Preliminary Summary Report from Water, Sediment and Fish samples collected at the TVA Ash Spill by Appalachian State University, Appalachian Voices, Tennessee Aquarium and Wake Forest University

Background

At 1 AM on December 22, 2008 a sixty-foot earthen dike securing five decades of coal fly ash in a retention pond at the Tennessee Valley Authority (TVA) Kingston Fossil Plant gave way. Most of an estimated 4.1 million cubic meters (1.1 billion gallons) of saturated coal fly ash fluidized and flowed catastrophically from the pond covering a total land and former aquatic area of ≈300 acres with deposits up to 10 meters deep. This ash spill is the largest volume industrial spill in US history. Approximately 90% of the catastrophic flow entered the Emory River[1] where the force of the flow pushed a large wave of ash, stranding and burying unknown numbers of fish and mussels on the shoreline and under mounds of ash. From the point of the spill the Emory River flows into the Clinch River two river miles downstream. The combined flows of the Clinch and Emory Rivers then flow into the impounded Tennessee River at Watts Bar Reservoir four river miles further downstream.

Executive Summary

We have estimated that the initial ash flow into the Emory River contained ~3830 tons of the 10 most toxic elements present in fly ash. Preliminary analyses of ash, water, sediments, and fish tissues collected from near the spill site 18 days following the dike failure revealed the following:

1) The total recoverable toxic elements arsenic, barium, cadmium, lead, and selenium were at elevated levels below the ash spill.
2) Ash and ash-laden sediments from the river had arsenic levels that exceeded the EPA residential removal action limits; while selenium levels were increased dramatically downstream of the spill.
3) Fish body burdens of selenium were at and beyond the thresholds of toxicity for reproduction and growth.
4) Histological study of fish gills shows a normal organization in the reference site fish. However, fish exposed to ash related toxic elements show several histological alterations indicative of toxic element stress.

The preliminary data suggest the selenium in the fish tissues may be the result of legacy selenium.[2,3] Within the next few months dissolved selenium concentrations in water can be expected to increase as a result of the spill. Selenium body burdens in fish will probably

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[1] Roane County Community Meeting, March 5, 2009, Chuck Head, Tennessee Department of Environment & Conservation , Powerpoint presentation on soil and ash monitoring . (http://www.state.tn.us/environment/kingston/pdf/comm_guid/030509RoaneCoMtgSoilAsh.pdf)

[2] The TVA Phase I Emory River Dredging Plan indicates that normal plant operations discharge 2.84 lb of dissolved selenium per day, equivalent to >41,000 pounds over the service life of the plant through December 2008.(Attachment 1- KIF Dredging Material Flow Analyses Summery, normal ash pond discharge of selenium in both tables included on page 5 of 7).

[3] TVA 2007 Toxics Release Inventory states that 1111 lbs of selenium compounds were released in 2007, which is equivalent to 3.04lbs per day (http://www.tva.gov/environment/air/kingston.htm#tri)

increase as the selenium released into the aquatic system is absorbed and bioaccumulated in the food web.

Methods

Seven locations were sampled for 17 elements on the Emory, Clinch and Tennessee Rivers on January 8 and 9, 2009 by a combined team of investigators from Appalachian State University, Appalachian Voices and the Tennessee Aquarium (see Figure 1). Conductivity and dissolved oxygen were collected in the field using a calibrated Orion 5 Star Plus Portable Multimeter. Turbidity was also analyzed in the field using a calibrated Hach 2100P portable turbidimeter that meets ISO 7027 turbidity measurement standards. Grab samples of surface water were collected and analyzed for total and dissolved toxic elements. Sediment samples were collected using an Ekman dredge sampler.

FIGURE 1. Appalachian State University /Appalachian Voices/Tennessee Aquarium Sampling Sites January 8 and 9, 2009. Tennessee River sampling location at TRM 567 not depicted.

Fish were collected using a boat electrofisher. The following report provides the summary results of water, sediment and fish tissue testing that was conducted on January 8 and 9, 2009.

