

HYDROZINCITE BIOPRECIPITATION: A PROMISING TOOL FOR BIOREMEDIATION OF WATERS CONTAMINATED BY HARMFUL METALS. HYDROCHEMICAL FACTORS AND MORPHOLOGICAL FEATURES OF THE BIOMINERALIZATION PROCESS

DANIELA MEDAS

Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, Via Trentino 51, 09127 Cagliari, Italy

INTRODUCTION

Since their appearance on the Earth's surface, cyanobacteria play key roles in the biosphere, particularly in the areas of element biogeochemical cycling, mineral transformations and evolution, bioweathering, and soil and sediment formation (Hazen *et al.*, 2008; Hazen & Ferry 2010; Gadd, 2010). Biomineralization refers to the processes by which organisms form minerals. Mineral precipitation mediated by microorganisms can be useful to decontaminate polluted soils and waters (Cutting *et al.*, 2010). In fact, bioremediation is the application of biological systems to the clean-up of organic and inorganic pollution, with bacteria and fungi being the most important organisms for reclamation, immobilization or detoxification of metallic pollutants (Gadd, 2010).

In the Ingurtosu mining district (Sardinia, Italy), harmful metals concentrations in waters of the Naracauli stream, the main stream of the area, are abated by the seasonal bioprecipitation of hydrozincite, $Zn_5(CO_3)_2(OH)_6$. Hydrozincite precipitation (Podda *et al.*, 2000; Zuddas & Podda, 2005) is promoted by a microbial community made up of a filamentous cyanobacterium (*Scytonema* sp.) and a microalga (*Chlorella* sp.). Hydrozincite could be used in a controlled process to attenuate metal pollution in mining waters. Information on chemical and environmental conditions that promote the biomineralization process and the role of bacteria on mineral morphology is fundamental for the development of remediation strategies at contaminated sites. This work aims to investigate the variables controlling the biomineralization process, and the hydrochemical factors that affect hydrozincite precipitation.

STUDY AREA

The Ingurtosu area is located in south-western Sardinia (Italy). It is characterized by Palaeozoic rocks forming a landscape of moderate relief and generally gentle slope. The Naracauli stream flows from Punta Tintillonis at 552 m a.s.l. down to the west, to the Mediterranean Sea. Its pattern is structurally controlled, and receives drainage from four tributaries: Rio Pitzinurri, Rio Sa Roa, Rio Bau, and Rio Sciopadroxiu (Pala *et al.*, 1996). The flow regime is typically torrential, with large flow variations between wet (from October to April) and dry (from May to September) seasons.

The Ingurtosu Pb-Zn deposit (Sardinia, Italy) was exploited for about a century until 1968. Ore deposits are classified as hydrothermal base-metal and industrial-mineral veins, some of which were among the most important mining reserves of Sardinia. The main ore minerals were galena and sphalerite; chalcopyrite and pyrite were present in small amounts. Gangue minerals were quartz and Fe-bearing carbonates (ankerite and siderite; Cavinato & Zuffardi, 1948). Calcite and dolomite were locally found in the gangue. During mining activities, the mining-related waste materials and flotation tailings (Fig. 1) were deposited near the flotation plants or along the valleys, resulting in a large, downstream dispersion of contaminated materials.

MATERIALS AND ANALYTICAL METHODS

Water samples

Water sampling campaigns were carried out in the Naracauli stream catchment area from 2009 to 2011, under different seasonal conditions. Locations of the water sampling stations are shown in Fig. 1. These samples

comprise waters from the Naracauli stream (from NS-100 to NS-5300) and tributaries (D, F, G and H), waters from the Ledoux gallery (E) and waters from tailings drainages (A, B and C). For the investigation of short-term chemical variations in the Naracauli stream, at station NS-420 water sampling was performed over a 45-h period: from h 20:00 to h 17:00 two days later (June 13 - 15, 2011; 14 samples). The station NS-420 was chosen because it is the site characterized by the highest amount of hydrozincite precipitation in the Naracauli stream. Temperature, *pH*, redox potential (Eh), electrical conductivity (EC), alkalinity and dissolved oxygen were measured on site; water was filtered (0.4 μm, Nuclepore 111130), and acidified for metal analyses. Anions were determined by ionic chromatography, and cations by ICP-OES, and/or by ICP-MS. To evaluate the accuracy and precision of trace elements, the SRM 1643e and ES-L-1 standard reference solutions were used. The ionic balance was always less than ±10%. The computer program PHREEQC was used for equilibrium calculations (Parkhurst & Appelo, 1999).

