Influences of Emergent Macrophytes on the Quality of Water Contaminated with Coal Ash Leachates

by

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Executive Summary

Coal combustion currently remains as one of the leading sources of energy in the world. The potential environmental contamination from the storage of coal ash has been highlighted in recent years by large spills from power plant retention ponds in Tennessee and North Carolina. These incidents, as well as permitted coal ash leachate discharge, make it important to understand the influences of coal ash on the ecology and biogeochemistry of aquatic ecosystems.

Wetland ecosystems are particularly of interest, as they serve as reservoirs and transformers of pollutants in the landscape, and constructed wetland treatment systems (CWTS) have been used in the past to treat coal combustion waste. Emergent macrophytes play a pivotal role in CWTS and are often a defining feature of natural wetland habitats that can contribute to the removal of pollutants from the water column through a range of biological and chemical processes. This study explored the explored the effects of two emergent macrophyte species, *Juncus effusus* and *Eliocharis quadrangulata*, on the physicochemical properties and trace element concentrations of water contaminated with coal ash.

A greenhouse study was performed with wetland microcosms dosed with leachates of fly ash derived from high sulfur and low sulfur coal sources. Microcosms were planted with *J. effusus*, *E. quadrangulata* or were unplanted to control for the presence of plants. Both types of leachate increased the electric conductivity (Ec) of microcosm water relative to controls received reverse osmosis water. High sulfur leachates increased water pH while low sulfur leachates decreased water pH. Both leachates significantly elevated boron and lithium concentrations in microcosm water and high sulfur leachates also elevated molybdenum significantly. The highest boron concentrations measured in the study exceeded several aquatic toxicity thresholds.

The macrophytes did not display any signs of toxicity, but did appear to exert an influence on the water chemistry. The presence of either species reduced the Ec of microcosm water significantly more than when plants were not presence. Both species also appeared to increase the removal efficiency of trace elements from the water column compared to microcosms with no macrophytes.

The findings of this study indicate that emergent macrophytes are tolerant to aquatic coal ash pollution and could potentially reduce associated perturbations in water quality. Their presence in aquatic ecosystems downstream from coal ash discharges could help maintain ecosystem integrity or they may be effectively utilized in CWTS for coal power plant wastewater. Further studies are needed to evaluate the influence of higher volumes of leachate contamination, bioaccumulation of trace elements in macrophytes and the speciation of trace elements from coal ash leachates.

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I. Introduction

A. Coal Ash Contamination Risks

Coal combustion is a main source of electricity in most parts of the world, accounting for 39% in the United States (EIA 2015), 81% in China, 71% in India and approximately 40% worldwide, as of 2012 (WCA 2014). The process of its combustion creates large amounts of coal combustion residuals (CCR). (Dellantonio et. al., 2010). CCRs contain various toxic trace metals (Patra et. al., 2012), as well as concentrated radionuclides (USEPA 2014), but are currently not classified as a toxic substance by the United States Environmental Protection Agency (USEPA 2014).

The large volume of CCRs stored in the United States has raised environmental concerns, highlighted by the Duke Energy Dan River Spill of 2014 and the Tennessee Valley Authority (TVA) Kingston Fossil Plant Spill in 2008. Many of the trace elements contained in CCRs are highly leachable from the particle surface (Ruhl et. al., 2010), so water released from retention ponds, either from unplanned spills or permitted discharge contains trace elements (Lemly 2012). CCR contamination can cause ecotoxicological risks, including reduced survival and slowed development in aquatic organisms (Rowe et. al., 2002; Lemly 2012). Additionally, some of the leachable metals in coal ash include Cr, Hg, As, and Se, all known to have toxic effects on wildlife and humans (Ruhl et. al., 2010). Coal burning power plants are also issued NPDES permits allowing them to discharge regulated amounts of CCR wastewater into public water.

Due to the prevalence of CCR contamination of surface and ground waters, it is important to understand how the potentially elevated levels of heavy metals will affect aquatic ecosystems as well as develop low cost effective remediation methods to reduce toxicity in downstream waters. This dual approach will help inform management decisions when dealing with CCR use and disposal.

B. Potential Role of Wetlands

Wetlands are intermediate ecosystems that are influential in biogeochemical cycling and can serve as sources, sinks or transformers of contaminants (Mitsch & Gosselink 2015; Reddy & Delaune 2008). Slow water flow, fluctuating reduction oxidation potential and specially adapted flora and fauna all contribute to making wetlands areas of high biochemical cycling. To this end, constructed wetlands are often used in waste water treatment (Vymazal 2011), and on occasion have been created at coal combustion facilities to decontaminate CCR wastewater (Ye et. al., 2001).

Emergent macrophytes are often influential in the decontamination of wastewater by treatment wetlands as they alter the chemical cycling of their environment with physiological adaptations such as radial oxygen loss (Preussler et. al., 2015; Bhatia et. al., 2014). Emergent macrophytes are thought to facilitate the removal of soluble contaminants from the water column through various means (Sundberg-Jones & Hassan 2007; Mitch & Gosselink 2015).

The presence of aerenchyma in their vascular tissue allows radial oxygen loss (ROL) in the rhizosphere, which creates microhabitats for aerobic bacteria (Mitch & Gosselink 2015; Preussler et. al., 2015; Reddy & Delaune 2008). The close proximity of anaerobic and aerobic zones that form in the saturated rhizosphere increases localized redox gradients that enhance chemical cycling, allowing the oxidation of metals into precipitates or the degradation organic pollutants (Bhatia & Goyal 2013; Reddy & Delaune 2008). Additionally, macrophytes can be physical stabilizers, immobilizing precipitates and contaminants sorbed to sediments, and serving as a substrate for periphyton biofilms, which are involved in contaminant sequestration (Sundberg-Jones & Hassan 2007).

Macrophytes can also sequester contaminants directly through their roots and translocate them to their above ground tissue (Bhatia & Goyal 2013). The efficiency of contaminant sequestration in plant tissue as a removal process is debatable however. Studies of constructed wetland treatment systems (CWTS) indicate that usually 0.1% to 3% of the contaminants removed from wastewater are sequestered by plants (Ye et. al., 2001), with averages leaning toward the lower end.

C. Research Objectives

There is limited literature on wetland interactions with CCR leachate, and an increasing need to understand its influence on aquatic ecosystems. Thus it is important to understand how emergent macrophytes influence the chemistry of water bodies that have been exposed to CCRs in order to assess management strategies that minimize human health and environmental risks. To address this, the study analyzed if emergent macrophytes are effective at reducing concentrations of heavy metals and other water chemistry perturbations in CCR disturbed water.

Juncus effusus and *Eliocharis quadrangulata* were grown in wetland microcosms and dosed with coal ash leachates in order to examine the effects on water chemistry and the environmental partitioning of heavy metals from high sulfur and low sulfur coal sources. These species were chosen because they are native to North Carolina and Tennessee and environmental assessments of CWTS at TVA coal facilities commonly found these species growing (Brodie 1990), indicating an innate tolerance to CCR leachate. Comparing the two coal types is important, because they are derived from different geographical locations and have different potential for re-use.

II. Methods and Materials

A. Microcosm Setup

The experiment was performed in the Duke University Biology Research Greenhouse in a room with a temperature of 20-25 C and a photoperiod of 14 hours of light per day. Twenty seven microcosms were made in white, 18.9 liter high density polyethylene (HDPE) buckets and soil acquired from a single source in the North Carolina piedmont. The soil was pasteurized and mixed to homogenize and reduce the differences in soil texture and microbiomes in the microcosms. Each microcosm was filled with soil 13 to 18 cm from the bucket rim and was planted with either 5 individuals of *Juncus effusus* or *Eliocharis quadrangulata* or 4 individuals of *Scheonoplectus tabernaemontani* over a 3 day period. The plants were acquired from Mellow Marsh Farm in Siler City, NC. *E. quadrangulata* and *S. tabernaemontani* were bare root plugs and *J. effusus* were root plugs in soil. Each microcosm was watered with approximately 0.7 liters of RO water once a day.

