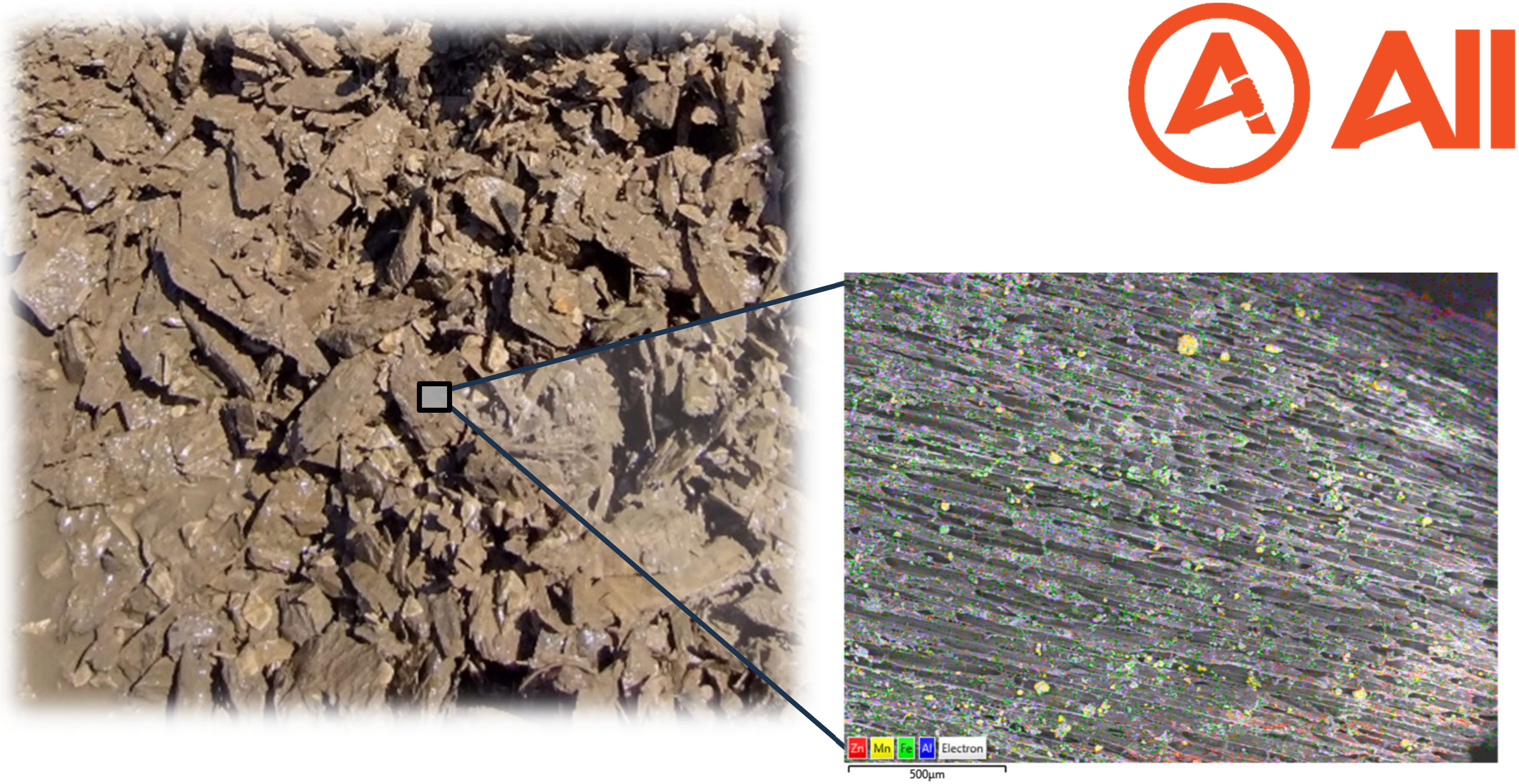

A toolbox for characterizing organic media in passive biotreatment cells

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Contributors

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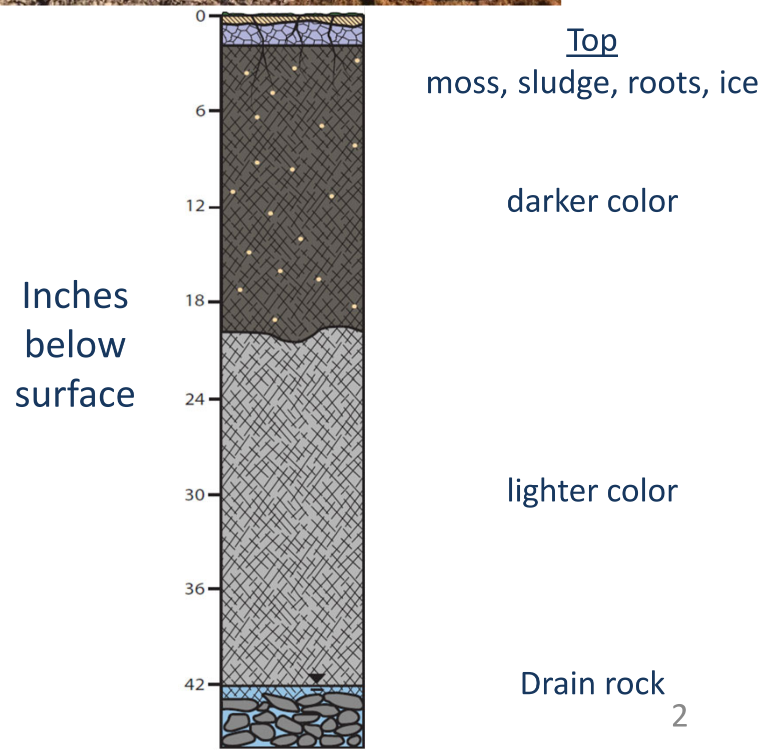
[4]Astragalus Environmental (Rico, CO)

Passive Biotreatment Cells

- Often referred to as
  - Sulfate-reducing bioreactors
  - Vertical flow ponds
  - Biocells

- Biotreatment cells used in the studies I'll discuss:
  - Vertical, downward flow, with water level near the media surface - sometimes below
  - Initial media composed of wood chips (65% by vol.), wood shavings (25%), composted steer manure (5%), and chopped alfalfa hay (5%)
  - Circumneutral water (pH 6.5 - 7.0), elevated in Zn, Cd, Cu, Fe, Mn, with seasonal variation
  - Operated for ~10 year

- More details of the site and water:
  - Moore, T., et al. Passive Treatment of Circumneutral Mine Drainage from the St. Louis Mine Tunnel, Rico, CO: Part 1 – Case Study: **Characteristics of the Mine Drainage**. *Mine Water and the Environment* (2022).
  - Dean, D., et al. Passive Treatment of Circumneutral Mine Drainage from the St. Louis Mine Tunnel, Rico, CO: Part 2 – **Vertical Biotreatment Train Pilot Study**. *Mine Water and the Environment* (2022).



