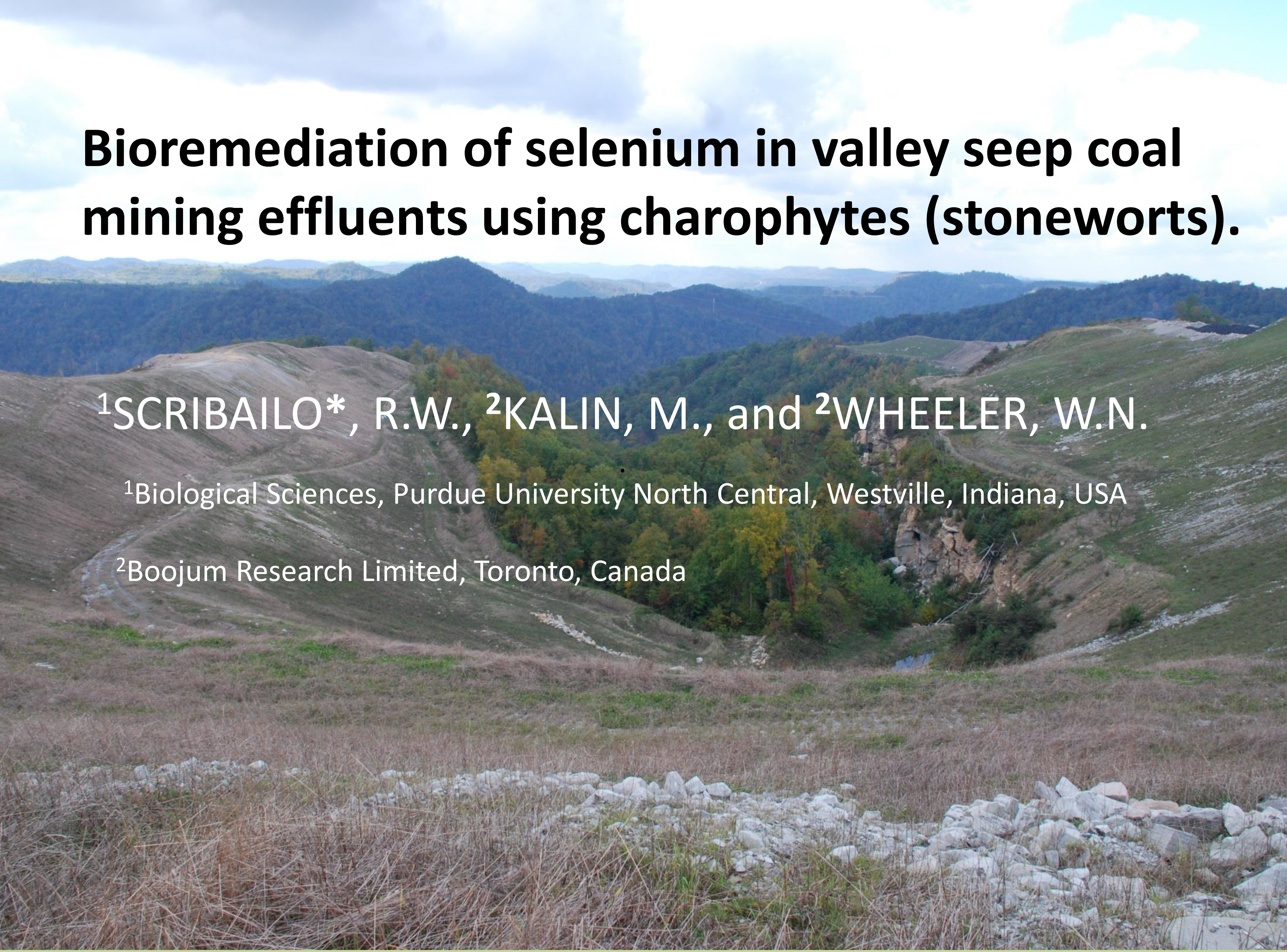
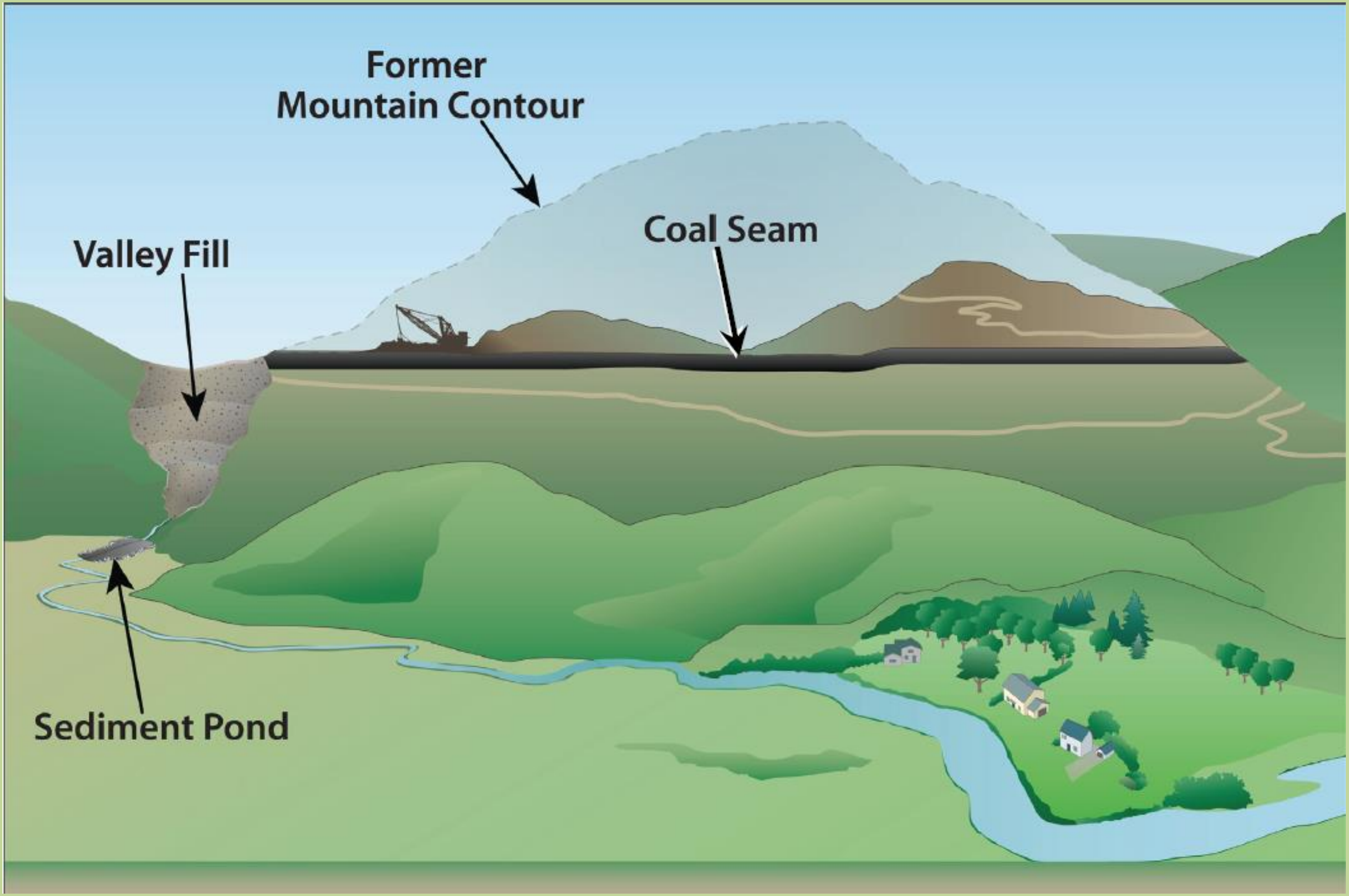
Bioremediation of selenium in valley seep coal mining effluents using charophytes (stoneworts).