WATER QUALITY

Surface water grab samples were obtained and analyzed by ICP-AES for total and dissolved elements using EPA Standard Methods SW-846-6010C and 200.7(Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma – Atomic Emission Spectrometry). The results from the water samples for dissolved metals (samples were filtered in the field and acidified) indicated no exceedances for the toxic elements of concern. Table 1 illustrates the results for total recoverable metals in which five toxic elements were found at levels above water quality standards and/or water quality criteria. It should be noted that these standards are based on dissolved metal water levels not total recoverable metals.

TABLE 1. Total recoverable elements summary observations of Jan 8 and 9 surface water samples, reported in ppm (mg/L). Water quality standards are based on either drinking water (DW) or Tennessee acute or chronic aquatic life criteria levels (all of which are based on dissolved values, not total recoverable).

Metals	ERM 3.3	ERM 2.2	ERM 1.6	ERM 0.1	CRM 4.6	CRM 3.3	TRM 567	WQ Standard (based on dissolved metals fraction only)	Comments
Arsenic	BDL	2.667	0.009	BDL	BDL	BDL	BDL	0.010	260x DW standard at spill site, diminishes downstream
Barium	0.030	7.313	0.120	0.079	0.075	0.066	0.083	2.0	3.65x DW standard at spill site, diminishes downstream in Emory River
Cadmium	0.0005	0.0151	0.0004	0.0004	0.0005	0.0005	0.0005	0.005	3x for DW standard at spill site, diminishes downstream
Lead	BDL	0.243	0.006	0.003	BDL	0.005	BDL	0.015	16x DW standard at spill site, diminishes downstream
Selenium	BDL	0.038	0.004	BDL	0.009	0.007	BDL	0.020 and 0.005	1.9x TN acute aquatic life criteria, 7.6 x TN chronic aquatic life criteria

Note 1: BDL = Below detection limits of lab instruments
Note 2: Yellow highlighted values indicate an exceedance of a water quality standard.

Water Chemistry

Exceedingly high levels of turbidity were recorded at Emory River mile 2.2 (ERM 2.2) on January 8 (see Table 2 below). A visible plume of ash filled surface water was observed

TABLE 2. Turbidity, dissolved oxygen and conductivity: summary observations of Jan 8 and 9 surface water samples.

	ERM 3.3	ERM 2.2	ERM 1.6	ERM 0.1	CRM 3.3	Comments
Turbidity (NTU)	21	40,550	152	44	No data	ERM 2.2 had turbidity levels that were 266 times higher than ERM 1.6 and > 2000 times higher than the upstream reference site at ERM 3.3
Dissolved Oxygen (%)	88.1	78.6	95.6	98	82	Lowest dissolved oxygen level occurred at ERM 2.2
Conductivity (µS/(cm)	54.4	263-289	58.2	54.7	188	Turbidity fluctuated between 263 and 289 at ERM 2.2.

flowing through the ash on land and into the Emory River, likely as a result of recent rainfall. Ash was fully suspended throughout the water column at this sample location. To see images (video) of this highly turbid water, please go to http://www.youtube.com/watch?v=ykwcQPf_9fc&feature=channel.

When the research team returned to the same location the next day (January 9), a much lower volume of water flow was observed entering the Emory River from the accumulated ash. Unlike the previous day, the runoff water was stratified with a layer of clear water at the surface and an ash filled layer of highly turbid water at the bottom.

Water quality conclusions: High levels of turbidity and conductivity and five of seventeen toxic elements were found at levels that were elevated in comparison to water quality standards or aquatic life criteria, including arsenic, barium, cadmium, lead and selenium. All of these occurred at ERM 2.2, the sampling location directly in the area of the ash spill.

Samples collected upstream and downstream of the ash spill area did not exhibit water quality violations for toxic elements. This suggests that the most elevated levels of toxic elements are localized in the area of the ash spill. In order to quantify the impact of toxic elements on water quality, it is imperative for samples to be collected in the immediate area of the ash spill.

Due to the stratification of ash observed in the Emory River on January 9, future water samples should be collected at several depths in the water column. During low flow conditions the ash seems to remain close to the bottom of river. Under higher flow conditions the erosive energy of the water will disturb and distribute the ash throughout the water column.

