INTERACTIONS BETWEEN MINERALS, CONTAMINANTS, AND MICROORGANISMS IN ACID MINE DRAINAGE SETTING

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INTRODUCTION

In sulphide or coal mining areas the weathering of sulphides caused by the atmospheric agents and microorganisms generates an important acidification of waters (even to negative pH values), a massive release of transitional and heavy metals in the aquatic and pedogenetic systems, and an extensive precipitation of secondary minerals, mainly as oxidation crusts, efflorescent salts, and colloidal precipitates (Blowes *et al.*, 2014). The acidic and metal polluted solutions are known as Acid Mine Drainage (AMD), and are considered as one of the main pollutants of water in many countries that have historic or current mining activities (Simate & Ndlovu, 2014). The process of AMD generation is extremely complex since it involves chemical, biological, and electrochemical reactions, which vary with environmental conditions. The extension of the impacted area depends on a combination of many physical, chemical, and biological factors (Weisener, 2003).

The role played by secondary minerals in the immobilisation of inorganic pollutants (*i.e.*, metals and metalloids) is crucial from an environmental point of view. Many factors, such as water chemistry, primary mineral assemblage, temperature, and moisture content influence the mineralogy and chemistry of secondary minerals (Nordstrom & Alpers, 1999).

Secondary minerals can scavenge elements from solutions in different ways, such as structural incorporation, sorption, or coprecipitation, basically depending on their crystal structure, thermodynamic constant, surface charge, and other factors. AMD varies significantly from site to site, and spans a wide range of pH, temperature and oxygen content. Such variations inevitably mean that a variety of acidophilic microorganisms, such bacteria, fungi, and algae, with different physiological properties can be found in AMD. These microorganisms can play a role both in the mobilisation of contaminants from minerals or in their immobilisation, through processes such as biomineralisation, bioaccumulation, and biosorption. It is possible to state that the interactions between minerals, contaminants, and microorganisms in AMD play a key role in determining the dispersion of the inorganic contaminants in the surrounding environments.

The studied area is the abandoned Cu mine of Libiola (North-West Italy), where the alteration of sulphides provokes intense processes of AMD, leading to the circulations of very acidic (pH down to 2.6), metal-rich (Electrical Conductivity up to 8000 µS) solutions (Marini *et al.*, 2003). In particular, on the basis of their pH and Eh values, mine waters of the Libiola area were divided in Red Waters (high Eh-very low pH), Intermediate Waters (high Eh-low pH), and Blue Waters (low Eh-high pH). From these waters, a wide range of minerals, mainly colloidal precipitates, form (Carbone *et al.*, 2013). While several minero-chemical studies have led to a good characterisation of the precipitates occurring in the mine area, few investigations on microbiota have been carried out in the Libiola mine area. Plants received more attention (Marsili *et al.*, 2009; Roccotiello *et al.*, 2010), whereas among microorganisms the most studied were macro- and micro-fungi, especially in waste-rock dumps (Marescotti *et al.*, 2013; Zotti *et al.*, 2014).

The aim of this thesis was to characterise the relationships between minerals, metals, and microorganisms in the colloidal precipitates of the Libiola mine site, as minerals within colloidal precipitates play a key role in the mobility of metals in the surrounding ecosystems for their capacity to retain metals in the solid fraction. The role of microorganisms, which has been highlighted in waste-rock dumps in the Libiola mine, has never been studied before in the colloidal precipitates. In order to fill this gap of knowledge, a characterisation of microorganisms in the main precipitate types has been carried out.

MATERIALS AND METHODS

Materials

At the Libiola mine site, the Eh-pH conditions of mine waters lead to the formation of several different precipitates, which represent a continuous series. With rising pH values, the precipitation of Fe-rich (2.4 < pH < 4.4), Al-rich (4.5 < pH < 6.7), and Cu-rich precipitates (6.4 < pH < 7.5) takes place. Five different groups of colloids were distinguished, basing on their colour and mine waters pH. Fe-rich precipitates are divided in ochreous precipitates from red waters (2.4 < pH < 2.8) and ochreous precipitates from intermediate waters (3.7 < pH < 4.4). Al and Cu-rich colloids were instead classified as milky-white precipitates from intermediate waters (4.5 < pH < 6.7) and greenish-blue precipitates from blue waters (6.4 < pH < 7.5), respectively. The last group of precipitates was named "mixing precipitates", which are generated by the confluence of AMD in the uncontaminated, weakly alkaline Gromolo Torrent (≈ 8), the main watercourse of the area. Moreover, synthetic samples of woodwardite, one of the minerals of greenish-blue precipitates, were obtained and their affinity towards Rare Earth Elements (REEs) tested, as in greenish-blue precipitates anomalously high concentrations of such elements were measured.

