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Study of the Interactions Between Microbial Communities and Clay Minerals in Contaminated Environments: Implications for Bioremediation Processes

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Abstract

The Reconquista River, the second most polluted river in Argentina, accumulates large amounts of organic matter and persistent toxic pollutants in its sediments. These conditions drive the selection of microorganisms with the potential to degrade contaminants and facilitate bioremediation. Notably, these microorganisms form biofilms on clay minerals, enabling them to survive under extreme conditions and alter the properties of the sediments.

This study explores the interactions between microorganisms, contaminants, and clays in degraded sediments, with the goal of improving bioremediation processes, specifically focusing on the bioleaching of heavy metals from contaminated sediments.

Samples were collected from two representative sites in the Reconquista River:

- ❑ Troncos del Talar (TT) (34°27'22.1"S, 58°35'55.3"W): highly contaminated.
- ❑ Dique Roggero (DR) (34°41'0.5"S, 58°51'29"W): used as a control site.

Indigenous bacteria were isolated from TT, including metal-bioleaching species (S-oxidizing and A. ferrooxidans). These bacteria were incubated with clay fractions extracted from TT to promote biofilm formation and assess physicochemical changes in the sediments. Using the data obtained from these experiments, heavy metal bioleaching assays in sediments from the TT area were optimized at the laboratory scale. Various systems were tested, combining different substrate alternatives in culture media and bacterial biofilm formation. The most effective system, achieving the highest percentage of heavy metal extraction, was subsequently scaled up to a pilot-scale adapted air-lift reactor.
Based on the results obtained thus far, the long-term objective is to optimize the bioleaching process by reducing the volume of bioleaching solutions required for metal extraction. Additionally, the goal is to design a system that minimizes energy consumption in the reactor and overall resource utilization, enhancing the sustainability and efficiency of the process.

Conclusions

- ➢ Clear indicators of contamination were identified at the TT site compared to the DR control site, primarily due to high levels of organic matter, sulfides, sulfates, and metals. According to the Canadian Sediment Quality Guidelines, the TT site exceeds established limits for Zn, Cu, and Cr.

- ➢ Interactions between bacterial biofilms and clay fractions demonstrated physicochemical alterations:

  - ▪ XRD Diffractogram: A slight shift in peak position and increased peak width between control clay fractions and those with biofilms may indicate expansion of illite-smectite layers and structural disorder in the mineral.
  - ▪ FTIR Spectrum: Changes observed in peaks corresponding to –OH bonds between control clay fractions and those with bacterial biofilms suggest alterations at the level of surface functional groups in the clays.
  - ▪ Particle size: A reduction in the particle size of the TT clay fraction was observed after contact with bacterial biofilms, which could indicate degradation of clay particles.
  - ▪ SEM images: This technique confirmed the formation of microbial biofilms on clay fractions.

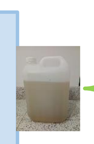
- ➢ Bioleaching assays for heavy metals from TT sediments showed that the most effective system combined both bacterial species (*A. ferrooxidans* and *S-oxidizing bacteria*) with biofilm formation on the substrates used in the growth medium (2K+S growth medium). This system was scaled up to a pilot-scale reactor, achieving higher percentages of heavy metal extraction compared to the laboratory scale.

- ➢ **The bioreactor technology reduced the bioleaching time for heavy metals from contaminated sediments from 250 days to just 30 days compared to previous works by our group. Additionally, this approach minimized the frequency of measurements required during the process, thereby reducing the consumption of reagents associated with monitoring various physicochemical parameters. This advancement contributes to making the bioleaching process more sustainable.**

- ➢ **Managing large volumes of bioleachate, recovering metals from these solutions and identifying sustainable and productive uses for bioremediated sediments pose significant challenges. Future efforts will focus on solving these problems so that bioremediation processes can be greener, and the waste generated can be used as a resource within a circular economy.**

Results

Water samples



TT: **pH:** 7,1; **[SO_4^{2-}]:** 58 mg/L; **Hardness:** 366 g/L; **Turbidity:** 147 NTU; [Cu], [Zn], [Fe], [Cr], [Cd] y [Ni]: <LOQ#

DR: **pH:** 8,3; **[SO_4^{2-}]:** 77 mg/L; **Hardness:** 220 g/L; **Turbidity:** 252 NTU; [Cu], [Zn], [Fe], [Cr], [Cd] y [Ni]: <LOQ#

 # LOQ = Limit of Quantification < 0,1ppm

TT: Contaminated site
DR: Control site

Sediments samples

TT: **pH:** 7,3; **Eh:** -330 mV; **[SVA]*:** 390 mg/kg; **[SO_4^{2-}]:** 2160 mg/kg; **Hum.:** 71%; **Organic matter:** 19,3%; **S_{BET}:** 16.0 m^2/g; **Size$_{(75\% cum.)}$:** 12,53 ±0,63 µm; **Textural composition:** clay silty loam (36% clay, 50% silt, and 14% sand).

