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Carol M. Babyak^a; Shea R. Tuberty^b; Bethany D. Carter^c; Amanda Gibbs^a; Derek Routhier^a; Christie Woodruff^b; Anna George^d; Donna Lisenby^e

^a A.R. Smith Department of Chemistry, Appalachian State University, Boone, NC ^b Department of Biology, Appalachian State University, Boone, NC ^c Department of Chemical and Biomolecular Engineering, Clemson University, Clemson, SC ^d Anna George, Tennessee Aquarium Conservation Institute, Chattanooga, TN ^e Donna Lisenby, Upper Watauga RiverKeeper, Boone, NC

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Sequential Extraction of River Sediments Impacted by a Recent Coal Fly Ash Slide

Carol M. Babyak¹,
Shea R. Tuberty²,
Bethany D. Carter³,
Amanda Gibbs¹,
Derek Routhier¹,
Christie Woodruff¹,
Anna George⁴,
and Donna Lisenby⁵

¹A.R. Smith Department of Chemistry, Appalachian State University, Boone, NC

²Department of Biology, Appalachian State University, Boone, NC

³Department of Chemical and Biomolecular Engineering, Clemson University, Clemson, SC

⁴Anna George, Tennessee Aquarium Conservation Institute, Chattanooga, TN

⁵Donna Lisenby, Upper Watauga RiverKeeper, Boone, NC

ABSTRACT A four-step sequential extraction method and inductively coupled plasma optical emission spectroscopy (ICP-OES) were used to determine the trace element distribution in river sediments impacted by a recent coal fly ash slide. The extractable, carbonate-bound, iron/manganese oxide-bound, and organic-bound phases were extracted. A large number of trace elements were investigated, but As, Co, Cr, Cu, Ni, and Zn had the best relative standard deviations (RSDs), usually less than 50%. Better reproducibility (less than 16%) was obtained for a standard reference material (SRM) that indicated that sample heterogeneity caused the large RSDs observed for the sediments. Analysis by ICP-OES was challenging because of the high salt concentrations in the extract. Calibration standards were matrix-matched, and some extracts were diluted to decrease the salt concentration. The percentage of extractable elements was smaller at the impacted sites compared to the upstream site; however, the concentrations of extractable elements were larger at the impacted sites. The largest exchangeable concentrations of As, Cr, and Cu were observed at the impacted sites and were 4.6, 1.1, and 5.5 mg/kg, respectively. Contrarily, the largest exchangeable Co and Zn concentrations were observed upstream of the spill, which indicated that these elements were more tightly bound to fly ash compared to uncontaminated sediments. The majority of extractable As was bound to iron and manganese oxides and may be liberated under reducing conditions. Students in a senior-level environmental chemistry course performed most of this work. The entire course was devoted to this research, and students benefited from exploring one problem in great depth.

KEYWORDS coal fly ash, ICP-OES, river sediment, sequential extraction

INTRODUCTION

Teaching in the Environmental Chemistry Laboratory

Recent research in chemical education indicates that undergraduate laboratory courses should be based on authentic inquiry and problem-solving as opposed to a series of unrelated cookbook-style experiments.^[1] Problem-based learning (PBL)^[2–5] and service-learning (SL)^[6] are two examples of teaching methods that engage students by immersing them in

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Address correspondence to Carol M. Babyak, A.R. Smith Department of Chemistry, Appalachian State University, Boone, NC 28608. E-mail: babyakcm@appstate.edu

real-world problems relevant to their lives or communities. Students enrolled in PBL or SL laboratories tend to be more motivated and are more likely to master skills such as operation of instrumentation, teamwork, and communication.^[7–13]

Environmental chemistry laboratories are readily adaptable to PBL and SL (see Wenzel and Austin^[14] and references therein). At Appalachian State University, we offer an upper-level environmental chemistry course and have taught it using PBL and SL. In the past, students have worked with the Agricultural Extension Office to develop analytical methods for the detection of pesticides used in the local Fraser fir tree farming industry. This paper describes our most recent project in which students collaborated with the Upper Watauga RiverKeeper and a biology faculty member on the analysis of river sediments impacted by a recent coal fly ash slide.

Coal Fly Ash Slide

On December 22, 2008, approximately one billion gallons of coal fly ash burst from a retention pond at the Tennessee Valley Authority's (TVA's) Kingston Fossil Plant (KIF) near Harriman, TN. Fly ash is a by-product of coal combustion and is composed primarily of the oxides of silicon, aluminum, iron, calcium, and magnesium, but also contains trace levels of potentially toxic elements including mercury, cadmium, arsenic, selenium, lead, molybdenum, nickel, zinc, and boron depending on the type of coal burned.^[15,16] Fly ash from the ruptured storage pond entered the Emory River roughly 2 miles upstream of its confluence with the Clinch River, which flows into the Tennessee River. The spill site and downstream locations have been sampled extensively by many scientists, and most recently Ruhl et al.^[17] reported on the potential environmental impacts of the spill, which include the release of toxic and radioactive elements into the atmosphere, contamination of stagnate water via leaching of toxic elements from fly ash, and formation of methylmercury in anaerobic river sediments. In this paper, we use the method of sequential extraction to investigate the potential mobilities and bioavailabilities of trace elements from contaminated sediments in the Emory River using sequential extraction.

Sequential Extraction

Elemental analysis of sediment samples is typically performed by acid digestion, which yields total trace element concentrations. Although total concentrations are useful when trying to assess the impact of an incident such as the TVA ash slide, they reveal little about the mobilities or bioavailabilities of trace elements to the aquatic ecosystem.

The method of sequential extraction can provide information about the distribution of trace elements in a sample, and hence their mobilities and bioavailabilities to aquatic systems. In sequential extraction the sample is extracted in steps, with a different extraction solvent used in each step. The extracted trace elements are operationally defined based on the solvents used.^[18,19] Sequential extraction usually starts with a gentle solvent such as water to extract the most mobile or labile elements, and ends with solvents that aggressively attack the sample to liberate relatively nonlabile elements. Each solvent is selected to attack a specific phase of the sample, for example carbonate, crystalline iron oxide, amorphous iron oxide, sulfide, or organic. The solubilities of trace elements in the extraction solvent also determine which trace elements are extracted.