After 21 days, the survivorship in the *S. tabernaemontai* was low so the species was removed from the experiment. The individuals were removed from the microcosms, then the soil of the 9 microcosms was re-homogenized in a large bucket and the microcosms were refilled with the soil 13 to 18 inches from the bucket rims. These microcosms were subsequently used as controls to evaluate effects from the absence of plants.

After 45 days, three of the *E. quadrangulata* microcosms that had multiple plugs die were replanted with an addition 1-3 plugs. The *J. effusus* microcosms had 100 percent survival rates.

After 77 days, each microcosm was flooded with RO water so that the water level was approximately 8 cm from the rim of the bucket. The water level was refilled to 8 cm from the rim 1-2 times a week for the remainder of the experiment to account for evaporation.

B. Fly Ash Leachate

Fly ash from high sulfur and low sulfur coal sources was acquired from two separate power plants. Leachates were made based on EPA Method 3051. An initial batch of leachates was made using a 10:1 and 20:1 liquid volume to solid mass ratio for both types of ash as well as a DI water blank. The 10:1 volume to mass was used to make the rest of the experimental leachate because of its higher trace element concentrations.

Moisture content of each fly ash was determined by weighing a 3 to 5 gram sample, placing it in an aluminum tray, drying in an oven at 95 C for 24 hours and reweighing. The moisture content was calculated with equation (1). Moisture content was used to calculate the desired wet mass to add with equation (2)

(1) Moisture Content = (initial mass – final mass) / (initial mass)

(2) Mass of fly ash to add = (moist mass) * (moisture content)

For each type of fly ash, approximately 300 grams of fly ash and 3 liters of deionized (DI) water, corrected for moisture content, was added to a polypropylene (PP) container. The PP containers were placed in a Precision reciprocal shaker bath and mixed at 180 rpm for 24 hours. The PP containers were then removed and the contents were allowed to settle for at least 30 minutes. A two-liter filter flask was attached to an air vacuum and fitted with a plastic Millipore filter. The contents of the PP containers were filtered through the apparatus with a 45 µm Whatman nylon membrane filters then poured into a HDPE bucket. Each type of leachate was filtered and placed in a separate HPDE bucket. A third HDPE bucket was filled with reverse osmosis (RO) water from the greenhouse, which had been used to water the microcosms during the length of the experiment.

C. Dosing

1. Pulse dose

Twenty five days after the microcosms were flooded, each one was dosed with 1 liter of leachate for their assigned treatments. Each leachate was mixed in its storage bucket with a wooden stirring rod for 10 minutes, then 50 ml of leachate was added to HDPE bottles corresponding to each microcosm. The leachates were then stirred for one minute and an additional 50 ml of leachate was added to each dosing bottle. The process was repeated until approximately 250 ml of leachate was added to each bottle. This process was repeated for the low sulfur leachate, high sulfur leachate and reverse osmosis water treatments, and the contents each dose bottle was added to its corresponding microcosm. The pipette was rinsed twice with DI water in between each treatment. Doses of 250 ml were added four times, once every four hours leading to approximately 1 liter of leachate being added to each microcosm over a 16 hours period.

2. Press dose

Sixty one days after the microcosms were flooded, a press dose, where a dose was applied once a day, was performed over a 10 day period. These dosing's were performed on the same microcosms with the same treatment they had received in the pulse dose 35 days earlier. The leachate bucket was stirred for 10 minutes, then a 250 ml PP bottle was used to scoop approximately 250 ml of leachate from the top of the bucket into another 250 ml PP bottle, then labeled for a corresponding microcosm. In between every three bottles, the leachate was stirred for one minute.

This was repeated for 9 microcosms of a given treatment, then the scoop bottle was rinsed three times with DI water, and the process was repeated for each treatment. The contents of each dosing bottle was then added to its corresponding greenhouse microcosm. Doses were prepared and added for 10 consecutive days, leading to approximately 2.5 liters of leachate being added over a 10 day period.

D. Microcosm Measurements

1. Plant Height

The height of plants in the microcosms were measured once a week with a nylon coated measuring tape beginning 16 days after the microcosms were setup. Each *J. effusus* microcosm contained 5 individuals for the duration of the experiment, but *E. quadrangulata* reproduced vegetatively via rhizomes throughout the experiment so only the 5 tallest individuals in each *E. quadrangulata* microcosm were measured.

2. Stem Density

The number of stems in each microcosm was counted once a week alongside height measurements beginning 59 days after the microcosms were setup.

3. Electric Conductivity (Ec)

The Ec and temperature of the water in each microcosm and was measured once a week beginning 6 days after flooding using and Orion model 122 conductivity meter and Orion 012210 conductivity cell. The frequency of Ec measurements was increased to 2 or 3 times a week at the beginning of the press dose until 17 days after the completion of the press dose at which point Ec was measured only once a week. Once a week the probe was calibrated in a standard KCl solution for reference.

4. PH

The pH measurements of microcosm water were taken beginning 6 days after flooding using a Hannah Instruments (HI) 98121 combo pH and ORP probe. The probe was tested in reference solutions of pH 4, 7, and 10 before measurements were taken. The probe was calibrated with pH 4 and pH 7 reference solutions when the readings deviated strongly from the reference solutions. The probe was periodically cleaned by soaking in HI 7061L electrode cleaning solutions for 30 minutes and rinsing thoroughly in with DI water followed by placements in HI 70300 storage solution.

E. Elemental Analysis

1. Sample Collection

Water samples were collected from each microcosm 0, 10, 32, 46, and 89 days after the first dosing. Samples were collected with a 20 ml pipette and 45-50 ml of sample were placed in 50 ml centrifuge tubes. The pipette tip was placed 5-10 cm beneath the water surface near the middle of the microcosms to avoid surface and edge effects. The pipette tip was rinsed twice with DI water in between each sample collection. After all microcosms were sampled, the centrifuge tubes were immediately stored at 4 °C.

2. Sample Processing

Approximately half of each water sample collected from the microcosms were filtered through a plastic Millipore apparatus fitted with a 45 µm Whatman nylon membrane filter into a 50 ml centrifuge tube using a pyrex filter flask attached to a vacuum.

In between each sample the filter paper was discarded and the Millipore apparatus was rinsed with a 0.1 N HCl, then rinsed three times with DI water. A new filter was added to the Millipore apparatus and the process was repeated for all of the water samples collected. Before analysis on ICP-MS, 2 ml of water sample was diluted to 10 ml with a trace metal grade acid solution containing 2% HNO_3 and 0.5% HCl solution.

F. Data Analysis

Quantitative data were analyzed using R statistical package software, utilizing t-tests when comparing two sample groups, analysis of variance (ANOVA) for comparing multiple sample groups with multiple factors, and analysis of covariance (ANCOVA) for comparing multiple samples groups with multiple factors and continuous variables. When data strongly deviated from normality, Kruskall-Wallis tests were used instead of ANOVAs.

1. Plant Growth and Biomass

Plant growth was analyzed by comparing weekly increases in height and stem density over the course of the experiments with linear regression models evaluating changes over time between treatment types. Differences in growth between the two species was analyzed using a Welch two sample t-test. Total dry biomass of plant material in each microcosm after harvest was analyzed using a two-way analysis of variance (ANOVA) evaluating species type and treatment.

Two multiple linear regressions were performed for each species comparing height growth or stem density growth to time and treatment type.

2. Water physico-chemical properties

Water Ec and pH were each analyzed in two separate regression models for the two different dosing periods in order to increase statistical clarity. Data were analyzed using multiple regression ANCOVA including time as a continuous independent variable and species and treatment as independent factors.