[1]SCRIBAILO*, R.W., [2]KALIN, M., and [2]WHEELER, W.N.

[1]Biological Sciences, Purdue University North Central, Westville, Indiana, USA

[2]Boojum Research Limited, Toronto, Canada



A watershed view of a mountaintop mine and valley fill.

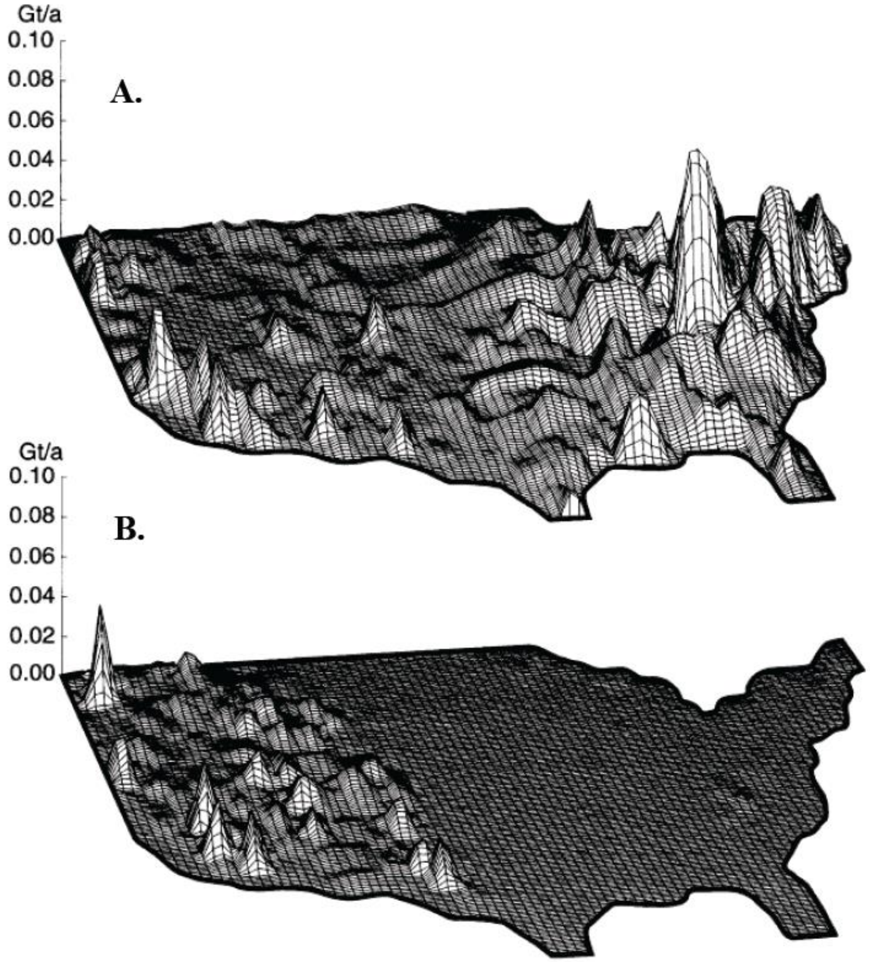


Figure 8. Earth movement by humans and streams. Maps of the United States showing, by variations in peak height, the rates at which earth is moved in gigatonnes per annum in a grid cell measuring 1° (latitude and longitude) on a side, by (A) humans and (B) rivers.

Source: Hooke (1999), used with permission from the publisher.

Impact of Mountain-top Mining

- The United States Environmental Protection Agency (EPA) estimates that by 2012 coal mining activities mines will have impacted 6.8% of the largely forested 4.86-million-hectare portion of the Appalachian Coalfield Region (ACR) within West Virginia (WV), Kentucky, Virginia, and Tennessee.

- Based on complete utilization of fill permits issued between 1992 and 2002, the EPA estimated that 1,944 km of headwater streams were buried during this period and predicted that the extent of buried stream length will double to almost 4,000 km by 2012.

- It is estimated that 22% of streams in West Virginia were biologically impaired as of 2012.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Selenium Toxicity and Chemistry

- Selenium, although an essential dietary element for most organisms in very low doses, causes developmental deformities in many invertebrates, and animals such as fish and salamanders.

- Selenium exists in different oxidation states including oxyanions [selenate (SeO_4^{2-}) and selenite (SeO_3^{2-})], reduced selenium [selenide (Se^{2-})] and elemental selenium (Se).

- Selenate and selenite oxyanions, common in oxidizing environments such as the potentially exposed mine materials in valley fill areas, are the most mobile and toxic of the selenium species.

ARIES Consortium

- ARIES (Appalachian Research Initiative in Environmental Science) was formed involving seven universities.
- One of the phases of this grant involved investigations of possible bioremediation methods to reduce selenium concentrations to acceptable levels.
- In discussions, the possibility of using charophytes for this process was attractive for several reasons. The "Chara process" has been used successfully by Boojum research for bioremediation clean up processes at mining locations around the world.

What are Charophytes?