Overall goals of analyses

- How long will the media last?
  - Predicting, planning, monitoring
- Can we make it last longer?
  - Supplementing nutrients, chemistry, hydrology
- Why is a biotreatment cell not working?
  - Biochemistry, environmental conditions, hydrology
- How deep are the sequestered metals?
  - Understanding and disposing media at end of life
  - Designing improved biotreatment cells

“Toolbox of Techniques”

Physical separation of media and fines

- Differentiating media and sequestered material

Bulk solid chemical analyses

- Total metals
- Nutrients (e.g., TOC, ammonia, TKN, S, P)

Sequential extractions

- Organic solvents for lignocellulosic/carbon forms
- Tessier-like extractions for minerals and metals

Imaging and elemental mapping

- Scanning electron microscopy, energy-dispersive X-ray spectroscopy for particle size and elemental distribution

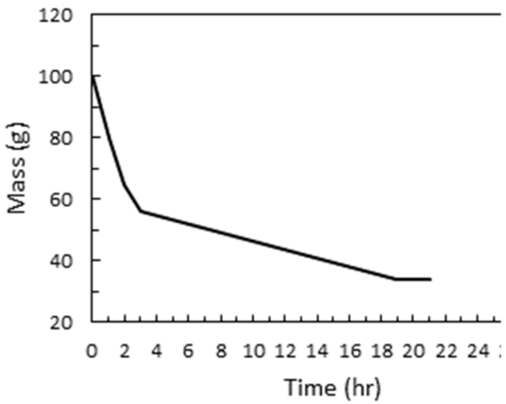
Leachate analyses

- Synthetic Precipitation Leaching Procedure (SPLP)
- Toxic Characteristic Leaching Procedure (TCLP)

Separating media and fines

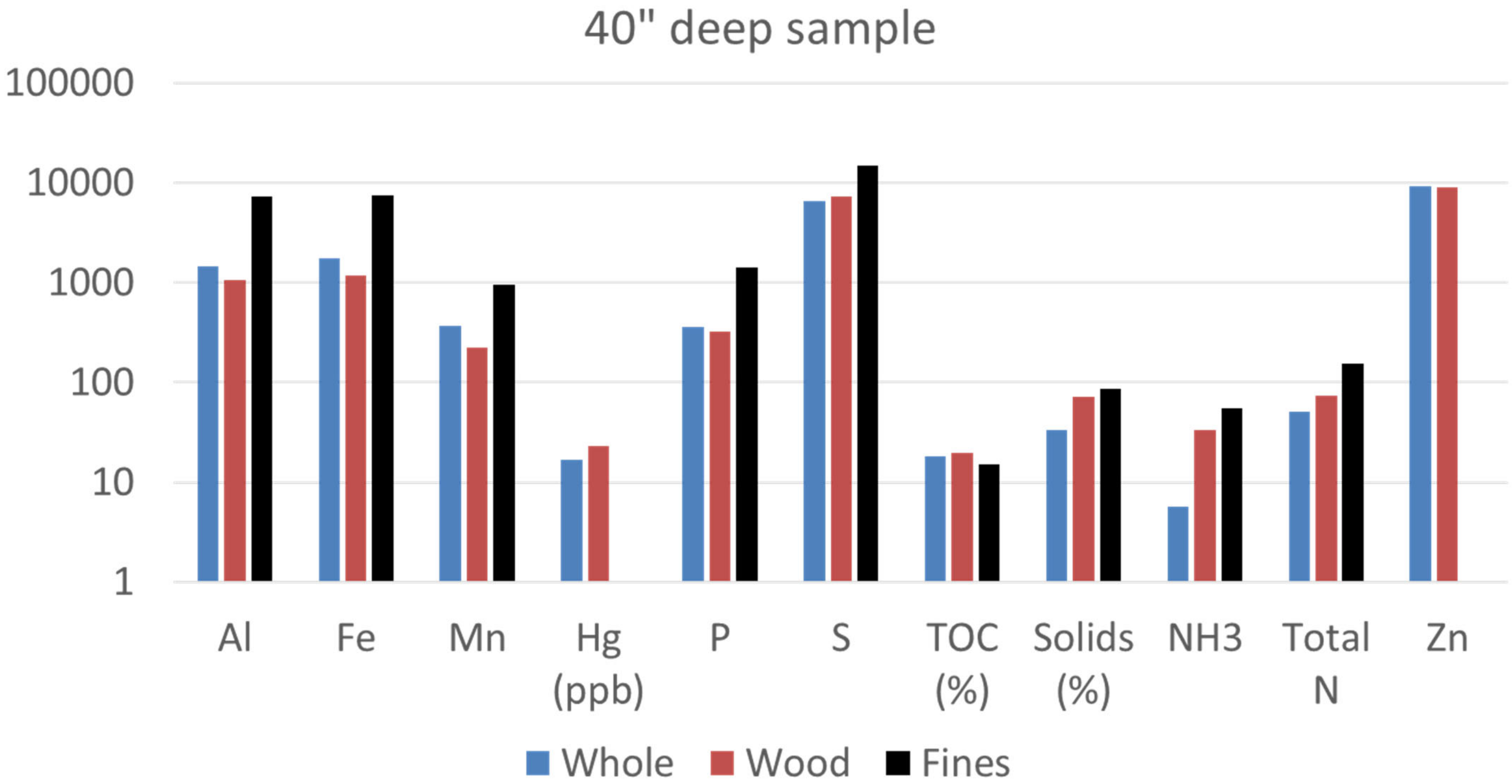
Goal: identify sequestered material and surface nutrients - not bulk media variability