RIVER SEDIMENT

River sediment was collected in 6 locations using an Ekman dredge grab sampler. Ash was observed in all samples except the upstream sample collected at Emory River mile 3.3 (ERM 3.3). The sediments were analyzed for seventeen toxic elements by ICP-AES following microwave assisted nitric acid digestion (EPA Standard Methods SW-846-6010C and 200.7). Table 3 contains sample results that are a cause for concern or exceed residential removal action limits.

TABLE 3. Sediment sample total metals summary observations, reported in mg/kg dry sediment (ppm).

Metals	ERM 3.3	ERM 2.2	ERM 1.6	ERM 0.1	CRM 3.3	TRM 567	Comments
Arsenic	4.073	111.897	65.985	27.360	42.254	12.437	2.86 times the residential removal action limit for arsenic at ERM 2.2
Selenium	0.513	3.859	6.385	3.816	2.82	1.828	Test sites below the ash spill had selenium levels in sediment that ranged between 3.5 to 12.4 times higher than the upstream reference site at ERM 3.3

River sediment conclusions: Arsenic and selenium are accumulating at high levels in river sediment in the vicinity of the ash spill and downstream of the spill site. Emory River sediment at ERM 2.2, 1.6 and 0.1 contained arsenic levels that ranged between 455 to 6.7

times higher than arsenic levels in sediment upstream of the spill at the ERM 3.3 'reference site'. Although we are using the 'clean' sediments we found near the shore at ERM 3.3 as a sediment reference sample, we have subsequently found ash in the main channel at this location. Therefore, the true reference values for sediment levels of toxic elements could be even lower. In contrast, sediment samples collected from the Tennessee and Clinch rivers exhibited arsenic levels that ranged between 3 and 10.3 times higher than the arsenic levels at the ERM 3.3 reference site. For sediment levels of selenium at ERM 2.2, 1.6, and 0.1 the levels were 7.5, 12.4, and 7.4 times higher, respectively, than the ERM 3.3 reference samples (0.5 ppm). Sediment collected downstream at CRM 3.3 and TRM 567 showed elevated levels of selenium that were 5.5 and 3.5 times higher than the reference site, respectively. Interestingly, the highest level of selenium was observed downstream of the spill site at ERM 1.6 which had twice the selenium as the actual spill site at ERM 2.2.

FISH COMMUNITY ASSESSMENT

The Emory River fish community was sampled using a boat electrofisher. Four locations were shocked for 500 shocking seconds each (see table 4). The majority of collected specimens were returned to the Emory River unharmed after being identified to genus and species and briefly inspected for external morphological anomalies.

TABLE 4. Number of fish species and number of fish collected from the Emory River: summary observations of Jan 8 and 9 samples. Fish were collected for 500 shocking seconds at each location.

	ERM 3.3	ERM 2.2	ERM 1.6	ERM 0.1
# Species Found	9	5	8	6
# Fish Found	55	13	32	111

In addition, ERM 2.2 was shocked for another 1000 shocking seconds during the afternoon of January 9. Nine individuals from 3 species were caught, bringing the total at that site to 7 species and 22 individuals. Three dead fish were observed downstream of the spill, but we were not able to discern a cause of mortality due to their advanced state of decomposition.

Fish Community Conclusions: Even with 3 times more shocking seconds at ERM 2.2, the site still had the fewest number of individual fish. In addition, several fish at and downstream of the spill site were observed with scrapes and lost scales. Both are conditions that may be attributed to physical abrasion from ash exposure or to stress from degraded water quality. Figure 2 illustrates that the gut, intestines and anal cavity of one channel catfish was filled with ash that accounted for almost 8% of its body weight (34 grams of ash).

FIGURE 2. A channel catfish collected from ERM 2.2 weighed 439 grams and contained 34 grams of ash in the gut, intestines and anal cavity, which means 7.7% of the body weight of the catfish was ash.