III. Results

A. Plant Growth and Biomass

Plant growth and biomass analysis between the two species was performed by comparing weekly increases in height and stem density over the course of the experiments as well as the total dry biomass of plant material in each microcosm after harvest (fig. 1).

For height growth and stem density growth, there were no significant trends over time, and only one regression model, height growth in *E. quadrangulata*, had a positive r squared value, which was still low at 0.0413 (Table 1). The only significant trend observed was in *E. quadrangulata* height growth in which the low sulfur leachate treatment had higher growth than high sulfur leachate or RO water treatments (B = 0.703, $p < 0.01$).

J. effusus had significantly lower height growth than *E. quadrangulata* (t=2.136, df=221, p = 0.034), while the was no significant difference in stem growth between the two species (t=1.327, df=187, p=0.186).

Table 1. Summary of linear regression models comparing plant growth for each species to time elapsed.

Species	Measurement	R^2	df	F	p
J. effusus	Height	-0.0179	3,113	0.322	0.81
E. Quadrangulata	Height	0.0413	3,113	2.67	0.051
J. effusus	Stem Density	-0.005	3,113	0.806	0.493
E. Quadrangulata	Stem Density	-0.0133	3,113	0.491	0.689

A significant difference was found between the average biomass of the two species (F=9.71, df=1, $p<0.01$), with *J.effusus* having a mean of 45.7 g, while *E. quadrangulata* had a mean of 35.8 g. There was no significant difference between treatments (F=0.652, df=2, p=0.536), but *J. effusus* appeared to have higher biomass in leachate treatments while *E. quadrangulata* had similar biomass in all treatments (fig. 1.)

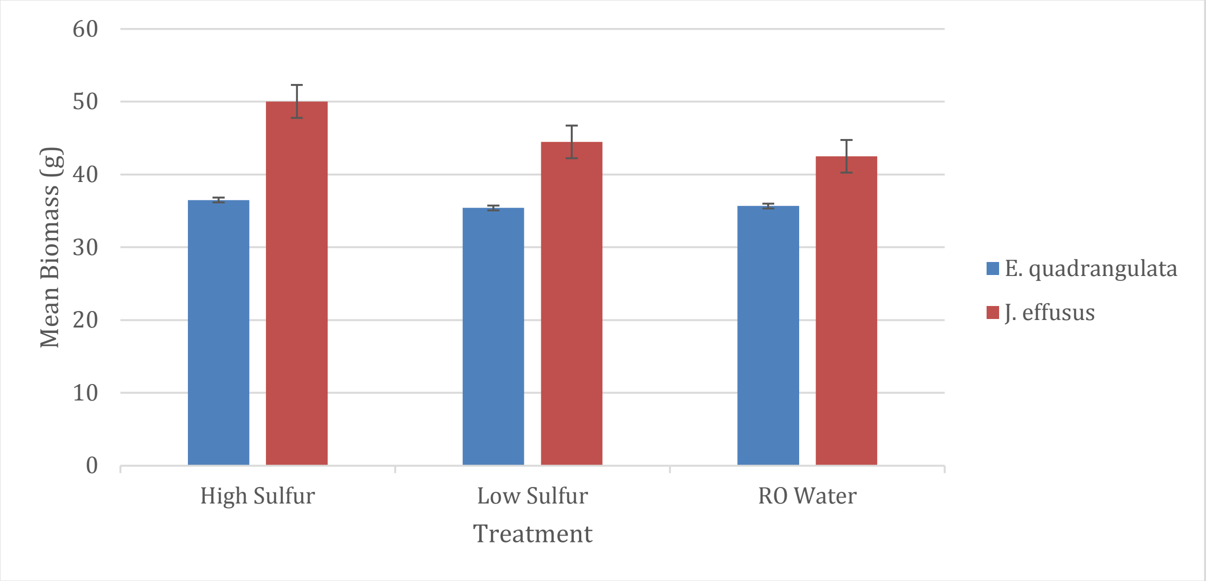


Figure 1. Mean dry biomass of plant material harvested from each microcosm compared between treatments (High sulfur fly ash leachate, low sulfur fly ash leachate and reverse osmosis water) and plant species.

B. Water Physico-Chemical Properties

The Ec and pH of microcosm water was analyzed separately for the two time periods of each dosing in order to assess the effects of each dosing and to increase statistical clarity. Data were analyzed using multiple regression ANCOVA including time as a continuous independent variable and species and treatment as independent factors.

1. Electric Conductivity (Ec)

For the first dosing period, Ec changed significantly relative to all variables analyzed, decreasing with time, decreasing with the presence of either plant species and decreasing with treatment type (table 2). For the second dosing period, Ec changed significantly relative to all variables analyzed, decreasing with time, decreasing with the presence of either plant species and decreasing with treatment type (table 2).

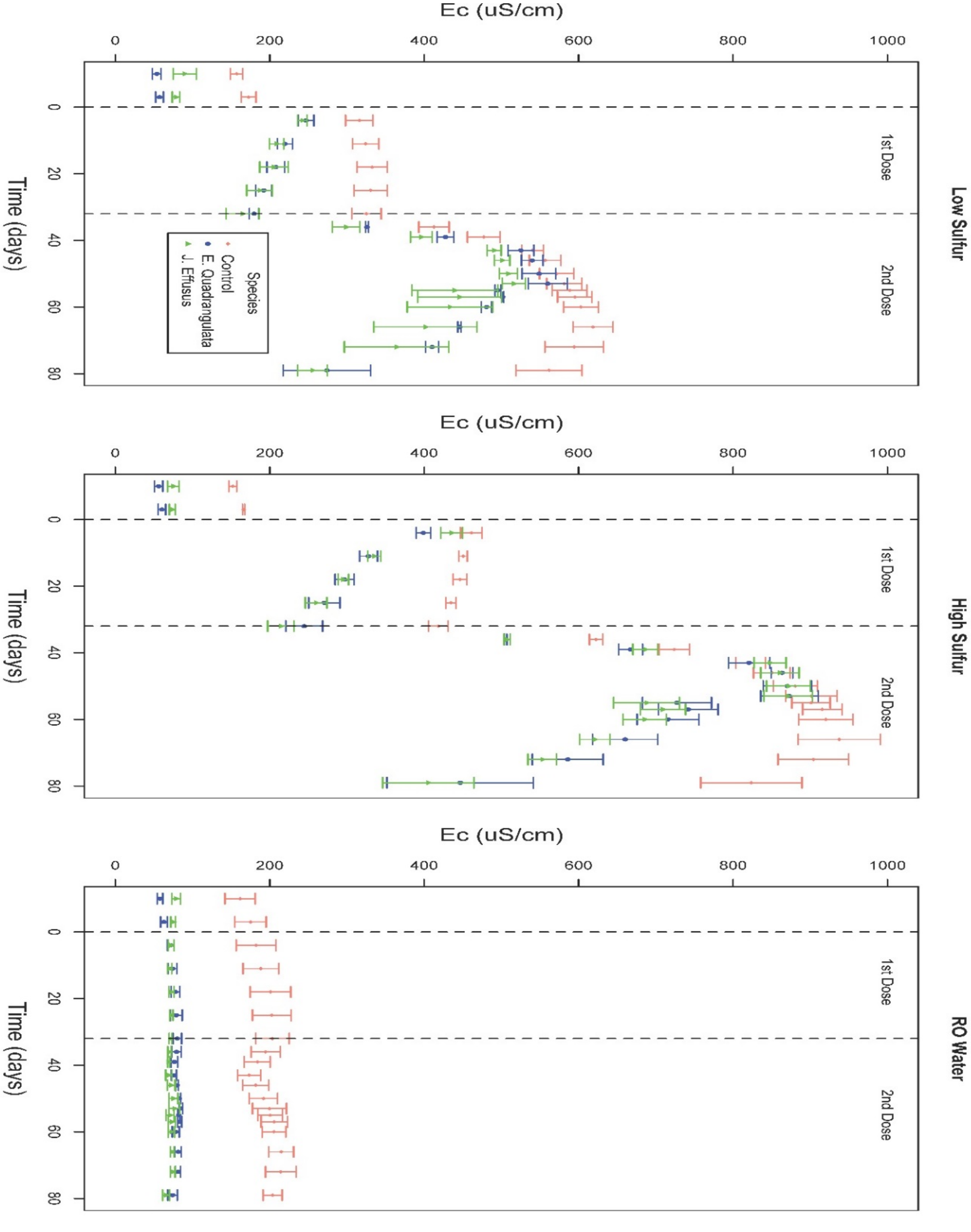


Figure 2. Electric conductivity (Ec) over time for high sulfur fly ash, low sulfur fly ash, and reverse osmosis water treatments in planted and control microcosms. Dashed vertical lines when dosing began. The second dosing ended on day 42.