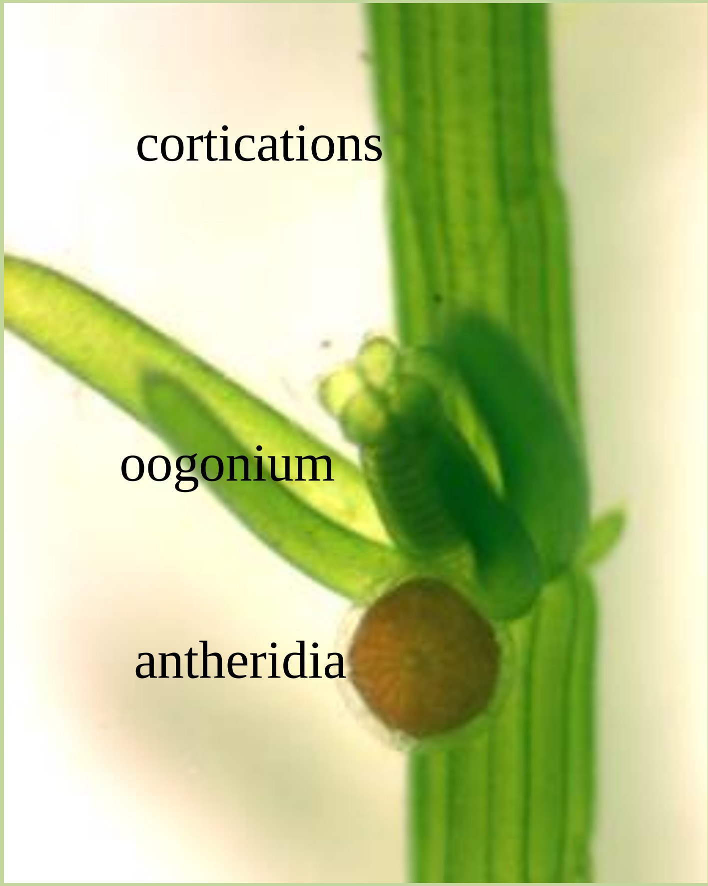
- Charophytes are macrophytic algae.
- They are the most advanced algae group and are thought to have given rise to the land plants.
- They are the most widespread plant group in the world.
- They occur in in almost every kind of submerged aquatic environment including marine and freshwaters habitats.
- They are strongly associated with calcareous alkaline waters and are rare in acidic waters.