Tessier et al.^[20] described one of the first sequential extraction methods used on sediment samples. It was a five-step extraction and began by targeting loosely bound trace elements that could be easily extracted through ion exchange. The remaining four steps targeted the carbonate, iron/manganese oxide, organic, and residual phases of the sediment by selectively dissolving them. Since then, many sequential extraction procedures have appeared in the literature along with some review articles.^[18,19] In the early 1990s, a standardized sequential extraction method was published^[21,22] and later revised^[23] by the Community Bureau of Reference (BCR) of the Commission of the European Communities. Table 1 shows the sequential extraction methods that have been performed recently on coal fly ash samples.^[24–31]

Compared to acid digestion, sequential extraction is more time-consuming and labor-intensive. The extract is usually separated from the sample by centrifuging, and it is difficult to quantitatively remove all of the supernatant. Some extraction

TABLE 1 Comparison of Sequential Extraction Methods Used Recently on Coal Fly Ash Samples^a

Reference	24 ^b	25	26	27	28	29 ^c	30 ^e	31 ^e
Sample description	1 g coal, ash	1 g landfilled coal fly ash	4 g coal, fly ash, slag	4 g fly ash	0.5 g fly ash	1 g reference ash, soil, or sediment	1 g reference fly ash	2 g coal fly ash
Water extractable	—	—	20 mL H ₂ O-continuous shaking for 15 hr	20 mL distilled H ₂ O-continuous shaking for 15 hr	—	—	40 mL deionized H ₂ O-continuous shaking for 16 hr	40 mL deionized H ₂ O-continuous shaking for 18 hr
Exchangeable	8 mL 1-M MgCl ₂ (pH 7.0)-continuous shaking for 1 hr	8 mL 1-M NH ₄ OAc (pH 7.0)-continuous shaking for 1 hr	20 mL 1-M NH ₄ OAc (pH 7)-continuous shaking for 15 hr	20 mL 1-M NH ₄ OAc (pH 7, NH ₄ OH)-continuous shaking for 15 hr	20 mL 1-M NH ₄ OAc (pH 6.5)-continuous shaking for 16 hr	—	—	—
Carbonate-bound (Acid-Soluble) ^c	8 mL 1-M NaOAc (pH 5, HOAc)-continuous shaking for 5 hr	8 mL 1-M NH ₄ OAc (pH 5.0, HOAc)-continuous shaking for 5 hr	20 mL 1-M NH ₄ OAc (pH 5)-continuous shaking for 15 hr	20 mL 1-M NH ₄ OAc (pH 5, NH ₄ OH)-continuous shaking for 15 hr	0.5-M HOAc (pH 2.7)-continuous shaking for 16 hr	40 mL 0.11-M HOAc-continuous shaking for 20 hr	40 mL 0.11-M HOAc-continuous shaking for 16 hr	40 mL 0.11-M HOAc-continuous shaking for 16 hr
Fe/Mn bound (Reducible)	20 mL 0.04-M NH ₂ OH · HCl in 25% (v/v) HOAc-occasional shaking at 96°C for 6 hr	8 mL 0.04-M NH ₂ OH · HCl in 25% (v/v) HOAc-occasional shaking at 96°C for 6 hr	20 mL 0.1-M NH ₂ OH · HCl in 25% HOAc-96°C for 15 hr	0.04-M NH ₂ OH · HCl in 25% (v/v) HOAc-occasional shaking 96°C for 6 hr	0.1-M NH ₂ OH · HCl (pH 2, HNO ₃)-continuous shaking for 16 hr	40 mL 0.1-M NH ₂ OH · HCl (pH 2, HNO ₃)-continuous shaking for 20 hr	40 mL 0.1-M NH ₂ OH · HCl (pH 2, HNO ₃)-continuous shaking for 20 hr	40 mL 0.1-M NH ₂ OH · HCl (pH 2, HNO ₃)-continuous shaking for 16 hr
Bound to organics/sulfides (Oxidizable)	3 mL 0.02-M HNO ₃ and 5 mL 30% H ₂ O ₂ (pH 2, HNO ₃)-occasional shaking at 85°C for 2 hr; 3 mL H ₂ O ₂ (pH 2, HNO ₃)-occasional shaking at 85°C for 3 hr; 5 mL 3.2-M NH ₄ OAc in 20% (v/v) HNO ₃ diluted to 20 mL w/ deionized water-continuous shaking for 0.5 hr	3 mL 0.02-M HNO ₃ and 5 mL 30% H ₂ O ₂ (pH 2, HNO ₃)-occasional shaking at 85°C for 2 hr; 3 mL H ₂ O ₂ (pH 2, HNO ₃)-occasional shaking at 85°C for 3 hr; 5 mL 3.2-M NH ₄ OAc in 20% (v/v) HNO ₃ diluted to 20 mL w/ deionized water-continuous shaking for 0.5 hr	—	5 mL 8.8-M H ₂ O ₂ (pH 2-3, HNO ₃)-85°C for 1 hr; Repeat previous step; 25 mL 1-M NH ₄ OAc (pH 2, HNO ₃)-continuous shaking for 16 hr	5 mL 8.8-M H ₂ O ₂ (pH 2-3, HNO ₃)-85°C for 1 hr; Repeat previous step; 25 mL 1-M NH ₄ OAc (pH 2, HNO ₃)-continuous shaking for 16 hr	10 mL 8.8-M H ₂ O ₂ (pH 2-3)-occasional shaking for 1 hr, followed by 1 hr at 85°C; 10 mL 8.8-M H ₂ O ₂ (pH 2-3)-85°C for 1 hr; 50 mL 1-M NH ₄ OAc-continuous shaking for 20 hr	10 mL 30% H ₂ O ₂ -continuous shaking for 20 hr; 10 mL 30% H ₂ O ₂ -continuous shaking for 1 hr, followed by 1 hr at 85°C; 10 mL 30% H ₂ O ₂ -85°C for 1 hr; 50 mL 1-M NH ₄ OAc (pH 2, HNO ₃)-continuous shaking for 16 hr	10 mL 30% H ₂ O ₂ -continuous shaking at room temp, followed by 1 hr at 85°C; 10 mL 30% H ₂ O ₂ -85°C for 1 hr; 50 mL 1-M NH ₄ OAc (pH 2, HNO ₃)-continuous shaking for 16 hr
Residual	— ^d	10 mL 16-M HNO ₃ -digest at 120°C to near dryness	—	HF:HClO ₄ :HNO ₃ (2:1:1) in a PTFE digestion bomb at 125°C	3.75 mL HNO ₃ , 2.5 mL HClO ₄ -100°C for 2 hr	—	—	Hot plate digestion w/ HNO ₃ :HCl:HF (2:5:15)