2. PH

For the first dosing period, pH changed significantly based on treatment type and with the presence of *E. quadrangulata*, but there were no significant trends with time or the presence of *J. effusus* (table 2). For the second dosing period, pH changed significantly relative to all variables analyzed, increasing with time, decreasing with the presence of either plant species and decreasing with treatment type (table 2).

Table 2. Multiple linear regression equations and summary statistics for Ec and pH change over time after the first and second dosing. Factor values in the equations are relative to microcosms with no plants and hi sulfur leachate dosing treatments (Low-S = low sulfur leachate dosing, RO Water = reverse osmosis water control, E. Quad = *E. quadrangulata* planted, J. Eff = *J. effusus* planted).

		MLR Equation	r^2	df	F	p
Dose 1	Ec (µS/cm)	Ec = 471 - 1.94 * Day – 107.1 * factor(Low-S) - 238.1 * factor(RO Water) - 123.3 * factor(E. Quad) - 127.3 * factor(J. Eff)	0.93	5, 129	384	<0.01
	pH	pH = 6.70 - 0.002 * Day – 1.39 * factor(Low-S) - 0.757 * factor(RO Water) + 0.352 * factor(E. Quad) + 0.077 * factor(J. Eff)	0.73	5, 129	70	<0.01
Dose 2	Ec (µS/cm)	Ec = 1097 – 3.94 * Day – 277.6 * factor(Low-S) - 658.6 * factor(RO Water) - 125.7 * factor(E. Quad) - 148.8 * factor(J. Eff)	0.95	5, 264	856	<0.01
	pH	pH = 7.12 + 0.011 * Day – 2.31 * factor(Low-S) - 0.932 * factor(RO Water) - 0.488 * factor(E. Quad) + 0.542 * factor(J. Eff)	0.83	5, 264	135	<0.01

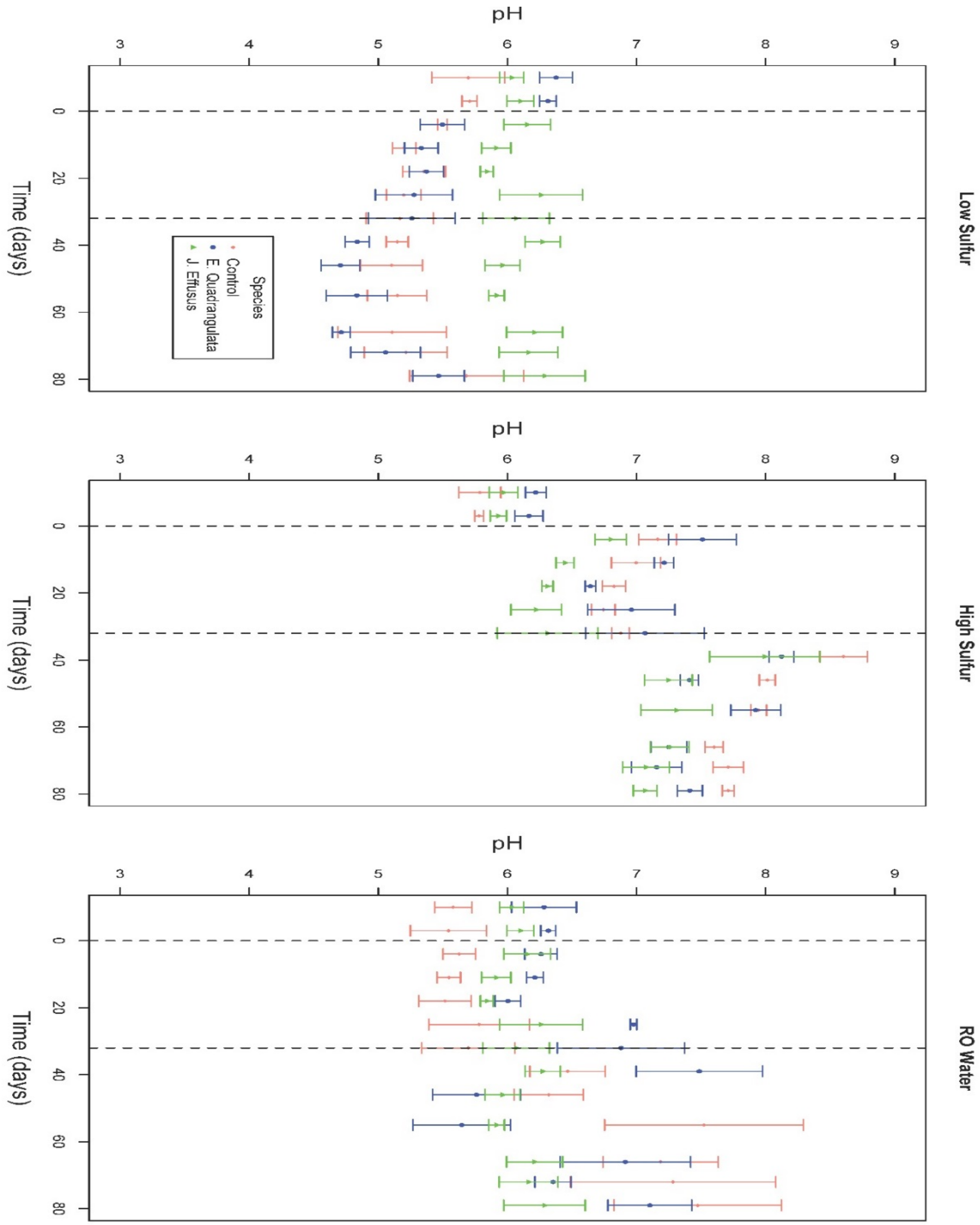
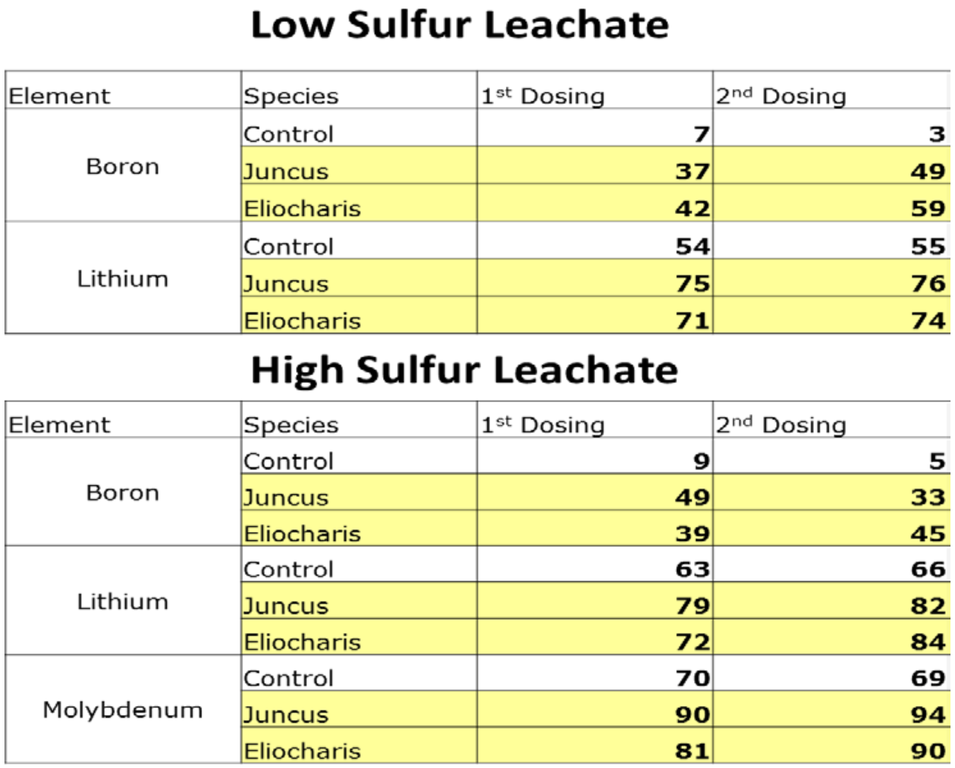