Charophyte Vegetative

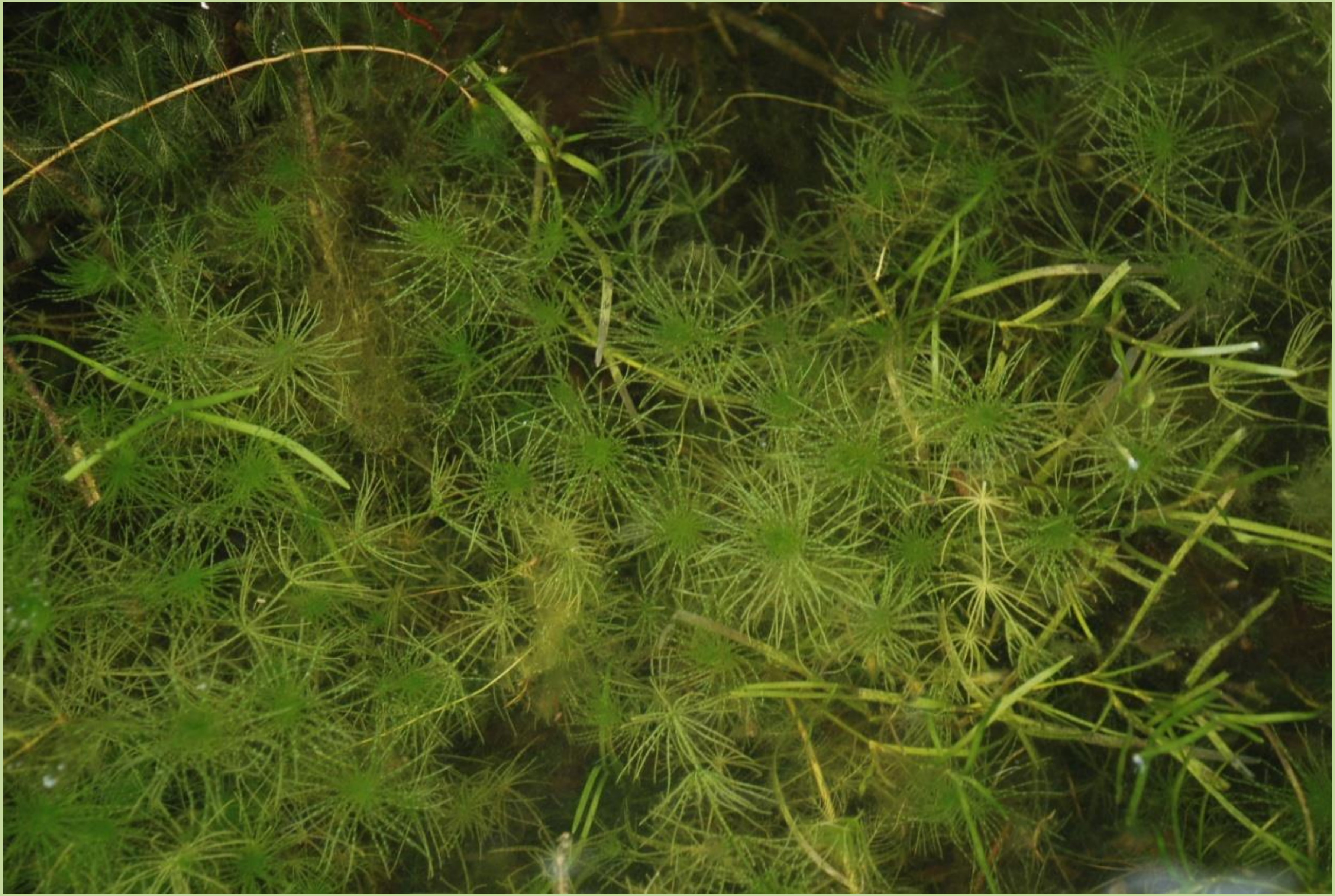
Charophyte Morphology

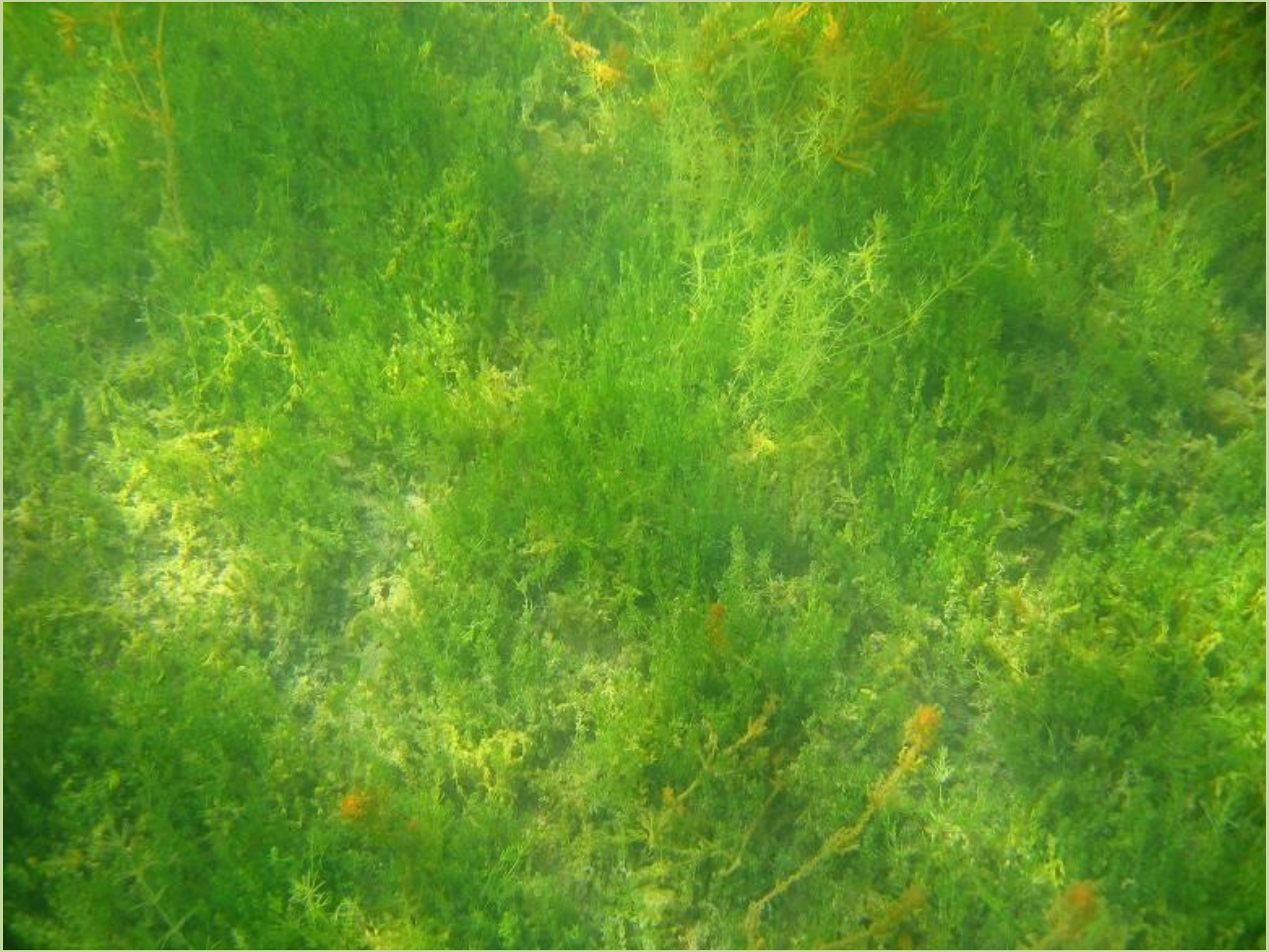
Charophytes reproduce by male and female spores.

Most charophytes are monoecious (have both sexes on the same plant).



Gametangia





Charophyte Importance in Wetlands and Lakes

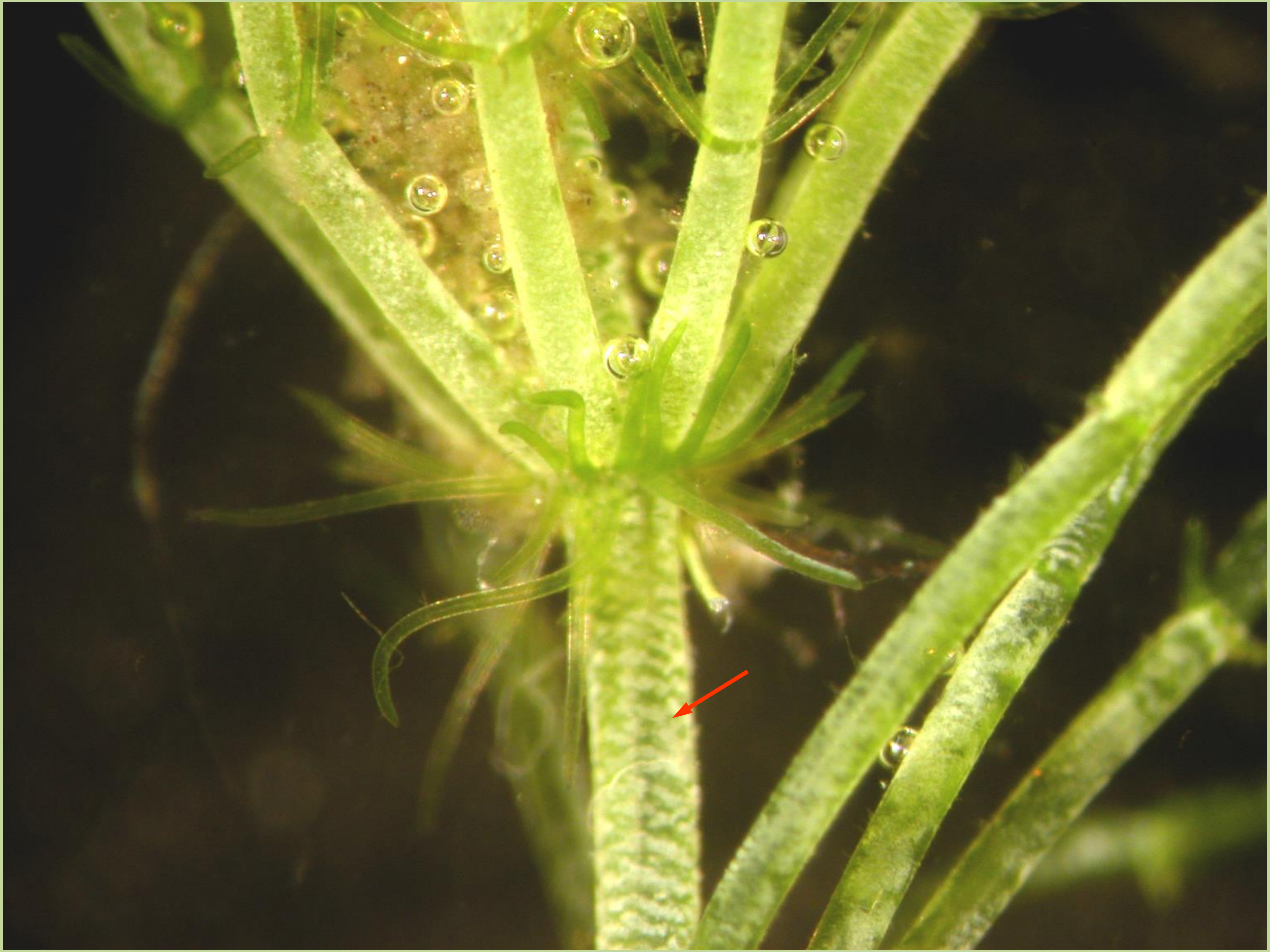
- Charophytes consolidate bottom sediments
- They reduce wave action affects
- They provide habitat and food for invertebrates
- They provide spawning habitat and protective cover for fish
- They improve water clarity by removing minerals from the water during photosynthesis.