^aAll extractions were performed at ambient temperature unless stated otherwise.^bBased on Tessier's method.^cThis phase was also referred to as the surface-bound oxide phase in references 26 and 27.^dIn the original Tessier method, the residual fraction was extracted with a 5:1 mixture of HF:HClO₄.^eBased on the BCR method.

solvents do not specifically attack one phase of the sample,^[18,19] and trace elements can be redistributed among the different phases during extraction.^[18,19,32] As shown in Table 1, a variety of extraction schemes have been used, which makes comparison of results difficult. There is also a lack of standard reference materials (SRMs) with known trace element distribution.^[18] In spite of these drawbacks, there is general agreement that sequential extraction has led to a better understanding of trace element bioavailability and mobility in the environment.^[18,19]

Purpose

Sequential extraction and inductively coupled plasma optical emission spectroscopy (ICP-OES) were used to determine extractable trace elements in river sediments impacted by a recent coal fly ash slide. We analyzed the extracts for Ag, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se, Tl, V, and Zn, but report results only for As, Co, Cr, Cu, Ni, and Zn, because the best reproducibilities were achieved for these elements. The bulk of this work was performed by three students in a senior-level environmental chemistry course and was continued by one student during summer research.

MATERIALS AND METHODS

Study Site Description

Sediment samples were collected from four locations in the Emory River and two in the Clinch River as shown in Fig. 1. A sample was also collected from the Tennessee River downstream of its confluence with the Clinch River. The spill site is ERM (Emory River Mile) 2.2, and was very shallow due to the large volume of ash that had spilled into the river. Visual inspection of the sediment sample at this site revealed that it consisted entirely of ash. Ash was observed at all locations downstream of the spill, although the ash layer was much thinner at the most downstream site in the Clinch River. The downstream samples at ERM 1.6, ERM 0.1, and CRM 3.3 consisted of mixtures of ash and sediment.

Sample Collection

Sediment samples were collected in January 2009 from boats using an Ekman dredge sampler. A small

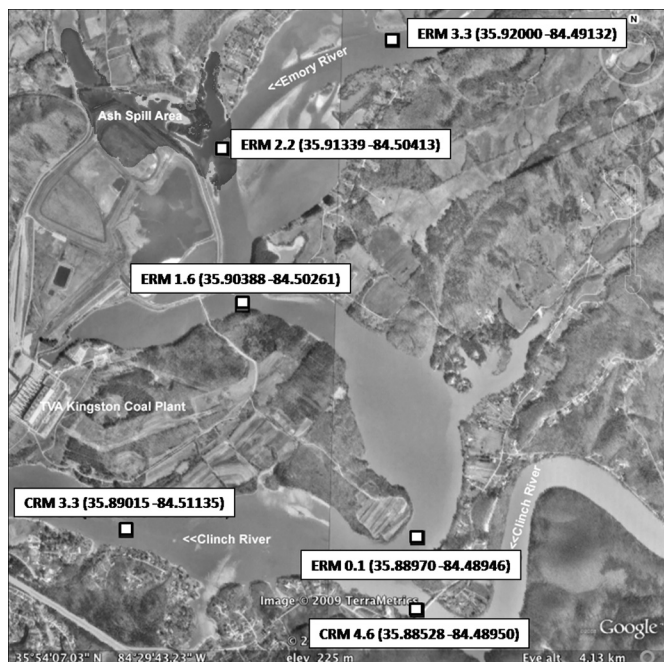


FIGURE 1 Sediment sampling locations. GPS coordinates are provided in parentheses. ERM = Emory River Mile. CRM = Clinch River Mile. Miles are measured from the mouths of each river.

core was removed from the dredged sample using a 50-mL polypropylene centrifuge tube and placed into a sterile Whirlpack bag. Samples were transported back to the lab on ice in coolers, frozen overnight at -20°C , and freeze-dried using a lyophilizer (Labconco, Model 77500, Kansas City, MO).

Glassware Washing

All glassware was washed either by acid-leaching overnight with 20% (v/v) nitric acid or rinsing with 50% (v/v) nitric acid. Nitric acid (EMD Chemicals, Gibbstown, NJ) of OmniTrace[®] grade was used throughout for cleaning, extraction, and spectroscopy.

Reagents

A 1,000 ppm multielement stock solution (BDH Aristar Plus) containing Ag, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Tl, V, and Zn, and individual single-component stock solutions containing 1,000 ppm As (EMD, CertiPur), Se (Ultra-Scientific, N. Kingstown, RI), and Mo (Ultra-Scientific) were used to prepare calibration standards. All reagents used in the sequential extraction were reagent grade, except for the ammonium acetate and hydrogen peroxide, which were HPLC-grade and technical grade,

respectively. Water with a resistance equal to or greater than 18 ohms from a Barnstead Nanopure system was used throughout.

Determination of Total Elements Using Microwave-Assisted Acid Digestion

A 0.5-g aliquot of dried sediment was weighed on an analytical balance (± 0.1 mg) and placed into a digestion vessel (CEM, Matthews, NC) along with 10 mL of concentrated nitric acid. The digestion vessel was capped with pressure plug inserted and loaded into the microwave reactor (MARSSXpress, CEM, Matthews, NC). Samples were digested using EPA method 3051. Briefly, the microwave was set to use 100% of 1600 watts for a 5.5-min ramp up to 175°C followed by a 10-min holding period at 175°C. Samples were allowed to cool, caps were removed, and samples filtered through 9-cm qualitative 413 filter paper (VWR, Cat. No. 28310-048) held in a glass funnel and collected in 50-mL volumetric flasks. The sample volume was brought up to 50 mL with deionized water, and then transferred to a clean 50-mL centrifuge tube for storage until ICP-OES analysis.

