

NM WRRI Student Water Research Grant 2024-2025 Final Report

1. Student Researcher: Raphael Rivadavia

Faculty Advisor: Jose M. Cerrato

2. Project title: Chemical and Microbial Processes Affecting Metal Mobility in the Sediment/Water Interface of an Acid Mine Drainage-Affected Stream

3. Description of research problem and research objectives.

Acid mine drainage (AMD) is a widespread environmental issue caused by mining activity. It is characterized by an acidic pH (below 4) and high concentrations of sulfate and metals, which threaten ecosystems and require billions of dollars to clean up. A clear example of this is the Animas River watershed in Colorado, where the 2015 Gold King Mine spill released 3 million gallons of AMD into the Animas River, contaminating a critical water source for human consumption and agriculture for several communities in Durango, CO, Farmington, NM, and the Southern UTE reservation.

The mobility of metals in AMD-affected streams is controlled by biogeochemical processes, which are influenced by seasonal variations in redox conditions, pH, and microbial activity. Dynamic redox conditions, often driven by seasonal changes such as snowmelt or drought, can lead to the mobilization of metals from sediments. Microbial communities play a critical role; in oxic environments, they can catalyze iron oxidation, leading to the sequestration of metals, whereas in anoxic environments, they can cause the reductive dissolution of metal oxides, releasing metals back into the water.

A critical gap in existing research is the understanding of how dynamic shifts in redox conditions affect both metal mobility and microbial communities in AMD-impacted streams. The novelty of this study lies in its investigation of how desorption and ion exchange reactions influence the lability of lead and copper from sediments under different redox, pH, and electron donor conditions. It also reports on the presence and shifts of metal-tolerant fungal species in these sediments, a group often overlooked in similar studies.

The objective of this study was to investigate how redox conditions influence the mobility of lead, copper, and iron from AMD-affected sediments. The research used controlled laboratory experiments with synthetic river water and sediments, subjecting them to redox cycles to track metal

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release. The findings have important implications for developing effective bioremediation strategies and for the potential recovery of critical minerals.

4. Description of methodology employed.

Water and sediment samples were collected from Cement Creek in Silverton, Colorado, in September of 2021 and 2023 (Figure 1). On-site measurements of pH, temperature, and electrical conductivity were taken. Water samples were collected and filtered for various analyses, including anions and metals. Sediment, limited to the top 5 cm due to the rocky substrate, was collected using a flame sterilized shovel to prevent contamination. The samples were transported to a lab and stored at 4 °C before homogenization.

Laboratory microcosm experiments were performed in 500 mL bottles to simulate the natural environment. Each bottle contained a 1:10 sediment-to-water ratio, using 35 grams of dry sediment and 350 mL of synthetic river water (SRW) that mimicked the chemical composition of Cement Creek water (Table 1). To stimulate microbial activity, a buffered sodium acetate solution was added to some of the microcosms, while a control group received no acetate. All experimental units were subjected to three sequential five-day redox cycles—oxic, anoxic, and oxic—to capture short-term reactions driving metal mobilization.

Throughout the experiments, the microcosms were agitated in an orbital shaker (120 RPM) and monitored for changes in pH and oxidation-reduction potential (ORP). Samples were taken at 0, 2, 24, and 120h from the beginning of each redox phase to monitor aqueous metal concentration. The collected samples were also used to characterize the microbial communities. Fungal communities were identified using both a culture-based approach with specific growth media and next-generation sequencing, a key method given the study's focus on an often-overlooked group of microorganisms. This involved DNA extraction and amplification, followed by sequencing using an Oxford Nanopore Technologies sequencer.

Additional extraction experiments were conducted to assess the lability of lead and copper from the sediments at two different pH levels (3.4 and 6.4), representing conditions in Cement Creek and downstream in the Animas River, respectively. The microstructure and composition of the sediments were also analyzed using a suite of advanced methods, including X-ray diffraction and various forms of electron microscopy. Finally, PHREEQC modeling was used to simulate the

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chemical speciation of metals in both the microcosm and extraction experiments, providing deeper insight into the observed reactions.

5. Description of results; include findings, conclusions, and recommendations for further research.

Analysis of Cement Creek Field Samples. Water from Cement Creek was found to have an acidic pH of 3.4 ± 0.1 and a high concentration of sulfate ($581 \pm 17.61 \text{ mg L}^{-1}$), consistent with a mining-affected area (Table 2, 3). Analysis also showed high levels of calcium, iron, and manganese, likely from local mineral weathering. While copper was present at $25.18 \pm 5.81 \text{ } \mu\text{g L}^{-1}$, lead was detected at a concentration of $5.64 \pm 0.14 \text{ } \mu\text{g L}^{-1}$, which is below the EPA's drinking water action level of $15 \text{ } \mu\text{g L}^{-1}$.

The sediments had high concentrations of iron ($230,442 \pm 17,076 \text{ mg kg}^{-1}$), over four times the average crustal concentration (Table 4). They also had significant amounts of aluminum, copper, manganese, and lead. Electron microscope analysis of sediments revealed a wide range of grain sizes and different compositions, with larger particles rich in iron and smaller ones composed of aluminosilicates (Figure 2). X-ray diffraction (XRD) confirmed the presence of goethite and various aluminosilicate minerals (Figure 3). Scanning Transmission Electron Microscopy (STEM) revealed distinct mineral morphologies, including thin platelets of Al-silicates and faceted crystals rich in iron, sulfur, and potassium, with detectable amounts of lead (Figure 4, 5). This composition and morphology are consistent with the mineral jarosite, which can scavenge heavy metals.

Effect of Redox Conditions on Metal Mobilization. The microcosm experiments successfully established oxic and anoxic conditions, as confirmed by oxidation reduction potential (ORP) measurements. During the anoxic phase, aqueous iron concentrations spiked significantly due to the reductive dissolution of iron phases (Figure 6). For instance, in the treatment without acetate, Fe levels increased 143-fold. In contrast, oxic conditions led to a sharp decrease in aqueous iron as it precipitated out of the water. Interestingly, lead mobilization was *immediate* from the start of the experiments and appeared to be independent of redox conditions, Fe-solubilization, and carbon amendment. Its concentration ranged from 11 to $52 \text{ } \mu\text{g L}^{-1}$, exceeding the EPA's drinking water action level (Figure 6).