DR: **pH:** 8,0; **Eh:** 246 mV; **[SVA]:** 2 mg/kg; **[SO_4^{2-}]*:** 300 mg/kg; **Hum.:** 32%; **Organic matter:** 3.9%; **S_{BET}:** 59.2 m^2/g; **Size$_{(75\% cum.)}$:** 17,39±0,68 µm; **Textural composition:** *clay loam (28% clay,49% silt y 23% sand).*

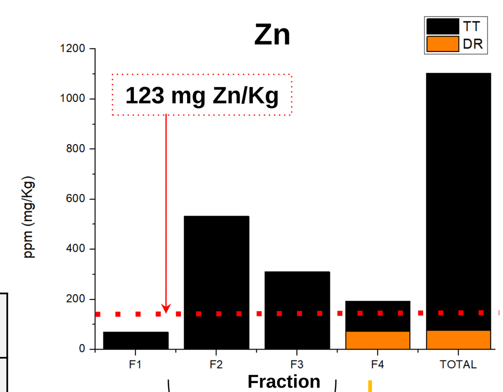
** SVA: Acid volatile sulfide*

SEDIMENTS: Sequential extraction using the BCR method and quantification of Zn, Cr, and Cu by AAS

Permissible limit according to the Canadian sediment quality guideline.**

F1. Weak acid agents; Metals associated with carbonates and silicates
F2. Reducing agents; Metals associated with Fe and Mn oxides
F3. Oxidizing agents; Metals associated with organic matter and sulfides
F4. Residual; Metals associated with structural components of the sediment
TOTAL. F1+F2+F3+F4

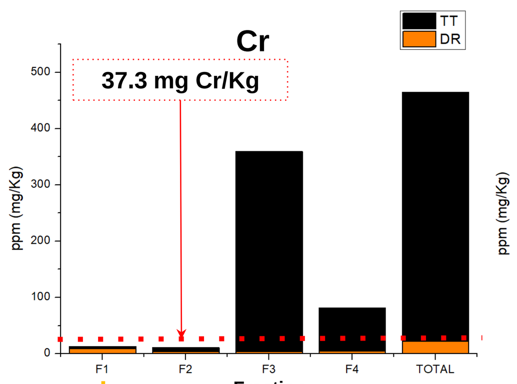
***Canadian Sediment Quality Guidelines for the Protection of Aquatic Life. https://ccme.ca/en/resources/sediment*



TT: Zn is associated with Fe oxides, organic matter, and sulfides.

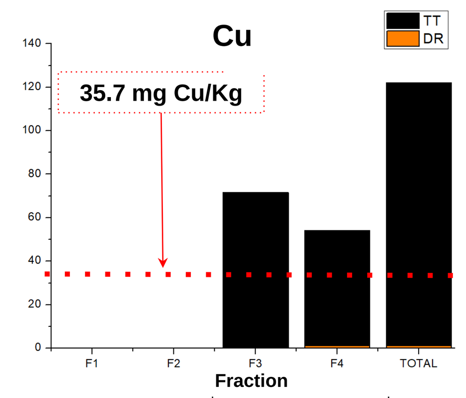
Lithogenic Zn in DR

[Fe (TT)]: 9275 mg/Kg
[Fe (DR)]: 431 mg/Kg



TT: Cr is associated with organic matter and sulfides.

Cr is associated with carbonates in DR.



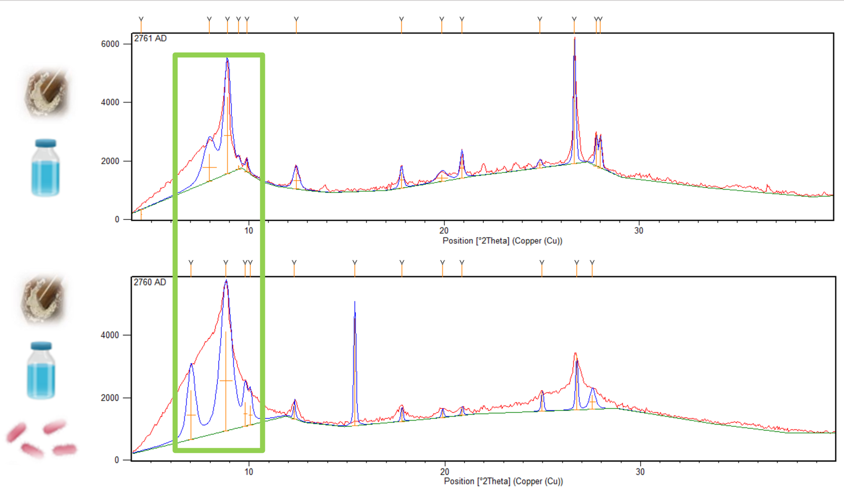
TT: Cu is associated with organic matter, sulfides, and the lithogenic phase.

