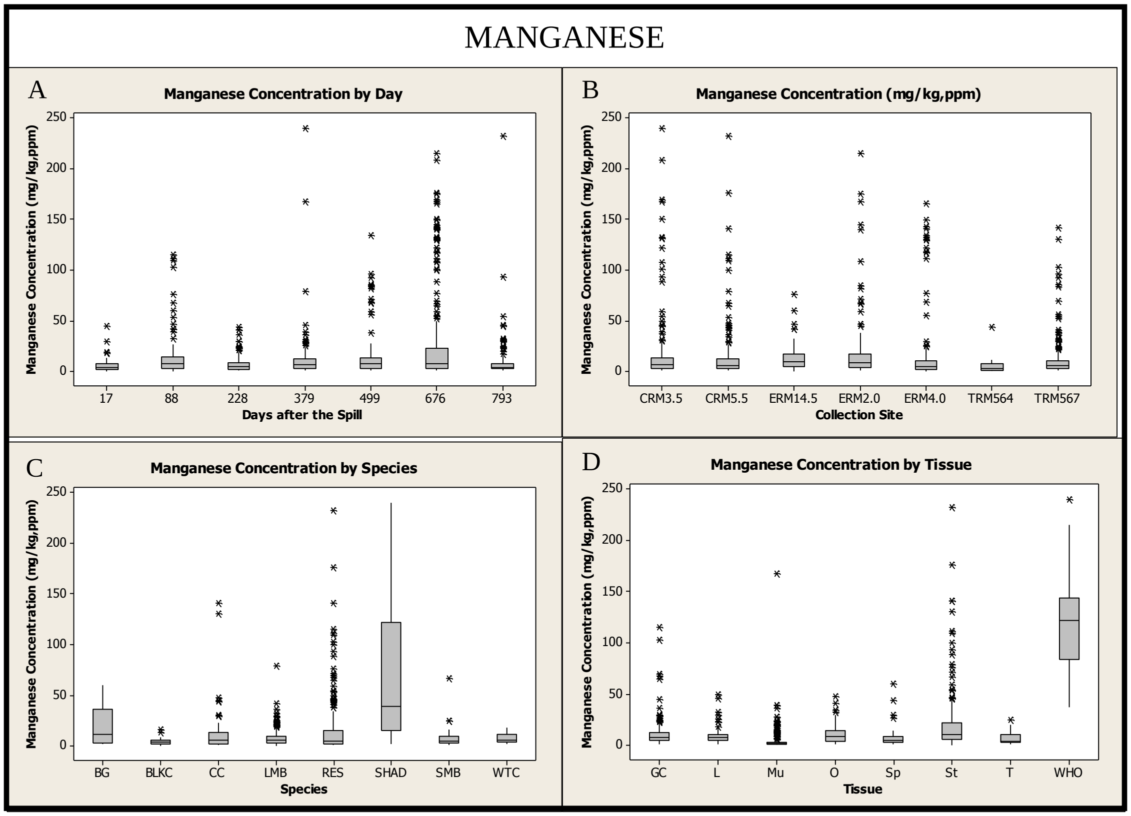

Figure S4. Manganese concentration in fish collected over the two year period at collection sites surrounding the TVA coal ash spill. A) Manganese body burden by collection date; 17 (January 2009), 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011). B) Manganese concentration by sampling location; ERM 14.5 (Emory River Mile 14), ERM 4 (Emory River Mile 4), ERM 2 (Emory River Mile 2), CRM 3.5 (Clinch River Mile 3.5), CRM 5.5 (Clinch River Mile 5.5), TRM 567 (Tennessee River Mile 567), and TRM 564 (Tennessee River Mile 564). C) Manganese body burden by fish species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad), SMB (Smallmouth Bass), and WTC (White Crappie). D) Manganese concentrations by tissue; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), T (testes), and WHO (whole body).