Sequential Extraction Procedure

The method described by Tessier et al.^[20] was used with the small change that 2 g of sediment were used in order to obtain a more representative aliquot from the sample. The volumes of extraction reagents were increased accordingly. Two to six replicate extractions were performed for each sample. Although the Tessier method does not determine the most bioavailable water-extractable fraction, we chose the Tessier method over the other methods in Table 1 because it could be completed in 3 days compared to 1 week for some of the other extraction schemes.

Step 1: Exchangeable. The dried sediment sample was weighed and transferred to a graduated 50-mL polypropylene centrifuge tube, and 15 mL of 1-M MgCl_2 were added. The sample was shaken on an orbital shaker (VWR Scientific, West Chester, PA) for 1 hr at 250 rpm, which was high enough to keep the ash suspended in solution. The supernatant was separated from the ash by centrifuging at 5,000 rpm

for 30–45 min in a VWR Clinical 200 centrifuge. The supernatant was carefully removed using a disposable plastic pipette and preserved with concentrated nitric acid. At first, the supernatant was transferred directly to a clean centrifuge tube and the graduations on the tube were used to determine the total extract volume; however, we eventually transferred the supernatant to a volumetric flask and diluted to the mark with water in order to know the total extract volume more accurately. The preserved extracts were stored in acid-washed plastic vessels at ambient temperature until ICP analysis. The sediment sample was saved for the next step of the extraction.

Step 2: Bound to carbonate (acid-soluble). To the sediment from step 1, 15 mL of 1-M sodium acetate (pH 5 adjusted with acetic acid) were added. The sample was shaken for 5 hr and centrifuged as described above. The supernatant was removed, preserved, and stored for ICP analysis.

Step 3: Bound to iron and manganese oxides (reducible). To the sediment from step 2, 40 mL of 0.04-M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25% (v/v) acetic acid were added. The sample was heated uncovered in a VWR water bath at 96°C for 6 hr with occasional stirring. The sample was allowed to cool and centrifuged as described earlier. The supernatant was removed, preserved, and stored for ICP analysis.

Step 4: Bound to organic matter and sulfides (oxidizable). To the sediment from step 3, 6 mL of 0.02-M HNO_3 and 10 mL of acid-stabilized 30% H_2O_2 were added. The sample was heated while uncovered in a water bath with occasional stirring at 85°C for 2 hr. The sample was removed from heat and allowed to cool. Another 6 mL of acidified H_2O_2 were added, and the sample was heated with occasional stirring for additional 3 hr. At this point, much of the extract volume had evaporated. The sample was allowed to cool, and then 10 mL of 3.2-M ammonium acetate in 20% (v/v) HNO_3 were added. The addition of acetate prevented readsorption of extracted metals through complexation with the acetate anion.^[20] The sample was diluted to 20 mL using deionized water and shaken for 30 min at ambient temperature. The supernatant was removed, preserved, and stored for ICP analysis as described previously.

Step 5 of the extraction (residual elements) was not performed due to safety concerns associated with HF and HClO_4 . In addition, the elements

extracted in this step are the least bioavailable. Landsberger et al.^[24] also did not perform this step of the extraction on coal fly ash samples (Table 1).

Determination of Elemental Concentrations in Extracts by ICP-OES

Standard Preparation

Calibration standards (0.01–2 mg/L) were initially prepared in 1% (v/v) HNO₃, but this was changed to a matrix that matched the extract. For example, when analyzing extracts from step 1 (exchangeable), we prepared standards in acidified 1-M magnesium chloride in order to match the matrix of the preserved extract. This was done because higher background emission intensities were observed for the extracts compared to standards prepared in HNO₃ during ICP-OES analysis. Bruder-Hubscher et al.^[33] and Ianni et al.^[34] also matrix-matched calibration standards for ICP-OES analysis of extracts from municipal waste incinerator bottom ash and certified reference sediment material, respectively.

Analysis of Extracts Using ICP-OES

All extracts were analyzed on a simultaneous Varian (Palo Alto, CA) 710 ES axial ICP-OES with CCD detector. A Cetac autosampler with 15-mL sample tubes was connected to the peristaltic pump. A concentric glass nebulizer (Seaspray, Varian) and glass cyclonic spray chamber were used for sample introduction. An internal standard of 2-mg/L yttrium was added to all standards and samples via the sample introduction system. The plasma power was 1.00 kW, the argon flow rate was 15.0 L/min with an auxiliary flow of 1.50 mL/min, and the nebulizer pressure was 300 psi. The following wavelengths were used: As had 188.980, Co had 238.892, Cr had 267.716, Cu had 327.395, Ni had 231.604, and Zn had 213.857. The concentrations in the first steps of the extraction were expected to be low, so a 60-s read time was used in order to improve detection limits. This meant that only one replicate measurement of emission intensity could be acquired, because almost the entire sample volume in the autosampler tubes was consumed during the measurement period. In addition, for most extracts, the total volume was 15–25 mL, which limited the

TABLE 2 Instrument Detection Limits (μg/L) for Each Element in Each Extraction Matrix

Matrix	As	Co	Cr	Cu	Ni	Zn
1-M MgCl ₂ pH = 1 (HNO ₃)	9	4	3	1	10	24
0.5-M NaOAc pH = 1 (HNO ₃)*	51	3	11	22	12	59
0.04-M NH ₄ OAc, 25% (v/v) HOAc, pH = 1 (HNO ₃)	13	1	0.8	0.8	3	13
1-M NH ₄ OAc, 8% (v/v) HNO ₃	6	0.6	0.3	0.5	11	7

*This matrix was diluted by a factor of 2 for ICP analysis.

number of replicate measurements per extract. However, multiple sediment samples were extracted.