Effect of pH on Metal Mobilization: In further extraction experiments, both lead and copper were found to be highly mobile at an acidic pH of 3.4, reaching concentrations of up to $27 \text{ } \mu\text{g L}^{-1}$ and 75

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$\mu\text{g L}^{-1}$, respectively (Figure 7). This is because the high concentration of H^+ ions at this pH (~ 4.0 mM) drives ion exchange reactions that release Pb^{2+} and Cu^{2+} from the sediment surfaces. Conversely, at a circumneutral pH of 6.4, the release of these metals was significantly lower, suggesting that pH-dependent ion exchange is the primary mechanism for their mobilization. Additionally, modeling indicated that the decreased release of lead and copper at pH 6.4 could be due to the precipitation of solid mineral phases.

Microbial Communities in Microcosms. Microbial diversity (alpha diversity) was observed to shift with changes in redox conditions and the addition of acetate (Figure 8). The composition of the fungal community also changed. The genera *Aspergillus* and *Malassezia* were consistently present, while *Trichoderma* and *Fusarium* appeared in specific phases (Figure 9). The presence of these fungal genera, particularly *Trichoderma* and *Fusarium*, is notable as they are known to tolerate and interact with heavy metals. The study's findings suggest a potential role for fungi in metal mobility within these environments. However, due to challenges with DNA extraction, a limitation acknowledged in the study, definitive conclusions about the influence of redox and carbon amendment on the microbial communities could not be drawn.

Future Research. To further understand metal mobility in AMD-impacted streams, future studies should investigate the specific functions and interactions of fungi. While this study identified metal-tolerant fungal genera, it was limited by low DNA yield, which restricted its ability to draw definitive conclusions. Further research is necessary to better understand how dynamic shifts in redox conditions influence both metal mobility and the microbial community, including fungi. Ultimately, this will help in the development of more effective bioremediation strategies and in the potential recovery of critical minerals.

6. Provide a paragraph on who will benefit from your research results. Include any water agency that could use your results.

This research benefits diverse stakeholders concerned with acid mine drainage impacts on water quality. Most importantly, communities living alongside affected waterways, such as the Animas and San Juan Rivers, will benefit from a greater understanding of the risks posed by lead, copper, and iron mobilization. In New Mexico, agencies like the New Mexico Environment Department (NMED) and the New Mexico Office of the State Engineer (NMOSE) can use the findings to improve water quality standards, monitoring, and remediation plans. Nationally, the Environmental

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Protection Agency (EPA) and the United States Geological Survey (USGS) can utilize these results to inform best practices for managing and mitigating acid mine drainage in other affected regions. Furthermore, private mining businesses and related industries can leverage this knowledge to develop more sustainable practices, minimizing their environmental impact and ensuring regulatory compliance, ultimately protecting vital water resources.

7. Describe how you have spent your grant funds. Also provide your budget balance and how you will use any remaining funds. If you anticipate any funds remaining after September 30, 2025, please contact Carolina Mijares immediately (575-646-7991; mijares@nmsu.edu).

A budget modification was requested and accepted by NMWRRI, as detailed in Table 5. All grant funds (\$7,500) were spent. The modification eliminated the original allocations for Salary (\$2,400), Fringe Benefits (\$480), and Equipment (\$600), and reduced the Other category. These adjustments allowed for the creation of a new Travel budget and increased allocations for Supplies and Services to cover specific project costs.

The increased Services budget (\$5,508) paid for technical services, specifically DNA sequencing analysis. The new Travel budget (\$461) covered overnight sample shipping and conference registration fees for the AEESP 2025 Conference and the 69th Annual New Mexico Water Conference. The expanded Supplies budget (\$1,274) was used to purchase a micropipette set and microbial growth media.

Table 5 - Summary of Budget Revisions and Final Expenditures

Budget	Original	Change	Revised
Salary:	\$2,400	(\$2,400)	\$0
Fringe Benefits:	\$480	(\$480)	\$0
Health Insurance:	\$0	\$0	\$0
Travel:	\$0	\$461	\$461
Supplies:	\$320	\$954	\$1,274
Services:	\$3,000	\$2,508	\$5,508
Equipment:	\$600	(\$600)	\$0
Other:	\$700	(\$443)	\$257
Total:	\$7,500	\$0	\$7,500

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8. List presentations you have made related to the project.

- The 69th New Mexico Water Conference was held in November 2024 at the Buffalo Thunder Resort in Pojoaque, NM
Title: Biogeochemical Processes Affecting Metal Mobility Under Oxic/Anoxic Conditions in a Stream Impacted by Acid Mine Drainage
- Oral presentation: April 25, New Mexico Geological Society Spring Meeting 2025 – New Mexico Tech – Socorro, New Mexico, USA
Title: Lead, Copper, and Iron Mobility in Oxic/Anoxic Conditions Under Acid Mine Drainage
- Poster presentation: May 20-22, AEESP Spring 2025 – Duke University, Durham, North Carolina, USA
Title: Effect of pH on chemical and microbial processes influencing metal mobility in a stream impacted by acid mine drainage

9. List publications or reports, if any, that you are preparing. For all publications/reports and posters resulting from this award, please attribute the funding to NM WRRI and the New Mexico State Legislature by including the account number: NMWRRI-SG-FALL2024.

Research manuscript **submitted** to the **American Chemical Society - Environmental Science & Technology Water journal on June 2025.**

Title: *Biogeochemical Processes Affecting Metal Mobility Under Oxic/Anoxic Conditions in a Stream Impacted by Acid Mine Drainage*

Status: First round of reviews submitted on September 23rd 2025

10. List any other students or faculty members who have assisted you with your project.

- Carl Abadam
- Katelin Fisher
- Abdul-Mehdi Ali
- Angelica Saenz-Trevizo
- Adrian Brearley
- Gregory Bonito

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- Marc Friedman
- Geisianny Moreira
- Geoffrey Willians
- Jay Kramer
- Carmen Atchley
- Debora Rodrigues
- Lurima Faria

11. Provide special recognition awards or notable achievements as a result of the research including any publicity such as newspaper articles, or similar.