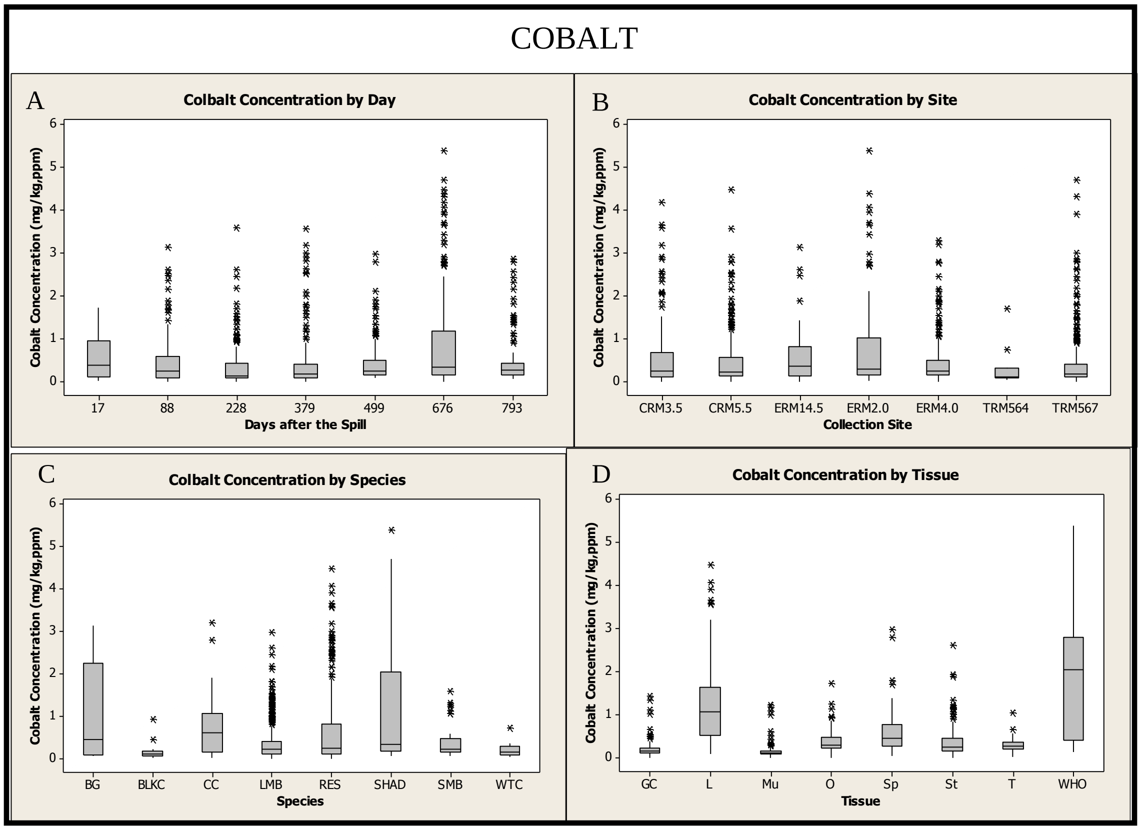

Figure S5. Cobalt concentration in fish collected over the two year period at collection sites surrounding the TVA coal ash spill. A) Cobalt body burden by collection date; 17 (January 2009), 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011). B) Cobalt concentration by sampling location; ERM 14.5 (Emory River Mile 14), ERM 4 (Emory River Mile 4), ERM 2 (Emory River Mile 2), CRM 3.5 (Clinch River Mile 3.5), CRM 5.5 (Clinch River Mile 5.5), TRM 567 (Tennessee River Mile 567), and TRM 564 (Tennessee River Mile 564). C) Cobalt body burden by fish species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad), SMB (Smallmouth Bass), and WTC (White Crappie). D) Cobalt concentrations by tissue; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), T (testes), and WHO (whole body).