Instrument detection limits (IDLs) were determined by measuring the emission intensities of seven blanks and taking three times the standard deviation.^[38] As shown in Table 2, the highest IDLs were observed in the matrix from step 2, and were probably related to the high salt concentration. Others^[34,36] have also observed variable detection limits in the different steps of sequential extraction. Most of the extract concentrations were above the IDLs. In steps 1 and 2, there were four instances when one replicate out of six was below the IDL. To calculate the average concentration in these cases, the nondetectable concentration was estimated as one-half of the IDL. When this estimate was averaged with the other replicates, the average concentration was above the IDL in all four instances.

The high dissolved solid concentration in the extracts was challenging to analyze by ICP-OES. It was difficult to keep the torch lit, because the inner injection cone became clogged with salt. In addition, thick white salt deposits were observed on the cone interface. This required regular removal of the torch and cone for cleaning. The extract from step 2 was particularly difficult to analyze due its high salt (sodium acetate) concentration; therefore, we diluted this extract by a factor of two, which allowed the torch to stay lit. Others have diluted extracts to a total dissolved solids concentration of less than 0.5%^[35] or 2 g/L^[36] prior to analysis by ICP-mass spectrometry (MS). Hall et al.^[37] also diluted sequential extracts of reference soils and sediments by a factor of 50 prior to ICP-MS. Although dilution was

necessary for the step 2 extracts, we tried to avoid diluting the extracts so the concentrations remained above IDLs.

RESULTS AND DISCUSSION

Total Elemental Analysis

Total element concentrations obtained by microwave-assisted acid digestion along with standard deviations and percent relative standard deviations (RSDs) are provided in Table 3. The largest RSDs were observed at ERM 3.3 (upstream of the spill), for all elements except zinc. Although the sample here consisted of sediment only—as opposed to the ash-sediment mixtures observed at some downstream sites—reproducibility was poor and probably affected by the lower concentrations of trace elements in the sample.

Total element concentrations were greater at ERM 2.2 (spill site) compared to ERM 3.3 (upstream) for all elements except copper. In general, trace element concentrations decreased downstream of the spill, but the standard deviations for concentrations at some downstream sites make distinction of them difficult. For example, the average total zinc

TABLE 4 Trace Element Composition of Fly Ash Samples Collected From a Storage Pond and Ash Flow at the KIF

Element	Mean ^a	Max ^a	ERM 2.2 ^b
As	46.2	113.0	112
Co	11.5	16.5	28
Cr	22.2	34.8	52
Cu	45.2	73.4	228
Ni	22.7	34.2	51
Zn	37.7	59.8	64

Units are mg/kg.

^aData are from TVA.^[15]

^bOur data from sediments collected at ERM 2.2 are shown for comparison.

concentration decreased moving downstream from ERM 2.2 to ERM 0.1, but the standard deviations make it impossible to distinguish the concentrations at these sites. Interestingly, total zinc concentrations were higher in the Clinch and Tennessee Rivers compared to the Emory River. Total arsenic concentrations decreased moving downstream from ERM 2.2 to ERM 1.6, but were indistinguishable at ERM 0.1 and CRM 3.3 due to overlapping standard deviations.

In general, the total element concentrations that we determined in sediments at the spill site (ERM 2.2) were larger than those reported by TVA^[15] for stored and spilled ash (Table 4). We determined a total As concentration of 112 mg/kg, while TVA reported an average of 46.2 mg/kg; however, the maximum As concentration reported by TVA was 113.0 mg/kg, which is much closer to our value. In general, our values were reasonably close to the maximum concentrations reported by TVA. The largest difference was for copper, where we determined a concentration of 228 mg/kg, while TVA reported a maximum concentration of 73.4 mg/kg. These differences may be explained by the sampling locations, collection dates, and the heterogeneous nature of coal fly ash.

Sequential Extraction

Reproducibility

Element concentrations expressed in mg/kg and percent from each step of the sequential extraction are provided in Tables 5 and 6 for the Emory River sites. Data are not presented for the Clinch and Tennessee River samples because in many cases

TABLE 3 Total Element Concentrations (mg/kg) in Sediment Obtained by Microwave-Assisted Acid Digestion

Phase	ERM 3.3	ERM 2.2	ERM 1.6	ERM 0.1	CRM 3.3	TRM 567
As	4.1	112	66	27	42	12
s	4.7	19	11	13	16	11
% RSD	115	17	17	46	38	89
Co	3.7	28	20	23	23	20
s	2.3	2	2	2	3	2
% RSD	60	6	12	11	11	10
Cr	4.7	52	39	33	33	25
s	1.4	3	0.6	1	1	2
% RSD	30	5	1	3	2	8
Cu	462	228	428	465	202	242
s	307	92	201	292	48	141
% RSD	66	40	47	63	23	58
Ni	6.9	51	38	31	30	20
s	4.1	1	7	2	2	3
% RSD	59	3	18	7	7	17
Zn	19	64	58	54	74	68
s	1	4	5	3	5	1
% RSD	7	7	8	6	7	1

The average, standard deviation (s), and percent relative standard deviation (RSD) for three replicate digestions are shown.