- New Mexico Geological Society Annual Spring Meeting – Student Travel Award - New Mexico Tech, Socorro, New Mexico - Apr. 2025
- ASCE - New Mexico Section Fall Conference - Graduate Student Award September 2024
- Center for Water and the Environment Mini-Conference, University of New Mexico, Albuquerque, New Mexico - Oct. 2024 - 3rd place poster. September 2024.

12. Provide information on degree completion and future career plans. Funding for student grants comes from the New Mexico Legislature and legislators are interested in whether recipients of these grants go on to complete academic degrees and work in a water-related field in New Mexico or elsewhere.

Currently in the seventh of eight semesters of their doctoral studies, the student intends to work in the private sector of the mining industry in New Mexico or elsewhere in the US/world after completing their Ph.D.

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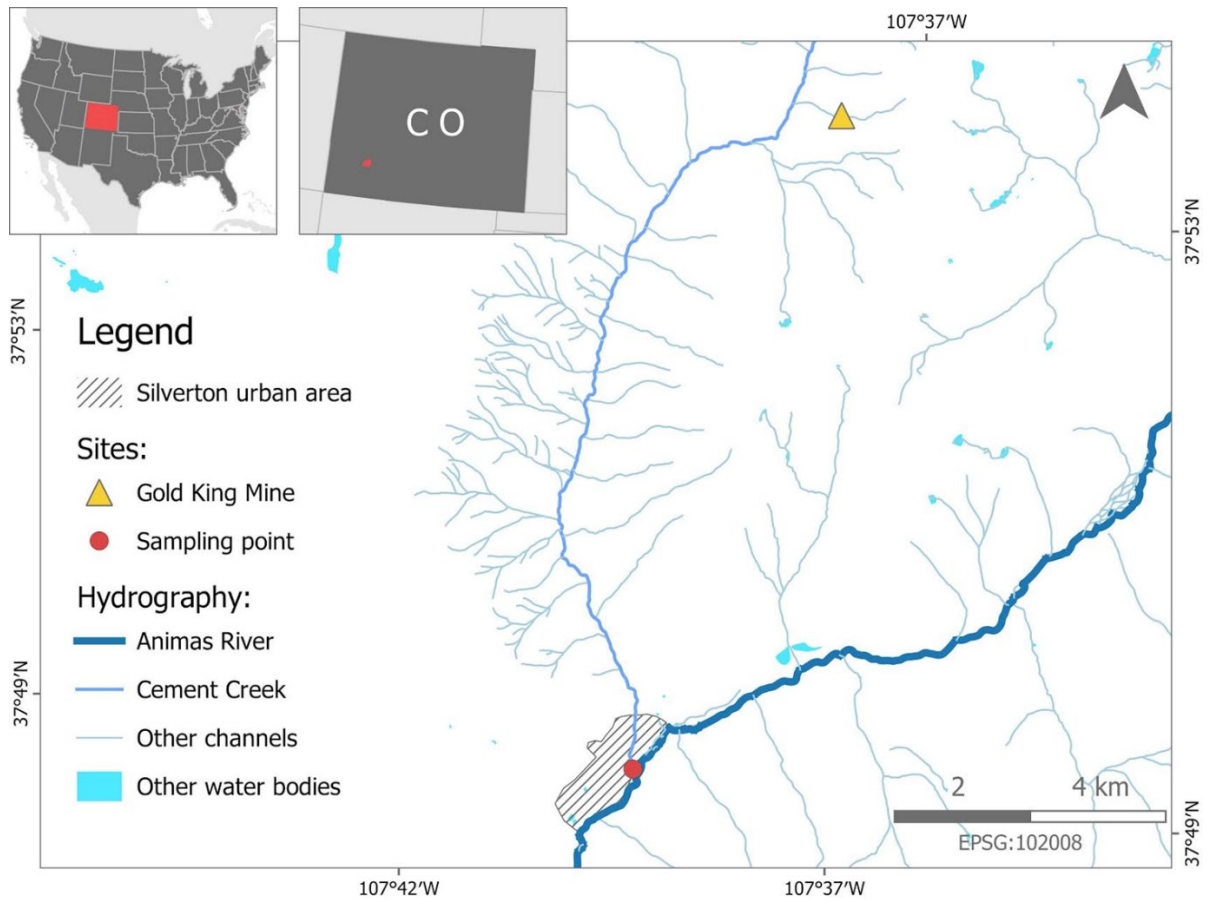


Figure 1. Map of the Animas River watershed near Silverton, Colorado, showing the location of the Gold King Mine, the sampling point, and key hydrographic features, including the Animas River and Cement Creek. The Silverton urban area is highlighted, along with other water bodies and channels in the region.

