INVESTIGATING POTENTIAL TOXICITY OF TVA KINGSTON FOSSIL PLANT COAL FLY ASH TO LARVAL AND JUVENILE FATHEAD MINNOWS USING EXTENDED ELUTRIATE BIOASSAYS

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INTRODUCTION

On December 22, 2008, over 5 million cubic yards of fly ash were released to the Emory River, TN from the Tennessee Valley Authority (TVA) Kingston Fossil Plant. Remediation and recovery efforts for this event are currently underway. The present study was conducted to decrease the uncertainty associated with potential biological impacts to fish due to removal of the fly ash from the Emory River by dredging.

METHODS

Field Collection

- All field samples collected on June 12, 2009.
- Emory River Reference water collected approximately 12 miles upstream of TVA Kingston Fossil Plant.
- Fly ash collected from the Emory River at five discrete locations and composited.
- Fly ash and water also collected from the Ash Recovery Ditch (Sluice Channel) on the site of the TVA Plant.

Elutriate Preparation

- Elutriates created with Emory River (EMR-EL) and Sluice Channel fly ash (SLC-EL).
- 10 g/L (wet weight) of fly ash added to respective site water, aerated for 10 d, and allowed to settle for one hour.
- This extended elutriate mixing time (10 d versus the normal 1 h) was used to conservatively represent extreme conditions for the release, dissolution, and changes in metal speciation that might occur during and after dredging.
- 100, 50, and 10% dilutions of both EMR-EL and SLC-EL along with respective site waters used in fathead minnow (*P. promelas*) bioassays. Juvenile fish exposed to EMR-EL (**Fig.1**). Larval fish exposed to EMR-EL and SLC-EL (**Fig 2**).

Fathead Minnow Elutriate Bioassays

	Larval Bioassay	Juvenile Bioassay
Fish Age	9 days	3 months
Duration	10 days	10 days
Chamber	300 ml beakers	4 L aquaria
Replication	5 replicates	5 replicates
Fish per chamber	10	5
Feeding	*Artemia* nauplii, every other day	Fish flakes, every other day
Aeration	3-5 bubbles per second	Rigorous
Static Renewal	Days 3 and 7	Days 3 and 7
Endpoints	Survival, Growth	Survival, Growth, LSI, Bioaccumulation

Fig. 1 – Juvenile fathead minnow elutriate bioassay

Fig. 2 – Larval fathead minnow elutriate bioassay

Gill Histopathology

- Gills were removed from 10 juvenile fish from both the EMR-EL 100% treatment and the Control.
- Gills were fixed in Bouin's solution for ~24 h at 4 °C, rinsed with 1X PBS 2 times for 2 h, dehydrated using an ethanol gradient, cycled through Clearify rinses, and embedded in paraffin.
- Efforts were made to position the gills to facilitate sectioning oriented in cross section to the filaments and transverse to the gill.
- 5 µm sections were prepared with a rotary microtome.
- Hematoxylin and eosin (H&E) staining performed.
- Gill filament width and other pathologies (e.g., desquamation, lamellae fusion and, chloride cell hypertrophy, epithelial hyperplasia) assessed.

Bioaccumulation

- Metal bioaccumulation determined in juvenile fish not used for analysis of gill histopathology.
- Fish homogenates digested by US EPA Method 3050B.
- As, Ba, Cr, Se, V quantitated in fish tissue by ICP-MS.
- Hg quantitated by cold vapor atomic fluorescence following US EPA Method 7474.
- Whole body burdens of metals compared to relevant screening criteria and literature tissue residue values (TRVs).

RESULTS AND DISCUSSION

- Mean measured total suspended solids in the 10-d 100% EMR-EL and SLC-EL bioassays were 493.7 ± 37.7 mg/L and 455.3 ± 17.7 mg/L, respectively.
- No significant reductions in survival observed in either the juvenile bioassay (EMR-EL) or the larval bioassays (EMR-EL and SLC-EL) (**Figs. 3 & 4, Table 1**).
- Larval *P. promelas* growth did not occur relative to initial weights of fish, potentially due to the reduced feeding ration (once every other day), precluding assessment of the larval growth endpoint.

Fig. 3 – Larval *P. promelas* survival in the EMR-EL10-d bioassay

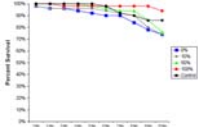


Fig. 4 – Larval *P. promelas* survival in the SLC-EL10-d bioassay

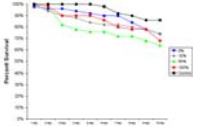
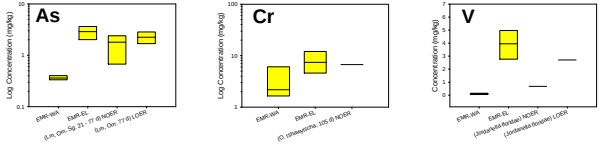


Table 1 – Results from the EMR-EL 10-d juvenile *P. promelas* bioassay

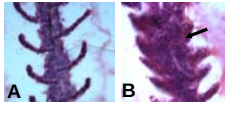
Concentration	Percent Survival	Length (mm)	Biomass (mg)	Liver mass (mg)	Liver Somatic Index
Control	100 ± 0	NA	NA	NA	NA
0%	100 ± 0	4.2 ± 0.3 (7%)	888 ± 188 (21%)	16.83 ± 4.84 (28%)	1.9 ± 0.6 (30%)
10%	100 ± 0	4.0 ± 0.4 (10%)	796 ± 173 (22%)	16.04 ± 4.03 (25%)	2.0 ± 0.3 (18%)
50%	100 ± 0	4.3 ± 0.2 (5%)	939 ± 138 (14%)	18.46 ± 5.04 (27%)	1.9 ± 0.5 (24%)
100%	100 ± 0	4.2 ± 0.3 (8%)	943 ± 167 (18%)	20.04 ± 8.09 (40%)	2.2 ± 0.8 (37%)

Fig. 5 – Tissue As, Cr, and V concentrations in fish exposed to Emory River Water (EMR-WA) and the EMR-EL compared to literature TRVs. Lm = *Lepomis macrochirus*. Om = *Oncorhynchus mykiss*. Sg = survival and growth.



- Metal concentrations in whole fish homogenates were found to be largely attributable to metal in the fish gut, as ingested fly ash. Gut metal concentrations were 50-2800 times higher than in the rest of the fish with the gut removed.
- Arsenic (As) concentrations in whole fish tissue were near literature TRVs (**Fig. 5**). However, this was a conservative assessment as the less toxic arsenate (As(V)) dominated in the elutriates, and TRVs were based on the more toxic arsenite (As(III)).
- Cr was at concentrations in whole fish tissue near the no observable effects residue (NOER) from literature studies (**Fig. 5**).
- V concentrations in whole fish tissue exceeded literature based screening values (**Fig. 5**), but the elevated concentrations are likely the result of fly ash in the gut.
- Hg and Se concentrations in tissues were below relevant TRVs.
- Some gill pathologies were observed in EMR-EL 100% treated fish (**Fig. 6**). One instance of lamellar fusion was noted in gills from control fish.
- However, problems with fish gill section positioning in several replicates resulted in reduced sample size, precluding definitive interpretation of gill histopathology.

Fig. 6 - **A.** Control fish showing no gross signs of toxicity. **B.** Treated fish with possible epithelial hyperplasia (arrow).



CONCLUSION

In this weight of evidence approach where water and solid phase chemistry (data not shown), toxicity, and bioaccumulation data are integrated, results suggest little potential for toxicity to related fish species due to fly ash removal dredging activities given the extreme conditions represented by oxidized elutriates in this study.

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