FISH TISSUE ANALYSIS

Largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*) and red ear sunfish (*Lepomis microlophus*) were retained for laboratory analysis from two locations in the Emory River (ERM 3.3 and ERM 2.2) and a reference site at TRM 568. Each fish was dissected with the liver, spleen (not sunfish), muscle, gastric caecum (not catfish), stomach and reproductive organs analyzed separately for seventeen toxic elements. Each sample was freeze dried before microwave assisted nitric acid digestion and analysis by ICP-OES, the results are reported in mg of element per kg of dry tissue weight (ppm). Fish gills were removed and fixed in 4% paraformaldehyde in phosphate buffer. Gills were paraffin processed and microtome sectioned at a thickness of 7 microns. Sections were stained in hematoxylin and eosin and examined under a light microscope.

The gill morphology of the fish collected from the reference site is similar to that of other teleost fish species[4] (Figure 3A). The gill is made up of rows of filaments from which vertically arise the lamellae. The lamellae are the site of oxygen absorption and are lined by a very thin epithelium composed of mainly pavement cells. Below that epithelium are lamellar blood sinuses, where oxygen is transported by the red blood cells to the systemic circulation. Between the lamellae, the filament is lined by a layered epithelium composed of several cell types (chloride, mucous and pavement cells). This tissue is responsible for ion exchange and is considered to function in a similar manner as the human kidney maintaining water and ion balance. Bass and catfish collected from the ERM 2.2 and 3.3 sites showed clear evidence of altered gill morphology such as a marked lifting of the lamellar epithelium, edema (swelling) in the filamental epithelium and an intense lamellar vasodilation (expansion of the blood vessels). The gills of the catfish also exhibited lamellar fusion in numerous areas as a result of filamental epithelial cell proliferation (abnormal tissue growth)(Figure 3B-D). This morphological finding has previously been described in numerous studies in which catfish have been exposed to environmental metal and pesticide pollution. [5,6]

Interstitial edema is one of the more frequent responses observed in gill epithelium of fish exposed to heavy metals. The lifting of lamellar epithelium is another histological change observed, probably induced by the incidence of severe edema. Edema with lifting of lamellar epithelium could serve as a mechanism of defense, because separation of epithelium from the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream. Also edema, epithelial lifting, and lamellar fusion are thought to be defense mechanisms that reduce the branchial superficial area in contact with the external water and toxic elements.[5] Both of these defense responses would also greatly reduce the efficiency of the gills to absorb oxygen.

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[4] Wilson J. M. & Laurent P. 2002. Fish gill morphology: inside out. J. Exp. Zool., 293:192-213.
[5] Figueiredo-Fernandes A., Ferreira-Cardoso J.V., Garcia-Santos S., Monteiro S.M.,Carrola J., Matos P. & Fontaínhas-Fernandes A. 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesquisa Veterinária Brasileira* 27*(3):103-109.*
[6] Ortiz, J B, Gonzalez de Canales, M L, Sarasquete (2003) Histopathological changes induced by lindane (γ-HCH) in various organs of fishes. Sci. Mar., 67 (1) 53-61.