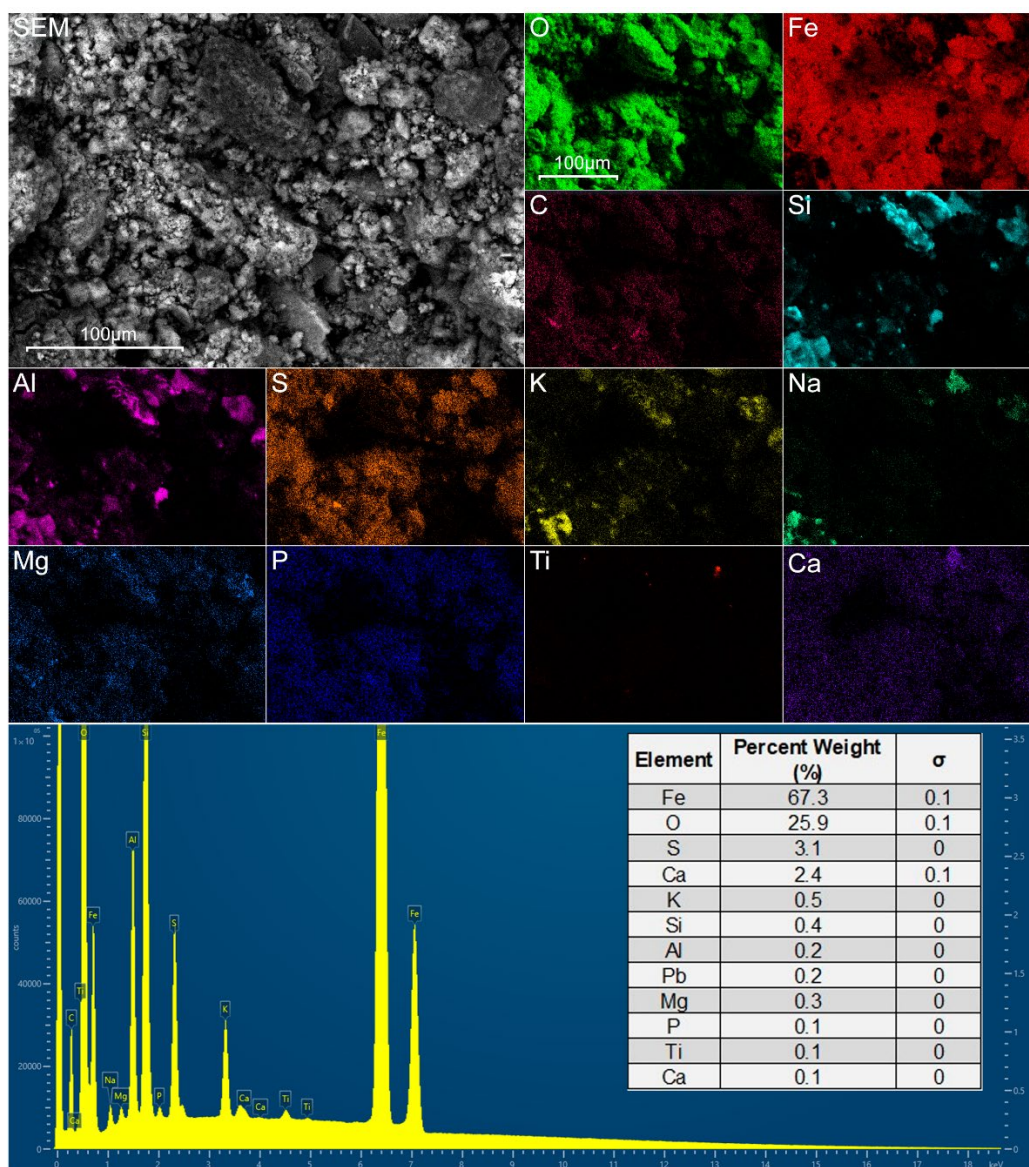


Figure 2. Scanning Electron Microscopy and Energy-Dispersive X-ray Spectroscopy (SEM-EDXS) mapping of sediment samples. The SEM image (upper left) reveals irregular aggregates with a range of grain sizes, with smaller particles ranging from 3 ~ 17 μm , and larger particles ranging from 32 ~ 87 μm . The larger particles are dominated by Fe, O, and S, suggesting the presence of Fe oxides, oxyhydroxides, and sulfates. Smaller particles exhibited Al, Si, and O, indicating aluminosilicates. The EDXS maps also detect trace amounts of Na, K, P, Ca, Mg, and Ti, likely associated with smaller or non-distinct particles, with Ti appearing in isolated regions. Elemental weight percentages and standard deviations (σ) are presented in the table, and the EDXS spectrum at the bottom confirms these elements' presence and relative abundances, supporting the complexity and heterogeneity of the sediment composition. This preliminary analysis suggests a diverse mineralogy, morphology, and sizes, justifying further STEM analysis on sieved samples.

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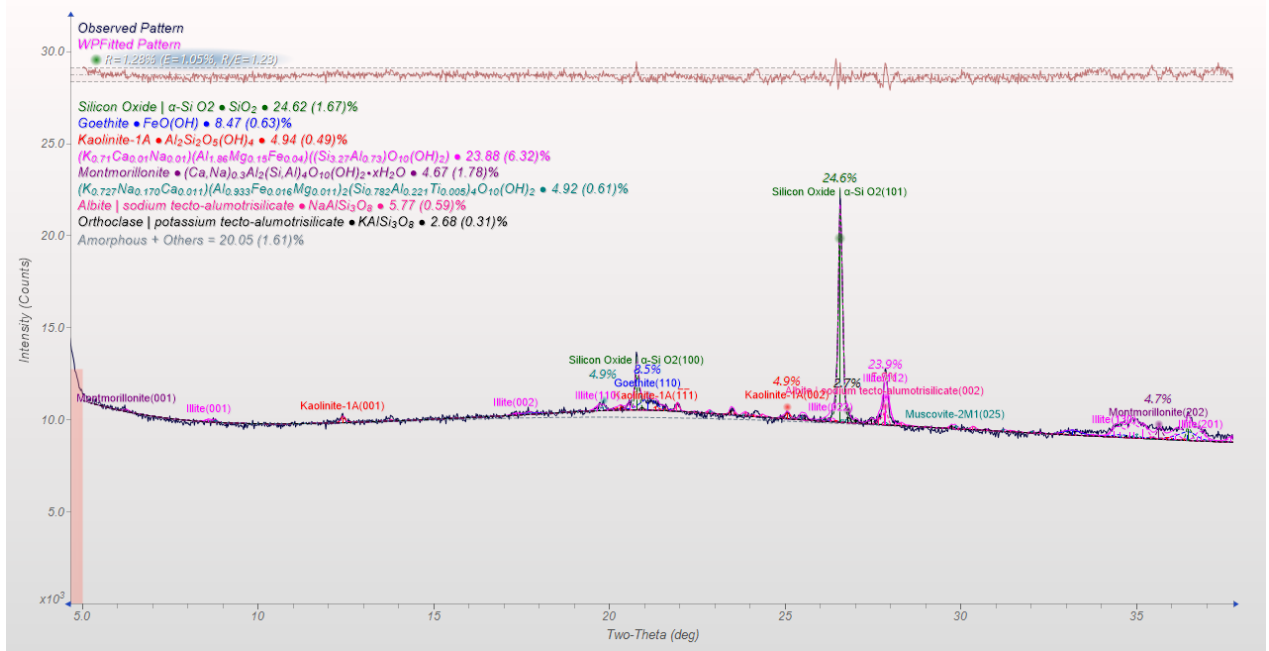


Figure 3. X-ray diffraction (XRD) pattern of sediment sample from Cement Creek. The observed pattern (black) is fitted against known mineral phases (colored text). Identified phases include quartz (SiO₂), goethite (FeO(OH)), kaolinite (Al₂Si₂O₅(OH)₄), illite (K_{0.65}Al₂(Al_{1.65}Si_{3.35}O₁₀)(OH)₂), montmorillonite ((Na,Ca)_{0.3}(Al,Mg)₂Si₄O₁₀(OH)₂·nH₂O), orthoclase (KAlSi₃O₈), muscovite (KAl₂(AlSi₃O₁₀)(OH)₂), and albite (NaAlSi₃O₈). The fitted pattern indicates the presence of both crystalline and amorphous phases, with quartz and goethite being the dominant minerals.

