BIOGEOCHEMICAL INTERACTIONS IN THE APPLICATION OF BIOTECHNOLOGICAL STRATEGIES TO MARINE SEDIMENTS CONTAMINATED WITH METALS

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Abstract: Sediment contamination in coastal areas with high anthropogenic pressure is a widespread environmental problem. Metal contaminants are of particular concern, since they are persistent and cannot be degraded. Microorganisms can influence metal mobility in the sediment by several direct and indirect processes. However, the actual fate of metals in the environment is not easily predictable and several biogeochemical constraints affect their behaviour. In addition, the geochemical characteristics of the sediment play an important role and the general assumptions for soils or freshwater sediments cannot be extended to marine sediments. In this paper we analysed the correlation between metal mobility and main geochemical properties of the sediment. Although the prediction of metal fate in sediment environment, both for *ex-situ* bioleaching treatments and *in-situ* biostimulation strategies, appears to require metal-specific and site-specific tools, we found that TOM and pH are likely the main variables in describing and predicting Zn behaviour. Arsenic solubilisation/increase in mobility appears to correlate positively with carbonate content. Cd, Pb and Ni appear to require multivariate and/or non-linear approaches.

Key words: contaminated sediment, metals and arsenics, *ex-situ* bioaugmentation, *in-situ* biostimulation, conceptual models.

1. Introduction

Marine coastal ecosystems and shelf seas are ultimate repository for contaminants in the environment (MAJONE *et al.,* 2014; MICHELI *et al.*, 2013). Chemical pollutants represent a threat for the environment and the human health, so that the European Parliament has included them among the descriptors of quality status of European seas (Descriptor 8 in the EU Directive 2008/56/EC, i.e. MSFD: Marine Strategy Framework Directive). At this regards, heavy metals are of particular concern due to their non-biodegradability, persistence and toxicity: high concentrations of metals result in deterioration of the water quality, with long-term implications on ecosystem and human health (FÖRSTNER and WITTMANN, 1979; NOGALES *et al.*, 2011; PASTORELLI *et al.*, 2012; FATOKI and MATHABATHA, 2001; JÄRV *et al.*, 2014).

The remediation of contaminated sediments represents a challenge of great concern, especially in connection with the recent interest in biotechnological approaches, which would offer environmentally friendly, cost-feasible strategies and larger acceptance by the society. However, the studies on the bioremediation of contaminated sediments have focused mainly on the organic component of the contamination, even with the production of a fair amount of patents. Conversely, the

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contamination of aquatic sediments by metals has been object of fewer studies and it represents one of the main challenges in the bioremediation field (AKCIL *et al.*, 2014). The discrepancy between metals and organics is very likely due to the incomplete understanding of the complex behaviour of metals in environmental matrices (among themselves, the sediment) which is, in turn, affected by geochemical and biological processes (WARREN and HAACK, 2001). The fundamental understanding of such key processes and of how the complex linkages among them can control the metals (and semi-metals) behaviour appears as an essential precursors to the determination of successful (bio)-remediation strategies for contaminated sediment.

Bioleaching is a bio-hydrometallurgical technique where chemolithotrophic Fe/S oxidizing bacteria produce chemical species with high metal leaching power (mainly protons and ferric ions; VERA *et al.*, 2013; SAND *et al.*, 2001; SCHIPPERS and SAND, 1999). This technique is well-established in mining industry but it has been also considered for metal removal from contaminated sediments (BRIERLEY and BRIERLEY, 2001; CHARTIER *et al.*, 2001; CHEN and LIN, 2004; SABRA *et al.,* 2011). Bacteria involved in bioleaching modify dramatically pH and ORP conditions, thus bioleaching has to be applied as *ex-situ* strategy for dredged materials, in view of sediment beneficial reuse (e.g. building industries or in beaches nourishment; BORTONE *et al.*, 2004; LEE, 2000; AHLF and FÖRSTNER, 2001). Nevertheless, many factors affect the real applicability of bioleaching techniques for sediment clean-up purposes: the type and concentration of the substrata, the ratio solid:liquid during the treatment, the type of microorganisms involved and not least the geochemical characteristics of the sediments (BEOLCHINI *et al.*, 2013; CHEN and LIN, 2004; BRIERLEY and BRIERLEY, 2001). We have recently studied the overall effect of these factors, with a particular focus on geochemical properties of marine sediments, where information is still limited (FONTI *et al.*, 2013a).