The microorganisms studied were bacteria, microfungi, and diatoms, each isolated from the colloidal precipitates. Bacteria were identified by means of PCR on the 16s gene, whereas microfungi were isolated and identified by the morphology of their colony and by DNA. Finally, diatoms were identified at the microscope.

Methods

All the precipitate samples were characterised by X Ray Diffraction (XRD), InfraRed (IR) and Mössbauer (MS) spectroscopy, ThermoGravimetry-Differential Thermal Analysis (TG-DTA), and Inductively Coupled Plasma (ICP) analyses. Selected samples underwent further analyses. Bulk Leaching Tests (BLT) and Sequential Extraction (SE) were applied in order to understand the possibility that contaminants could be remobilised from the precipitates. When possible, the metal uptake methods and release potentials were determined for all the precipitates. Two different and complementary SE procedures, Modified BCR and DOLD (Dold, 2003; Rauret *et al.*, 1999), were used. Synchrotron light techniques, as X-ray Absorption Spectroscopy (XAS) and Pair Distribution Function (PDF), were also used on amorphous to low-crystallinity materials (especially Al-, Cu-rich precipitates, and mixing precipitates) at the European Synchrotron Radiation Facility (ESRF) in order to obtain information on the relationships between minerals and contaminants.

Synthetic woodwardite was prepared using a coprecipitation method derived from the synthesis of synthetic analogues of hydrotalcite (Reichle, 1986) and zincowodwardite (Ardau *et al.*, 2012). The doping of this phase with selected Light REE (La, Ce, and Nd) and Heavy REE (Y and Gd) was also performed in order to study the relationships between REEs and woodwardite.

Selected microfungi strains, isolated from greenish-blue precipitates, were tested for Cu and Zn tolerance and bioaccumulation. For these tests, several $5 \cdot 10^8$ per mL conidial inocula suspensions (20 mL deionised water and 10 μL of tween 80) were prepared by scraping the surface of 14 days old cultures with a loop using Burker Chamber. After the tolerance tests, on two strains belonging to the genera *Penicillium* and *Trichoderma*, XAS spectra at the Cu k-edge were collected in order to study the speciation of this metal.

RESULTS AND DISCUSSIONS

Mineralogical and chemical characterisation

The chemical state of the system (*i.e.*, Eh-pH values) deeply influences the mineralogy of the authigenic phases. In particular, it is possible to conclude that the higher the pH values, the higher the concentrations of metals other than Fe, which on the contrary tend to decrease with increasing pH.

Ochreous precipitates from red waters are formed mainly by goethite (FeOOH) + jarosite $[KFe_3(SO_4)_2(OH)_6]$ or schwertmannite $[Fe_8O_8(OH)_{8-x}(SO_4)_x]$. The concentrations of contaminants in these precipitates are quite low, with only Cu reaching significant concentrations (up to 2812 mg kg^{-1}). Ochreous

precipitates from intermediate waters are amorphous materials, different from ferrihydrite, which is the usual amorphous Fe phase reported from AMD (Jambor & Dutrizac, 1998). The concentration of trace elements slightly rose, with Cu reaching 4893 mg kg^{-1}.

Milky-white precipitates are formed by allophane [$Al_2O_3(SiO_2)_{1.3-2} \cdot 2.5-3H_2O$] or basaluminite [$Al_4(SO_4)(OH)_{10} \cdot nH_2O$], with mixtures of these two amorphous to low-crystallinity minerals which could not be excluded. The bulk chemistry is dominated by Al and Si. Compared to ochreous precipitates, Al-rich precipitates are characterised by generally higher concentrations of Cu (reaching > 1 wt.%), Zn, and REEs, whereas they have lower or comparable amounts of elements such as Mn, Ni, Co, Cd, Pb, V, As, and Sb.

Greenish-blue precipitates mineralogy varies from samples completely composed by allophane to samples with (hydro)woodwardite [$Cu_{1-x}Al_x(SO_4)_{x/2}(OH)_2 \cdot nH_2O$]. The bulk chemistry is dominated by Al, Cu, and Si, but also Zn reaches important concentrations (up to more than 1 wt.%), along with Cd and REEs.