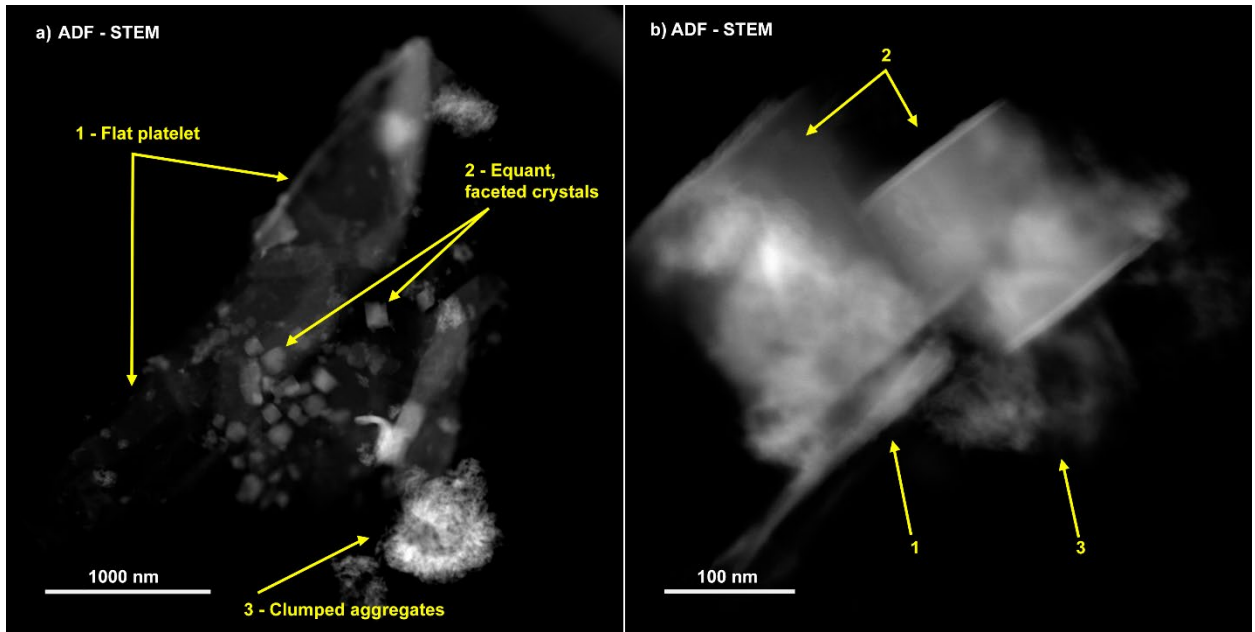


Figure 4. Annular Dark-Field STEM micrographs of a Cement Creek sediment sample exhibiting a mixture of euhedral and anhedral phases. **a)** Diverse morphologies are observed, including a thin, flat platelet (arrow 1), smaller, equant, faceted crystals (arrow 2), and irregular, clumped aggregates (arrow 3); **b)** Magnified view of a different area showing all three morphologies: a thin platelet (arrow 1), an equant, faceted crystal with well-defined edges (arrow 2), and an irregular, clumped aggregate (arrow 3).