TABLE 5 Sequential Extraction Results for Emory River Sediment Samples

ERM	Phase	As		Co		Cr		Cu		Ni		Zn	
		mg/kg	s	mg/kg	s	mg/kg	s	mg/kg	s	mg/kg	s	mg/kg	s
3.3	Exchangeable	0.20	0.05	0.6	0.2	0.11	0.04	1.7	0.8	0.6	0.1	6.2	2.5
	Carbonate bound	3.4	0.6	0.4	0.1	0.18	0.05	0.5	0.3	0.4	0.1	5.2	1.3
	Fe/Mn oxide bound	0.15	0.05	0.5	0.1	0.17	0.03	0.09	0.04	0.7	0.1	1.7	0.8
	Organic/sulfide bound	1.3	0.5	1.1	0.1	1.5	0.1	2.0	0.9	2.7	1.0	3.0	0.4
	Sum	5.1		2.6		2.0		4.3		4.4		16	
	Unextracted	0		1.1		2.7		458		2.5		2.9	
2.2	Exchangeable	4.2	3	0.10	0.06	0.8	0.7	4.0	2.6	0.5	0.1	1.8	1.0
	Carbonate bound	16	2	0.22	0.04	1.7	0.2	9.2	2.5	0.5	0.1	5.1	1.0
	Fe/Mn oxide bound	10	3	1.1	0.5	2.8	0.5	3.9	1.8	1.5	0.7	3.4	1.5
	Organic/sulfide bound	0.3	0.1	0.9	0.2	3.3	1.2	9.6	1.1	2.8	0.7	32	41
	Sum	30		2.3		8.6		27		5.3		42	
	Unextracted	82		26		43		201		45		22	
1.6	Exchangeable	4.6	2.3	0.3	0.1	1.1	0.8	5.5	2.8	0.6	0.1	5.2	0.4
	Carbonate bound	13	2	0.3	0.1	0.9	0.2	8.4	2.4	0.4	0.1	5.3	1.3
	Fe/Mn oxide bound	11	2	1.1	0.4	2.8	0.6	2.6	1.6	1.6	0.6	3.8	1.4
	Organic/dulfide bound	1.3	0.5	2.6	*	1.5	0.8	2.0	0.9	2.7	1.0	9.4	2.7
	Sum	30		4.3		6.3		18.5		5.3		24	
	Unextracted	36		16		33		410		33		34	
0.1	Exchangeable	0.6	*	0.4	*	0.09	*	0.9	*	0.8	*	5.4	*
	Carbonate bound	2.6	1.8	0.8	0.5	0.6	0.5	2.6	0.9	1.1	0.4	8.6	4.3
	Fe/Mn oxide bound	3.4	0.8	1.1	0.6	1.6	0.8	1.6	0.2	1.3	0.5	6.7	2.1
	Organic/sulfide bound	8.9	6.8	2.5	0.7	4.5	1.4	12	9	4.0	1.6	8.7	2.7
	Sum	15.5		4.8		6.8		17		7.2		29	
	Unextracted	12		18		26		448		24		25	

"Sum" is the sum of the exchangeable, carbonate-, Fe/Mn oxide-, and organic-bound concentrations. "Unextracted" was calculated by subtracting "Sum" from the total element concentration determined by microwave-assisted acid digestion.

* $n = 2$, so no standard deviation could be calculated.

only two replicates were performed for these sites, and standard deviations were not obtained. As shown in Table 7, RSDs ranged from 7 to 84%. Larger RSDs were observed at ERM 0.1 for As, Co, Cr, and Ni, probably because the sample was a nonuniform mixture of ash and sediment. In general, most RSDs were less than 50%. There were 20 samples with RSDs less than 30%, 76 samples with RSDs less than 50%, and 14 samples with RSDs greater than 50%. It should be noted that even worse RSDs were obtained for the elements Ag, Cd, Mo, Pb, Se, Tl, and V, which is why we do not draw conclusions about their fractional distribution in these samples.

Our RSDs are much larger than those reported by others, and can be explained by the heterogeneous nature of the samples.^[20,36,37,39,40] After sample collection and drying, we did not take any special steps to homogenize the sediments, such as grinding and sieving, because we did not want to alter

the trace element distribution in the sample; even oven-drying can alter trace element distribution.^[41] In addition, the dried ash-laden sediments were light and fluffy, and there were safety concerns about students grinding and sieving these samples and possibly breathing in the dust. Landsberger et al.^[24] used this method to extract coal fly ash, but did not indicate if the samples were homogenized and did not provide reproducibility data. Others^[30,31] generally observed RSDs less than 15% in extracts of certified coal fly ash samples, except in the first step, where the concentration was lower than in other steps. Marin et al.^[36] explained the poor reproducibility in the organic-bound fraction of reference marine sediments by the more complex chemical reactions that occurred during this step and a matrix effect from ammonium acetate. We observed three instances when the RSD exceeded 50% for the organic-bound fraction in the data set shown in Tables 5 and 6.

TABLE 6 Percentages of Extracted Elements From Emory River Sediment Samples

ERM	Phase	As	Co	Cr	Cu	Ni	Zn
3.3	Exchangeable	5	16	2	0.4	9	33
	Carbonate bound	83	11	4	0.1	6	27
	Fe/Mn oxide bound	5	14	4	0.02	10	9
	Organic/sulfide bound	32	30	32	0.4	39	16
	Sum (Extractable)	125*	71	42	0.9	64	85
	Unextracted	0	29	58	99	36	15
2.2	Exchangeable	4	0.4	2	2	1	3
	Carbonate bound	14	0.7	3	4	1	8
	Fe/Mn oxide bound	9	4	5	2	3	5
	Organic/sulfide bound	0.3	3	6	4	6	50
	Sum (Extractable)	27	8	16	12	11	66
	Unextracted	73	92	84	88	89	34
1.6	Exchangeable	7	1.5	3	1	2	9
	Carbonate bound	20	1.5	2	2	1	9
	Fe/Mn oxide bound	17	6	7	0.6	4	7
	Organic/sulfide bound	2	13	4	0.5	7	16
	Sum (Extractable)	46	22	16	4	14	41
	Unextracted	54	78	84	96	86	59
0.1	Exchangeable	2	2	0.3	0.2	3	10
	Carbonate bound	10	4	2	0.6	4	16
	Fe/Mn oxide bound	13	5	5	0.3	4	12
	Organic/sulfide bound	33	11	14	3	13	16
	Sum (Extractable)	58	22	21	4	24	54
	Unextracted	42	78	79	96	76	46

Percentages were calculated by dividing concentrations in mg/kg for each step by the total concentration determined by microwave-assisted acid digestion.

*The sum of the extractable fractions was greater than the total concentration obtained by microwave-assisted acid digestion.