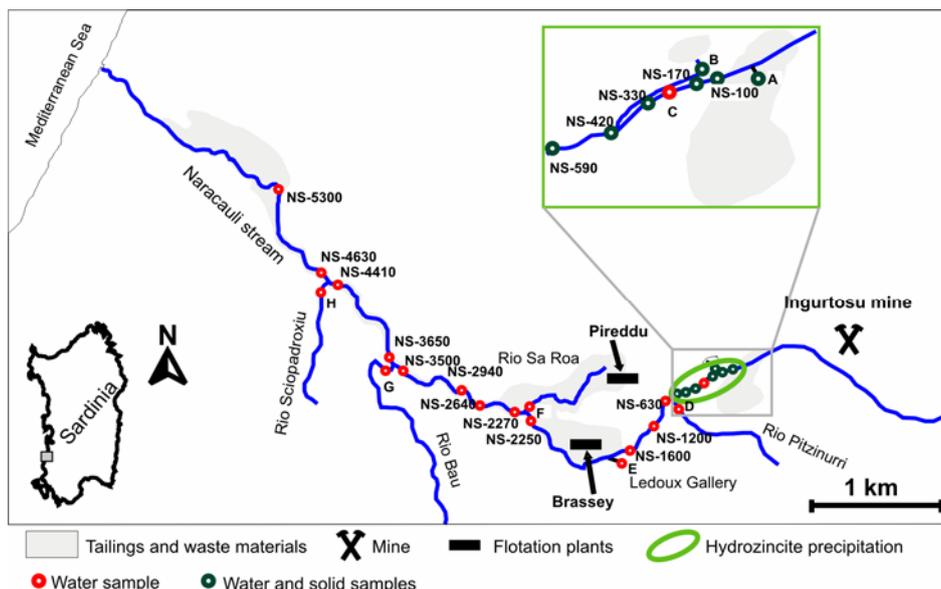


Fig. 1 - Map showing the distribution of mining-related residues (grey areas), the flotation plants (black rectangles), the stations where hydrozincite bioprecipitation occurs (green ellipse), and the location of water (red and green circles) and solid (green circles) samples.

Solid samples

From 2009 until 2011, about 30 hydrozincite samples were collected at several sites in the Naracauli stream (Fig. 1), under different seasonal conditions. The Fig. 2 shows some samples collected from station NS-100 to NS-590, where hydrozincite consists of crusts with variable thickness and colour (white, ochre, green) depending on the amount of iron-oxides and organic matter. Hydrozincite precipitates either on inorganic substrates like stones (Figs. 2a and b) or on organic substrates such as roots (Fig. 2c), leaves and twigs (Fig. 2d).

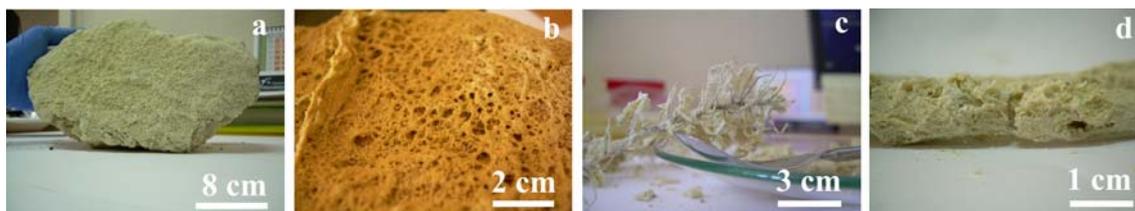


Fig. 2 - Photos of some solid samples collected from station NS-100 to NS-590: a) and b) show hydrozincite precipitated on stones; c) and d) show hydrozincite precipitated on roots and twigs, respectively.

X-ray diffraction (XRD) analysis was performed using conventional θ - 2θ equipment (Panalytical) with Cu K α wavelength radiation ($\lambda = 1.54060 \text{ \AA}$). Elemental characterization of hydrozincite was performed on 0.5 g of each selected sample by acid digestion (for more details see Medas, 2011). Microscopic surface features were investigated using a scanning electron microscope (SEM QUANTA 200, FEI).