Finally, mixing precipitates were composed by completely amorphous materials, with small amounts of cuprite (Cu_2O). XRD diagram and chemical analyses showed that this amorphous phase is different from that occurring in ochreous precipitates from intermediate waters. Thermal analyses (TG-DTA) showed the probable presence of some structural OH, suggesting that ferrihydrite should not be taken into account.

The bulk chemistry is dominated by Fe, followed by concentrations in the order of several percentages of Al and Cu. Among trace elements, Zn is the only one reaching important concentrations, in the order of thousands of mg kg^{-1}. Rietveld refinement with the addition method (Balić-Žunić, 2002) showed that mixing precipitates were detectable in significant concentrations in the superficial sediments (first 2 cm) of the Gromolo Torrent, downstream from the mine area. In particular, mixing precipitates were constantly around 90% in the sampling points near the mine area and were also present in high concentration (57%) at the mouth of the torrent in the Ligurian Sea. These precipitates deeply influence the bulk chemistry of the superficial sediments of the torrent, which are enriched in elements such as Al, Cd, Co, Cu, Fe, and Zn. Therefore, mixing precipitates play an important role in contaminants dispersion outside mine area.

The synthesis of woodwardite was successful and led to the formation of a Cu-Al layered structure. The stoichiometry of the synthetic samples is comparable to woodwardite, althoug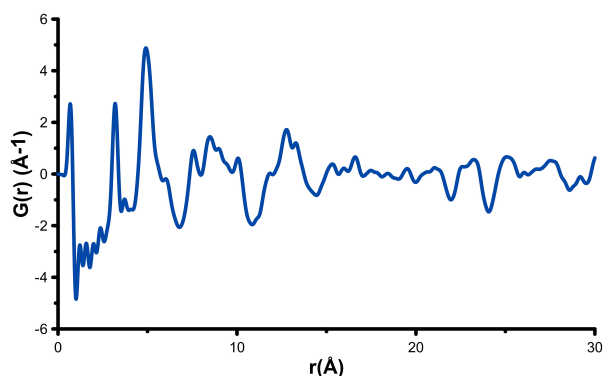h some problems remain with the position and the concentration of sulphate. This phase has an affinity towards REEs, as proved by XRD diagrams and SEM observations, with the exception of La and of Ce^{4+}, resulted from the partial oxidation of Ce^{3+} during the synthesis routine. X-Ray Diffraction showed the presence of a turbostratic structure, and a model for different polytypes with a different number of layers (from 1 to 4) was developed. EXAFS and PDF derived <Cu-O> distance (1.98 Å ± 0.02Å) and <S-O> distance (1.45 ± 0.02Å) are compatible with published structural data of woodwardite (Fig. 1; Witzke, 1995). However, <Y-O> distance (2.37Å ± 0.02 Å) seems too large to be compatible with the

Fig. 1 - PDF diagram of a woodwardite synthetic sample.

size of the octahedral layer. This suggests that Y could be hosted in the interlayer, making woodwardite a promising material for the recovery of REEs from solutions.

Minerals-contaminants interactions

The combined results from BLT and SE showed that in ochreous precipitates from red waters (goethite + jarosite) metals are either strongly bound to the solid fraction, probably hosted in the structure of minerals (Cd, Pb, Mn, Cr, Cu, V, Mn, and Pb), but significant amounts of some metals (Co, Ni, and Zn) are in the water-soluble fraction, probably hosted by sulphates formed by pore water evaporation. Only S was easily leached

using a low ionic strength reactant such as deionised water. The combined application of modified BCR and DOLD SE procedures allowed recognising also the probable presence of separated Mn-oxyhydroxides, as already found by Scheinost *et al.* (2001). Adsorption process plays a minor role, if any, in the immobilisation of contaminants. This statement is supported by the results from Rietveld refinement, which indicates for goethite a value for *b* higher than the theoretical one, suggesting Fe substitution by larger cations.

The ochreous precipitates from red waters (schwertmannite) showed little differences, with Cr mainly released in the third step, probably for its oxidation from Cr^{3+} to the more soluble Cr^{6+}. The only element with an important sorbed fraction was Cu ($\approx$ 3.5%). Ochreous precipitates from intermediate waters (amorphous), tested with BLT, showed a similar behaviour compared to schwertmannite-dominated ochreous precipitates from red waters. Unfortunately, due to the paucity of their occurrence in the Libiola mine, it was impossible to collect enough materials of these precipitates for SE procedures.