Sahuquillo et al.^[42] used certified reference lake sediment to investigate sources of error in the BCR method and found that reproducibility was improved when the pH of the hydroxylamine hydrochloride solution was lowered from 2.0 to 1.5. A more acidic solution had the ability to extract a higher

concentration of elements and to resist changes in pH from alkaline samples. In their work with high-carbonate soils, Sulkowski and Hirner^[43] recommended repeating the extraction if the pH of an extract rose by more than 0.5 unit during any step of the extraction. Although we did not measure the pHs of our extracts, controlling the pH may be important for coal fly ash, which can be alkaline, depending on the type of coal burned.

We extracted a standard reference material (SRM) to verify if sample heterogeneity explained our poor RSDs. As shown in Table 8, the largest RSD in any step of the SRM extraction was 16%, indicating that better precision can be obtained for a well-mixed sample.

Extractable and Nonextractable Elements

In this section, we discuss the extractable and nonextractable elements in Emory River sediments. We define the *extractable concentration* as the sum of the exchangeable, carbonate-, iron/manganese oxide-, and organic-bound concentrations, because trace elements can be liberated from these phases by changes in environmental conditions such as pH, oxidizing-reducing potential (ORP), or microbial activity.^[19] The nonextractable concentration was calculated by subtracting the extractable concentration from the total concentration obtained by microwave-assisted acid digestion. The nonextractable fraction is essentially the residual fraction, but we prefer the term *nonextractable* because we did not experimentally perform the residual extraction as described by Tessier et al.^[20]

As shown in Table 6, a striking difference between the upstream (ERM 3.3) and impacted sites (ERM 2.2, 1.6, and 0.1) is the smaller percentage of extractable

TABLE 7 Averages and Ranges of RSDs for Sequentially Extracted Elements at Each Site

Element	ERM 3.3		ERM 2.2		ERM 1.6		ERM 0.1	
	avg. RSD	range	avg. RSD	range	avg. RSD	range	avg. RSD	range
As	28	18–40	32	14–62	31	14–49	58	25–77
Co	22	7–33	36	20–54	23	7–35	45	29–57
Cr	20	8–37	38	13–84	31	8–74	54	32–82
Cu	45	41–47	38	11–65	47	28–62	41	15–73
Ni	26	12–36	29	17–47	29	17–40	40	38–43
Zn	32	15–45	39 ^a	20–52 ^a	24	7–37	38	31–50

The average and range are reported for the four steps of the sequential extraction.

^aThe RSD of the organic-bound fraction was 129%, but not included in the average or range.

TABLE 8 Sequential Extraction Results for SRM 2711 Montana Soil

Phase	As	Co	Cr	Cu	Ni	Zn
Exchangeable	2.6	0.05	nd	0.9	nd	0.9
S	0.1	0.01	—	0.1	—	0.1
% RSD	3	12	—	5	—	6
Carbonate Bound	5.5	0.61	nd	4.1	1.1	19
S	0.3	0.03	—	0.2	0.1	0.4
% RSD	5	5	—	5	7	2
Fe/Mn Oxide Bound	0.6	3.0	0.75	14	3.3	105
S	0.1	0.2	0.03	0.6	0.2	2
% RSD	16	6	4	4	6	2
Organic Bound	9.4	0.60	1.6	19.2	2.0	21
S	0.3	0.01	0.1	0.3	0.2	0.6
% RSD	4	2	7	2	8	3

Units are mg/kg. nd = not detected.

elements at the impacted sites. In addition, for all elements except copper and zinc, the percentage of extractable elements increased moving downstream from the spill site (ERM 2.2) to ERM 0.1. At ERM 3.3, the extractable fraction ranged from 42 to 125% (excluding copper), while at ERM 2.2 it ranged from 8 to 27% (excluding zinc). Although there were smaller *percentages* of extractable elements at the impacted sites, the *concentrations* of extractable elements were in fact larger at the impacted sites. For example, 27% of arsenic was in the extractable form at the spill site compared to 125% at the upstream site; however, 30 mg/kg of arsenic was extracted from the spill site, while only 5.1 mg/kg were extracted from the upstream site. In other words, small percentages of extractable elements at the impacted sites corresponded to relatively large concentrations, because the volume of ash was so large there.

Most of the sediment samples collected from the Emory River (both impacted and upstream) had the majority of trace elements sequestered in the nonextractable phase. Notable exceptions were observed at ERM 3.3, where the largest arsenic fraction was carbonate-bound (83% or 3.4 mg/kg), the largest zinc fraction was exchangeable (33% or 6.2 mg/kg), and the largest nickel fraction was organic-bound (39% or 2.7 mg/kg).

In general, the literature indicates that the residual fraction comprises greater than 50% of coal fly ash. For example, Landsberger et al.^[24] reported 93% of Cr was in the residual fraction in Illinois coal fly ash, and Smeda and Zyrnicki^[30] reported 68–74%

residual Cr in certified reference fly ash; these values compare well to the 84% nonextractable Cr that we found at the spill site. In their work with coal fly ashes from Brazil, Spain, and China, Fernandez-Turiel et al.^[27] reported residual fractions ranging from 81 to 95% for Cd, Co, Cu, Ni, Pb, Sb, and Zn. Petit and Rucandio^[29] reported 65% residual Cd in reference fly ash, while Wadge and Hutton^[25] reported 55% and 98% residual Cd and Pb, respectively, in landfilled coal fly ash. The residual fractions of Cr, Ni, Pb, and Zn were greater than 75% in coal fly ash collected from a Bulgarian power plant.^[31] These data imply that many trace elements are strongly incorporated into the ash structure, and may explain our observation that the extractable fraction increased in moving downstream where less ash was observed in the sediment.

Element Distribution in the Extractable Fractions

Although trace elements are strongly incorporated into the ash and sediment structures, it is not unreasonable to suppose that as ash deposits are transported downstream where they encounter different environments such as pH, ionic strength, or oxidation reduction potential (ORP), these new environments may change the distribution of trace elements in the ash deposit.^[19] In this section, we compare the concentrations of trace elements in each fraction at the four sampling sites in the Emory River, and restrict our discussion to the extractable fractions.