Interaction Between Clay Fractions and Bacterial Biofilms: Physicochemical Characterization

XRD Diffractogram

Clay Fraction TT + Growth Medium 0K+S (Control)

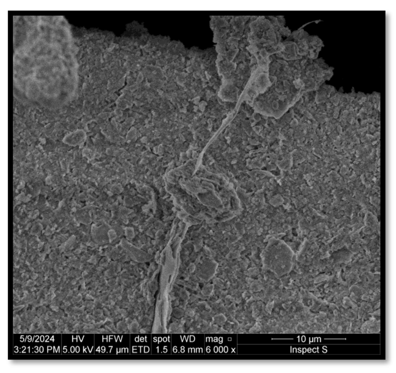
Clay Fraction TT + Growth Medium 0K+S + S-oxidizing bacteria



The signals in the diffractogram between 10 and 14 2-theta suggest a slight shift in the peak positions of illite-smectite in the samples with bacteria compared to the control, as well as a slight increase in peak width.

Similar results were observed for the interaction between the clay fraction TT and *A. ferrooxidans* bacteria.

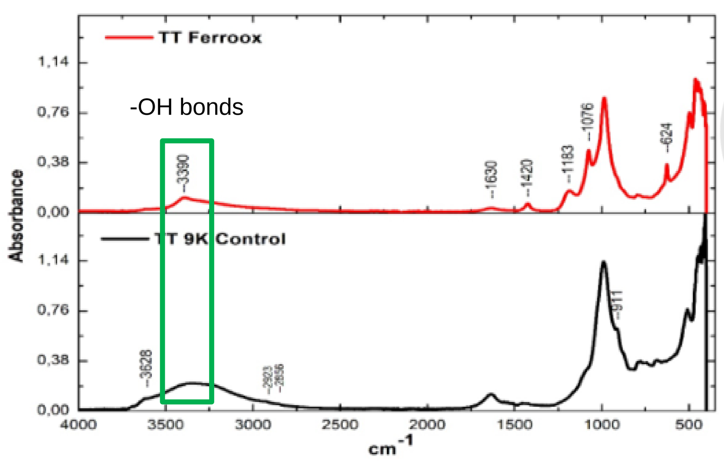
SEM images confirmed the presence of biofilm formation in the clay fraction TT



Particle size analysis

The particle size of the clay fraction TT was reduced from 6.6 to 3.1 µm due to interaction with *A. ferrooxidans*. When in contact with S-oxidizing bacteria, it was reduced from 5.2 µm to 3.8 µm.

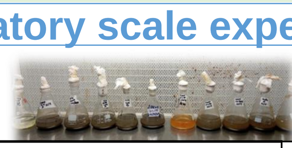
FTIR Spectrum



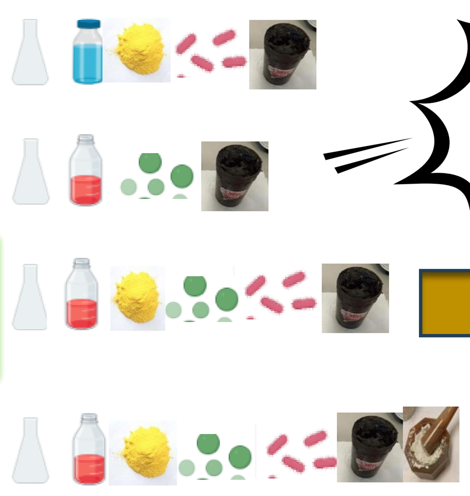
Changes in functional groups of the clay fraction TT due to interaction with *A. ferrooxidans* bacteria. Similar results were obtained for S-oxidizing bacteria.

Bioleaching experiments

Laboratory scale experiments



One-month experiment	% metals bioleached from sediments
0K+S growth medium + S-oxidizing bacteria + TT sediment	Zn: 18% Cu: 29% Cr: 22%
2K growth medium + *A.Ferrooxidans* bacteria + TT sediment	Zn: 19% Cu: 20% Cr: 15%
2K+S growth medium + *A.Ferrooxidans* and S-oxidizing bacteria + TT sediment	**Zn: 25% Cu: 40% Cr: 44%**
2K+S growth medium + *A.Ferrooxidans* and S-oxidizing bacteria + TT sediment + Clay fraction TT	Zn: 20% Cu: 29% Cr: 20%



Pilot plant scale experiment: Bioreactor

% metals bioleached from sediments

Zn: 100%
Cu: 67%
Cr: 44%

Scaling up the system

Adapted air-lift reactor

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