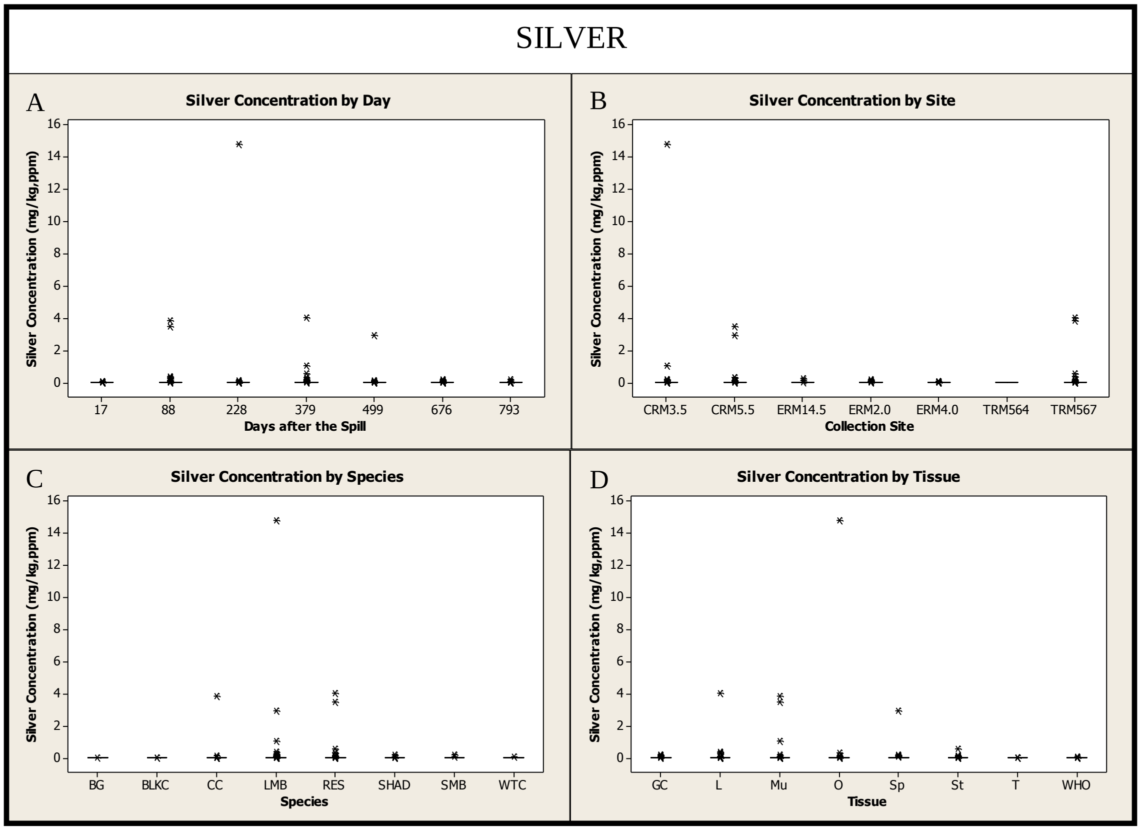

Figure S6. Silver concentration in fish collected over the two year period at collection sites surrounding the TVA coal ash spill. A) Silver body burden by collection date; 17 (January 2009), 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011). B) Silver concentration by sampling location; ERM 14.5 (Emory River Mile 14), ERM 4 (Emory River Mile 4), ERM 2 (Emory River Mile 2), CRM 3.5 (Clinch River Mile 3.5), CRM 5.5 (Clinch River Mile 5.5), TRM 567 (Tennessee River Mile 567), and TRM 564 (Tennessee River Mile 564). C) Silver body burden by fish species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad), SMB (Smallmouth Bass), and WTC (White Crappie). D) Silver concentrations by tissue; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), T (testes), and WHO (whole body).