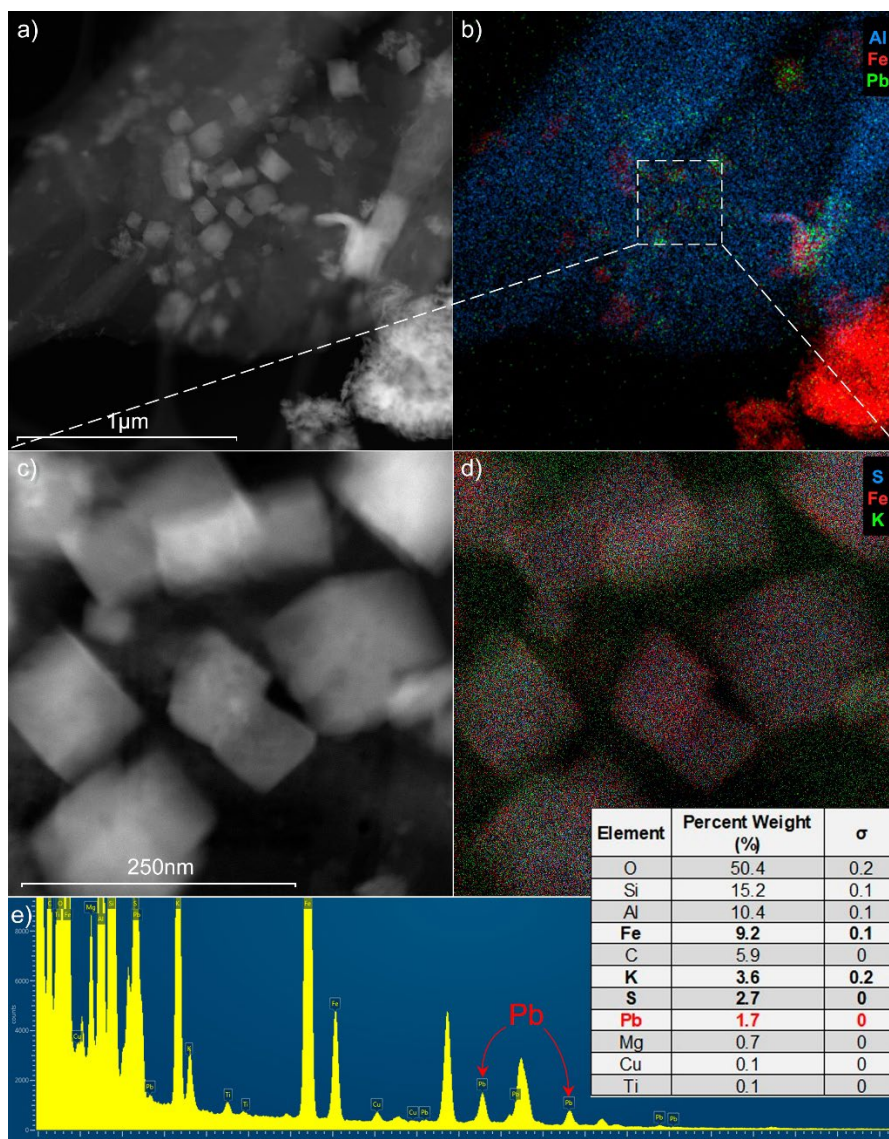


Figure 5. STEM-EDXS analysis reveals diverse mineralogy and confirms Pb-bearing jarosite in Cement Creek sediments. **a)** ADF-STEM micrograph shows various phases with distinct morphologies; **b)** Layered EDXS elemental map identifies thin platelets mainly composed of Al (blue), consistent with aluminosilicates. The equant, faceted crystals contain Fe (red) and Pb (green), suggesting Pb-bearing phases, and the clumped aggregates mainly contain Fe, iron oxides and oxyhydroxides; **c)** ADF-STEM close-up of faceted crystals (gray box in b); **d)** Layered EDXS elemental map of c) shows equant, faceted crystals mainly containing Fe-S-Pb, supporting the presence of Pb-bearing jarosite. **e)** EDXS spectrum and table with the percent weight of detected elements from c) confirm the presence of Fe, K, O, Pb, and S on the crystals. The ratios are consistent with Pb-bearing jarosite (Pb/Fe ratio < 0.166).

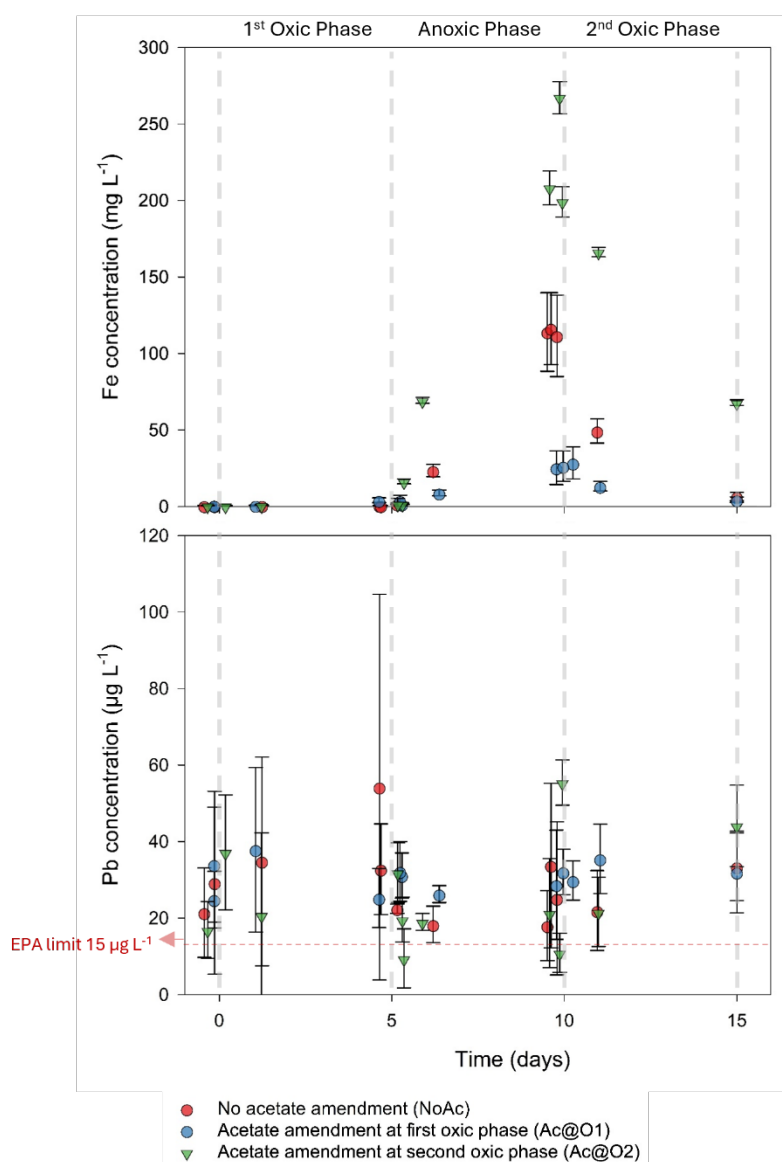


Figure 6. Aqueous metal concentrations over time by treatment and redox phase in microcosm experiments – ICP-OES and ICP-MS analysis. The top graph shows the concentration of aqueous Fe in mg L^{-1} , and the bottom graph shows the concentration of aqueous Pb in $\mu\text{g L}^{-1}$. Data points represent the mean of triplicate measurements, and error bars indicate standard deviation. Treatments are represented by different symbols and colors in the legend: Ac@_O1 (red circles) – Acetate amendment (5 mM) at the first oxidic phase; Ac@_O2 (blue squares) – Acetate amendment (5 mM) at the second oxidic phase; and NoAc (green triangles) – No acetate amendment.