Dehydrator, 40 °C for 24 hours



Shaker, 16-mesh screen

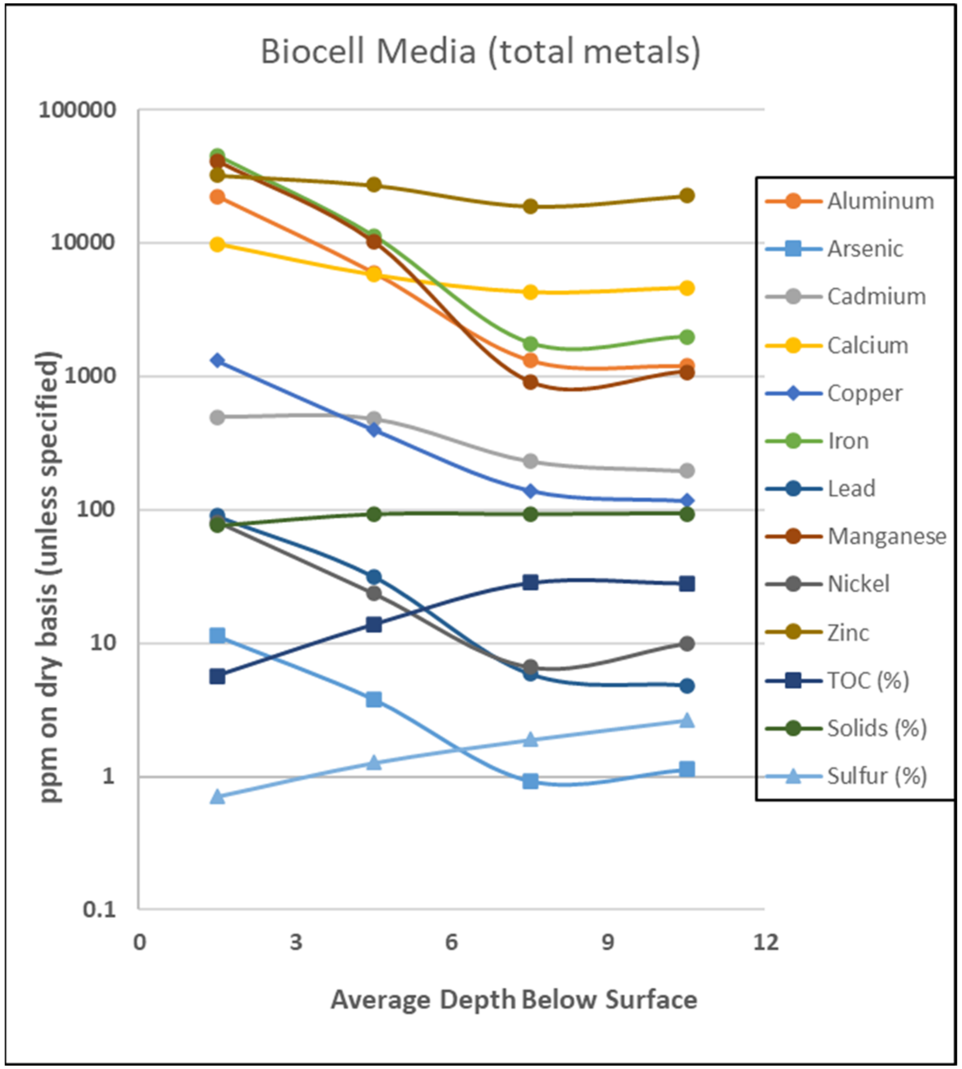
Wood Fraction vs. Fines



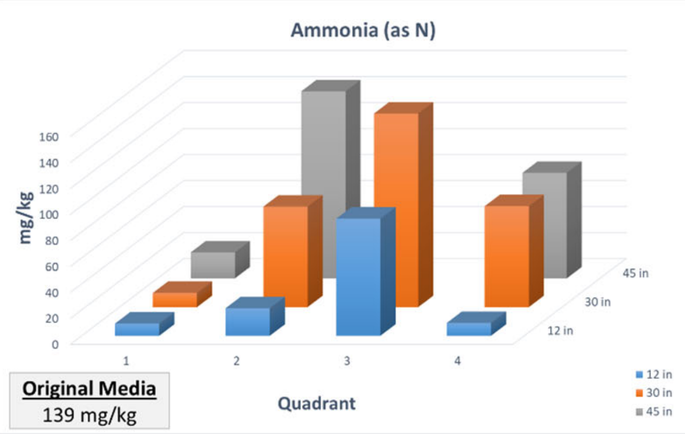
All values reported in ppm, unless stated

Solid Chemical Analyses

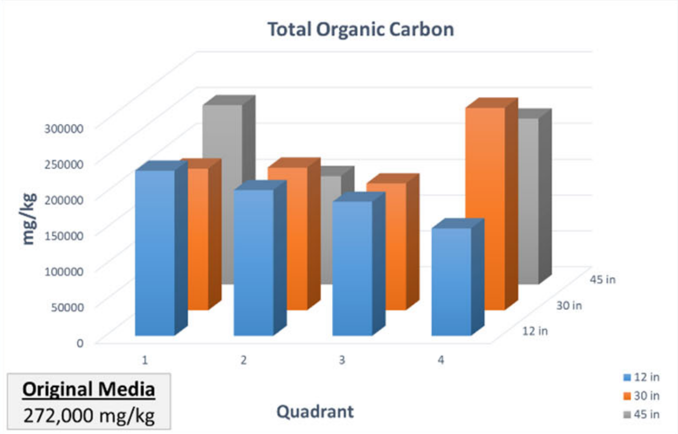
Spatial understanding of nutrients and metals