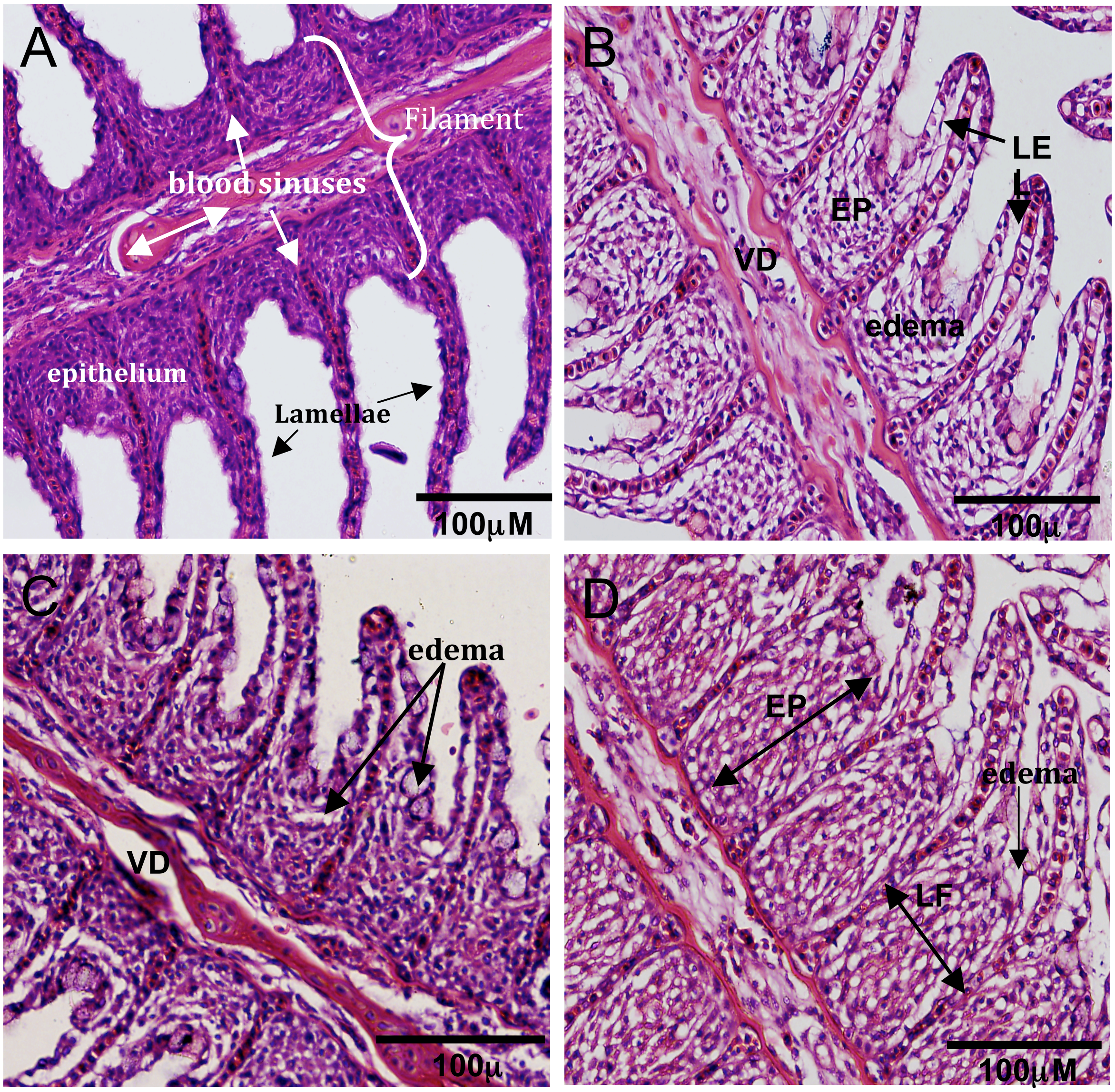


FIGURE 3. Histological sections of catfish gills (10x magnification) from, (A) the reference site at TRM 568 and, (B-D) the ash spill site at ERM 2.2. The reference gill (A) shows normal tissue arrangement, while the gills from the 3 ash exposed catfish (B-D) all express pathology consistent with toxic element exposure including edema, vasodilation (VD), epithelial proliferation (EP), lamellar epithelium lifting (LEL), and lamellar fusion (LF).

Importantly, elevated levels of selenium were observed 18 days after the spill even though it usually takes at least 30 days for bioaccumulation of this trace element to occur (Table 5).[7] From the elevated levels of selenium found in fish tissue at the spill site and the upstream reference site, it would appear that selenium has been bioaccumulating in the aquatic environment surrounding the Kingston coal plant prior to the ash spill. Based on the levels of selenium observed, the fish community is approaching a toxic threshold level for selenium assimilation. The levels of selenium in the fish are nearing a point at which reproductive failure begins. These reproductive impacts can begin at the toxic thresholds for selenium which are known to occur at approximately 8 ppm in muscle, 10 ppm in ovaries, 12 ppm in the liver and spleen, and 4 ppm in whole-body samples (all as dry weight). There is 100 % reproductive loss when ovaries contain 50-70 ppm selenium. Selenium levels at 2.5 ppb in water can be converted to 25 ppm selenium in fish through biomagnification.[7] Elevated levels of lead were also found in some fish samples (bass testes and spleen) although accumulated arsenic levels were not a concern.

TABLE 5. Mean tissue burdens (n=3) of selenium in fish collected Jan. 8 and 9. Note that spleens from redear sunfish were not large enough for analysis.