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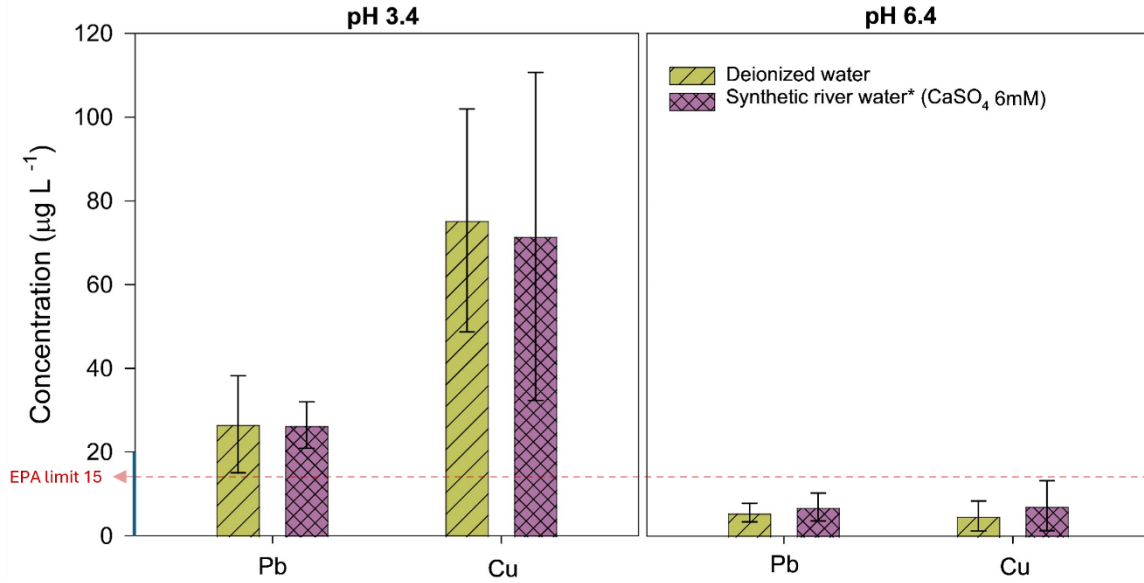


Figure 7. Lead and Cu concentrations in water exposed to sediments under pH 3.4 (left panel) and 6.4 (right panel). Light green bars with diagonal stripes represent samples from deionized water while the purple, cross-hatched bars represent samples from synthetic river water (SRW*), which contains 6 mM CaSO₄. Concentrations are shown in micrograms per liter (µg L⁻¹). Each bar is the average of three measurements, and the black vertical lines show the standard deviation.

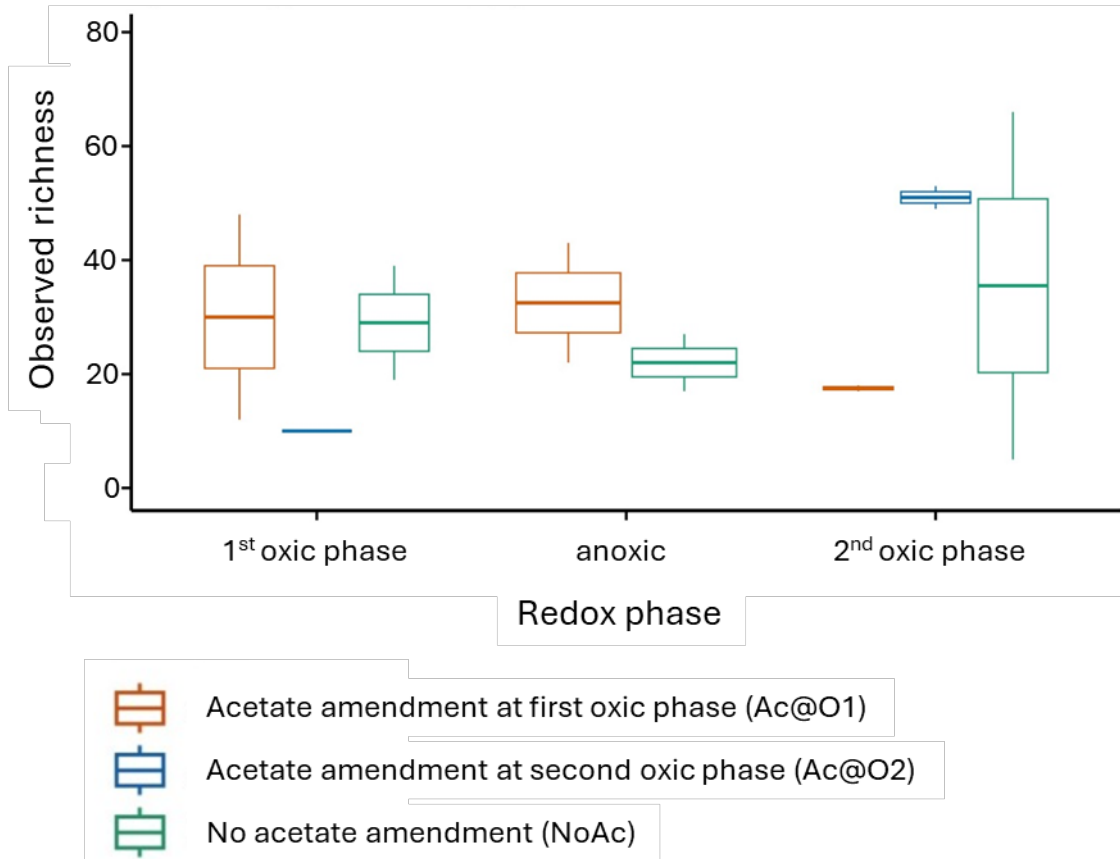
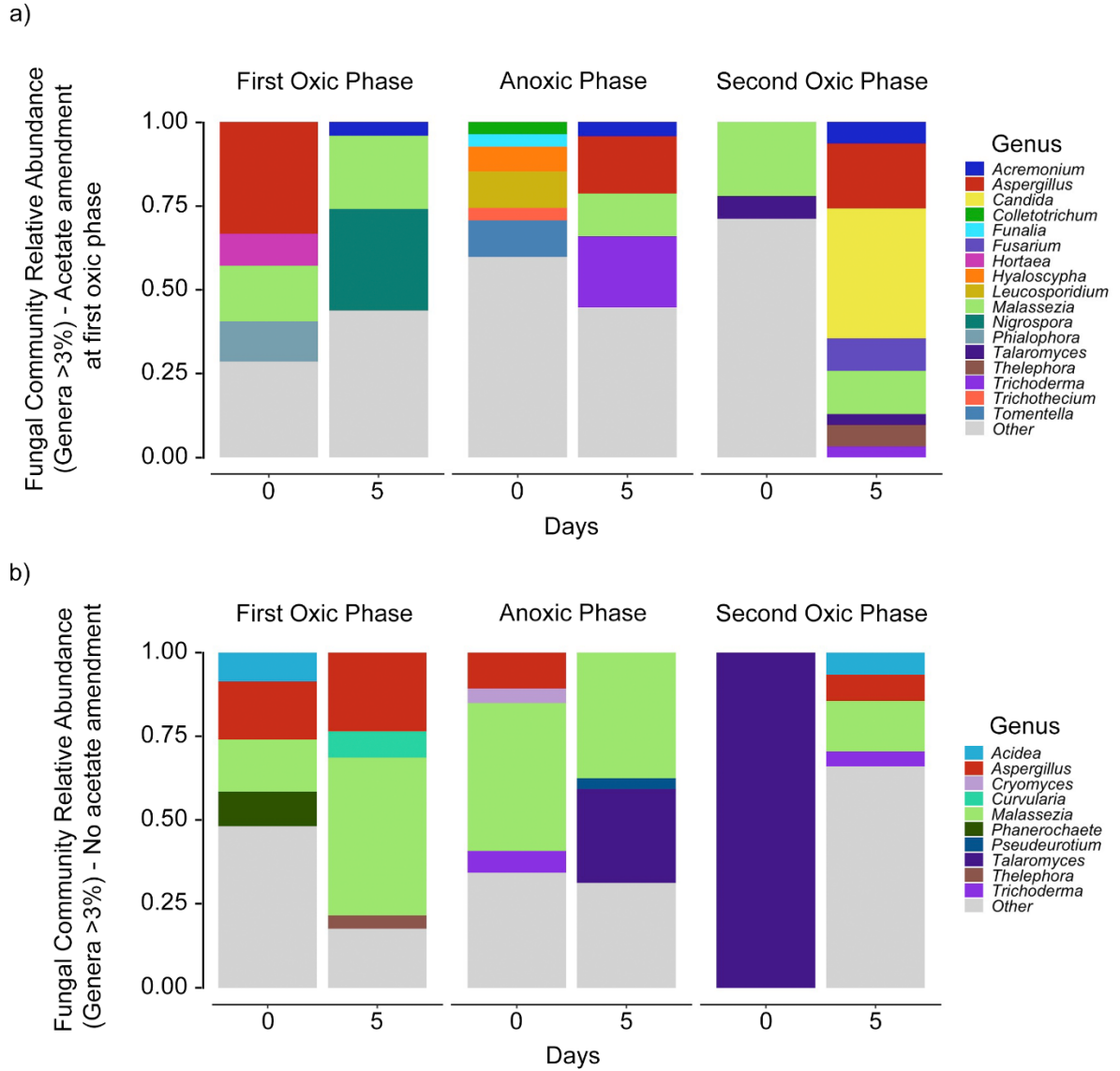


