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Microbial Mediated Zinc Removal From Mine Water: A Microcosm Study

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1. INTRODUCTION

The purpose of this work is to examine the process of natural attenuation observed in a mining-impacted stream with circum-neutral pH, the Rio Naracauli creek in the Pb-Zn orefield of Montevecchio-Ingurtosu in SW Sardinia, Italy. Microbial mats induce the precipitation of zinc in form of hydrozincite with also appreciable decrease of dissolved Pb, Cd, Ni and Cu in the stream waters (Podda et al., 2000; Zudda and Podda, 2005).

A bench-scale experiment was designed to study the biogeochemical mechanisms responsible for the observed natural attenuation, using cyanobacteria isolated from white precipitate material collected from the Rio Naracauli creek.

Figure 1. Occurrence of biomineralised mat in Rio Naracauli, Sardinia, Italy. (A) Stretch of stream where biomineralisation occurs in form of hydrozincite precipitation. (B) Detail of white mineralised mat. (C) Leaf encrusted by white precipitate.

2. MATERIAL AND METHODS

Preparation of bacterial culture

A number of stones and leaves were collected from the Rio Naracauli creek in Sardinia, Italy, in June 2007 and kept in a sealed container in the dark at 4ºC. The surfaces of this material were coated in a white precipitate which was used to cultivate a culture of presumptive cyanobacteria, using a 1:10 solution of BG11 medium.

Microcosm setup

Thirty sacrificial batch microcosms (Table 1) were set-up in Ehrlenmeyer flasks containing 150 ml water with chemistry similar to that in-situ at Rio Naracauli (Cl^- 93 mg/l; SO_4^{2-} 745 mg/l; HCO_3^- 27 mg/l; Na^+ 55 mg/l; K^+ 7.5 mg/l; Mg^{2+} 29 mg/l; Ca^{2+} 99 mg/l, Zn 300 mg/l).

Microcosms were stored at 17ºC with gentle agitation, inspected for growth of biofilm twice a week and sampled at intervals to assess microbial growth and hydrozincite precipitation and to assay aqueous chemical speciation.

TABLE 1 Microcosm set-up

Microcosm ID	Description	Contents	Quantity
A1.1, A1.2, A1.3, A1.4, A1.5	Sterile control	Zinc spike, 150 ml synthetic minewater, ~1 g dead leaf matter, cyanobacteria - autoclaved	5
A1.1D, A1.2D, A1.3D, A1.4D, A1.5D	Sterile control (duplicates)	Zinc spike, 150 ml synthetic minewater, ~1 g dead leaf matter, cyanobacteria - autoclaved	5
A2.1, A2.2, A2.3, A2.4, A2.5	Zinc spike - leaf substrate	Zinc spike, 150 ml synthetic minewater, ~1 g dead leaf matter, cyanobacteria	5
A2.1D, A2.2D, A2.3D, A2.4D, A2.5D	Zinc spike - leaf substrate (duplicates)	Zinc spike, 150 ml synthetic minewater, ~1 g dead leaf matter, cyanobacteria	5
A3.1, A3.2, A3.3, A3.4, A3.5	Zinc spike - glass beads	Zinc spike, 150 ml synthetic minewater, ~1 g silica sand, cyanobacteria	5
A3.1D, A3.2D, A3.3D, A3.4D, A3.5D	Zinc spike - glass beads (duplicates)	Zinc spike, 150 ml synthetic minewater, ~1 g silica sand, cyanobacteria	5

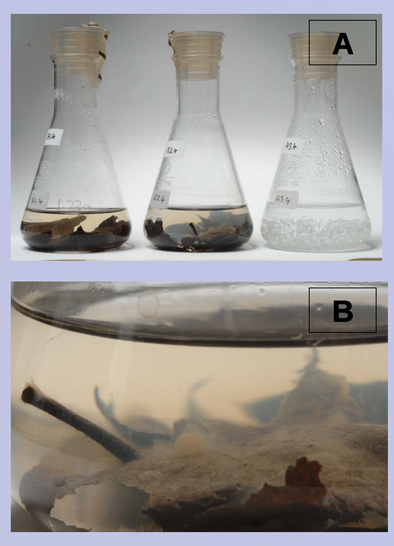

Figure 2. (A) From left to right: Microcosm A1: Sterile control; A2: Cyanobacteria + leaf; A3: Cyanobacteria +silica sand. (B) Detail of filamentous growths observed in A2.

3. RESULTS

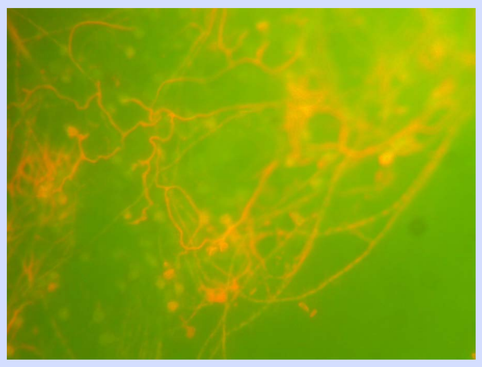

Figure 3. Filamentous bacteria, presumptive cyanobacteria, observed by epifluorescence microscopy in microcosms containing leaf matter (A2).