RESULTS AND DISCUSSION

Water geochemistry

The Table 1 shows the range of *pH* values, redox potential (Eh), electrical conductivity (EC), total dissolved solids (TDS), and saturation index (SI) with respect to calcite in water samples collected in the Naracauli stream catchment. Stream waters from Naracauli and tributaries, and waters from Ledoux Gallery have *pH* values near neutral to slightly alkaline (6.3-8.4), suggesting a buffering effect of carbonate minerals. Indeed, most waters are at equilibrium or slightly supersaturated with respect to calcite. Drainages from tailings have different characteristics: waters at stations A and B show the lowest *pH* values (6.2-7.0), and are undersaturated with respect to calcite. Waters at station C have *pH* values between 7.0 and 7.6 and are at equilibrium with respect to calcite. Redox potential values (mainly between 0.40 and 0.52 V) indicate oxidizing conditions. TDS varies significantly in the Naracauli stream catchment (0.3-2.8 g/l). The drainages from the tailings (stations A and B) have the highest TDS values (1.2-2.8 g/l); conversely, waters from tributaries (0.3-1.1 g/l) and Ledoux Gallery (0.4-0.5 g/l) show the lowest TDS values. Accordingly, the highest values of TDS in the Naracauli stream are observed upstream of the confluence with the Rio Pitzinurri (see Fig. 1), whereas TDS values decrease downstream due to dilution by less saline tributaries.

The Naracauli stream waters have a dominant Ca-Mg-sulphate composition, reflecting the composition of tributaries, the interaction with tailings banked close to this stream, and inputs from stations A, B, and C (see Fig. 1). Stations A and B, draining mine tailings, are characterized by waters showing a predominant Zn-sulphate composition. Waters at station C (tailing drainage) have a Ca-Mg-sulphate character and lower Zn concentrations, due to a different mineral composition in the waste dump, and because of the possible precipitation of hydrozincite, the saturation limit of which is always exceeded in the water samples at station C ($SI_{\text{hydrozincite}}$ in the range of 1 to 4).

Table 1 - Range of values of *pH*, redox potential (Eh), electrical conductivity (EC), total dissolved solids (TDS), and saturation index (SI) with respect to calcite in water samples collected in the Naracauli stream catchment area.

Type	<i>pH</i>	Eh	EC	TDS	SI_{calcite}
		[V]	[mS/cm]	[g/l]	
Naracauli stream	6.9 - 8.4	0.39 - 0.62	0.72 - 2.19	0.4 - 1.6	0.0 - 1.0
Tributaries	7.0 - 8.3	0.40 - 0.54	0.50 - 1.60	0.3 - 1.1	0.0 - 1.0
Tailings drainages	6.2 - 7.6	0.44 - 0.53	1.43 - 3.28	1.1 - 2.8	-2.0 - 0.5
Ledoux Gallery	6.3 - 8.1	0.44 - 0.46	0.69 - 0.86	0.4 - 0.5	0.1 - 0.6

The maximum Zn concentration in waters from the Naracauli stream catchment area attains several hundreds of mg per litre, whereas Cd and Pb concentrations are in the order of thousands of μg per litre, despite the near neutral to slightly alkaline *pH* values (6.2-8.4). Zn concentration in waters is positively correlated with Pb, Cd, Ni and Co concentrations. The strongest correlation was observed between Zn and Cd, with a constant Zn/Cd ratio (close to 100) among samples that could suggest the weathering of a relatively uniform composition of sphalerite from tailings and mine wastes. Waters from tributaries show the lowest concentrations of contaminants. The highest contents in harmful and toxic elements were observed in waters that drain flotation tailings and mine wastes. Metals concentrations change under different seasonal conditions. The highest

concentrations were observed under high-flow condition (October-April), probably due to the high runoff through the tailings and to aqueous transport of these metals in association with very fine particles, *i.e.* $< 0.4 \mu\text{m}$.

Biom mineralization: X ray diffraction study and chemical analysis

The XRD patterns confirm the dominant presence of hydrozincite in the Naracauli samples with quartz as accessory mineral. Patterns in the biomineral samples from the Naracauli stream show a peak broadening that suggests a lower crystallinity of these materials compared to the reference sample (sample Geo obtained from supergene Zn deposits, Malfidano Mine, Sardinia-Italy). This observation is in agreement with data previously shown by De Giudici *et al.* (2009).

Chemical analysis on hydrozincite samples has shown that the Zn content is in good agreement with the theoretical formula of hydrozincite (Jambor, 1964), notwithstanding the possible presence of other Zn-phases not determined by XRD. Pb, Cd, and Ni are the most abundant metals following Zn. This result is in agreement with previous observations showing that the precipitation of hydrozincite removes not only Zn from waters, but also other metals such as Cd, Pb, Ni and Cu (Podda *et al.*, 2000; Zuddas & Podda, 2005), and it is also consistent with the decrease in metals observed in water along the Naracauli stream.