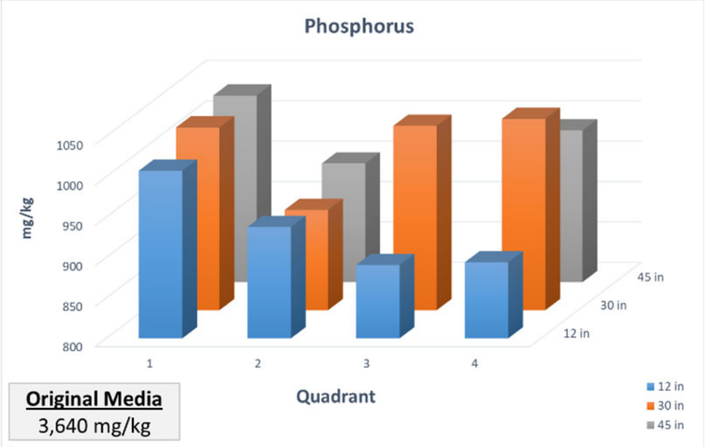
Nutrient Distribution



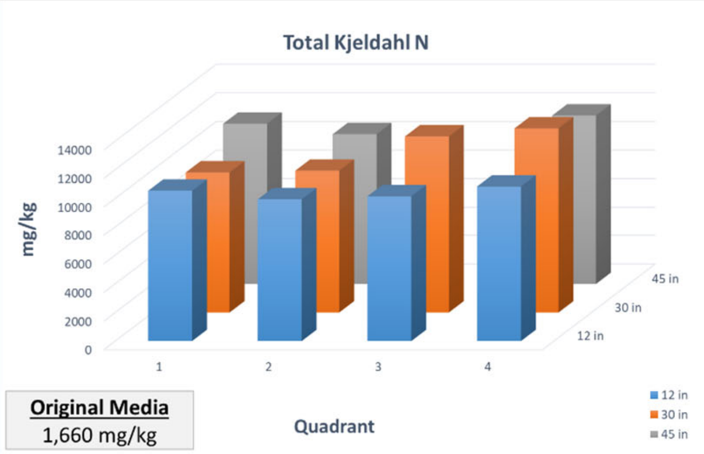
Original Media
139 mg/kg



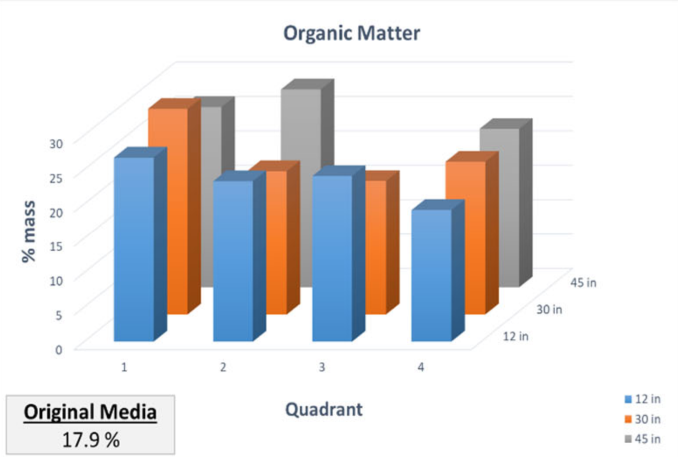
Original Media
272,000 mg/kg



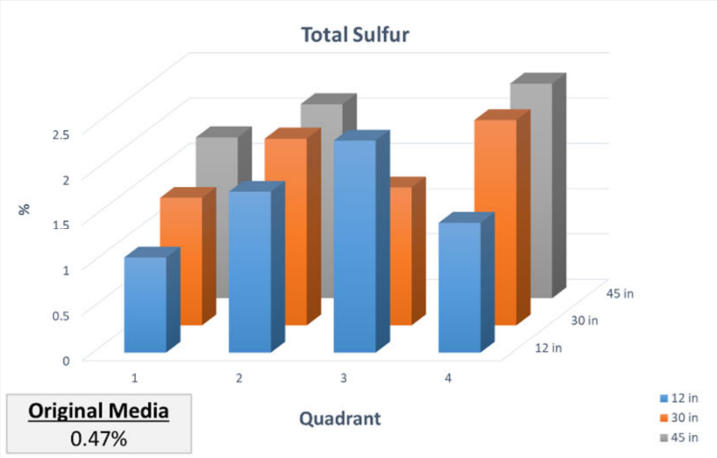
Original Media
3,640 mg/kg



Original Media
1,660 mg/kg



Original Media
17.9 %



Original Media
0.47%

Organic Sequential Extractions

Analyses conducted by Celignis Laboratory (Ireland)

Goal: Differentiate carbon forms

Sugars

- Total Sugar, Hexosans, Pentosans, Glucan, Xylan, Rabinan, Galactan, Mannan

Lignin

- Klason Lignin, Acid Soluble Lignin, Acid Insoluble Residue

Ash and Extractives

- Total Ash, Acid Insoluble Ash, Full Extractives, Water Soluble Extractive, Ethanol Soluble Extractives

Lignocellulosic Summary

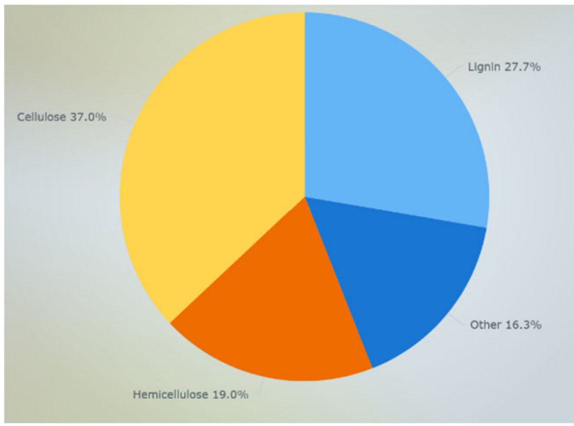
- Cellulose, Hemicellulose, Lignin

Organic Sequential Extraction examples

	Total Sugars	Glucan	Xylan	Mannan	Arabinan	Galactan	Rhamnan	Klason Lignin	Acid Soluble Lignin	Extractives	Ash
Initial Media	53.4	35.3	7.4	7.0	1.4	2.0	0.2	25.5	0.9	6.4	4.6
12”	21.9	13.9	3.2	3.0	0.6	1.0	0.2	20.8	1.2	4.3	57.8
30”	41.1	26.5	5.5	6.3	1.0	1.5	0.3	28.6	1.1	5.2	20.7
48”	42.9	27.5	5.1	7.6	1.0	1.6	0.2	28.6	1.1	5.5	17.2