Figure 3. PH over time for high sulfur fly ash, low sulfur fly ash, and reverse osmosis water treatments in planted and control microcosms. Dashed vertical lines when dosing began. The second dosing ended on day 42.

C. Metal Concentrations

Table 3. Mean percent reductions of trace metal concentrations in microcosm surface water dosed with high sulfur and low sulfur coal ash leachate.



Low Sulfur Leachate

Element	Species	1st Dosing	2nd Dosing
Boron	Control	7	3
	Juncus	37	49
	Eliocharis	42	59
Lithium	Control	54	55
	Juncus	75	76
	Eliocharis	71	74

High Sulfur Leachate

Element	Species	1st Dosing	2nd Dosing
Boron	Control	9	5
	Juncus	49	33
	Eliocharis	39	45
Lithium	Control	63	66
	Juncus	79	82
	Eliocharis	72	84
Molybdenum	Control	70	69
	Juncus	90	94
	Eliocharis	81	90

1. Lithium (Li)

Elevated Li concentrations were observed in microcosms receiving fly ash leachates (fig. 4). Li concentrations were higher in vegetated microcosms soon after dosing with a maximum mean of 75.1 µg/L in low sulfur *E. quadrangulata* microcosms (appendix 1). Samples taken several weeks after dosing showed Li concentrations were lower in vegetated microcosms. The reduction of Li from high and low sulfur leachates was similar for both species, ranging between 71% and 84%, while controls only reduced Li concentrations 54% to 63% (table 3). Kruskal-wallis tests showed significant relationships between Li concentrations and treatment type during the second, fourth and fifth measurements, and significant differences in the Li concentrations for each successive time point (appendix 2).

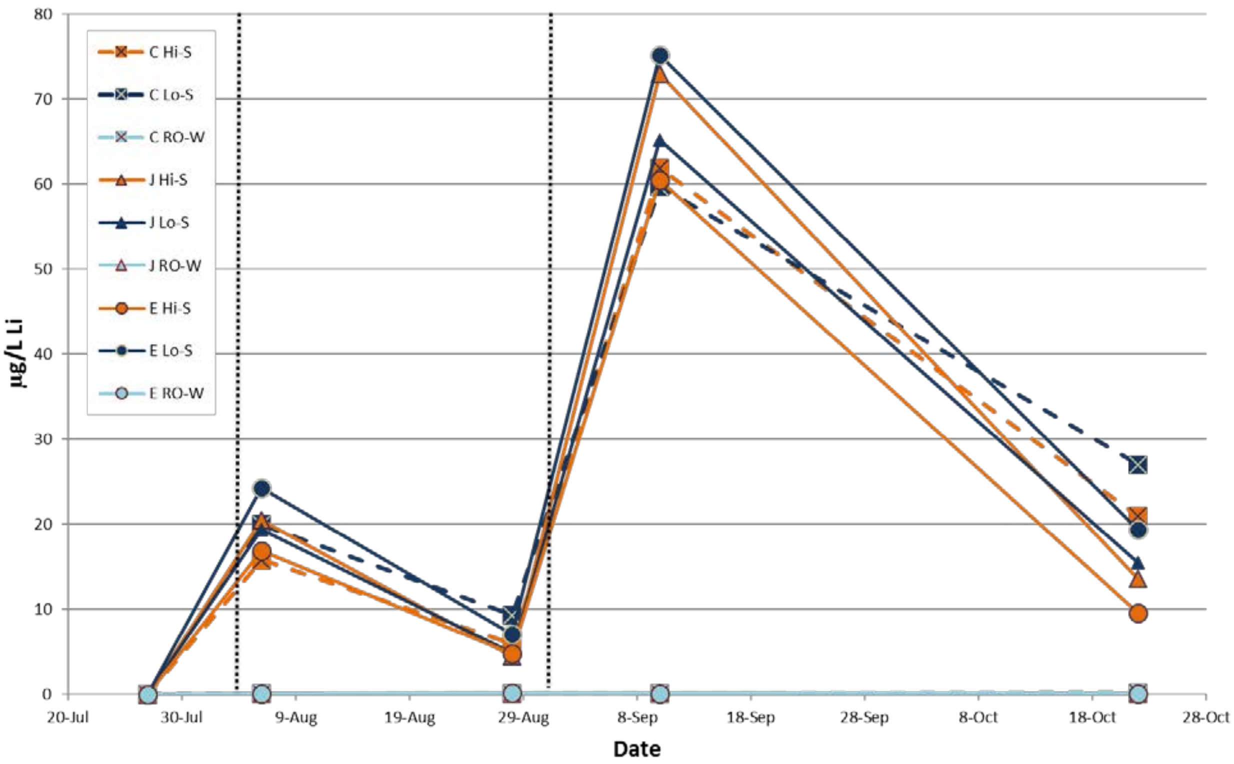


Figure 4. Lithium concentrations over time in wetland microcosms. Vertical dashed lines indicate dosing dates. The first letter in the legend represents species type, the following abbreviation represent treatment type, dashed lines represent microcosms with no plants (C = control, J = *J. effusus*, E = *E. quadrangulata*, Hi-S = high sulfur leachate, Lo-S = low sulfur leachate, RO-W = reverse osmosis water).

2. Boron (B)

Elevated B concentrations were observed in microcosms receiving fly ash leachates (fig. 4). High Sulfur leachates increased the B concentration more than Low Sulfur leachates, with maximums of 792.5 and 215.3 µg/L, respectively, while RO water microcosms had a maximum of 12.12 µg/L (appendix 1). Samples collected soon after dosing had similar B concentrations for each treatment and plant or control, while samples taken later after the dosing showed reduced B concentrations in vegetated microcosms (fig. 4). B reduction in control microcosms did not exceed 10%, while planted microcosm reductions ranged from 33.4% to 58.6% (table 3). Kruskal Wallis tests showed a significant relationship between B concentrations and treatment type for each time point as well as between time point intervals, except the interval between the second and third measurement. Because B concentrations changed very little with the addition of RO water, time interval tests were run only including leachate dosed microcosms, and all time intervals were found to be significantly different (appendix 2).