Biom mineralization: morphological study by optical and scanning electron microscopy analysis

Biom minerals are composite materials containing an organic matrix and nano- or micro-scale amorphous or crystalline minerals (Gilbert *et al.*, 2005). The Fig. 3a shows hydrozincite biomineralization observed by Confocal Laser Microscopy. Cyanobacteria can be recognized as a series of attached cells, often fluorescent, that result in filaments tens of micrometers long. The biomineralization consists of globules, external to the bacterial cells, which attach themselves one to another to form sheaths (Podda *et al.*, 2000). The Fig. 3b shows a hydrozincite sample collected at the beginning of precipitation, whereas the Fig. 3c shows hydrozincite collected several days after precipitation. In the first case, biomineral sheaths form a thin network that can be associated to the initial stage of the development of the biofilm. In the second case a three-dimensional structure is observed, that can be associated to the mature state of the biofilm. Larger magnification (Fig. 3d) shows that hydrozincite precipitates around organic matter filaments forming globules. Larger magnification (Fig. 3d) shows that hydrozincite precipitates around organic matter filaments forming globules.

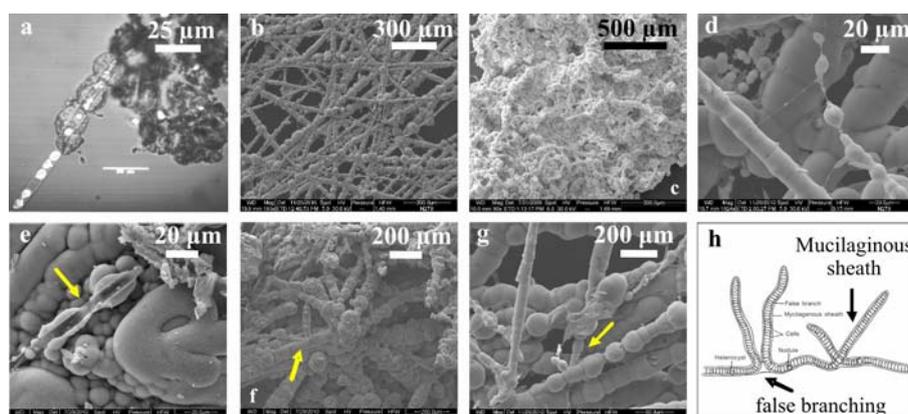
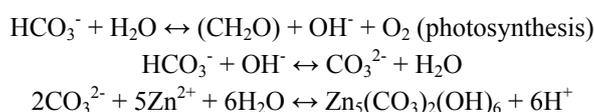


Fig. 3 - a) Image collected by Confocal Laser Microscopy in the transmission mode. Biomineralization forms globules external to the bacterial cells (by Podda *et al.* 2000, modified); b-g) Images collected by SEM; b: hydrozincite sample collected at the beginning of precipitation; c: hydrozincite sheaths form a three-dimensional structure; d: hydrozincite precipitates around organic matter filaments forming globules; e: a sheath section, showing how the biominerals encrust bacterial filament following its shape (yellow arrow); f and g: branches in the hydrozincite sheath are indicated by the yellow arrow. These branches result from false branching of the *Scytonema* sp. h) False branching in *Scytonema* sp. (by Margaret & Angela, 2007, modified).

To investigate the bacterial control on the hydrozincite shape and to assess if environmental conditions have any influence on biomineralization morphology, a morphological study was carried out on the Naracauli hydrozincite (Medas *et al.*, 2012a and b). The role of bacteria on the hydrozincite shape can be recognized in Figs. 3e-g; the Fig. 3e shows a sheath section, where the biomineral encrusts bacterial filament following its shape whereas in the Figs. 3f and g, the presence of a branch on the hydrozincite sheaths is clearly observed. *Scytonema* sp. is characterized by its unique type of branching known as false branching (Fig. 3h). The filament breaks up near a heterocyst and the broken end protrudes out of the sheath as a branch (Rajan, 2001). This typical shape is preserved also after the bioprecipitation of hydrozincite around the bacterial filaments, as shown in the Figs. 3f and g.