All values reported as % of total

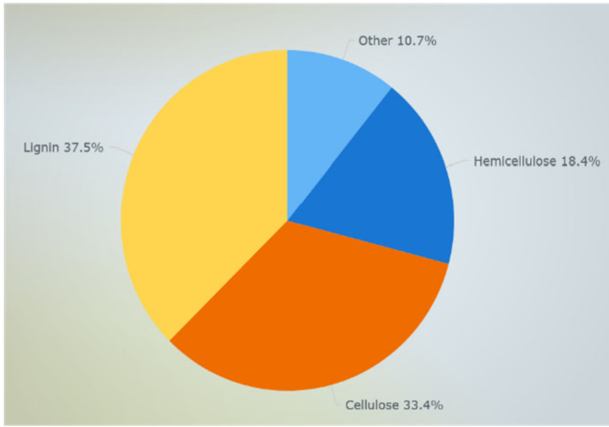
Initial → After ~10 years



Cellulose ⇩

Hemicellulose ⇔

Lignin ⇧

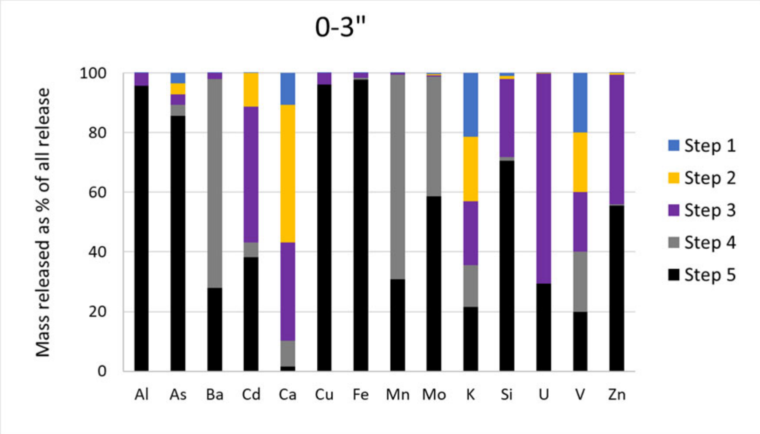


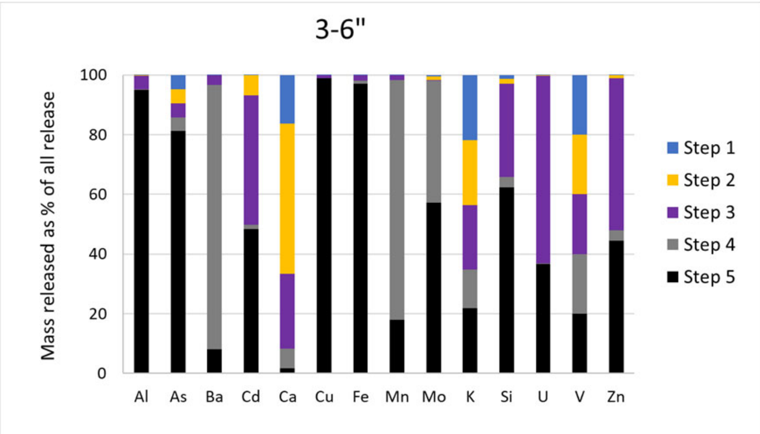
Sequential Extractions

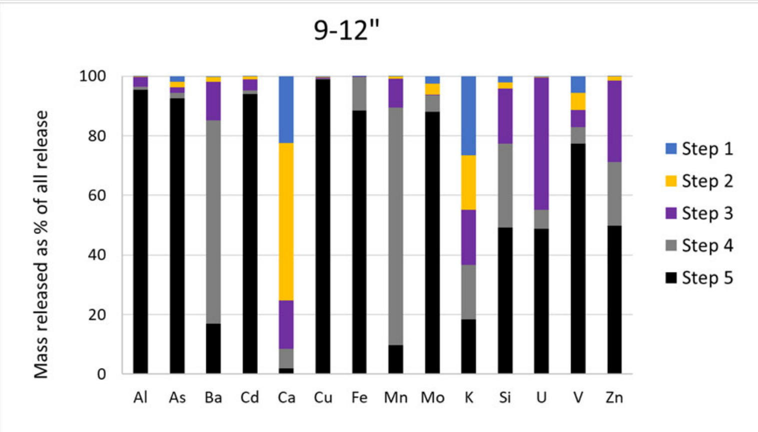
Tessier extraction procedure (black) from Tessier et al. (1979), with modifications based on Klock et al. (1986) (in red, to differentiate sulfide)

Step	Description	Reagent	Procedure
I	Readily Dissolved	Distilled/Deionized Water	
II	Exchangeable and Adsorbed	1 M MgCl2-6H2O	1. Weigh 2.0 g sample into 50-mL centrifuge tube. 2. Add 16 mL 1 M MgCl2-6H2O 3. Place on shaker table for 1 hour 4. Centrifuge on high for 30 minutes 5. Pipette supernatant into centrifuge tube for analysis 6. Add 16 mL deionized water into tube with sediment and hand shake one minute 7. Pipette off supernatant
III	Carbonate Metal	1 M NaOHAc	1. Add 16 mL of 1 M NaOHAc, pH 5 (adjusted with HoAc) 2. Shake 2.5 hours 3. Repeat steps 5 through 7 as in Step II
IV	Easily Reducible and Iron Oxides	0.1 M NH2-OH-HCl	1. Add 40 mL of 0.1 M NH3OH-HCl with 0.01 M HNO3 2. Hand shake for one minute 3. Place in oven at 96 +/- 3 degrees C for 6 hours. Hand shake every hour. 4. After 6 hours remove from oven and hand shake. Repeat steps 5 through 7 as in step 2
V-a	Sulfide Bound Material		(procedure adapted from Klock et al) weigh 1000 mg of sample into a 250-ml beaker. Add 40 ml of the Lefort aqua regia mixture (3:1 nitric acid--hydrochloric acid), cover, and heat on a steam bath **under a 2-day period to prevent drying the water bath (ACZ modification).** Uncover, evaporate to a syrupy consistency, and then add 30 ml of water. Cool, filter into a 100-ml volumetric flask, and wash with 10% nitric acid. Bring to volume with 10% nitric acid.
V-b	Organic Bound Metal	0.02 M HNO3 then 3.2 M NH4OAc	1. Add 6 mL of 0.02 M HNO3 2. Add 10 mL of 30% H2O2 adjusted to pH 2 with HNO3 3. Hand shake one minute 4. Place in oven at 85 +/- 2 deg C for 2 hours 5. Shake at end of one and two hours 6. Add 6 mL H2O2 (pH 2 with HNO3) and hand shake 7. Heat to 85 +/- 2 deg C for 3 hours. Shake every hour 8. Cool sample 9. Add 10 mL of 3.2 M NH4)Ac in 20% v/v HNO3 10. Add 8 mL H2O2 11. Place on shake table for 30 minutes 12. Repeat steps 5 through 7 as in Step II
VI	Residual Metal	HF/HNO3	1. After centrifuge step pipette off supernatant 2. Mix sample well with stir rod in test tube 3. Digest in microwave using HF/HNO3 digestion