It is possible to conclude that ochreous precipitates are good sinks for metals, although such metals do not reach important concentrations, and low dissolution or desorption should be expected. Environmental concerns in any case are still present, and attention should be paid to the long-term stability of minerals such as goethite and schwertmannite, which tend to transform with time in more stable phases (generally hematite). This process was simulated on the samples of ochreous precipitates with a low-T heat treatment (< 350 °C), which forced the formation of nanocrystalline hematite from the starting assemblages. BLT on low-T heat treated aliquots showed the remobilisation of high amounts of Al, Cd, Co, Cu, Mn, Ni, Pb, and Zn. Similar trends were observed in naturally aged samples (Cruz-Hernández *et al.*, 2016). One possible solution to this problem could be high-T heat treatment, which leads to the formation of more stable minerals and lowered significantly the release of elements from the aliquots tested with BLT.

The two selected SE procedures could not be applied to milky-white and greenish-blue precipitates, as such procedures were developed for Fe-rich ochreous precipitates, and would not be selective if applied to other materials. Therefore, in order to test the remobilisation of elements from these precipitates, only BLT were applied. As regards milky-white precipitates, BLT on basaluminite dominated sample showed that these precipitates are bad sinks for metals, as high concentrations of Cd, Co, Cu, Mn, Pb, and Zn were measured in deionised water leachates. The release of these elements was constantly above 10% of the starting concentration in the solid fraction, with Zn reaching the maximum release (73.84%). These results suggest that metals are loosely bound to the surface of the minerals, probably as outer-sphere complexes, and are readily remobilised even with deionised water. The occurrence of milky-white precipitates at the Libiola mine site is limited in space and time, and therefore these precipitates should not be considered a major threat for environmental quality.

The released concentrations of metals from greenish-blue precipitates were the highest among the tested precipitates, with Cu and Zn, in particular, reaching very high concentrations (611 and 596 mg kg⁻¹, respectively). Also Mn, Co, and, in a minor extent, Zn, Ni, and Cr showed higher concentrations compared to ochreous precipitates. Heat treatment at high temperature (900 °C) efficiently retained in the solid fraction high concentration of metals, preventing their dispersion in the environments due to dissolution/desorption reactions occurring to the primary assemblage. However, its efficacy seemed lower compared to the results obtained for ochreous precipitates.

The high concentrations of Al, Fe, Cu, and Zn in BLT leachates of mixing precipitates may indicate a partial dissolution of the precipitates during the experiment. SE procedure showed that metals are more distributed among the different fractions compared to ochreous precipitates, although usually the highest fraction for all metals (except Cr and Zn) is in the fraction bound to iron oxyhydroxides. This fact reflects the great capacity of amorphous materials to incorporate metals. On the contrary, Zn is preferentially adsorbed on the surface, while Cr is retained in the residual fraction. Contrarily to what observed in ochreous precipitate samples, adsorption plays an important role in immobilising contaminants, whereas water-soluble fraction is significantly lower. The combined use of two different SE procedures allowed to recognise the important role of copper oxide in the immobilisation of this element. BLT were performed also with seawater. The results showed that some

metals, such as Cd, Co, and Mn were released in considerably higher concentrations compared to BLT with deionised water, with Pb released also in high amounts in both experiments. Probably exchange reactions with sea water cations took place, as suggested by the high Cation Exchange Capability (CEC) observed for mixing precipitates.

XAS spectra collected on natural precipitate samples showed that Zn^{2+} and Cu^{2+} are the dominating forms inside the precipitates, although in mixing precipitates significant concentrations of Cu^{1+} were observed. In fact, in mixing precipitates the pre-edge features and the contemporary presence of the absorption edges of Cu^{2+} (8996 eV) and Cu^{1+} (8982 eV) indicated the presence of two oxidation states for Cu (Fig. 2). This fact was probably due to the abrupt mixing process between red waters and Gromolo Torrent and to the subsequent formation of cuprite.
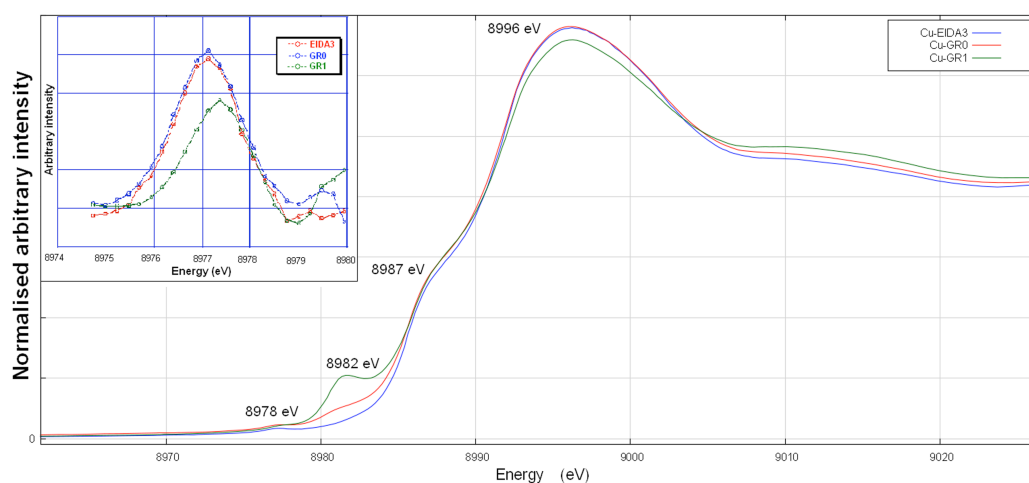