More detailed investigations were carried out on globules and sheaths, measuring the diameter distribution in some hydrozincite samples collected in 2009 during different seasonal periods. Hydrozincite samples collected in spring are characterized by globules having a diameter between 20 and 40 μm , with a mean diameter of 30 μm . In contrast, hydrozincite samples collected in late summer are characterized by few short-length sheaths, whose globules show a greater variability in diameter. About 90% of globules measured range between 30 and 80 μm , with a mean diameter of 50 μm . Considering influence of water flow, it was observed that hydrozincite sheaths precipitated under low flow condition have more or less a constant diameter, whereas under high flow conditions sheaths are very large and become thinner at the final ends.

Podda *et al.* (2000) described hydrozincite biomineralization as an epicellular biomineralization, considering that under natural conditions microorganisms are characterized by electronegative surfaces that can bind Zn^{2+} . Simultaneously, the CO_2 fixation from dissolved HCO_3^- and release of OH^- during photosynthesis lead to the shift of carbonate species equilibrium. This process results in a local oversaturation with respect to hydrozincite around the bacteria. The following reactions were proposed:



An *in vitro* investigation (De Giudici *et al.*, 2007), using cyanobacteria and water collected at Naracauli, showed different morphological and crystallinity characteristics between natural and *in vitro*-formed hydrozincite. For a significant hydrozincite precipitation to happen *in vitro* the presence of live cyanobacteria was required while cyanobacterial lysate or cyanobacterial exopolymers did not induce an effective hydrozincite biomineralization. These observations suggest a clear control of the *Scytonema* sp. both on hydrozincite precipitation and biomineral morphology in response to different environmental conditions. De Giudici *et al.* (2009) confirmed that Naracauli hydrozincite is a Biologically Controlled Mineralization (BCM), and revealed, by NMR analysis, the presence of peaks from organic biopolymers, most likely exopolysaccharides (EPS). It is in fact known that many cyanobacteria, including *Scytonema* sp., produce mucilaginous external layers (Li *et al.*, 2001) mainly made up of EPS. EPS has been shown to protect against solar radiation, dehydration, and phagocytosis and is a key component of biofilms, helping in maintaining their structural integrity (Phoenix & Konhauser, 2008). EPS filaments are clearly visible in SEM photos of Naracauli hydrozincite (see Figs. 3d, e, and g).

The composition and quantity of bacterial exopolysaccharides can change depending on natural conditions (De Carvalho & Fernandes, 2010). Taking into account the potential benefits and detrimental side effects of biominerals, they conspicuously appear to parallel those afforded by EPS (Phoenix & Konhauser, 2008). For example, Phoenix *et al.* (2001) revealed that mineralized bacteria, isolated from the Krisuvik hot spring (Iceland) have a marked resistance to UV compared to non-mineralized bacteria. Biomineralization is useful to attenuate the harmful wavebands of UV radiation and, simultaneous bacterial photosynthesis is not interrupted. The larger diameter observed for hydrozincite biomineralization collected in late summer can be

ascribed to a difference in the production of external mucilage sheath by cyanobacteria colonies in response to stress conditions (Li *et al.*, 2001).

Another control exerted by *Scytonema* sp. on the biomineralization is revealed by variations of hydrozincite morphology as a function of the water flow. All hydrozincite sheaths precipitated under low flow conditions have more or less a constant diameter, whereas under high flow conditions sheaths become thinner at the final ends. Purevdorj *et al.* (2002) showed that hydrodynamics influences structure and behaviour of the biofilms. In fact, biofilms grown under turbulent flow form filamentous streamers. The observed differences in hydrozincite shape can be ascribed to the influence of hydrodynamics on the structure of the biofilm and, consequently, on biomineral shape.

Variations in water geochemistry and optimum conditions of bioprecipitation

To investigate the variations in water chemical characteristics induced by hydrozincite precipitation, Zn content, alkalinity, pH, and hydrozincite SI have been plotted against distance in the part of the Naracauli stream where biomineralization occurs. Fig. 4a shows that Zn concentration decreases continuously from station NS-100 to station NS-590. This behaviour is the result of: *i*) the precipitation of hydrozincite, and *ii*) the dilution effect caused by the inflow of seep water (station C, see Fig. 1) characterized by high alkalinity and poor concentrations in zinc, as compared to waters from the Naracauli stream. Specifically, alkalinity in the Naracauli stream waters decreases from station NS-100 to station NS-170 (Fig. 4a) because of hydrozincite precipitation; between station NS-170 and station NS-330 the alkalinity increases because of alkaline seepage water (station C); further downstream alkalinity decreases because of hydrozincite precipitation. Increase in pH, observed in Fig. 4b, can be ascribed to the photosynthetic process due to the presence of cyanobacteria that releases OH⁻ in solution (Zuddas & Podda, 2005). In general, the highest quantity of bioprecipitation occurs at stations NS-420 and NS-590, accordingly, the highest values of hydrozincite SI are observed at these sites (Fig. 4b).

