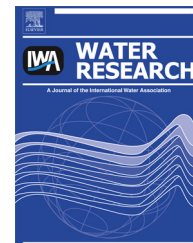




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# Influence of biogeochemical interactions on metal bioleaching performance in contaminated marine sediment

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## ABSTRACT

Bioleaching strategies are still far from finding real applications in sediment clean-up, although metabolic mechanisms governing bioleaching processes have been deeply studied and can be considered well established. In this study, we carried out bioleaching experiments, using autotrophic and heterotrophic acidophilic bacteria strains, and worked with marine sediments characterized by different geochemical properties and metal concentrations and speciations. The solubilization efficiency of the metals was highly variable, with the highest for Zn (40%–76%) and the lowest for Pb (0%–7%). Our data suggest that the role of autotrophic Fe/S oxidizing bacteria is mainly associated with the production and recycling of leaching chemical species, mainly as protons and ferric ions. Metal solubilization appears to be more related to establishing environmental conditions that allow each metal or semimetal to remain stable in the solution phase. Thus, the maintenance of acid and oxidative conditions, the chemical behavior in aqueous environment of each metal species and the geochemical characteristics of sediment interact intimately to influence metal solubilization in site-specific and metal-specific way.

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## 1. Introduction

Metal contamination in marine coastal sediments is a widespread environmental problem that is of major concern due to potential detrimental effects on the ecosystem and on human health. Indeed, metals do not necessarily persist in the sediment but they can enter the food web and/or be spread into the environment in response to redox changes that can arise from disposal actions or resuspension phenomena. Dredging operations periodically carried out in port areas produce very large volumes of contaminated sediments (e.g., around 100 to

200 million cubic meters per year in Europe, according to SedNet estimations; Bortone et al., 2004), that need to be correctly managed. Landfill disposal, confined aquatic disposal, or dumping at sea are still the most frequently applied management strategies all over the world, even though they are associated with several disadvantages, including: limited space capacity, high cost, and low environmental sustainability and compatibility (Dell'Anno et al., 2009; Rulkens, 2005). There is an urgent need for strategies that can reduce contaminant concentrations to threshold levels, to allow the re-use of dredged materials. In this

*Abbreviations:* DOM, dissolved organic matter; DSMZ, German Resource Centre for Biological Material; ORP, oxidation reduction potential; SMT, European Standards Measurements and Testing Programme; TOM, total organic matter; TPN, total prokaryotic number; US EPA, United States Environmental Protection Agency.

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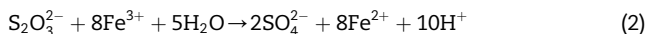
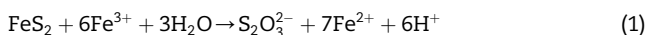
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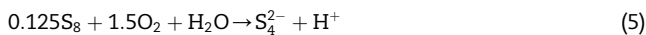
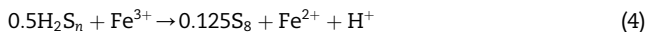
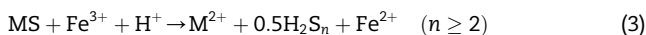
context, bioremediation offers potential alternatives to conventional techniques. Biologically mediated leaching (i.e., bioleaching) has been widely stated as a promising environmentally friendly technique for the remediation of metal-contaminated sediments (Brierley and Brierley, 2001; Chartier et al., 2001; Chen and Lin, 2004; Sabra et al., 2011). Bioleaching strategies are based on the metabolism of acidophilic chemoautotrophic bacteria that can oxidize elemental sulfur, reduced sulfur compounds and/or iron (i.e., Fe/S oxidizing bacteria). Their metabolic products are mainly sulfuric acid and ferric ions, which have high leaching power (Beolchini et al., 2012; Komnitsas and Pooley, 1991) and can solubilize metals in their reduced forms (i.e., especially sulfides) and those associated with acid-soluble ores.