Figure 8. Alpha Diversity (Observed Richness) by Microcosm Phase and Acetate Amendment. Box plots showing the observed richness of microbial communities in microcosm sediments across three redox phases (first oxic phase, anoxic phase, second oxic phase) for different acetate amendment conditions (Ac@O1 - acetate at first oxic phase, Ac@O2 - acetate at second oxic phase, NoAc - no acetate). Note the absence of data for the anoxic phase in the Ac@O2 treatment due to limitations in DNA sequencing quality.



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Table 1. Synthetic River water (SRW) recipe used in microcosm experiments.

Chemical Parameter	Synthetic River Water (mg L⁻¹)
Ca ²⁺	196.3
K ⁺	2.1
Mg ²⁺	10.8
Na ⁺	4.5
SO ₄ ²⁻	520.4
NO ₃ ⁻	0.1
Cl ⁻	1.0
F ⁻	1.3
pH	3.4

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Table 2. Physical chemical parameters of water samples collected at Cement Creek.

Parameter	Unit	Average
Dissolved oxygen	mg L ⁻¹	7.4 ± 0.1
Electrical conductivity	μS cm ⁻¹	1,293 ± 2.9
Dissolved organic carbon	mg L ⁻¹	1.62 ± 0.1
pH	-	3.4 ± 0.1
Alkalinity	mg L ⁻¹ as CaCO ₃	0

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Table 3. Total metal concentrations in the water samples presented as the average of three aqua regia digestions and the standard deviation.

Element	Unit	Average
Al		6.48 ± 0.02
B		0.07 ± 0.002
Ca		190.07 ± 1.50
Fe		7.18 ± 0.12
K		2.43 ± 0.04
Mg	(mg L ⁻¹)	11.03 ± 0.09
Mn		3.39 ± 0.03
Na		4.20 ± 0.05
Sb		0.11 ± 0.003
Si		14.29 ± 0.22
Sr		2.20 ± 0.02
Zn		1.43 ± 0.02
As		0.02 ± 0.001
Cu	(μg L ⁻¹)	25.18 ± 5.81
Ni		167.61 ± 8.38
Pb		5.64 ± 0.14
U		2.13 ± 0.24

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Table 4. Total metal concentrations in the sediment samples presented as the average of three aqua regia digestions and the standard deviation.

Element	Average (mg kg⁻¹)
Al	7128.03 ± 175.2
As	72.38 ± 5.80
Br	522.53 ± 37.6
Ba	24.67 ± 5.8
Ca	1,045.40 ± 173.18
Co	4.1 ± 0.2
Cu	52.65 ± 3.67
Fe	230,442.74 ± 17,076.96
K	821.62 ± 48.04
Li	11.58 ± 0.64
Mg	434.81 ± 28.06
Mn	242.77 ± 10.37
Mo	5.82 ± 0.40
Na	215.07 ± 76.60
Pb	215.09 ± 3.01
Sb	7.85 ± 0.90
Se	26.36 ± 1.44
Si	9.09 ± 1.77
St	54.31 ± 8.00
U	722.13 ± 48.22
V	58.69 ± 3.00
Zn	331.8 ± 203.96