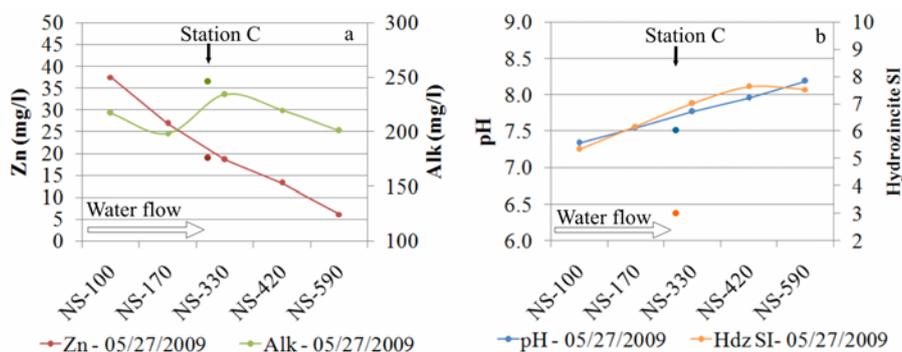


Fig. 4 - Variation in Zn concentration, alkalinity, pH and hydrozincite SI in waters where hydrozincite precipitates. Values from station C are also indicated.

The Fig. 5 shows the relation between Zn²⁺/CO₃²⁻ molar ratio and the SI with respect to hydrozincite. The Zn²⁺/CO₃²⁻ values in waters collected from March to November 2009 varied from 1 to 2100. The lowest values, between 1 and 10, were observed in May and June at sites where hydrozincite was more abundant (NS-420 and NS-590) and hydrozincite SI reached the highest values. After heavy rainfall, Zn²⁺/CO₃²⁻ values in the Naracauli stream waters increased up to 400 in spring and up to 2100 in autumn. Contrastingly, hydrozincite SI values decrease after rain events. This behaviour can be explained taking into account the effects of runoff flowing into the stream: high runoff carries high Zn contents which are leached from the mining-related wastes, and the acidic contribution of rain water causes the decrease in carbonate ions. Moreover, the Fig. 5 shows inverse relation between pH values and Zn²⁺/CO₃²⁻ molar ratio, probably reflecting a higher Zn mobility at lower pH. The

highest pH , the highest values of hydrozincite SI, and lowest Zn^{2+}/CO_3^{2-} values were observed in waters collected at stations NS-420 and NS-590 in late spring.

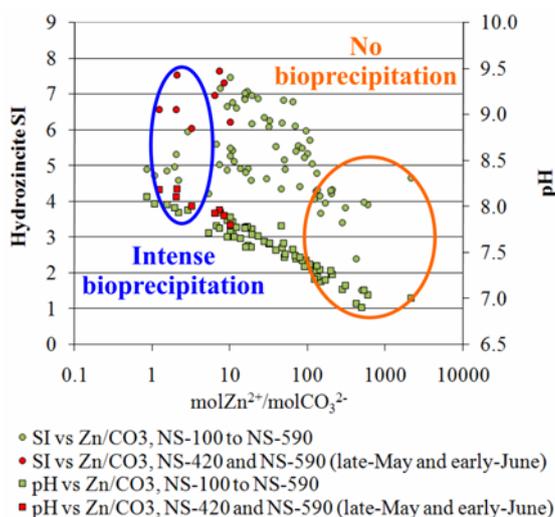


Figure 5 - Plot showing hydrozincite SI vs. Zn^{2+}/CO_3^{2-} molar ratio, and pH vs. Zn^{2+}/CO_3^{2-} molar ratio in samples collected during 2009. Stations NS-420 and NS-590 are the stations of more efficient biomineralization.