Fig. 2 - XANES region of three samples of mixing precipitates. In the inset: zoom on the pre-edge features.

Minerals-contaminants interactions

Since the present thesis was the first attempt to study the impact of microorganisms in the precipitates of the studied area, it was impossible to investigate all the different types of microorganisms in all samples. Therefore, it was decided to concentrate on few assemblages, chosen on the basis of the abundance of the precipitates and on the information which can be obtained from the microorganisms taken into consideration. We focused on bacteria assemblages in ochreous precipitates from red waters in order to obtain information on their role in AMD. The microfungi from greenish-blue precipitates from blue waters were studied in order to find fungal strains which could be good accumulators of metals. Finally, diatom assemblage of mixing precipitates of the Gromolo Torrent was studied, as these algae are reliable indicators of water quality and allowed assessing the dispersion of elements outside the mine area.

The bacterial assemblages in ochreous precipitates from red waters suggest a microaerobic environment, with low oxygen, rather than a complete anoxic, reducing environment. The difference with the oxidative, oxygenated AMD waters could be due to the fact that in a semisolid matrix, such as sediments, with different micro-niches that permit diverse environmental conditions, antithetic metabolisms can operate (Amils, 2016). The bacteria population was predominant, with only one OTU of archaea recognised. Among bacteria, primary autotrophs, such as *Acidithiobacillus* and *Leptospirillum* genera, were not widespread. On the contrary, Fe oxidisers and Fe reducing heterotrophs bloomed. Organic carbon, generally not present in high amounts in AMD, might be supplied by algae and vegetal litter, as supported by the presence in good amount of the genus *Cytophaga*, which is able to degrade cellulose. The oxidation/reduction cycle of Fe is at the base of the bacterial community. Further investigations should be performed, however, to verify if these bacteria are able to reduce Fe^{3+} from the solid fraction, leading to the leaching of minerals, or directly provoke the precipitation of Fe^{3+}

minerals by oxidising Fe^{2+} in solution. In general, Fe oxidising bacteria from the water column and in the precipitates oxidise Fe^{2+} with the indirect mechanism, helping in maintaining low pH values and favouring metals to remain in solution. Since few meters after the bacterial assemblage sampling point, red waters merge with the unpolluted Gromolo Torrent, Fe oxidisers might play an important role in the dispersion of elements from the mine site to the fluvial ecosystem.