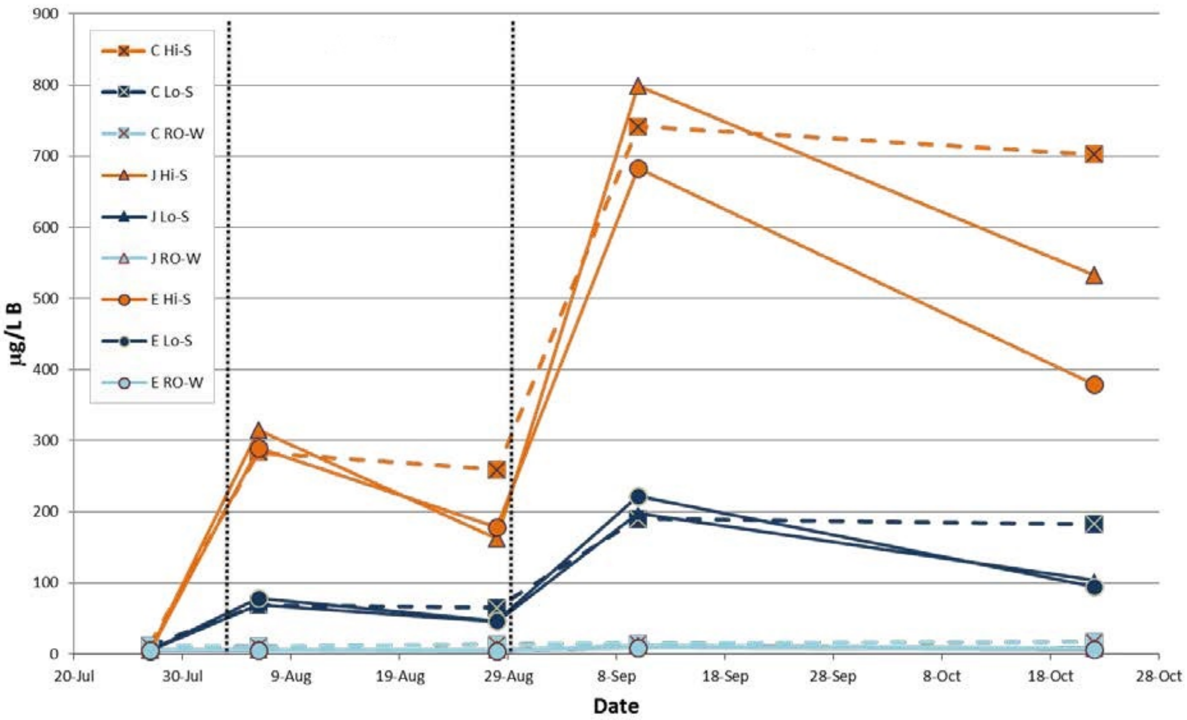


Figure 5. Boron concentrations over time in wetland microcosms. Vertical dashed lines indicate dosing dates. The first letter in the legend represents species type, the following abbreviation represent treatment type, dashed lines represent microcosms with no plants (C = control, J = *J. effusus*, E = *E. quadrangulata*, Hi-S = high sulfur leachate, Lo-S = low sulfur leachate, RO-W = reverse osmosis water).

3. Molybdenum (Mo)

Elevated Mo concentrations were observed in microcosms receiving high sulfur leachates while low sulfur leachates caused minimal increases in Mo (fig. 6). High sulfur leachates raised Mo concentrations as high as 220.7 µg/L, while low sulfur leachates only raised Mo to 13.1 µg/L (appendix 1). For high sulfur leachate dosings, planted microcosms reduced Mo 80.7-93.5% while controls reduced Mo by around 70% (table 3). Kruskal Wallis tests showed a significant relationship between Mo concentrations and treatment type for all but the first time point as well as for each time point interval, except the interval between the second and third measurement. Because Mo concentrations changed very little with the addition of RO water, time interval tests were run only including leachate dosed microcosms, and all time intervals were found to be significantly different (appendix 2).

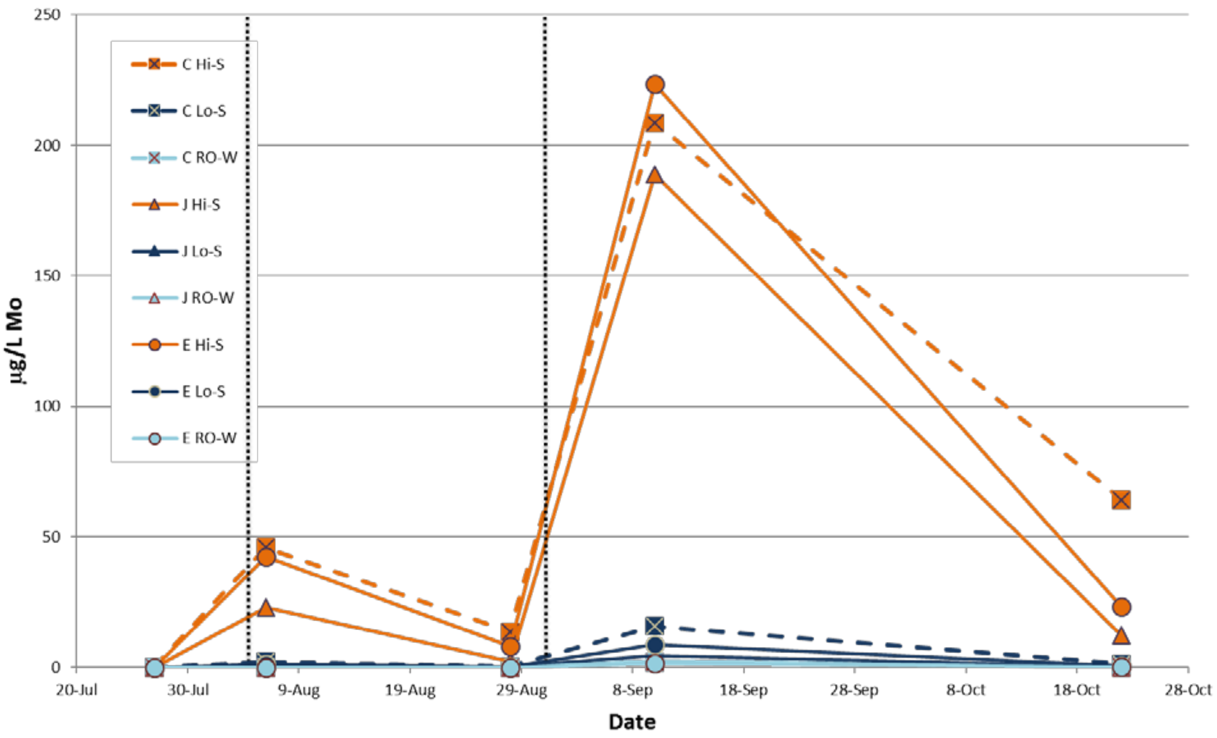


Figure 6. Molybdenum concentrations over time in wetland microcosms. Vertical dashed lines indicate dosing dates. The first letter in the legend represents species type, the following abbreviation represent treatment type, dashed lines represent microcosms with no plants (C = control, J = *J. effusus*, E = *E. quadrangulata*, Hi-S = high sulfur leachate, Lo-S = low sulfur leachate, RO-W = reverse osmosis water).

IV. Discussion

A. Water Chemistry Effects

The addition of both types of leachate was correlated to significant trends in microcosm water. Low sulfur leachates were related to increased Ec and ORP and reduced pH, as well as increased B and Li concentrations. High sulfur leachates were related to increased Ec and pH, as well as B, Mo, and Li concentrations relative to controls. Relative to low sulfur leachates, high sulfur leachates had greater increases in Ec and B concentration, and also showed elevated Mo concentrations. Low sulfur leachates did not elevate Mo concentrations, but had similar Li concentrations as high sulfur leachates and made water more acidic.

B. Plant Growth

All of the *J. effusus* root plugs survived the duration of the experiment, and as five distinct individuals form the original root plugs. A number of the *E. quadrangulata* root plugs died off early in the experiment, but after a few months the survivors began to reproduce through rhizomes throughout the rest of the experiment with most microcosms stabilizing at around 20 individuals.