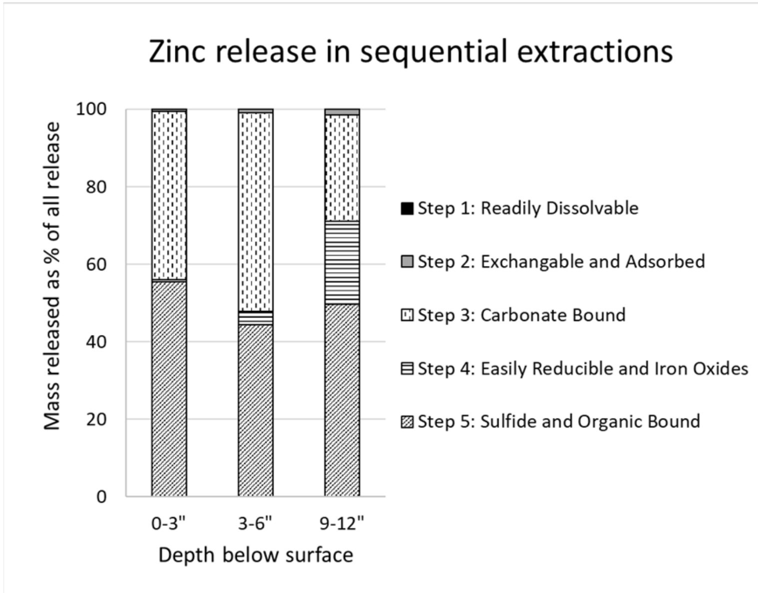
Sequential Extractions data example



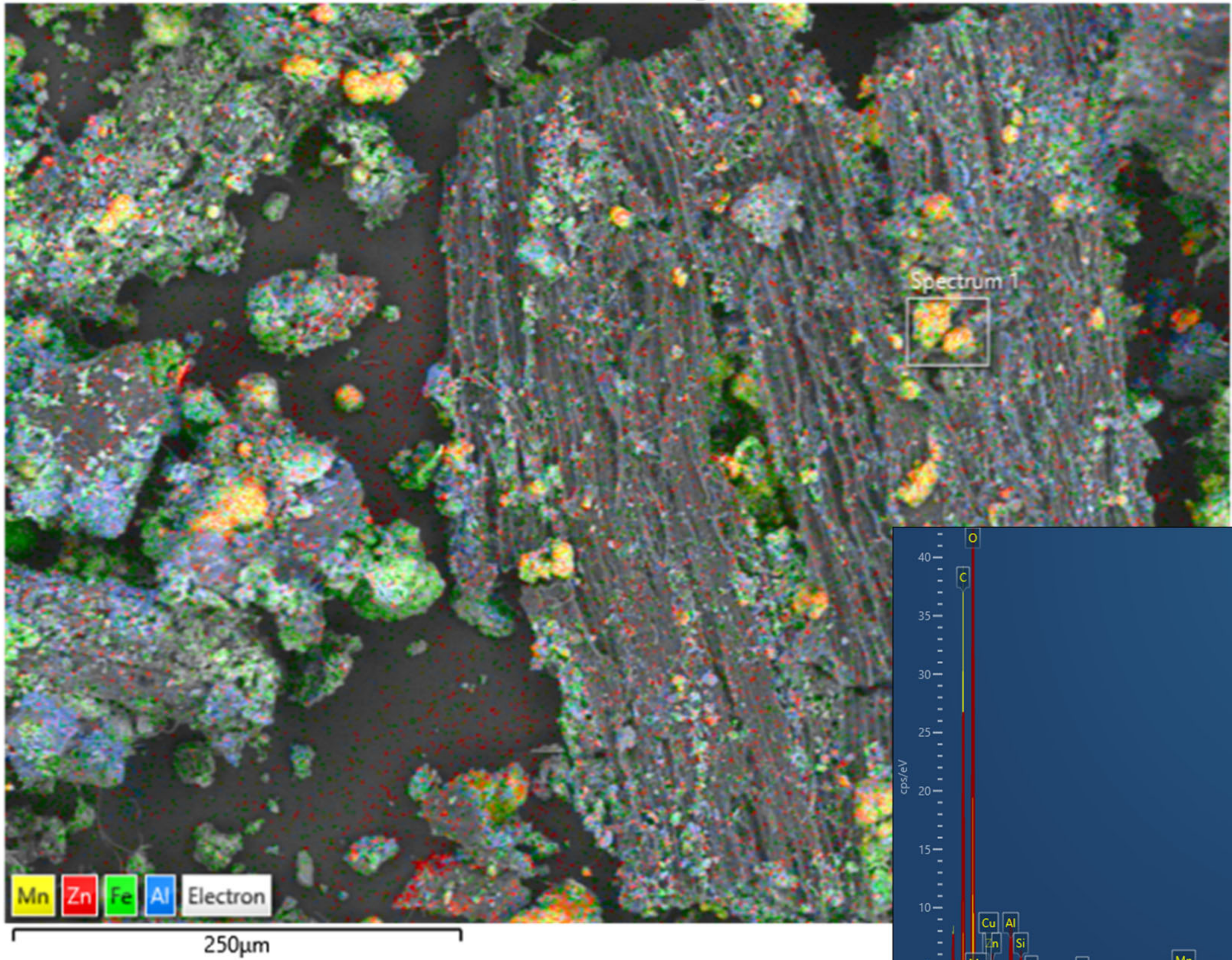


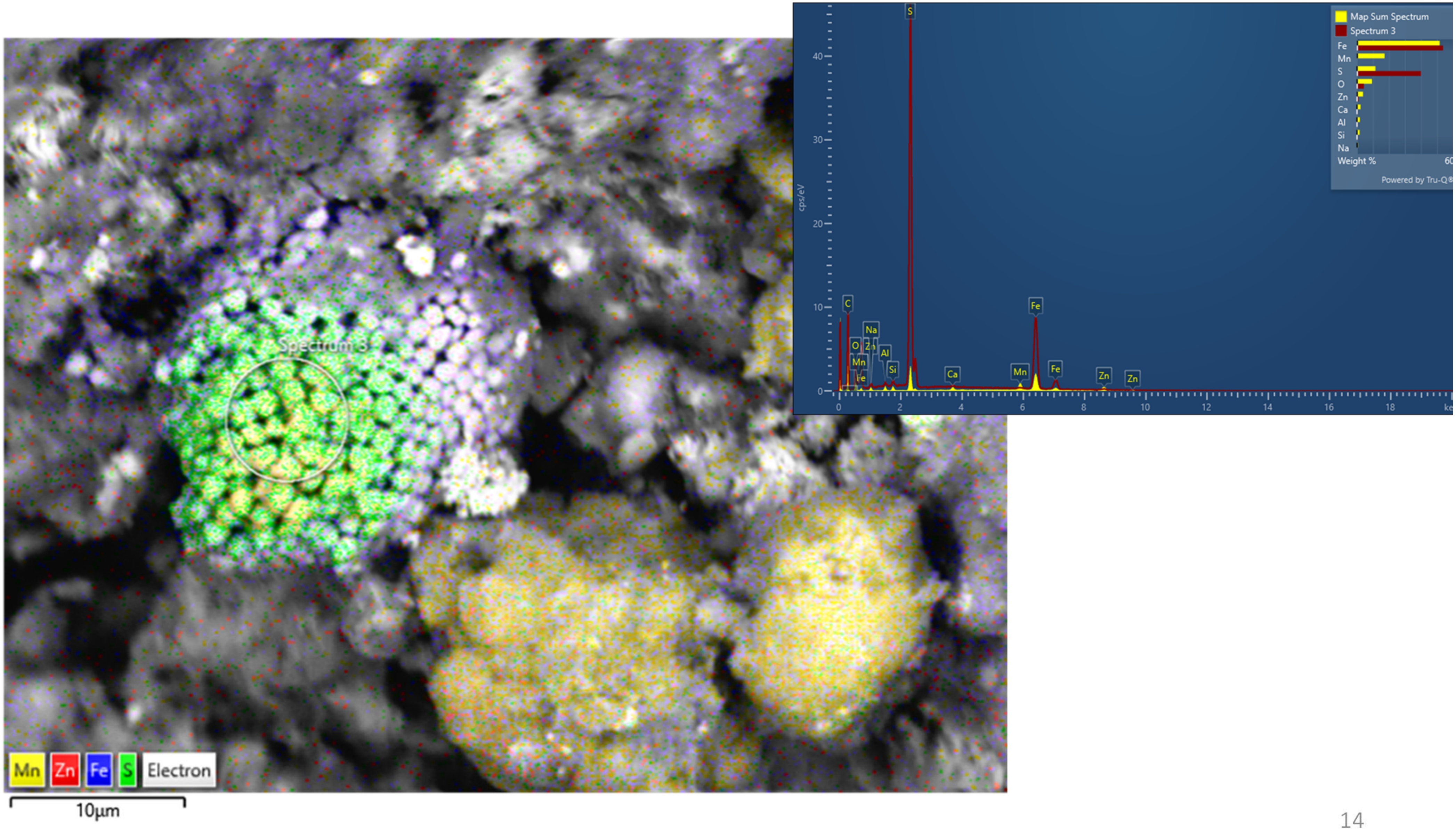


Key					
Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Readily Dissolved	Exchangable and Adsorbed	Carbonate Bound	Easily Reducible and Iron Oxides	Sulfide (a) and Organic (b) Bound	Residual Metal