Among the different fungal strains identified from greenish-blue precipitates, *Penicillium* spp. and *Trichoderma* spp. were the most abundant genera. Strains belonging to the genera *Mucor* spp., *Rhizopus* sp., *Absidia* sp., and some yeasts were also recognised. The genus *Penicillium* is often reported to be dominant in copper-contaminated environments (Gadd, 2006), and also *Trichoderma* is a good Cu tolerant and accumulator genus (Anand *et al.*, 2006). Differences in metals tolerance and growth response were observed with Zn-doped MEA between the different genera and among the same genus. The growth of *Trichoderma* sp. on Zn-doped MEA was almost inhibited, while on the contrary *Penicillium* sp. were able to grow normally with a substrate having 3000 mg kg^{-1} of Zn. Bioaccumulation test with *Penicillium* sp. and *Trichoderma* sp. isolated from greenish-blue precipitates were performed with Zn- and precipitates-doped MEA. The results indicated that both strains were good accumulators of metals, although *Penicillium* sp. invariably showed higher concentration of metals in the dried biomass. Both strains were able to reduce divalent to monovalent Cu, as testified by the XAS spectra collected at the Cu k-edge on the dried biomass (Fig. 3). As the higher the Cu^{1+}, the higher the total concentration of Cu in the biomass, it is possible to postulate that Cu reduction favours the bioaccumulation from these microfungi. The bioaccumulation of REEs was also tested, because this group of elements is of high economic interest, and in greenish-blue precipitates relatively high concentrations of REEs have been observed. The results showed that *Penicillium* sp. is more suitable for the recovery of LREEs and MREEs, while *Thricoderma* sp. is more selective for HREEs.
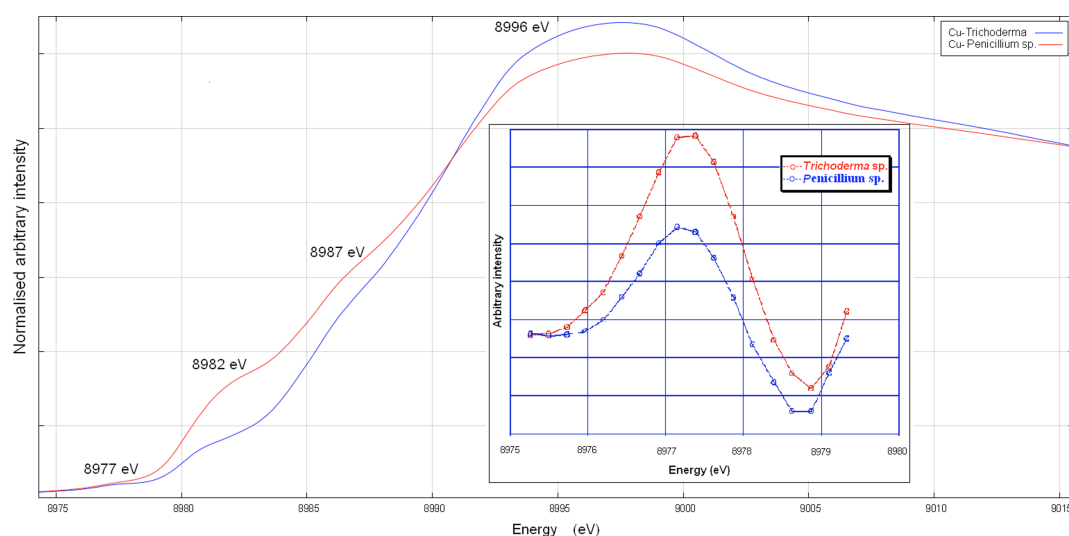

Fig. 3 - XANES spectra of the fungal strains grown on precipitate-doped MEA. In the inset: zoom on the pre-edge features.

In the Gromolo Torrent the diatom assemblage before the confluence of AMD is dominated by *Cymbella* and *Cocconeis* spp., typical of mountain areas and widespread in the Alpine region (especially in the northern side), and usually found in oligotrophic to mesotrophic environments not altered by human activities and with medium electrolyte content (Falasco *et al.*, 2013). Then, the diatom assemblages were characterised by a shift in composition and dominance toward more tolerant and cosmopolitan species, such as *Achnanthidium* spp and *Fragilaria* sp. It is worth noting that in the two sites immediately after the confluences of mine waters the number of diatoms decreased dramatically, indicating that probably metals concentration could have reached the mortality levels. The level of labile metals, the most easily accessible and dangerous for biota, detected by

Diffusion Gradient in Thin films (DGT) was very high, confirming this hypothesis. In the part of Gromolo Torrent flowing inside the mine area, *Achnanthidium* spp dominate the diatom assemblage, due to low pH and high metal concentrations (Fig. 4). Subsequently, the dominance of the assemblage shifts towards *Fragilaria* sp.,
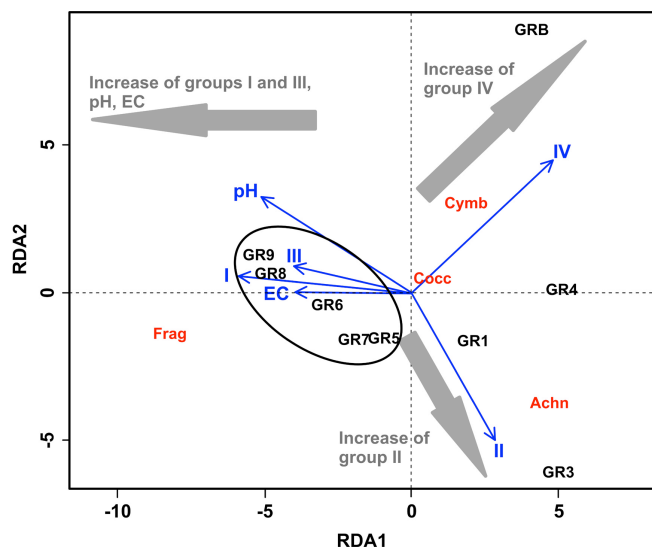