Charophyte photosynthesis

- Characean cells are able to generate spatially separated bands of high and low pH in the medium adjacent to their cell surface(Spear et al. 1969, Lucas and Smith 1973).
- When exposed to light, the cell surfaces show sharply peaked alkaline regions with pH values between 8.5 and 9.5, and acid regions with a pH of about 5.5.
- Cell walls calcify in the alkaline regions of their surface as plants take up bicarbonate for photosynthesis. During the process $CaCO_3$ encrustations may either be formed by precipitation due to alkalization of the medium (Spear et al. 1969) or involve active Ca^{2+} and H^+ ATPases (McConnaughey and Falk 1991; Schmolzer, Hoftberger and Foissner , 2011).

Charophyte mineral precipitation

- In addition to calcium (Ca), McConnaughey (1991) found strontium (Sr) and manganese (Mn) can also precipitate in alkaline regions.
- Boojum Research found *Chara* to hyperaccumulate Radium in ponds at Rabbit Lake in Saskatchewan.
- Siong and Asaeda (2009) showed that 17% of the total cadmium (Cd) accumulated by *Chara fibrosa* was carbonate-bound.



Charophytes as biomineralizers

The growth characteristics of charophytes aid in the biomineralization process. They take nutrients from the water up through their large cell walls and on the outside of the cell walls over the entire biomass.

They have no roots, but are simply anchored in the sediments. Their growth form is apical growth and basal decay, which means the plants grow from the top and degrade from the bottom, while sinking into the sediment.

In addition, the biomass collects debris and TSS on its surface, which contributes to water clarity.

Selenium and charophytes

- Water-soluble selenium could potentially be removed from the water by alkaline band precipitation during charophyte photosynthesis.
- Selenium has a high affinity for organic compounds especially when in the oxidized state.
- The development of bioremediation systems which can reduce selenium outputs from retention ponds to below compliance levels is a highly desired outcome for the coal mining industry.

Research Goals

To carry out a preliminary scoping study survey of valley fill retention ponds and determine:

- Inflow and outflow rates and selenium concentrations.
- Existing biotic components of these ponds to determine if significant selenium is being compartmentalized in a particular fraction.
- Determine if bioengineering might be a feasible option for reducing selenium outflow concentrations.

Research Methods

Valley seep ponds from Alpha Natural Resources (Black Castle 1, 2 and 3) were sampled on October 2nd and Cliff's Natural Resources (Cliffs-Dingus) Pond was sampled on October 3rd, 2012.

Continuous data logger flow meters were installed to determine inflow and outflow rates. Water samples were assessed for selenium and other elements.

pH and specific conductivity were measured for pond depth profiles.

Selenium was assessed at the University of Kentucky using ICP analysis of washed and dried material of known weight.

General Findings

- Most mines were far out of compliance on selenium outflow levels from valley seep ponds. Some ponds were actually exporting selenium at levels higher than inflows.
- pH values of ponds were in the 6.5 to 7.5 range.
- Specific conductivity was very high ranging from 2100 to 3500 µS cm^{-1} and increasing with depth.
- Aquatic plant species diversity was limited and varied considerably amongst the ponds visited.
- Several sites had existing charophyte populations.