E. quadrangulata had a higher plant height growth rate than *J.* effusus, possibly due to new rhizome offspring growing more vigorously. *J.* effusus had a higher average biomass. This increased biomass could be due to denser root mass or harder stem tissue.

None of the growth or biomass parameters were significantly related to leachate dosing except for an increase in *E. quadrangulata* height growth correlated with low sulfur leachate. The growth of *E. quadrangulata* may favor slightly more acidic conditions, have been stimulated by nutrients contained in the leachates, or be a stress response. Neither plant species displayed toxicity symptoms. This finding is not surprising as emergent macrophytes have been documented to have high tolerances to inorganic contaminants (Bhatia & Goyal 2013).

C. Plant Effects on Water Quality

Both macrophyte species appeared to reduce perturbations from fly ash leachates and facilitate the transition back towards pre dosing levels. The effects were especially pronounced for Ec, as well as B and Mo concentration. After dosings, B concentrations were never reduced more than 10% in unvegetated microcosms, while vegetated microcosms reduced the B concentrations by 33-59%. For both types of fly ash, vegetated microcosms reduced Ec by around 50% from peak levels at the final measurements while non vegetated microcosms had fairly constant Ec levels during all measurements after dosing. In all microcosms, pH moved back towards neutral, but vegetated microcosms increased this effect.

These findings suggest that the presence of emergent macrophytes helps ameliorate the impacts of coal ash contamination. B and Mo are both plant nutrients so the macrophytes may be pulling these elements out of the water column via root absorption and translocating them into plant tissue or storage vacuoles. The increased Ec reduction with plants present confirms previous studies that macrophyte treatment wetlands reduce Ec (Maine et. al., 2009). Potential mechanisms behind these reductions in charged soluble elements include rhizofiltration, phytostabilization, and phytoaccumulation (Rai 2009; Bobadilla et. al., 2013).

D. Toxicity Potential

A. Boron

Boron can be a problematic aquatic contaminant with World Health Organization (WHO) guidelines recommending drinking water standards as low as 0.5 mg/l (Hilal et. al., 2011). Predicted no effect concentrations (PNEC) of boron to aquatic organisms can range from 0.18 mg/l to 1.3 mg/l (Dyer, 2000; Schoderboeck et. al., 2011). Boron is very water soluble and is one of the more abundantly leachable elements associated with coal ash (Ruhl et. al., 2014) so its potential to contaminate aquatic environments as a result of coal ash discharge should be considered.

Efforts have been put into using CWTS to treat boron contamination in wastewater, but the efficacy of these systems at B removal is often 30% or less (Ye et. al., 2001; Turker et. al., 2014). The reduction of B in vegetated microcosms in this study ranged from 33%-59%, though the microcosms were not flow through reactors and the boron doses were relatively low compared the levels in CWTS studies.

B. Molybdenum

Molybdenum is an essential element in animals and plant that has reported toxicity problems with grazing ruminants (Neunhauserer et. al., 2000) as well as potential reproductive defects in male humans (Meeker et. al., 2008). Molybdenum is readily absorbed when ingested in a water soluble form (Vyskocil & Viau 1999), so increased concentrations from high sulfur fly ash could be problematic for wildlife or humans if it reached drinking water. The highest concentration found in this study was 221 µg/l, lower than most aquatic toxicity thresholds which tend to be greater than 100 mg/l (Davies et. al., 2005).

C. Lithium

Lithium is a light metal that tends to occur at low levels in surface waters (Tanner 1995) that can begin demonstrating aquatic toxicity as low as 0.15 mg/l (Kszos et. al., 2003). The highest Li concentration measured in this study was 0.072 mg/l, below toxicity thresholds. While lithium is used in pharmaceutical medications in humans, the concentrations prescribed to patients can be potentially toxic (Aral & Vecchio-Sadus 2008), so increased Li in drinking water could be especially hazardous to the health of lithium users.

E. Implications

Much of the concern over aquatic coal ash contamination has focused on well know heavy metals like Cr, As and Se. The conservative dosing levels in this study did not raise any of these elements above 1 µg/l, but even when these contaminants were not elevated, elements like B, Li, and Mo were significantly elevated. These elements are much less ubiquitous than the afore mentioned heavy metals, which indicates that CCRs need to be examined as a source of lesser-known inorganic contaminants.

The apparent ability of *J. effuses* and *E. quadrangulata* to reduce trace element concentrations and pH and Ec disturbances indicates that the presence of these plants and other emergent macrophytes could be crucial to managing potential threats of aquatic CCR pollution. Additionally, the apparent resistance of the plants to toxicity from the fly ash leachate indicates these species could be effective in CWTS. These findings also reinforce the results of the TVA CCR CWTS projects, and suggest that wetland treatment systems could be useful in managing CCR waste and power plants.

In the future, elemental analysis of soils and plant tissue from the microcosms will be analyzed to evaluate the storage of trace elements in soil over time and sequestration potential in plant stems and roots.

To further understand the relationships between macrophytes, CWTS and coal waste management, follow up studies could evaluate more environmentally representative microcosms, such as flow through reactors, as well as dose response studies of plant growth and sequestration. Sampling macrophytes growing in natural settings near CCR contamination, such as downriver from the Eden plant on the Dan River, could be used to analyze trace metal sequestration in natural environments and compare it to trace element levels in plants from greenhouse studies.

V. References

Aral, H, Vecchio-Sadus, A. 2008. Toxicity of lithium to humans and the environment-A literature review. Ecotoxicology and Environmental Safety, 70:349-56.

Bhatia, M, Goyal, D. 2014. Analyzing remediation potential of wastewater through wetland plants: a review. Environmental Progress & Sustainable Energy, 33:10.1002.

Bobadilla, M, Aliaga, E, Yupanqui, E, Palomino, E. 2012. A phytoremediation approach using *Calamagrostis ligulata* and *Juncus imbricatus* in the Andean wetlands of Peru. Environmental Monitoring and Assessment, 185:323-34.

Brodie, GA. 1990. Constructed wetlands for treating acid drainage at Tennessee Valley Authority coal facilities. Proceeding of the International Conference on the Use of Constructed Wetlands in Water Pollution Control, 461-470.

Davies, TD, Pickard, J, Hall, KJ. 2005. Acute molybdenum toxicity to rainbow trout and other fish. Journal of Environmental Engineering and Science, 4:481-5.

Dellantonio, A, Fitz, WJ, Repmann, F, Wenzel, WW. 2010. Disposal of coal combustion residues in terrestrial systems: contamination and risk management. Journal of Environmental Quality, 39:761-75.

EIA. 2015. "What is U.S. electricity generation by energy source?" U.S. Energy Information Administration. 31 Mar. 2015. Web. 21 Apr. 2015. www.eia.gov/tools/faqs/faq.cfm?id=427&t=3

Kszos, LA, Beauchamp, JJ, Stewart, AJ. 2003. Toxicity of lithium to three freshwater organisms and the antagonistic effect of sodium. Ecotoxicology, 12:427-437.

Lemly, DA. 2012. Wildlife and the coal waste policy debate: proposed rules for coal waste disposal ignore lessons from 45 years of wildlife poisoning. Environmental Science & Technology, 46:8595-600.

Maine, MA, Sune, N, Hadad, H, Sanchez, G, Bonetto, C. 2009. Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. Journal of Environmental Management, 90:355-63.

Meeker, JD, Rossano, MG, Protas, B, Diamond, MP, Puscheck, E, Daly, D, Paneth, N, Wirth, JJ. 2008. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. Environmental Health Perspectives, 116:1473-9.

Mitsch, William J., and James G. Gosselink. *Wetlands*. 5th ed. Hoboken: Wiley, 2015. Print.

Neunhauserer, C, Berreck, M, Insam, H. 2001. Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation. Water, Air, and Soil Pollution, 128:85-96.