Biological treatments based on the exploitation of the autochthonous microbial assemblages are gaining increasing prominence in bioremediation of a variety of environmental; anaerobic biodegradation matrices such as wastewaters, soils and sediments contaminated has shown a great potential for *in-situ* applications for the abatement of persistent organic pollutants in anoxic contaminated marine sediments (HARITASH and KAUSHIK 2009; VAN HULLEBUSCH *et al.*, 2005). As concerns metal contaminants, sulphate reducing bacteria within the sediment can decrease metal mobility by generating sulphides (GADD, 2004; JIANG and FAN, 2008). Nevertheless, other microbe-mediated redox processes occur in marine sediment environment and may affect the fate of metals. Moreover, the fate of metals in the sediment depends upon the balance between immobilization (i.e., redox transformations, precipitation, adsorption and intracellular uptake) and mobilization processes (i.e., redox reactions, leaching, volatilization by methylation and chelation/ complexation) and microorganisms can largely affect these processes.

We found previously that metal behaviour during sediment bioremediation depends upon several chemical and biological processes, of which the effects interacts together and varies on the basis of metals to be involved and of the geochemical characteristics of the sediment. In particular, when sediment bioremediation consists in *ex-situ* bioleaching strategies (aimed at solubilizing metals and decreases their concentrations), the main factors affecting metal removal are i) intrinsic properties of metals, ii) carbonate content in the sediment (or its acid-neutralizing capacity), iii) metal partitioning iv) other sediment properties (e.g. composition of sediment organic matter, mineralogical composition, availability of soluble ligands), v) the presence of microbial consortia able to establish environmental conditions favourable for metal stability in the solution phase (i.e. low pHs, high ORP), vi) the presence of key growth substrate; the final balance is highly site-specific and metal-specific. Similarly, during *in-situ* bioremediation actions (aimed at stimulating indigenous biodegradative microbial functions) metal contaminants are affected by i) intrinsic properties of metals, ii) metal partitioning, iii) total organic matter, iv) other geochemical properties of the sediment, v) the microbial functions in the autochthonous community, vi) the selectivity of biostimulation (e.g. type of amendants, oxygen concentration).

In this paper, interactions metal-microbe-sediment observed during biostimulation of the autochthonous microbial community and during bioleaching as two biological remediation strategies are analysed together in order to improve our knowledge about biogeochemical processes occurring during bioremediation of sediments, likely one of the more complex environmental matrices.

Fig. 1. Geographical location of the three seaports from which studied sediment samples come from.

2. Materials and methods

2.1 Experiments

Two biotechnological strategies of sediment remediation experiments are discussed in this paper: 1) bioaugmentation with acidophilic microbial consortia, aimed at bio-mobilizing metals from the sediment (i.e. bioleaching), and 2) biostimulation of the autochthonous microbial community in the sediment, aimed at investigating the potential in metal bio-immobilization. Sediment samples were collected in three Italian commercial seaports: Piombino (Tyrrhenian Sea), Livorno (Tyrrhenian Sea) and Ancona (Adriatic Sea; Fig. 1). In this paper, we refer to sediment samples as Sediment A, Sediment B and Sediment C, respectively.