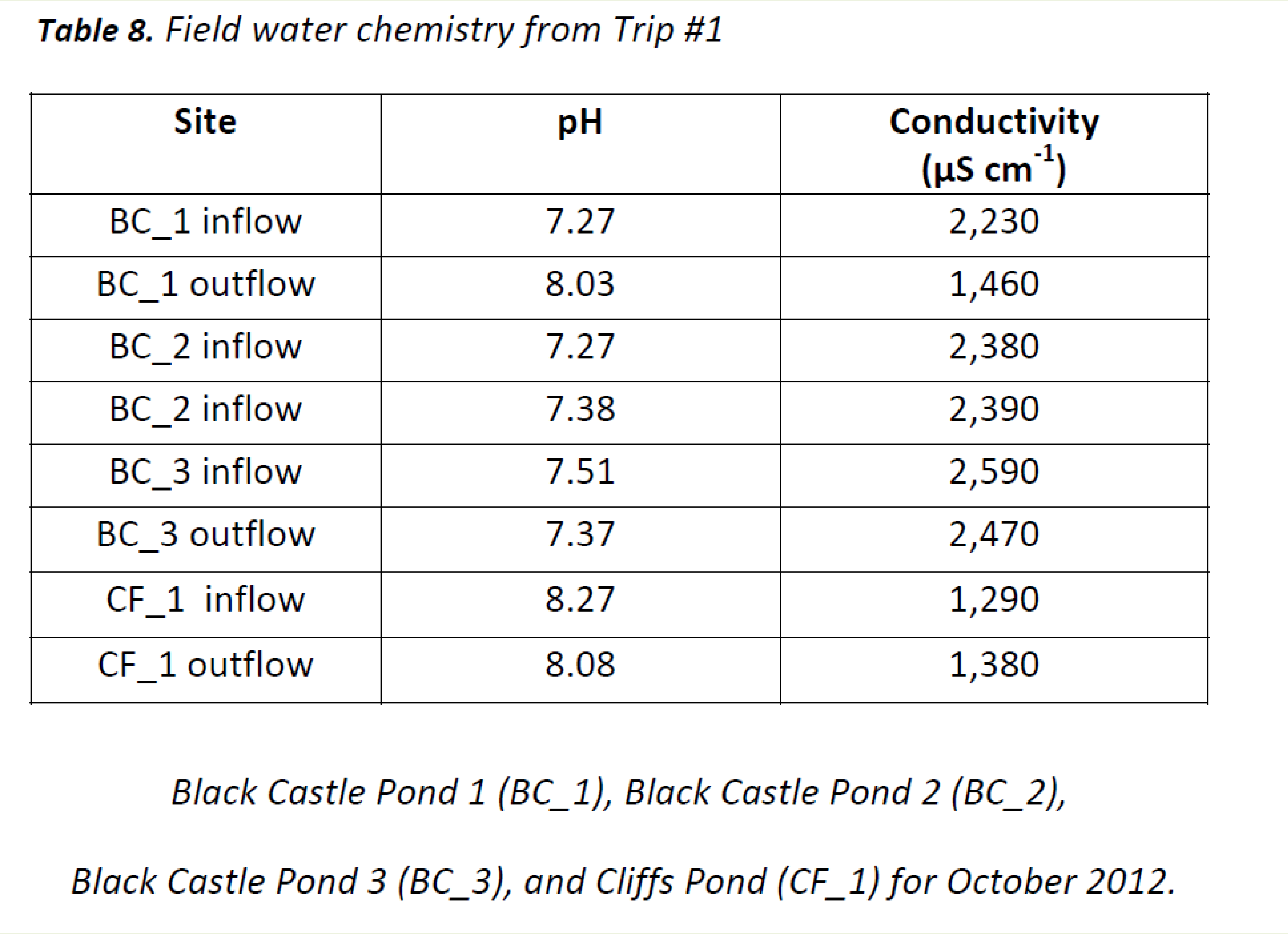


Table 8. *Field water chemistry from Trip #1*

Site	**pH**	**Conductivity (μS cm^{-1})**
BC_1 inflow	7.27	2,230
BC_1 outflow	8.03	1,460
BC_2 inflow	7.27	2,380
BC_2 inflow	7.38	2,390
BC_3 inflow	7.51	2,590
BC_3 outflow	7.37	2,470
CF_1 inflow	8.27	1,290
CF_1 outflow	8.08	1,380

Black Castle Pond 1 (BC_1), Black Castle Pond 2 (BC_2),

Black Castle Pond 3 (BC_3), and Cliffs Pond (CF_1) for October 2012.

***Table 1**. Dimensions of the Black Castle Pond 1 pond*

Watershed area	60.7 ha
Storage capacity (volume)	24,400 m3
Area	1858 m2
Max Depth	4.5 m

Vegetation was very sparse covering only about 20% of the bottom as the pond was dredged in 2012.

The main emergent plant was the narrow-leaf cattail with a small number of plants of *Scirpus* sp. and the only two submerged plants were leafy pondweed and water grass.

Although there was no immediately apparent bottom film there was considerable floating decomposing detritus.

The water was very opaque with lots of suspended fine silt and the bottom could not be seen below about two feet.

ALPHA NATURAL RESOURCES - Black Castle 1 pond

Typha latifolia

Luziola fluitans (water grass)

Black Castle Pond 2 and 3

Table 2. *Dimensions of the Black Castle Pond 2*

Watershed area	127.5 ha
Storage capacity (volume)	23,313 m^3
Area	1742 m^2
Max Depth	7.1 m

Table 3. Dimensions of the Black Castle Pond 3.

Watershed area	127.5 ha
Storage capacity (volume)	24,400 m^3
Area	5806 m^2
Depth	7.8 m

Black Castle Pond 2 and 3

BC 2 is deep (7.1 m) with only shallow edges which support dense populations of narrow-leaf cattail with extensive epiphytic growth on the old stocks and the silt curtain. The main submerged species were the small leaved pondweed and an unidentified pondweed. Only few small patches of common stonewort or *Chara*, were present.

Black Castle Pond 3 is a deep pond with vegetation near the perimeter but more gently sloping near the inflow. The pond contains abundant Sago pondweed at both ends as well as locally dense patches of *Chara*. Submerged vegetation was 70% the former and 30% *Chara* cover sparsely on the bottom at 7.8m. Both species were coated with periphyton and trapped considerable debris.

Black Castle 2

Stuckenia pectinata –sago pondweed

Cliffs-Dingus Pond

Table 4. *Dimensions of the Cliffs Pond.*

Watershed area	59.9 ha
Storage capacity (volume)	7401 m_3
Area	669 m_2
Depth	3.9 m

The Cliffs-Dingus Pond was by far the most highly vegetated; with a maximum depth of about 3.9 m. Eighty percent of the pond was vegetated. This vegetation was about 40% sago pondweed, 40% small pondweed and 20% water grass but no Chara was found (Table 5). The most dominant alga was *Oscillatoria* but this was the only pond to also have small amounts of the green algae *Mougeotia* and *Spirogyra*. The scarcity of green algae may also simply be a seasonal effect since this was mid-October.