	ERM 3.3 Mean Selenium (mg/kg or ppm)		**ERM 2.2 Mean Selenium (mg/kg or ppm)**
Fish Species	**Largemouth Bass**	**Redear Sunfish**	**Channel Catfish**
Liver	5.12	7.92	4.81
Spleen	19.57	-	11.45
Muscle	4.90	5.17	3.19
Gonad	8.63	8.64	11.86

Due to this surprising result, additional fish tissue analysis is needed upstream and downstream of the TVA ash spill in order to quantify the breadth and scope of elevated selenium levels in fish surrounding the Kingston coal plant. Additionally, fish should be collected from much further upstream of the ash spill in order to find a reference fish population that has not been impacted by decades of discharges from the ash ponds at the Kingston coal plant.

Fish Tissue Conclusion: Selenium concentrations in important fish species in the Emory River are already at toxic thresholds (the tipping point). This means that the river ecosystem cannot tolerate further assimilation of selenium from the ash spill. There is no margin of safety and additional selenium uptake will result in bioaccumulation to levels that severely impact fish reproduction. At the site of the spill, we observed numerous fish with clogged gills. Healthy fish gills will look feathery and display a dark red color, but the fish closest to the spill site had gills that were dark brown, clumped and filled with ash and mucus. Fish absorb oxygen directly from the water across their gills, so if the gills are bunched and coated with sediment or ash, the fish will suffocate. Histopathology of fish gills demonstrated that edema, epithelial proliferation, and lamellar epithelial lifting and fusion had occurred in ash exposed fish, which may serve the fish as a defense response to waterborne toxins but will reduce the efficiency of oxygen absorption as well.

Scanning Electron Microscopy Analysis of Floating Ash Particles (Cenospheres)

Plumes of floating coal ash waste (cenospheres) were observed downstream of ERM 2.2. Surface water samples containing these floating ash particles were collected for further analysis by scanning electron microscopy. Some of the sample was digested in nitric acid and dried while the other was left in its raw state as it existed in the Emory River and dried. The acid washed sample consisted of relatively homogeneous, smooth round particles of silicon

[7] Lemly, A. D.; Selenium Assessment in Aquatic Ecosystems: A Guide for Hazard Evaluation and Water Quality Criteria; Springer-Verlag New York, April 2002.

and aluminum oxide glasses that ranged in size from 5-100 microns in diameter (see Figure 4).

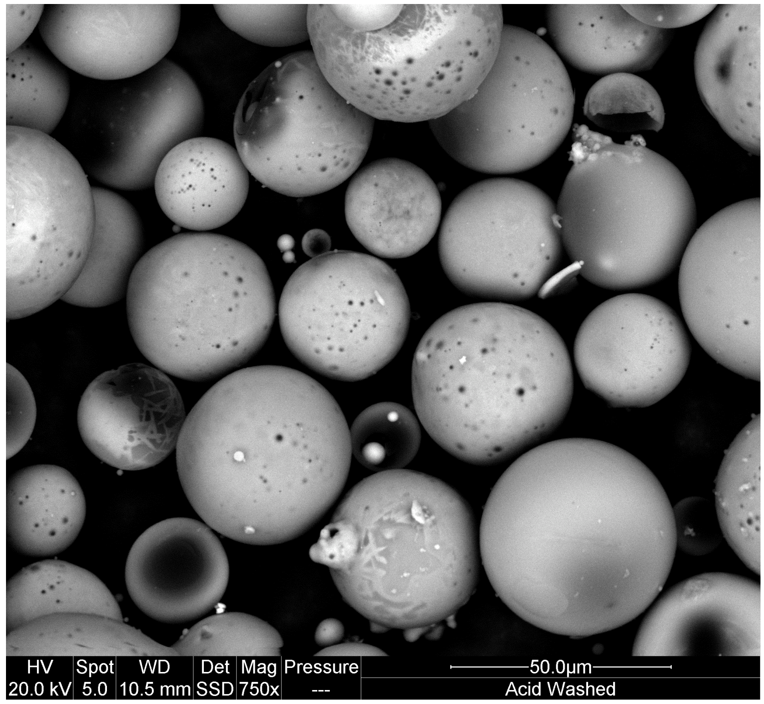


FIGURE 4. Acid washed cenospheres collected from the Emory River below the TVA ash spill. These have no iron oxide coatings to which toxic elements can adhere.