The solubilization of metal sulfides by Fe/S oxidizing bacteria has long been described as a process based on two independent mechanisms: a 'direct mechanism' (i.e., the direct enzymatic oxidation of the sulfur moiety of the metal sulfide) and an 'indirect mechanism' (i.e., the non-enzymatic metal sulfide oxidation by Fe(III) ions combined with enzymatic (re-)oxidation of the resulting Fe(II) ions; Sand et al., 2001). However, it is now generally accepted that the 'direct mechanism' of biological metal sulfide oxidation does not exist. Conversely, the true effectors for metal solubilization from ores are the products of bacterial metabolism, previously known as the 'indirect mechanism' (Rohwerder et al., 2003). According to Rohwerder and Sand (2007), the Fe/S oxidizing bacteria approach the mineral surface by creating a biofilm, in a 'contact sub-mechanism', while some planktonic bacteria cells remain floating in the bulk solution ('non-contact sub-mechanism'). In either case, the dissolution of metal-bearing minerals can follow two different reaction pathways, which depend on the acid-solubility of the sulfides involved: acid-insoluble metal sulfides (e.g., pyrite, molybdenite, tungstenite) are exclusively oxidized via electron extraction (the 'thiosulfate pathway'), while acid-soluble metal sulfides (e.g., sphalerite, galena, arsenopyrite, chalcopyrite, hauerite) are dissolved by the combined actions of Fe(III) oxidative attack and proton attack (the 'polysulfide pathway'). These two pathways can be simplified using the following equations (Schippers and Sand, 1999):

Thiosulfate pathway:



Polysulfide pathway:



As shown in Equations (1)–(5), protons are generated in both of these pathways, and so the pH is lowered.

In our previous study (Beolchini et al., 2009), we demonstrated that at least when working with low solid content (i.e., 20 g/L), Fe/S oxidizing bacteria can be applied to dredged marine sediments with high efficiency for Ni, Cu, Zn and Cd

removal. We also suggested that heterotrophic Fe-reducing bacteria can support leaching bacteria (i.e., Fe/S oxidizing bacteria) increasing the metal solubilization. Nevertheless, the applicability of bioleaching to sediment clean-up purposes is affected by several factors, including the kind and concentration of the substrata, the ratio solid:liquid, the kind of microorganisms involved, and not least, the geochemical characteristics of the sediments (Brierley and Brierley, 2001; Chen and Lin, 2004). These factors thus need to be further investigated, especially in marine sediments, where information is still limited (Akinci and Guven, 2011; Beolchini et al., 2009, 2013).

In the present study, we investigated factors that can influence the performance of bioleaching strategies for the removal of metals from contaminated marine sediments. We worked with sediment samples characterized by different geochemical properties and metal concentrations and speciations. The sediment characteristics are expected to affect the interactions among metals, the sedimentary matrix and the leaching strains, with cascade effects on the bioleaching performances. The results obtained are also used to outline, for the first time, a conceptual model that describes the main biogeochemical interactions that need to be considered for the definition of efficient bioleaching strategies to be applied to contaminated marine sediments.

## 2. Materials and methods

### 2.1. Sediment samples

Sediment samples were collected in the Mediterranean Sea from the ports of Livorno (43° 33' 53.39" N, 10° 18' 2.79" E; Tyrrhenic Basin, Italy), Piombino (42° 55' 54.58" N, 10° 32' 34.13" E; Tyrrhenic Basin, Italy) and Ancona (43° 37' 29.8194" N, 13° 29' 56.94" E; Adriatic Basin, Italy), hereafter referred as sediments A, B and C, respectively. After their collection, the sediments were sieved to <2 mm, to remove gravel, homogenized and then stored at 4 °C until their use.

The sediment grain size was determined by sieving techniques. The mineralogical characterization was performed using an X-ray powder diffractometer (Philips X Pert 1830). The water, total carbonate and total organic matter (TOM) in the sediment samples were determined as already described by Rocchetti et al. (2012). Details about the procedures here used are given in the [Supplementary On-line Materials](#).

### 2.2. Metal partitioning among the geochemical fractions of the sediment

We used the three-step sequential selective extraction procedure by the European Measurements and Testing (SMT) program (Quevauviller, 1998). According to this scheme, we considered the metals that were associated with four sediment fractions: (i) the acid soluble fraction, as the metals associated with the exchangeable fraction and metals in the carbonate-bound fraction; (ii) the reducible fraction, as the metals associated with Fe/Mn oxides; (iii) the oxidizable fraction, as the metals bound to high-molecular-weight organic compounds and to sulfides; and (iv) the residual

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