Diel cycles in dissolved metals concentrations in the Naracauli stream

Diel cycles in dissolved Zn, Co, Ni and Mn were found to occur in a selected station along the Naracauli stream. The highest change in concentration was observed for Zn: the difference between the minimum (3 mg/l) and maximum (4.7 mg/l) Zn concentrations is 1.7 mg/l (about 35%). The minimum values occurred at h 17:00 and maxima between h 02:00 and h 05:00. The timing of diel cycles in Co and Ni is very similar to that for Zn, but the ranges of Co and Ni concentrations are much smaller than Zn. Increased nocturnal concentrations could result from a combination of geochemical and biological processes. Considering the relations among temperature, pH , and Zn contents, temperature and pH would seem to be the parameters that control variations in Zn concentration. Water temperature shows a well defined diurnal cycle. Maximum water temperature was observed between the h 13:00 and h 17:00. Water temperature varies due to change in air temperature and incident solar radiation. The pH values vary between 7.7 and 8.1; the highest values were observed during the sunny hours and the lowest during the night or early morning. The diel pH cycle derives from photosynthesis (predominant during the day) and respiration (predominant during the night). Obtained results may be explained by adsorption-desorption reactions (onto colloids, carbonates, bacterial surfaces and biofilms) and/or different rates of mineral precipitation between the morning and the night time. Diurnal metal cycles should be taken into account to evaluate environmental conditions, potential risks and cleanup of contaminated sites.

CONCLUSIONS

The innovative approach of this work was to combine geochemical and mineralogical investigations with biological information, specifically on the structure and development of biofilm under different water chemistry and flow conditions. Information on variables that control the biomineralization process is fundamental for the development of bioremediation strategies. This work will lay a solid foundation for designing future bioremediation methodologies for waters influenced by mining activities. In fact, the obtained results suggest that bioprecipitation of hydrozincite could be applied to attenuate metal pollution in mining environments where water acidification do not occur. According to field observations, correlated with speciation and equilibrium calculations, the optimum condition for hydrozincite precipitation occurs in late spring of rainy years, when the hydraulic regime in the stream reaches stationary conditions, and SI with respect to hydrozincite, and pH reach the highest values, in agreement with the higher stability of the hydrozincite solid phase in contact with slightly alkaline waters. Concomitantly, Zn^{2+}/CO_3^{2-} molar ratio reaches values close to 1, indicating that kinetic

processes have a role on the hydrozincite biomineralization process. Conversely, heavy rain events occurring in late spring appear to inhibit biomineralization, likely due to the decrease in the SI values resulting from the dilution effect of rain water.

The microbial inoculation together with the alterations and control of hydrochemical parameters may be an attractive approach for *in situ* bioremediation providing the achievement of the necessary conditions for hydrozincite precipitation are achieved.

Future research should be performed to verify if diel cycles are affected by seasonal conditions, and to clarify microbiological seasonal dynamics in the Naracauli stream, specifically the conditions that favour *Scytonema* sp. predominance, together with an appropriate understanding of microbe-metal-related reactions. This information will help to assess the best conditions to develop bioremediation techniques, and could allow for optimization of the biomineralization process by controlling the physico-chemical conditions of the contaminated area.