The untreated sample contained a heterogeneous mixture of miscellaneous coal ash particles, organic river materials (such as leaf debris), and cenospheres that appeared to have an iron oxide coating (see Figure 5). This coating is less than 5 microns thick, and easily fractures and sloughs off during spot analysis with the electron beam.

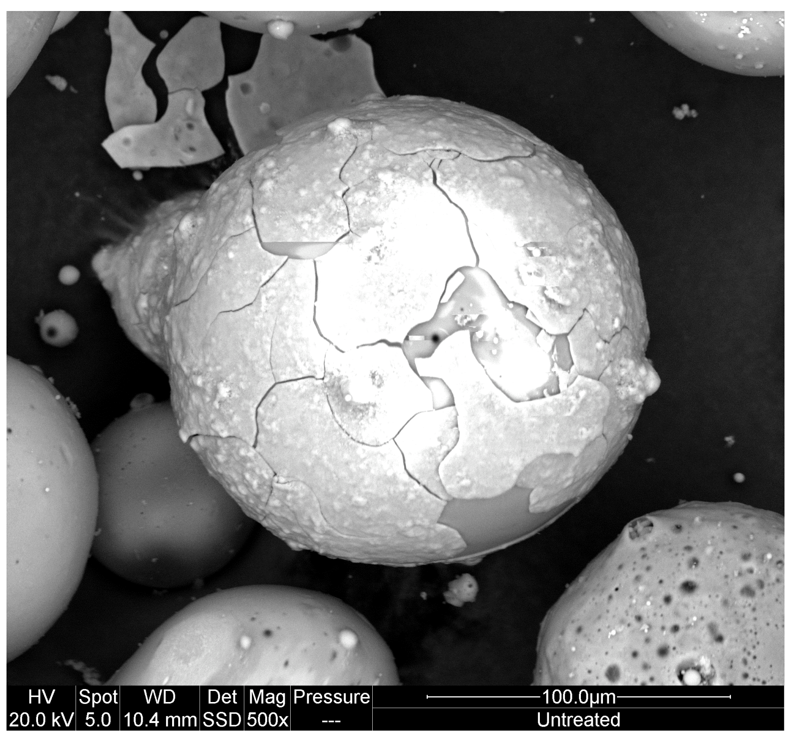
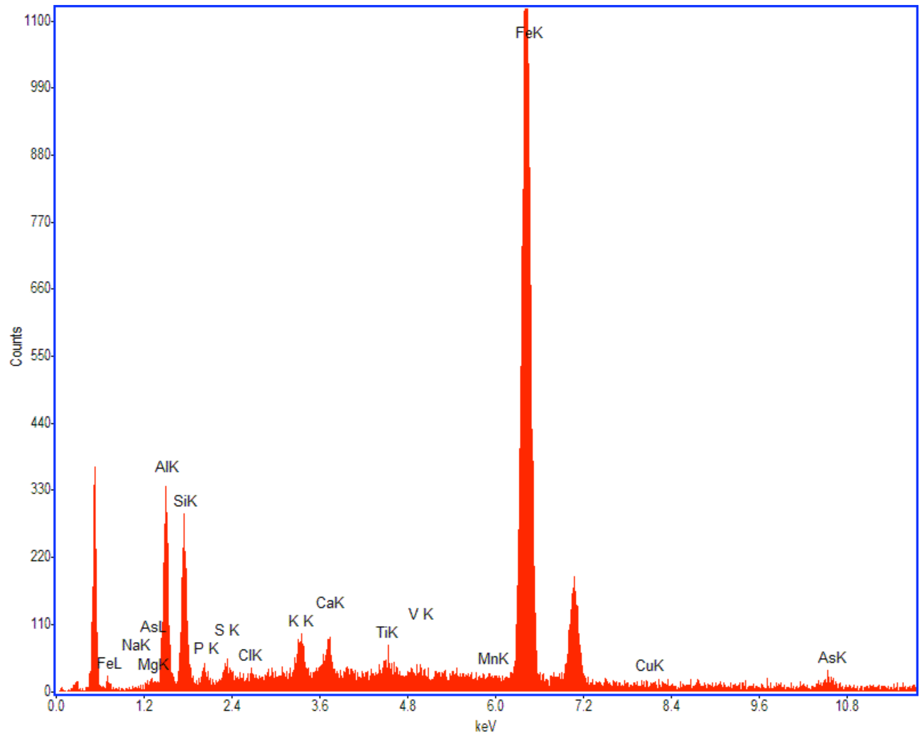