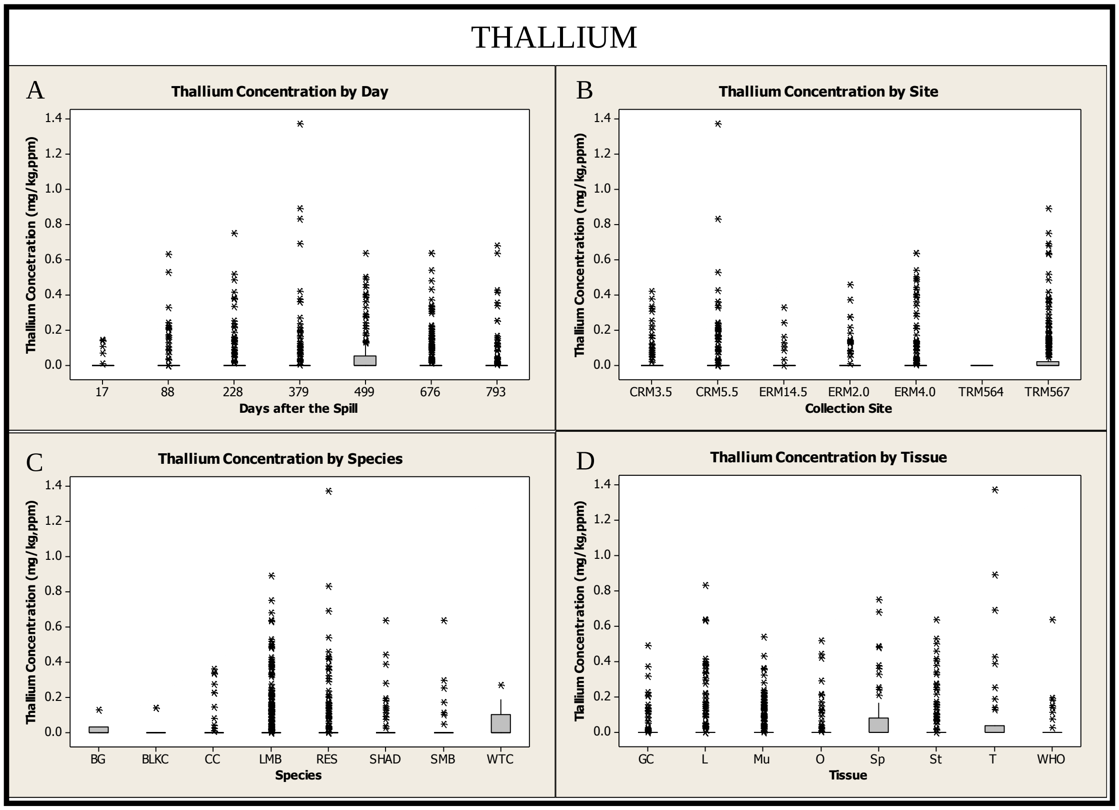

Figure S7. Thallium concentration in fish collected over the two year period at collection sites surrounding the TVA coal ash spill. A) Thallium body burden by collection date; 17 (January 2009), 88 (March 2009), 379 (January 2010), 676 (September 2010), and 793 (January 2011). B) Thallium concentration by sampling location; ERM 14.5 (Emory River Mile 14), ERM 4 (Emory River Mile 4), ERM 2 (Emory River Mile 2), CRM 3.5 (Clinch River Mile 3.5), CRM 5.5 (Clinch River Mile 5.5), TRM 567 (Tennessee River Mile 567), and TRM 564 (Tennessee River Mile 564). C) Thallium body burden by fish species; BG (Bluegill Sunfish), BLKC (Black Crappie), CC (Channel Catfish), LMB (Largemouth Bass), RES (Redear Sunfish), SHAD (Gizzard Shad), SMB (Smallmouth Bass), and WTC (White Crappie). D) Thallium concentrations by tissue; GC (gastric ceacum), L (liver), Mu (muscle), O (ovary), Sp (spleen), St (stomach), T (testes), and WHO (whole body).

Biographical Sketch

Daniel Lee Jackson was born the second of two sons to James and Teresa Jackson in Winston-Salem, NC. Growing up in Winston-Salem, Daniel enjoyed athletics like playing baseball, and wrestling. However, his true passion was nature, as he spent many weekends camping, hiking, fishing, or skiing with family and friends or with the Boy Scout troop to which he belonged. He attended Mount Tabor High School in Winston-Salem, during which time he earned his Eagle Scout award for conducting a stream restoration.

As a child, Daniel visited Boone, NC and fell in love with the mountains of western North Carolina and knew that Appalachian State University was where he wanted to attend college. During his undergraduate career, Daniel's course work followed the pre-medicine path, but after working with Dr. Shea Tuberty on several environmental projects, his childhood passion for nature was restored.

His graduate work examining the effects of contamination on fish populations was inherently interesting to him, and he hopes this work will ultimately lead to changes to our societal practices. At this point in his life, Daniel looks to find a career which would allow him to continue his education and further his experience, so that he can help educate others and be a leader towards environmental change.