REFERENCES

- Cavinato, A. & Zuffardi, P. (1948): Geologia della miniera di Montevecchio. *In*: “Notizie sull’industria del Piombo e dello Zinco in Italia”, Montevecchio Società Italiana del Piombo e dello Zinco, **1**, 427-464.
- Cutting, R.S., Coker, V.S., Telling, N.D., Kimber, R.L., Pearce, C.I., Ellis, B.L., Lawson, R.S., Van Der Laan, G., Patrick, R.A.D., Vaughan, D.J., Arenholz, E., Lloyd, J.R. (2010): Optimizing Cr(VI) and Tc(VII) remediation through nanoscale biomineral engineering. *Environ. Sci. Technol.*, **44**(7), 2577-2584.
- De Carvalho, C.C.C.R. & Fernandes, P. (2010): Production of Metabolites as Bacterial Responses to the Marine Environment. *Mar. Drugs*, **8**, 705-727.
- De Giudici, G., Podda, F., Caredda, A., Tombolini, R., Casu, M., Ricci, C. (2007): In vitro investigation of hydrozincite biomineralization. *In*: “Water Rock Interaction 12”, T.D. Bullen & Y. Wang, eds. Taylor & Francis, London, **2**, 415-419.
- De Giudici, G., Podda, F., Sanna, R., Musu, E., Tombolini, R., Cannas, C., Musinu, A., Casu, M. (2009): Structural properties of biologically controlled hydrozincite: An HRTEM and NMR spectroscopic study. *Am. Mineral.*, **94**, 1698-1706.
- Gadd, G.M. (2010): Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology*, **156**(3), 609-643.
- Gilbert, P.U.P.A., Abrecht, M., Frazer, B.H. (2005): The Organic-Mineral Interface in Biominerals. *In*: “Molecular Geomicrobiology”. J.F. Banfield, K.H. Nealson & J. Cervini-Silva, eds. Mineral. Soc. Am., Washington D.C. *Rev. Mineral. Geochem.*, **59**, 157-185.
- Hazen, R.M. & Ferry, J.M. (2010): Mineral evolution: Mineralogy in the fourth dimension. *Elements*, **6**(1), 9-12.
- Hazen, R.M., Papineau, D., Bleeker, W., Downs, R.T., Ferry, J.M., McCoy, T.J., Sverjensky, D.A., Yang, H. (2008): Mineral evolution. *Am. Mineral.*, **93**, 1693-1720.
- Jambor, J.L. (1964): Studies of basic copper and zinc carbonates. 1. Synthetic zinc carbonates and their relationship to hydrozincite. *Can. Mineral.*, **8**, 92-108.
- Li, P., Harding, S.E., Liu, Z. (2001): Cyanobacterial exopolysaccharides: their nature and potential biotechnological applications. *Biotechnol. Genet. Eng. Rev.*, **18**, 375-404.
- Margaret, E. & Angela, G. (2007): Algae. *In*: “Practical Manual for Botany”, New Age International Pvt. Ltd., **1**, 1-26.
- Medas, M. (2011): Hydrozincite bio-precipitation: a promising tool for bioremediation of waters contaminated by harmful metals. Hydrochemical factors and morphological features of the biomineralization process. PhD Thesis, Univ. of Cagliari.
- Medas, D., Cidu, R., Lattanzi, P., Podda, F., De Giudici, G. (2012a): Natural Biomineralization in the Contaminated Sediment-water System at the Abandoned Mine of Ingurtosu. *In*: “Bio-Geo Interactions in Metal-Contaminated Soils”, E. Kothe & A. Varma, eds. Springer-Verlag, Berlin Heidelberg, *Soil Biol.*, **31**, 113-130.
- Medas, D., Cidu, R., Lattanzi, P., Podda, F., Wanty, R.B., De Giudici, G. (2012b): Hydrozincite seasonal precipitation at Naracauli (Sardinia-Italy): hydrochemical factors and morphological features of the biomineralization process. *Appl. Geochem.*, in press, doi: 10.1016/j.apgeochem.2012.02.016.
- Pala, A., Costamagna, L.G., Muscas, A. (1996): Valutazione delle riserve idriche nei bacini dei Rii Piscinas e Naracauli (Sardegna Meridionale). *Boll. Soc. Geol. Ital.*, **115**, 717-735.
- Parkhurst, D.L. & Appelo, C.A.J. (1999): User’s guide to PHREEQC (version 2) - A computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations. U.S. Geol. Surv. Water-Resources Investigations Report, 99-4259.

- Phoenix, V.R. & Konhauser, K.O. (2008): Benefits of bacterial biomineralization. *Geobiology*, **6**(3), 303-308.
- Phoenix, V.R., Konhauser, K.O., Adams, D.G., Bottrell, S.H. (2001): Role of biomineralization as an ultraviolet shield: implications for Archean life. *Geology*, **29**(9), 823-826.
- Podda, F., Zuddas, P., Minacci, A., Pepi, M., Baldi, F. (2000): Heavy metal coprecipitation with hydrozincite $[\text{Zn}_5(\text{CO}_3)_2(\text{OH})_6]$ from mine waters caused by photosynthetic microorganisms. *Appl. Environ. Microbiol.*, **66**(11), 5092-5098.
- Purevdorj, B., Costerton, J.W., Stoodley, P. (2002): Influence of Hydrodynamics and Cell Signaling on the Structure and Behavior of *Pseudomonas aeruginosa* Biofilms. *Appl. Environ. Microb.*, **68**(9), 4457-4464.
- Rajan, S.S. (2001): Introduction to algae, 1st Ed. Anmol Publications Pvt. Ltd., New Delhi, 279 p.
- Zuddas, P. & Podda, F. (2005): Variations in physico-chemical properties of water associated with bioprecipitation of hydrozincite $[\text{Zn}_5(\text{CO}_3)_2(\text{OH})_6]$ in the waters of Rio Naracauli, Sardinia (Italy). *Appl. Geochem.*, **20**(3), 507-517.