FIGURE 5. Untreated cenospheres with secondary mineralization gel coating containing elevated levels of arsenic. Collected from the Emory River 18 days after the spill occurred.

Analysis of the coating by Energy Dispersive X-ray analysis (EDS) revealed detectable levels of arsenic (see Figure 6). Analyses of cenospheres without this iron oxide coating do not show detectable arsenic. It appears that the arsenic is adhering to the iron oxide coatings on the cenospheres, which are likely to be the products of weathering in wet ash ponds.

FIGURE 6. EDS spectrum of iron oxide coating showing detectable arsenic (AsK) in the lower right part of the image (analysis taken at 30kV, high vacuum, uncoated sample).



Due to analytical limitations of the SEM and a lack of appropriate arsenic standards, it is not possible at this time to quantify the exact amount of arsenic adhering to these coatings, but the relatively high detection limit of the SEM indicates that the average concentration is in the range of one part per hundred (1% or 10,000 ppm). Approximately 10% of the cenospheres collected from the river surface sample have these iron oxide coatings. These coatings are hypothesized to be the byproducts of many years worth of degradation and breakdown processes in wet ash basins. At this time, the physical characteristics of this iron oxide coating in an aquatic environment are not known. It is likely that in a wet environment these coatings are in the form of an iron oxide gel, which toxic elements can easily adsorb onto.

Conclusion electron microscopy: Further analysis of cenospheres and the iron oxide coating is continuing at the Appalachian State University electron microscopy lab. It is likely that arsenic is not the only heavy metal that adheres to the iron oxide coating on the ash particle, and further study using instrumentation with lower detection limits will be used to confirm this. Depending on the outcome of ongoing research, it may be determined that in contrast to the uncoated "young" cenospheres recently created by the combustion process, "old" cenospheres that come from aged wet ash basins are coated by this iron oxide gel. These gel coatings on floating cenospheres are analogous to microscopic rafts that can transport toxic elements into the aquatic environment upon their accidental release and/or washing into the river during high water events. However, it is clear from this initial analysis that further study of aged coal ash particles stored in a wet environment is needed before any solid conclusions can be drawn.

Overall Summary:

Eighteen days after the TVA ash spill, elevated levels of five toxic elements continued to seriously contaminate water, sediment or fish in the Emory River. Of all the tested constituents, arsenic and selenium appear to be the pollutants of greatest concern. Considering the baseline levels of toxins present and the amount released to the aquatic environment from the ash spill, further study of water quality, sediment and aquatic life is needed to evaluate the environmental impacts likely to unfold over time. The impacts are expected to be severe and persistent, especially for selenium. Fish are showing external signs of stress by way of gill and scale epithelial pathologies and body burdens of selenium are cause for great concern. The SEM images have demonstrated that the ash particles (cenospheres) that were released from the TVA ash ponds may have changed significantly since they were deposited there, specifically the mineral gel coatings that form on ash stored in ponds seems to be the main source of toxic elements measured in water and sediment samples. It should be noted that, to date, there has been no exploration of the impact of the TVA ash spill on phytoplankton, zooplankton, benthic macroinvertebrates, aquatic birds and small mammals. We conducted another and even more extensive collection of water, sediments and fish samples on March 20 and 21, 2009. This includes fish sampling from all collection sites, and bank to bank and vertical transects of water and sediments at all sites. The report on those data will be available in June.

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Note: to see the sampling crew, observe the electrofishing and sediment sampling process, please go to http://www.youtube.com/watch?v=ykwcQPf_9fc&feature=channel

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