Patra, KC, Rautray, TP, Tripathy, BB, Nayak, P. 2012. Elemental analysis of coal and coal ash by PIXE technique. Applied Radiation and Isotopes, 70:612-6.

Preussler, KH, Mahler, CF, Maranho, LT. 2015. Performance of a system of natural wetlands in leachate of a posttreatment landfill. International Journal of Environmental Science and Technology, 12:2623-38.

Rai, PK. 2009. Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophys. Critical Reviews in Environmental Science and Technology, 39:697-753.

Reddy, Ramesh K. and DeLaune, Ronald D. *Biogeochemistry of Wetlands*. Boca Raton: CRC Press, 2008. Print.

Rowe, CL, Hopkins, WA, Congdon, JD. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. Environmental Monitoring and Assessment, 80:207-76.

Ruhl, L, Vengosh, A, Dwyer, GS, Hsu-Kim, H, Deonarine, A. 2010. Environmental impacts of the coal ash spill in Kingston, Tennessee: an 18-month survey. Environmental Science and Technology, 44:9272-8.

Ruhl, L, Dwyer, GS, Hsu-Kim, H, Hower, JC, Vengosh, A. 2014. Boron and strontium isotopic characterization of coal combustion residuals: validation of new environmental tracers. Environmental Science and Technology, 48:14790-8.

Sundberg-Jones, SE, Hassan, SM. 2007. Macrophyte sorption and bioconcentrations of elements in a pilot constructed wetland for flue gas desulfurization wastewater treatment. Water Air and Soil Pollution, 183:187-200.

Turker, OC, Vymazal, J, Ture, C. 2014. Constructed wetlands for boron removal: a review. Ecological Engineering, 64:350-9.

USEPA. 2014. "EPA Announces First Regulations to Safeguard Disposal of Coal Ash." EPA Newsroom. 19 Dec. 2014. Web. 21 Apr. 2015. <http://yosemite.epa.gov/opa/admpress.nsf/bd4379a92ceceeac8525735900400c27/8d1b001db717edbf85257db3006b2747!OpenDocument>

USEPA. 2014. "Coal Fly Ash, Bottom Ash, and Boil Slag." Radiation Protection. 4 Feb. 2014. Web. 21 Apr. 2015. www.epa.gov/radiation/tenorm/coalandcoalash.html

Vymazal, J. 2011. Constructed wetlands for wastewater treatment: five decades of experience. Environmental Science and Technology, 45:61-9.

Vyskocil, A, Viau, C. 1999. Assessment of molybdenum toxicity in humans. Journal of Applied Toxicology. 19:185-192.

WCA. 2014. “Coal Statistics” World Coal Association. Sep. 2014. Web. 21 Apr. 2015. www.worldcoal.org/resources/coal-statistics

Ye, ZH, Whiting, SN, Qian JH, Lytle, CM, Lin ZQ, Terry, N. 2001. Trace element removal from coal ash leachate by a 10-year old constructed wetland. Journal of Environmental Quality, 30:1710-1719.

Appendix 1. Trace Element Concentrations

Table 4. Trace element concentrations in water samples collected from microcosms. Elements: B = boron, Li = lithium, Mo = molybdenum. Species: C = control with no plants, J = *J. effusus*, E = *E. quadrangulata*. Concentrations from left to right: Initial pre-dose sample, peak value after final dosing, final value before plant harvest, % reduction of concentration after first dose, % reduction of concentration after second dose.

Treatment	Element	Species	Background (ug/L)	Peak (ug/L)	Final (ug/L)	1st Dose Red. (%)	2nd Dose Red. (%)
RO Water	B	C	9.74	8.09	12.12	-0.277	-0.498
		J	4.24	6.06	3.84	0.055	0.367
		E	3.30	2.69	1.62	0.353	0.398
	Li	C	0.03	0.06	0.08	-0.638	-0.505
		J	0.00	0.10	0.02	0.392	0.758
		E	0.00	0.03	0.01	-4.330	0.768
	Mo	C	0.01	-0.67	0.20	12.616	1.304
		J	0.04	0.06	-0.02	0.080	1.264
		E	0.00	-1.10	0.00	-5.338	1.004
Low Sulfur Leachate	B	C	9.95	183.60	177.54	0.070	0.033
		J	3.97	191.97	98.56	0.366	0.487
		E	3.33	215.33	89.13	0.419	0.586
	Li	C	0.02	59.63	26.89	0.536	0.549
		J	0.01	65.13	15.41	0.749	0.763
		E	0.00	75.11	19.29	0.710	0.743
	Mo	C	0.01	13.10	1.19	0.840	0.909
		J	0.03	1.92	0.16	-0.026	0.918
		E	0.01	6.09	0.36	0.857	0.940
High Sulfur Leachate	B	C	10.53	735.14	697.34	0.089	0.051
		J	3.36	792.48	527.68	0.489	0.334
		E	2.75	676.39	374.67	0.388	0.446
	Li	C	0.01	61.79	20.83	0.626	0.663
		J	0.02	72.86	13.48	0.785	0.815
		E	0.01	60.44	9.46	0.720	0.844
	Mo	C	0.01	205.93	63.98	0.703	0.689
		J	0.05	186.16	12.14	0.901	0.935
		E	0.01	220.71	23.20	0.807	0.895

Appendix 2. Statistical Test Results for Trace Element Concentrations

Table 5. Non parametric Kruskall-Wallis test results for trace element concentrations compared to time and treatment. (A) Statistical differences between treatment types at a given time point. (B) Statistical difference between successive time intervals. (C) Same as (B) except RO water controls removed because trace element concentrations were not expected to change much from RO water dosings.

A) Time Point Treatment Effect

Element	Date	Chi Sq	df	p
B	28-Jul	0.956	2	<.001
	7-Aug	23.1	2	<.001
	29-Aug	23.1	2	<.001
	11-Sep	23.1	2	<.001
	23-Oct	23.1	2	<.001
Li	28-Jul	1.37	2	0.505
	7-Aug	20	2	<.001
	29-Aug	20.4	2	0.276
	11-Sep	17.4	2	<.001
	23-Oct	19.7	2	<.001
Mo	28-Jul	1.11	2	0.573
	7-Aug	23.1	2	<.001
	29-Aug	23.1	2	<.001
	11-Sep	23.1	2	<.001
	23-Oct	21.1	2	<.001

B) Time Interval Effects

Element	Dates	Chi Sq	df	p
B	7/29 - 8/7	22.6	1	<.001
	8/7 - 8/29	2.037	1	0.154
	8/29 - 9/11	7.42	1	<.01
	9/11 - 10/23	1.84	1	<.05
Li	7/29 - 8/7	39.8	1	<.001
	8/7 - 8/29	12.3	1	<.001
	8/29 - 9/11	12.3	1	<.001
	9/11 - 10/23	6.25	1	<.05
Mo	7/29 - 8/7	28.1	1	<.001
	8/7 - 8/29	0.704	1	0.401
	8/29 - 9/11	21.3	1	<.001
	9/11 - 10/23	12.6	1	<.001

C) Time Interval Effects w/o Control

Element	Dates	Chi Sq	df	p
B	7/29 - 8/7	26.3	1	<.001
	8/7 - 8/29	6.57	1	<.05
	8/29 - 9/11	18.2	1	<.001
	9/11 - 10/23	4.77	1	<.05
Li	7/29 - 8/7	26.3	1	<.001
	8/7 - 8/29	26.3	1	<.001
	8/29 - 9/11	17.2	1	<.001
	9/11 - 10/23	26.3	1	<.001
Mo	7/29 - 8/7	26.3	1	<.001
	8/7 - 8/29	4.36	1	<.05
	8/29 - 9/11	17.2	1	<.001
	9/11 - 10/23	9.03	1	<.01