The largest exchangeable arsenic concentrations were observed at the spill site (4.2 mg/kg), and downstream at ERM 1.6 (4.6 mg/kg). The exchangeable arsenic concentration then decreased further downstream at ERM 0.1 (0.6 mg/kg), but it should be noted that only two replicates were analyzed here, and no standard deviations were available (Table 5). At ERM 2.2 and ERM 1.6, 10 and 11 mg/kg of arsenic, respectively, were associated with the iron/manganese oxide phase, which is known to scavenge trace elements in aqueous environments^[20] and during coal combustion.^[26] Under anoxic conditions, this phase is thermodynamically unstable^[44–46] and often attacked by benthic organisms,^[47] which has the potential to release arsenic into the environment. It is difficult to comment on

the distribution of arsenic at ERM 0.1, because the RSD for the oxidizable phase was so large (76%).

Similar to arsenic, the largest exchangeable chromium and copper concentrations were observed at ERM 2.2 and ERM 1.6, and probably reflect the larger amounts of ash in these sediments. Contrarily, the largest exchangeable cobalt concentration was observed at the upstream site (ERM 3.3), where no ash was observed in the sample. The concentration of exchangeable zinc was also greater at ERM 3.3 compared to the spill site. These observations may reflect the degree to which cobalt and zinc were incorporated into the ash structure. Total metal analyses indicated that cobalt and zinc were in fact present at the spill site, but they appear to be less extractable by solvents used in the sequential extraction. Querol et al.^[26] explained the lower leachability of zinc from Spanish coal fly ashes by the incorporation of zinc into refractory aluminosilicate phases formed during coal combustion.

Exchangeable nickel concentrations were indistinguishable at all sites due to overlapping standard deviations, and were greater than the acid-soluble concentrations. Of the extractable phases, the majority of nickel was associated with the organic phase. Under oxidizing conditions or increased degradation of organic matter, nickel could be released into the environment.^[19]

Comparison of Our Results to the Literature

It is difficult to compare our results to those of others, because so many different methods have been used to sequentially extract coal fly ash (Table 1). Landsberger et al.^[24] who also used the Tessier method, reported that no arsenic was detected in the first four extracts of coal fly ash collected from power plants in Illinois. Bodog et al.^[28] reported 22 mg/kg exchangeable Cu in Austrian coal fly ash, while we found 4.0 and 5.5 mg/kg exchangeable Cu at ERM 2.2 and 1.6, respectively. Again, a direct comparison is difficult, because Bodog used a different extraction solvent and time to determine exchangeable copper (Table 1).

Our extractable Cr results are very similar to those reported by Lihareva^[31] for Bulgarian coal fly ashes extracted using the BCR method. We found 1.7, 2.8, and 3.3-mg/kg Cr in the carbonate, Fe/Mn

oxide, and organic phases, respectively, while Lihareva^[31] found 1.15, 2.02, 3.28 mg/kg in the respective phases. However, we found 0.5-, 1.5-, and 2.8-mg/kg Ni in the carbonate, Fe/Mn oxide, and organic phases, respectively, while Lihareva^[31] reported 12.6, 4.92, and 1.64 mg/kg in the respective phases.

We have collected various fish species from the study site and analyzed their muscle and organ tissues for trace elements. Work is underway to determine if there is a correlation between extractable trace element concentrations in sediments and trace element concentrations in fish.

Instructor Perceptions of the Laboratory Experience

Students were not formally evaluated about their experience in this course, but they appeared to be excited about and dedicated to this research project. They were motivated to work on a project that received a great deal of media attention. Although the breadth of the material covered during the semester was much less, students effectively learned just as much in studying one problem in greater depth. Students gained experience with an extraction method not normally covered in the undergraduate curriculum and had to overcome the challenges of running samples containing high salt concentrations on the ICP-OES.

The experience could have been improved if we had had more time for data analysis and interpretation, most of which was left up to the instructor. We performed all the extractions first, and by the end of the semester, many extracts requiring ICP-OES analysis had accumulated. Analyzing the extracts as they were generated may have allowed us to discover the reproducibility problems sooner.

IDLs should have been determined for all four extraction matrices before any samples were extracted or analyzed. More attention was initially paid to the extraction process and scheduling student responsibilities for each extraction step. When we finally began analysis of the extracts, we had to address the high background intensities of the samples (by matrix matching the standards) and the high salt concentrations in the matrix (by diluting the samples). This slowed our progress and left us with enough time to determine IDLs for two extraction matrices; the IDLs for the remaining two

matrices were completed by a summer research student. In spite of the slow progress, the students in this laboratory were able to optimize the ICP parameters, and this has been extremely valuable for the undergraduate students currently continuing this research.

CONCLUSION

We used the sequential extraction method developed by Tessier to investigate the potential mobilities and bioavailabilities of As, Co, Cr, Cu, Ni, and Zn in Emory River sediments that were impacted by an accidental release of coal fly ash. Trace elements at the spill site were found largely in the nonextractable phase, but the concentrations of extractable elements were larger compared to the upstream site. The largest exchangeable As, Cr, and Cu concentrations were observed at the spill site and just slightly downstream at ERM 1.1. The majority of extractable arsenic was associated with the iron/manganese oxide phase, which is thermodynamically unstable under reducing conditions, and could therefore serve as a source of arsenic under reducing conditions. Surprisingly, the largest exchangeable cobalt and zinc concentrations were observed upstream of the spill at ERM 3.3, which indicated that these elements were more tightly bound to fly ash than to uncontaminated sediments. The majority of extractable Ni was associated with the organic phase, which could be a source of Ni under oxidizing conditions. In general, we observed poorer reproducibility in the sequential extraction method than that reported in the literature, and were unable to report on many of the analyzed trace elements. However, analysis of a well-blended SRM indicated that reproducibility could be improved by homogenizing the sample prior to extraction. We recommend that calibration standards for ICP-OES analysis be prepared in the same matrix as the extract to eliminate differences in background emission intensities, and that the extract from step 2, which is in a matrix of acidified 1-M sodium acetate, be diluted by factor of two in order to prevent clogging of the torch and coating of the cone interface by salt deposits. A semester-long research project on a real-world problem was an effective way to teach environmental chemistry and gave students experience with unconventional samples and methods.

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