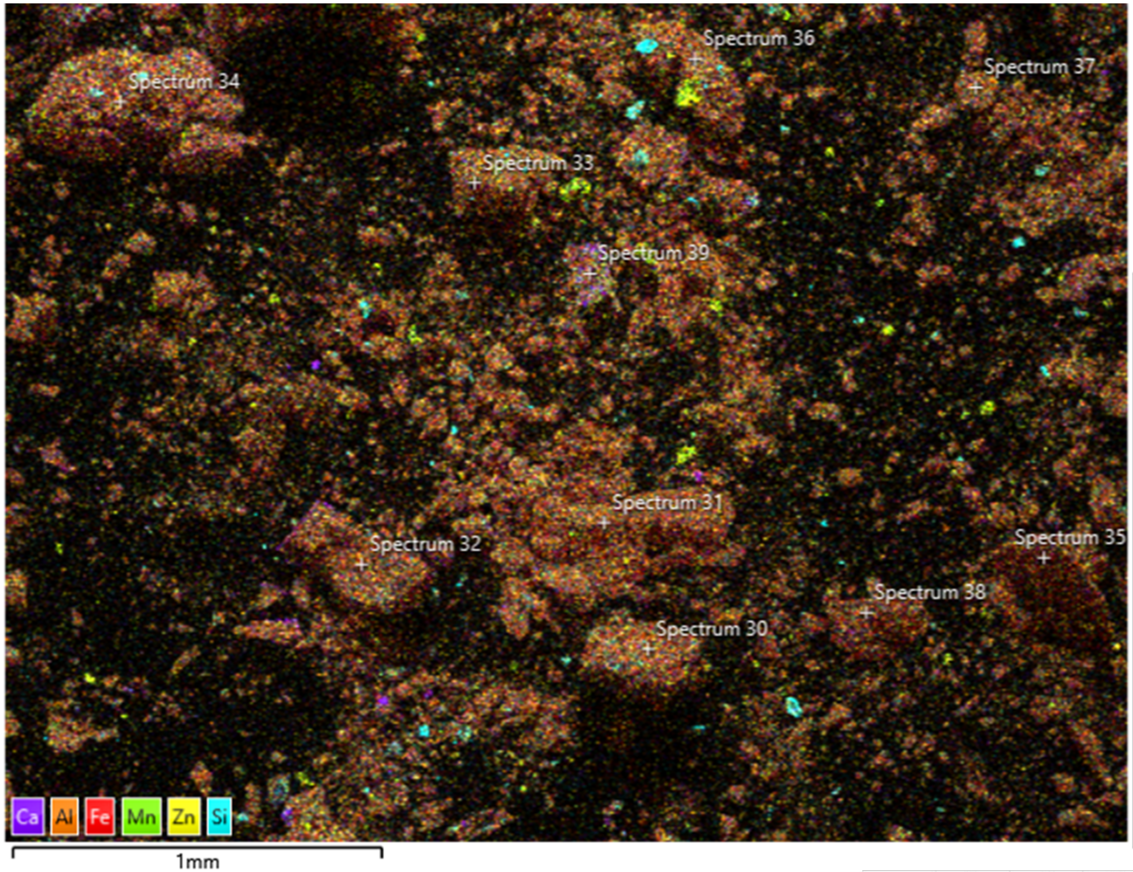


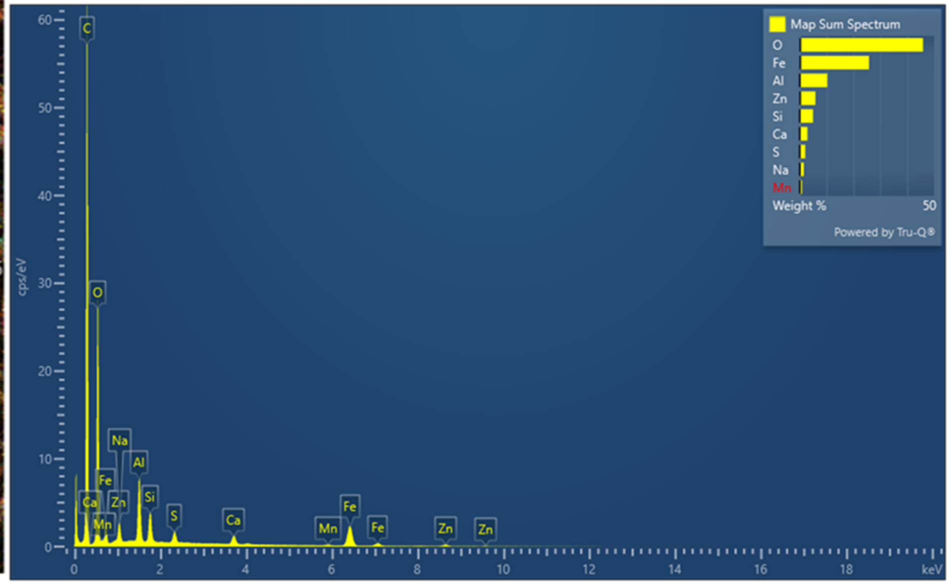
Imaging and chemical mapping




Spectrum 3
Mn
Zn
Fe
S
Electron
10µm
Map Sum Spectrum
Spectrum 3
Fe
Mn
S
O
Zn
Ca
Al
Si
Na
Weight %
cps/eV
C
Na
O
Mn
Al
Si
Ca
Fe
Zn