Fig. 4 - RDA on the diatom assemblages, metal concentrations in the precipitates, and physico-chemical parameters of waters (pH, EC). Metals (in blue) are grouped as reported in paragraph 3.1 (group I = Ca, Ba, and Sr; group II = Al, Cd, Co, Cu, Fe, Hg, Zn, and S; group III = K, P, Pb As, and Sb; group IV = Cr, Mg, Mn, Ni, Ti, V, and Zr). In the black circle are grouped moderately impacted sites.

probably as a result of the partial restoration of pH values and the lower concentration of metals in solution.

During the procedure of diatom identification, deformed (teratological) frustules of the species *Fragilaria rumpens* and *Achnantidium minutissimum* were frequently observed in samples downstream the mine area, testifying the detrimental role played by metals on the quality of the Gromolo Torrent waters and their dispersion outside mine area.

CONCLUSIONS

i) Ochreous precipitates are generally good sinks of metals, although in small amounts. The main way of immobilisation of metals is structural incorporation, as sorption is not favoured due to the fact that pH values are lower than the isoelectric points of the minerals present in such precipitates. The only way to remobilise significant concentration of metals is the destabilisation of the primary

assemblage (ageing process). This problem can be solved with heat treatment at high temperature (900 °C).

ii) Milky-white and greenish-blue precipitates are able to mobilise high concentrations of metals, but a significant part of these metals is easily remobilised using deionised waters. Probably, dissolution and/or desorption processes could be invoked to explain the release of metals in solution.

iii) Mixing precipitates are generally good sinks for a high amount of metals, although they are able to mobilise significant concentrations of elements during the interactions with seawater, probably for exchange reaction.

iv) Zn^{2+} and Cu^{2+} are the dominating form for these metals in the precipitates, although in mixing precipitates significant concentrations of Cu^{1+}, probably linked with the abrupt mixing process between red waters and Gromolo Torrent, were observed.

v) Woodwardite was successfully synthesised, and its affinity towards REEs (with the exception of La and Ce^{4+}) was demonstrated, making this mineral a promising material for REEs recovery.

vi) Bacterial assemblage of ochreous precipitates from red waters is composed mainly by heterotrophs supported by algae and vegetal litter. Fe oxidation/reduction cycle is at the base of this community, but the role of bacteria in the formation or leaching of secondary minerals should need further investigations. Bacteria seem to contribute to maintain low pH values, favouring the dispersion of metals outside the mine area.

vii) Strains of *Trichoderma* and *Penicillium* isolated from greenish-blue precipitates proved to be good candidates for bioremediation and recovery of Cu and Zn (only *Penicillium*), as well as REEs.

viii) Diatom assemblages showed a shift from a *Cymbella* and *Cocconeis* to an *Achnantidium* spp. and *Fragilaria* sp. dominance, indicating that changes in pH and metal concentrations provoked by the immission of red waters in the Gromolo Torrent have a great influence on periphyton community.

ix) DGTs showed that high concentrations of labile metals, and in particular of Cu and Zn, are present in Gromolo Torrent waters, leading to the presence of a significant number of teratological frustules of *Achnantidium minutissimum* and *Fragilaria rumpens*.

REFERENCES

Amils, R. (2016): Lessons learned from thirty years of geomicrobiological studies of Río Tinto. *Res. Microbiol.*, **167**, 539-545.

Anand, P., Isar, J., Saran, S., Saxena, R.K. (2006): Bioaccumulation of copper by *Trichoderma viride*. *Biores. Technol.*, **97**, 1018-1025.

Ardau, C., Frau, F., Dore, E., Lattanzi, P. (2012): Molybdate sorption by Zn-Al sulphate layered double hydroxides. *Appl. Clay Sci.*, **65-66**, 128-133.

Balić-Žunić, T. (2002): Quantitative powder diffraction phase analysis with a combination of the Rietveld method and the addition method. *Powder Diffr.*, **17**, 287-289.