Sediment samples were stored at 4 °C until their use. Five aliquots of each samples were treated with an excess of 10 % HCl to remove carbonates, washed with distilled water, dried (60 °C, 24 h) and then calcined at 450 °C for 2 h; total organic matter (TOM) was determined as the difference between dry weight of the sediment and weight of the residue after combustion. Water content was calculated as the difference between wet and dry weight. Content of (semi-)metals was determined after acid digestion ($HCl:HNO_3 = 3:1$, at 150 °C for 90 min) by ICP-AES, according to EPA procedure (US EPA, 2001). Metal partitioning was determined by the three-step selective sequential extraction (SSE) procedure by the European Measurements and Testing programme (FÖRSTNER, 1993; SALOMONS, 1993); in particular, four geochemical fractions of the sediment were considered: i) the exchangeable fractions and carbonate bound fraction; ii) Fe/Mn oxides fraction (i.e. reducible fraction) iii) organic and sulphide fraction (i.e. oxidizable fraction); and iv) the residual fraction, that is given by metals that remains in the solid residue (mainly in the crystalline lattice of primary and secondary minerals). Mineralogical composition was analysed by X-ray diffractometer (XRD; Philips X Pert 1830).

Table 1. Experimental plan of the bio-mobilization experiment (Bioaugmentation).

Factor	**Unit**	**Coded levels**			
		Factor code	**-1**	**0**	**1**
Sediment	-		Sediment A[1]	Sediment B[1]	Sediment C[1]
Inoculum	-		CTRL[2]	AUTO[2]	MIX[2]
Fe ($FeSO_4$)	g/L	IRON	-	0	8.9
Glucose	g/L	Glucose	-	0	0.1
S^0	g/L	sulfur	-	0	1.0

[1] Sediment A = samples coming from the port of Piombino (Tyrrhenian Sea, Italy); Sediment B = samples coming from the port of Livorno (Tyrrhenian Sea, Italy); Sediment C = samples coming from the port of Ancona (Adriatic Sea, Italy). See also Fig. 1.

[2] CTRL = abiotic control (no inoculum); AUTO = autotrophic strains (*Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans*); MIX = autotrophic and heterotrophic strains together (*At. ferrooxidans*, *At. thiooxidans*, *L. ferrooxidans* and *Acidiphilium cryptum*).

Experimentation followed full factorial plans, which factors and levels are shown in Tab. 1 and Tab. 2. In particular, bioleaching experiment (1) simulated a biological *ex-situ* sediment treatment aimed at removing metal contaminants; Fe/S oxidizing strains (*Acidithiobacillus ferrooxidans* DSMZ 14882T, *At. thiooxidans* DSMZ 14887T, *Leptospirillum ferrooxidans* DSMZ 2705T) and a heterotrophic Fe-reducing strain (*Acidiphilium cryptum* DSMZ 2389T) were used to inoculate a pre-acidified sediment slurry (i.e. 100 g/L in 9K medium, pH 2 with 5 M H_2SO_4; SILVERMAN and LUNDGREN, 1959); microcosms were added with $FeSO_4$ (‘Fe’: 0 or 8.9 g/L), elemental sulphur (‘S^0’: 0 or 1.0 g/L) and/or glucose (‘Glucose’: 0 or 0.1 g/L), according to the experimental plan (Tab. 1).

Table 2. Experimental plan of the biostimulation experiment.

Factor	**Unit**	**Coded levels**		
		Factor code	**0**	**1**
Na-Acetate	mM (C)	Ac	0	20
Lactose	mM (C)	Lac	0	20
Inorganic macronutrients	presence/absence[1]	N+P	no	Yes

[1] Inorganic macronutrients (i.e. $(NH_4)_2SO_4 + KH_2PO_4$): final concentrations were defined on the basis of the organic carbon content in the sediment, according to a C:N:P molar ratio equal to 100:10:1, optimal for microbial activity (BEOLCHINI *et al.*, 2010; MORGAN and WATKINSON, 1992).

Biostimulation experiment (2) simulated an *in-situ* sediment bioremediation treatment in which the indigenous microbial community is stimulated at degrading organic pollutants; in particular, 250 g/L sediment slurries (liquid medium was 0.2 µm pre-filtered artificial seawater) were added with sodium acetate, lactose and/or inorganic nutrients (Tab. 2) and then incubated in the absence of O_2 sources for 60 days, in the dark at room temperature (20 °C ± 1). Sodium acetate and lactose were selected as electron donors for stimulating reducing processes in the sediment (FINKE *et al.*, 2007; DELL’ANNO *et al.*, 2009), while we used ammonium sulphate and potassium phosphate as source of inorganic N and P. In both experiments, a particular attention was paid at carrying out control treatments.