Epifluorescence microscopy showed that the microcosms containing leaf matter were able to sustain an active population of bacteria for the duration of the experiment (226 days).
The microcosms containing bacteria and silica sand (A3) without the supplement of leaf material were only able to support a much smaller bacterial population in some of the microcosms, without any filamentous bacteria present. The autoclaved cultured flasks, held as a sterile control, did not support any bacterial growth.

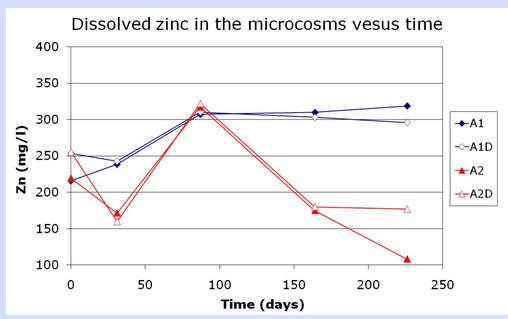

Figure 4. Observed trends of pH and zinc in solution during the microcosm experiment.

Curves showing zinc concentrations in solution versus time in the various microcosms. It is shown that the microcosms containing cyanobacteria with a source of carbon (A2) remove zinc from solution. No zinc decrease is observed in the sterile controls (A1) by the end of the experiment.

Curves showing solution pH versus time in the various microcosms. It is shown a pH increase in the microcosms containing cyanobacteria with a source of carbon (A2), indicative of photosynthetic activity. The pH in the sterile control (A1) microcosms remains mostly below 5.

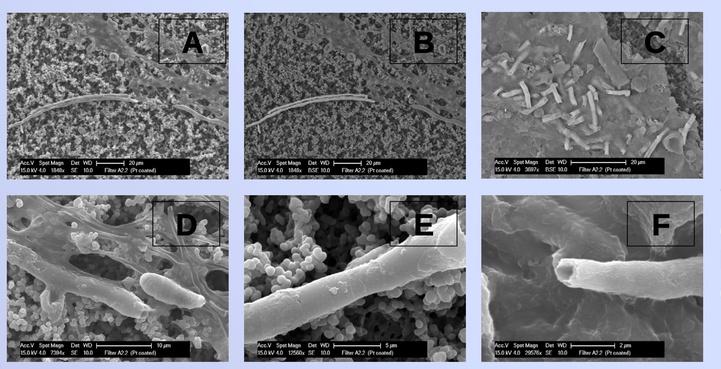

Figure 5. Scanning electron micrographs of the suspended materials from the microcosm flasks.

The SEM analysis shows common fibres, 10 to 50 um long (Fig.5), with brighter contrast in BSE mode (Fig.5B) and rich in zinc and phosphorous (Fig.5G). A tubular morphology of these filaments is shown at greater magnification (Fig.5D-E-F).

Similar shapes have been observed by Podda et al. 2000 and interpreted as inorganic encrustation, made by microspherical particles of hydrozincite, adhering to the bacterial sheath. The additional two control samples do not show any evidence of zinc-containing biological material.

At the end of the experiment dissolved organic matter (NPOC) concentrations (55-45 mg/l) in the cyanobacteria microcosms were significant lower than in the sterile control microcosms (370-180 mg/l), indicating the consumption of dissolved organic matter to support bacterial activity.

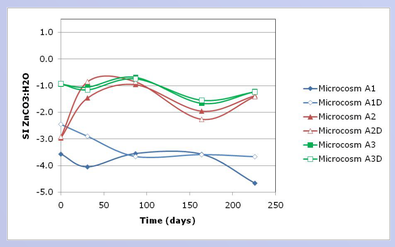

Figure 6. Saturation Indices for $ZnCO_3$:H_2O.

Calculation of saturation indices for a range of common Zn compounds indicate undersaturation, with both low pH and alkalinity limiting precipitation of carbonate phases (Fig.6).
Cell-surface microenvironments with steep gradients of pH and alkalinity, induced by photosynthesis, could drive localised precipitation of zinc carbonates (Fig.7) and, together with absorption onto the cell matrix, account for the observed decrease of zinc in the microcosm solutions.

Fig 7. Biomineralisation of hydrozincite through phytosynthesis

$$HCO_3^- + H_2O \rightarrow (CH_2O) + OH^- + O_2$$
$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$$
$$2CO_3^{2-} + 5Zn^{2+} + 6H_2O \rightarrow Zn_5(CO_3)_2(OH)_6 + 6H^+$$

CONCLUSIONS

The overall results produce more evidence supporting the role of cyanobacteria for bioremediation of metal contaminated waters. Sequential extraction studies are required to further understand the mobility and subsequent stability of metals associated with this type of microbial mats.

REFERENCES: Podda, F., et al., *Heavy metal coprecipitation with hydrozincite [Zn-5(CO3)(2)(OH)(6)] from mine waters caused by photosynthetic microorganisms. Applied And Environmental Microbiology, 2000,66(11): p. 5092-5098.*
Zuddas, P. and F. Podda, *Variations in physico-chemical properties of water associated with bio-precipitation of hydrozincite [Zn-5(CO3)(2)(OH)(6)] in the waters of Rio Naracauli, Sardinia (Italy). Applied Geochemistry, 2005, 20(3): p. 507-517.*