Cliff Natural Resources Mine

Potamogeton natans

Aelosoma hemprichi

Se removal per m^2

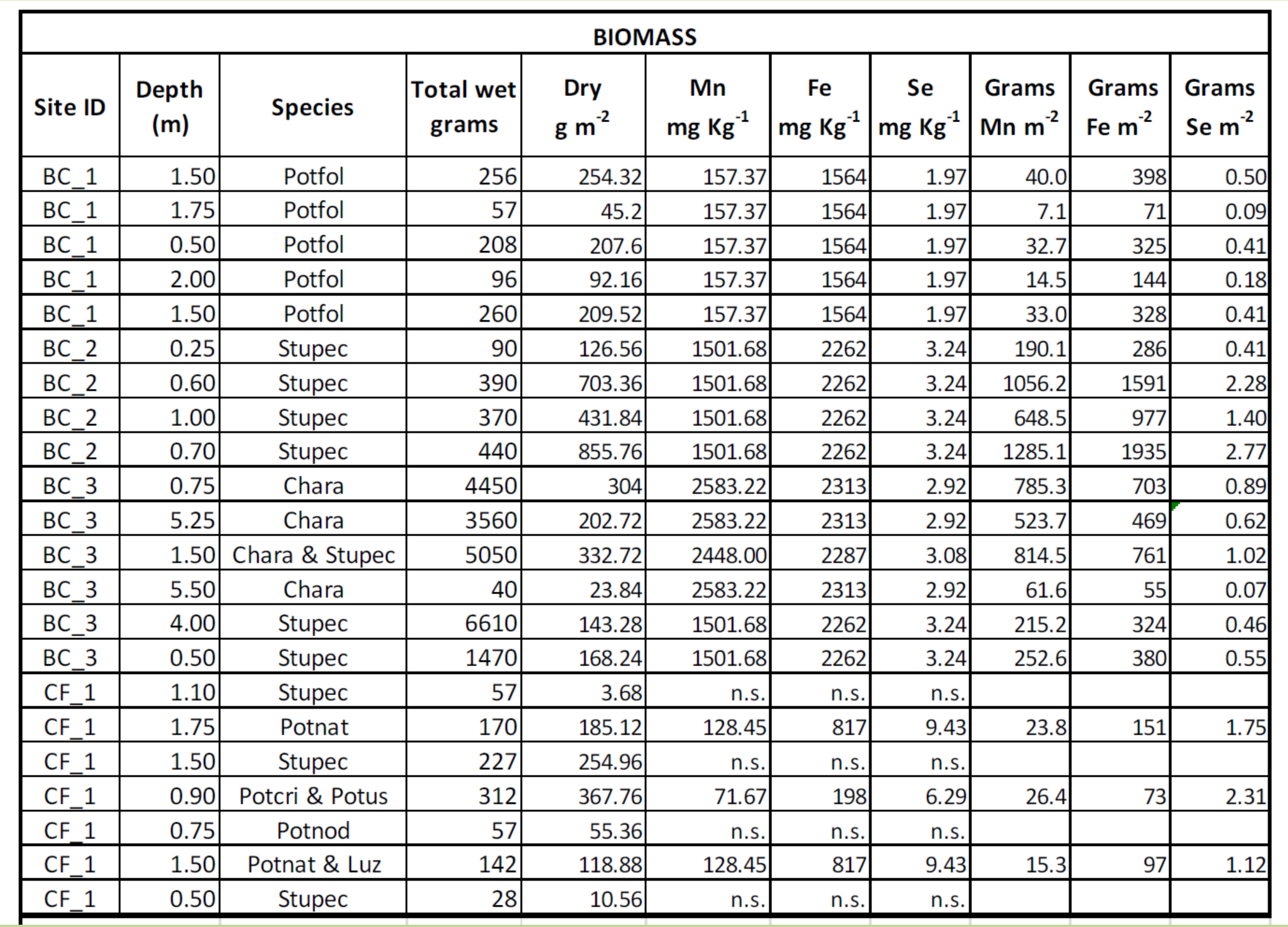
In June 2013 in the second field trip to the sites, we determined the coverage of each of the major vegetation types and took quadrat samples.

From these quadrats the density of the standing biomass of each of the vegetation components was determined at each location and at several depths.

Samples were dried, giving us dry biomass per square meter. A second subsample was dried and shipped to the analytical laboratory of Dr. Jason Unrine at the University of Kentucky to determine the elemental composition per gram.

Selenium removal per square meter was calculated.

Se removal per m^2



BIOMASS										
Site ID	Depth (m)	Species	Total wet grams	Dry g m^{-2}	Mn mg Kg^{-1}	Fe mg Kg^{-1}	Se mg Kg^{-1}	Grams Mn m^{-2}	Grams Fe m^{-2}	Grams Se m^{-2}
BC_1	1.50	Potfol	256	254.32	157.37	1564	1.97	40.0	398	0.50
BC_1	1.75	Potfol	57	45.2	157.37	1564	1.97	7.1	71	0.09
BC_1	0.50	Potfol	208	207.6	157.37	1564	1.97	32.7	325	0.41
BC_1	2.00	Potfol	96	92.16	157.37	1564	1.97	14.5	144	0.18
BC_1	1.50	Potfol	260	209.52	157.37	1564	1.97	33.0	328	0.41
BC_2	0.25	Stupec	90	126.56	1501.68	2262	3.24	190.1	286	0.41
BC_2	0.60	Stupec	390	703.36	1501.68	2262	3.24	1056.2	1591	2.28
BC_2	1.00	Stupec	370	431.84	1501.68	2262	3.24	648.5	977	1.40
BC_2	0.70	Stupec	440	855.76	1501.68	2262	3.24	1285.1	1935	2.77
BC_3	0.75	Chara	4450	304	2583.22	2313	2.92	785.3	703	0.89
BC_3	5.25	Chara	3560	202.72	2583.22	2313	2.92	523.7	469	0.62
BC_3	1.50	Chara & Stupec	5050	332.72	2448.00	2287	3.08	814.5	761	1.02
BC_3	5.50	Chara	40	23.84	2583.22	2313	2.92	61.6	55	0.07
BC_3	4.00	Stupec	6610	143.28	1501.68	2262	3.24	215.2	324	0.46
BC_3	0.50	Stupec	1470	168.24	1501.68	2262	3.24	252.6	380	0.55
CF_1	1.10	Stupec	57	3.68	n.s.	n.s.	n.s.			
CF_1	1.75	Potnat	170	185.12	128.45	817	9.43	23.8	151	1.75
CF_1	1.50	Stupec	227	254.96	n.s.	n.s.	n.s.			
CF_1	0.90	Potcri & Potus	312	367.76	71.67	198	6.29	26.4	73	2.31
CF_1	0.75	Potnod	57	55.36	n.s.	n.s.	n.s.			
CF_1	1.50	Potnat & Luz	142	118.88	128.45	817	9.43	15.3	97	1.12
CF_1	0.50	Stupec	28	10.56	n.s.	n.s.	n.s.			

Selenium versus flow Cliffs pond

The monitoring data for the **Cliffs Pond** is the best dataset available courtesy of Dr. Richard Warner, University of Kentucky.

Data ranged from January 2010 to January 2013. Flow (A) varied from 5 GPM (October 2010) to a high of 584 GPM (January 2013 data not shown as it was an outlier).

Over the same period, the selenium concentrations Figure 11 B at that outlet varied between 0.5 (April 2012) and 42 µg L-1 (October of 2010).

From the date graph (A), flows were definitely higher in 2012 and 2013 than in 2010. Selenium, however, seems to be decreasing with time (B).