Imaging and elemental mapping

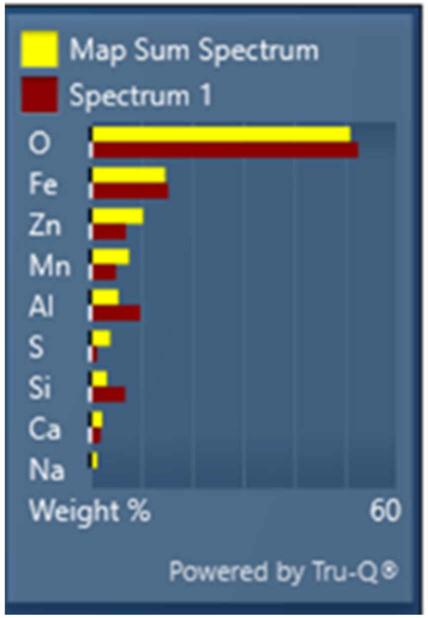




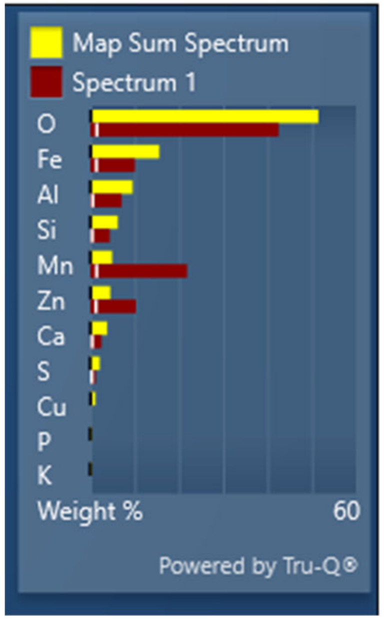
	C	O	Na	Mg	Al	Si	S	K	Ca	Mn	Fe	Cu	Zn
C	1												
O	-0.73374	1											
Na	0.02983	-0.22799	1										
Mg	-0.06424	0.248823	-0.07049	1									
Al	-0.55465	0.523825	-0.52133	-0.37988	1								
Si	-0.47616	0.425819	-0.52933	-0.42387	0.94491	1							
S	-0.12532	0.297334	-0.14561	0.989409	-0.25845	-0.30871	1						
K	0.322253	-0.24254	-0.0218	-0.04771	-0.1328	-0.02936	-0.06603	1					
Ca	-0.06484	0.137052	-0.13195	0.958461	-0.29636	-0.32151	0.970556	-0.02328	1				
Mn	0.145913	-0.37566	0.714455	-0.07075	-0.60671	-0.63449	-0.16086	0.065545	-0.13285	1			
Fe	0.289954	-0.49902	-0.37193	-0.32876	0.170872	0.291338	-0.29189	0.036512	-0.16485	-0.53752	1		
Cu	0.362797	-0.64731	-0.07467	-0.3498	-0.10376	-0.02359	-0.34836	0.032029	-0.27743	-0.11718	0.728102	1	
Zn	0.320518	-0.71014	0.614448	-0.29662	-0.53339	-0.52166	-0.3665	0.18214	-0.27538	0.846044	-0.09771	0.346153	1

Imaging and elemental mapping

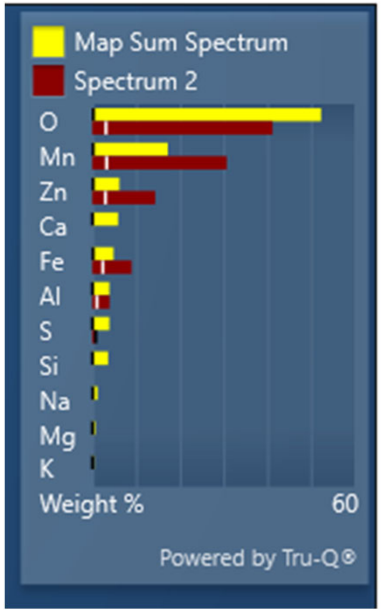
Yellow bars show total map sum spectra for EDXS at depths



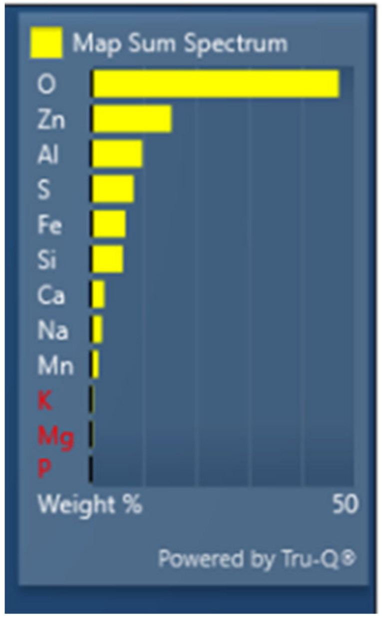
0-3”



3-6”



6-9”



9-12”

Leachate analyses

Methods

- Media from 0-1' and 3' deep was placed in uncovered and perforated 5-gallon buckets and stored at 70 °F
- At 30-, 60-, and 90-day intervals, samples were removed and the samples were analyzed using two leach methods
  - Toxicity Characteristic Leaching Procedure (TCLP, EPA 1311)
    - Used for waste characterization for landfills. EPA determination of toxicity.
  - Synthetic Precipitation Leaching Procedure (SPLP, EPA 1312)
    - Specific to soil, with a perspective of leaching into groundwater

Leachate results

TCLP Composite Results									
Depth	**Time (days)**	**Arsenic (mg/L)**	**Barium (mg/L)**	**Cadmium (mg/L)**	**Chromium (mg/L)**	**Lead (mg/L)**	**Selenium (mg/L)**	**Silver (mg/L)**	**Mercury (µg/L)**
	RCRA Limit	5.0	100	1.0	5.0	5.0	1.0	5.0	0.2 ug/L
0-1'	Fresh	0.0096J	0.18	0.4	ND	ND	0.0087J	ND	ND
	30	ND	N/A	0.8	ND	0.039J	ND	ND	N/A
	60	ND	0.159	0.5	ND	ND	ND	ND	ND
	90	ND	0.14J	0.4	ND	ND	ND	ND	ND
3'	Fresh	0.014J	0.19	0.0	0.0063J	0.020J	0.038J	ND	ND
	30	ND	N/A	0.022J	ND	ND	ND	ND	N/A
	60	ND	0.108	ND	ND	ND	ND	ND	ND
	90	ND	0.089J	0.032J	ND	ND	ND	ND	ND

SPLP Composite Results													
Depth	**Time (days)**	**As (µg/L)**	**Cd (µg/L)**	**Cr (µg/L)**	**Cu (µg/L)**	**Fe (µg/L)**	**Pb (µg/L)**	**Mn (µg/L)**	**Mo (µg/L)**	**Ni (µg/L)**	**Se (µg/L)**	**Ag (µg/L)**	**Zn (µg/L)**
0-1’	Fresh	ND	1.8J	ND	ND	35.8J	ND	4.7J	9.1J	ND	ND	ND	330
	30	ND	2.4J	ND	ND	57.2	ND	16.8	10.5J	ND	ND	ND	660
	60	ND	0.714	ND	ND	41.7	ND	22.4	ND	ND	ND	ND	49.2
	90	ND	5.4	1.0J	19	360	ND	50	2.2J	3.3J	ND	ND	640
3’	Fresh	ND	3.8	ND	8.1J	172	ND	10	9.6J	ND	ND	ND	526
	30	ND	19.8	ND	31.8	879	3.3J	221	9.3J	4.1J	ND	ND	2710
	60	ND	1.82	2.91	ND	166	ND	21	ND	ND	ND	ND	251
	90	ND	3.9J	1.2J	9.9J	160	ND	98	3.4J	8.5	ND	ND	1400

Summary of analytical toolbox

- Important to consider fines vs. whole samples
- Solid chemical methods (metals and nutrients) are great for
  - Spatial distribution and changes since initial media
- Sequential extractions further inform
  - How bioavailable is the carbon
  - With which major mineral phases are metals associated
- Microscopy and elemental mapping help visualize
  - Size and distribution of particles on the surface
  - Material associations
- Leachate analyses help us understand
  - Under what conditions will metals be released

Questions to dig deeper?

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