Blowes, D.W., Ptacek, C.J., Jambor, J.L., Weisener, C.G., Paktunc, D., Gould, W.D., Johnson, D.B. (2014): The geochemistry of Acid Mine Drainage. *In*: "Treatise on Geochemistry 2nd Edition", D.H. Holland & K.K. Turekian, eds. **11**, 131-190, Elsevier.

Carbone, C., Dinelli, E., Marescotti, P., Gasparotto, G., Lucchetti, G. (2013): The role of AMD secondary minerals in controlling environmental pollution: indications from bulk leaching tests. *J. Geochem. Explor.*, **132**, 188-200.

Cruz-Hernández, P., Pérez-López, R., Parviainen, A., Lindsay, M.B.J., Nieto, J.-M. (2016): Trace element-mineral associations in modern and ancient iron terraces in acid drainage environments. *Catena*, **147**, 386-393.

Dold, B. (2003): Speciation of the most soluble phases in a sequential extraction procedure adapted for geochemical studies of copper sulfide mine waste. *J. Geochem. Explor.*, **80**, 55-68.

Falasco, E., Piano, E., Bona, F. (2013): Guida al riconoscimento e all'ecologia delle principali diatomee fluviali dell'Italia nord occidentale. *Biol. Amb.*, **27**, 292 pp.

Gadd, G.M. (2006): Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycol. Res.*, **111**, 3-49.

Jambor, J.L. & Dutrizac, J.E. (1998): Occurrence and constitution of natural and synthetic ferrihydrite, a widespread iron oxyhydroxide. *Chem. Rev.*, **98**, 2549-2585.

Marescotti, P., Roccotiello, E., Zotti, M., De Capitani, L., Carbone, C., Azzali, E., Mariotti, M.G., Lucchetti, G. (2013): Influence of soil mineralogy and chemistry on fungi and plants in a waste-rock dump from the Libiola mine (eastern Liguria, Italy). *Period. Mineral.*, **82**, 141-162.

Marini, L., Saldi, G., Cipolli, F., Ottonello, G., Vetuschi Zuccolini, M. (2003): Geochemistry of water discharges from the Libiola mine, Italy. *Geochem. J.*, **37**, 199-216.

Marsili, S., Roccotiello, E., Carbone, C., Marescotti, P., Cornara, L., Mariotti, M.G. (2009): Plant colonization on a contaminated serpentine site. *Northeast. Nat.*, **16**, 297-308.

Nordstrom, D.K. & Alpers, C.N (1999): Geochemistry of Acid Mine Waters. *In*: "The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques and Health Issues", G.S. Plumlee & M.J. Longdon, eds. Society of Economic Geologists, 133-157.

Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, Ph. (1999): Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.*, **1**, 57-61.

Reichle, W.T. (1986): Synthesis of anionic clay minerals (mixed metal hydroxides, hydrotalcite). *Solid State Ionic*, **22**, 135-141.

Roccotiello, E., Zotti, M., Mesiti, S., Marescotti, P., Carbone, C., Cornara, L., Mariotti, M.G. (2010): Biodiversity in metal-polluted soils. *Fresen. Environ. Bull.*, **19**, 2420-2425.

Simate, G.S. & Ndlovu, S. (2014): Acid mine drainage: Challenges and opportunities. *J. Environ. Chem. Eng.*, **2**, 1785-1803.

Scheinost, A.C., Stanjek, H., Schulze, D.G., Gasser, U., Sparks, D.L. (2001): Structural environment and oxidation state of Mn in goethite-groutite solid-solutions. *Am. Mineral.*, **86**, 36-44.

Weisener, C.G. (2003): Novel spectroscopic techniques to characterize mine waste. *In*: "Environmental Aspect of Mine Wastes", J.L. Jambor, D.W. Blowes, A.I.M. Ritchie, eds. Mineralogical Association of Canada, Short Course Handbook, **31**, 181-202.

Witzke T. (1995): Untersuchung natürlicher sulfathaltiger hybrider Schichtstrukturen: Charaktereisierung, Systematik, Strukturmodellierung und Rietveld-Verfeinerung. PhD thesis, Martin-Luther-Universität Halle.

Zotti, M., Di Piazza, S., Roccotiello, E., Lucchetti, G., Mariotti, M.G., Marescotti, P. (2014): Microfungi in highly copper-contaminated soils from an abandoned Fe-Cu sulphide mine: Growth responses, tolerance and bioaccumulation. *Chemosphere*, **117**, 471-476.