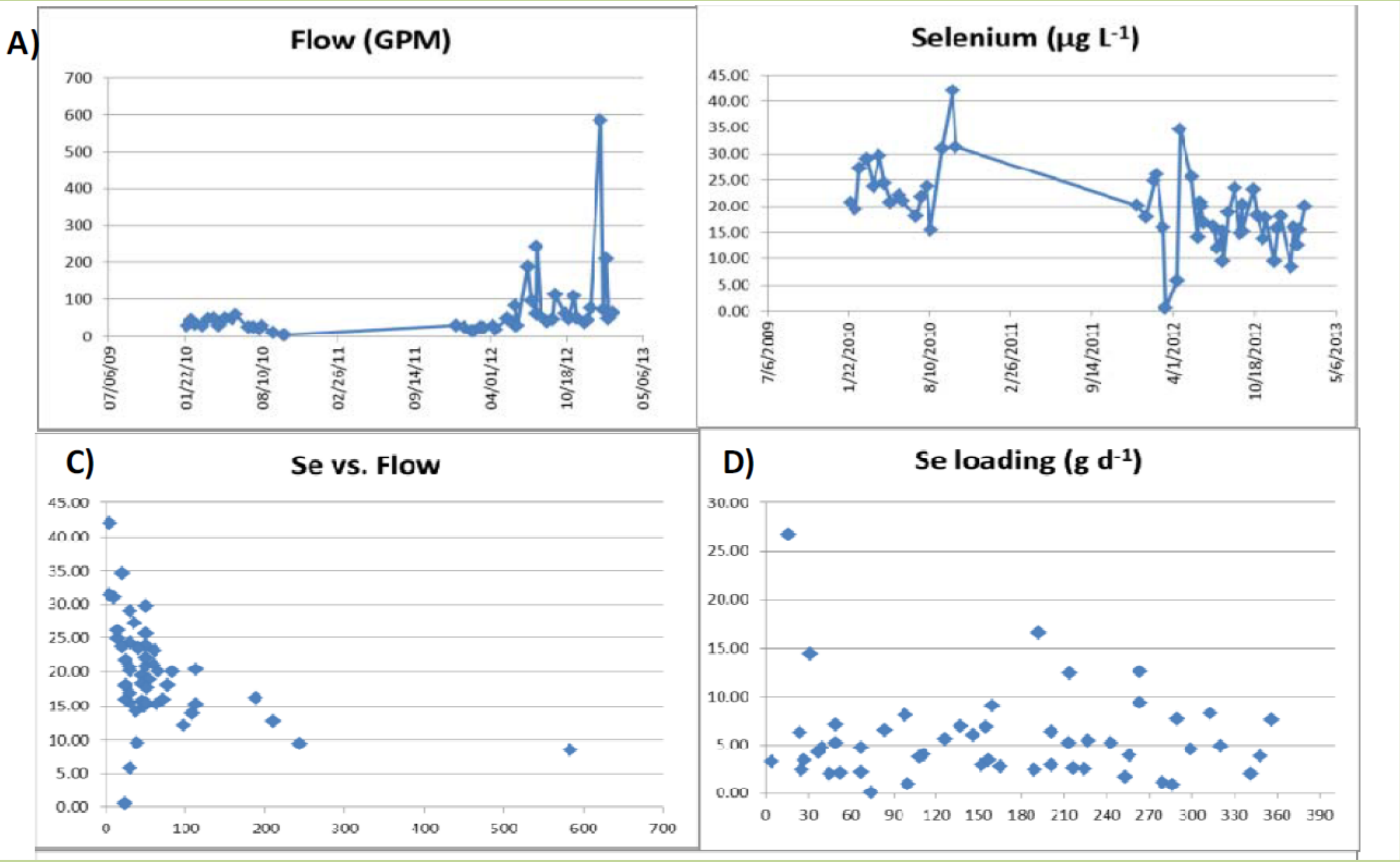
In graph C, we plotted flow vs. selenium concentration. The data are clumped heavily at the low flow end. Higher selenium concentrations are

associated with lower flows. **This suggests that precipitation events bring freshwater into the watershed, diluting the existing effluent.**

The selenium load is shown in Figure 11D. Loads varied between 27 g d-1 to

a low of 0.07 g d-1 resulting in annual loadings for 2010 (1090 g Se year-1), 2011 (1680 g Se year-1), 2012.

Selenium versus flow Cliffs pond



Monitoring data from Cliffs Resources. A) Flow measurements by date taken at sampling station (WV1016750). B) Selenium concentrations at the monitoring station by date. C) Selenium concentration graphed against flow rate. D) Selenium loading (g d-1) plotted by day of year.

Feasibility of using *Chara* for selenium removal

Black Castle Pond 3 had the most extensive *Chara* growth.

The average biomass was 177 dry grams of *Chara* per square meter. Since Black Castle Pond 3 is 0.58 ha in surface area, and *Chara* covers 50% of the area, *Chara* covers 0.29 ha or app. 2900 m^2.

Multiplying the 177 dry grams m-2 x 2900 m2, gives app. 514 kg of dry *Chara* mass in the pond. Since the *Chara* population sampled from Black Castle Pond 3 contained between 2.9 and 8.0 mg of Se per kg, we can multiply the total biomass in the pond by the selenium concentration.

Thus the standing biomass of 514 kg contained approximately 1.5 kg of selenium on the low side and about 4.1 kg of selenium on the high side.

Black Castle pond 3 selenium removal

Selenium concentrations are remarkably lower than the inflow reporting a reduction of 7 µg -L1. During the peak of the growing season in 2014 Se levels dropped below detectable limits for BC3. Similarly, sulfur, sulfate, vanadium and magnesium are also leaving the pond at lower concentrations than were reported for the inflow sample.

Do sulfur and selenium share similar dynamics?

According to Ralston *et al*. (2008) and many other bio-geochemists, selenium and sulfur behave in a chemically similar manner.

The reduction of sulfur (not sulfate) could well be related to the selenium concentration reductions.

Although it is at present only a speculation, both Black Castle Pond 2 and Black Castle Pond 3 show reductions in sulfur by about 1000 mg L-1 between inflow and outflow, and both have *Chara* populations.

Valley seep pond selenium removal

1.Requires a pond in which biota with a suitable growth form exists such as charophytes. Its growth needs to be promoted through seeding or transplanting such that it self perpetuates as the dominant vegetation.

2. Requires charophytes to remove (adsorb, absorb, and or volatilize) the selenium which occurs in the effluent. The preliminary study indicated this to be the case.

3. Requires a sufficiently long residence time that selenium has a chance to contact biota as it passes through the pond.

Valley seep pond selenium removal

4. Charophyte populations provide a selenium sink as their biomass retain selenium until they reach the sediments. The growth characteristics of charophytes ensure that once reaching the sediments the selenium will become immobilized as it biomineralizes.

5.Ponds cannot have such high turbidity that the opacity of the water prevents the establishment of submerged plant populations. New construction should utilize pre-treatment sediment traps for cleanout that will not require disturbance of plants in the treatment pond.

THANK YOU!