During the experiment, we measured pH and ORP using a pH/ORP meter (inoLab Multi 720, WTW), we determined the prokaryotic cell abundance (DANOVARO *et al.*, 2002), the concentrations of metals and As both in the liquid phase and in the sediment (US EPA, 2001). For the biostimulation experiment we also assessed changes in metal partitioning (as described above) and variations in the microbial community by coupling ARISA (Automated Ribosomal Intergenic Spacer Analysis; LUNA *et al.*, 2006) and metagenetic analyses (Next Generation sequencing; data analysis by MOTHUR pipeline; SCHLOSS *et al.*, 2009).

Additional details about experimental set-up, experimental conditions and analytical determinations are given in FONTI *et al.* (2013a) and FONTI *et al.* (2015).

2.2 Statistical analysis

For the statistical analysis, we introduced a new parameter M that described the partitioning among the four geochemical fractions of the sediment, for each metal investigated:

$$M = -Res^2 - 0.33*Ox^2 + 0.33*Redu^2 + Ex/Carb^2 \qquad (1)$$

where, for each metal investigated, “Res”, “Ox”, “Redu” and “Ex/Carb” represent the relative contribution of the residual, oxidizable, reducible and exchangeable/

carbonatic fractions, respectively, to the total content in the sediment. M>0 indicates a mobilization.

We used Student's t test (α=0.05) to compare Total Organic Matter content (TOM), carbonate content and metal partitioning (i.e. M_Zn, M_Cd, M_Cr, M_Ni, M_Pb and M_As) in the three sediments. We also carried out linear regression analyses (least square estimate) between metal solubilization efficiencies obtained during bioleaching experiment and sediment TOM, either carbonate content or metal partitioning (see eq.1). We used JMP® Statistical Discovery software (version 10.0.0, SAS Institute, Inc.) to carry out all the statistical analysis shown in this paper.

3. Results and discussion

3.1 Differences and similarities among marine sediment samples

Table 3 summarises the main geochemical characteristics of the three sediments. Sediment samples are of carbonatic nature, although also quartz and albite were very abundant minerals in sediment A. Such characteristic is congruent with Mediterranean coastal sediment properties (DELL'ANNO *et al.*, 2002; SPAGNOLI *et al.*, 2010; SCHIPPERS and JØRGENSEN, 2002). Compared with pristine coastal marine systems, the three sediments were rich in organic matter, with a TOM content even higher than in other polluted marine harbours (DELL'ANNO *et al.*, 2002).

Table 3. Main geochemical characteristics of sediment samples.

	Unit	**Sediment A** (Port of Piombino)	**Sediment B** (Port of Livorno)	**Sediment C** (Port of Ancona)
Mineral component	-	quartz, albite, calcite, alunite, hematite, Na/H/Zn silicates, clinochlore	quartz, calcite albite, K-feldspars, clinochlore, muscovite, dolomite	quartz, calcite albite, K-feldspars, clinochlore, muscovite, dolomite
Water	%	25 ± 1	36 ± 2	40 ± 1
Carbonates	mg/g	380 ± 10	500 ± 50	450 ± 10
TOM	mg/g	65 ± 5	32 ± 1	28 ± 1
As	ppm	48 ± 2	11 ± 1	10 ± 2
Zn	ppm	1 030 ± 70	170 ± 30	83 ± 3
Cu	ppm	37 ± 4	43 ± 7	33 ± 2
Cr	ppm	140 ± 50	124 ± 30	70 ± 10
Ni	ppm	29 ± 5	70 ± 10	40 ± 5
Cd	ppm	1.8 ± 0.5	0.50 ± 0.05	0.50 ± 0.01
Pb	ppm	200 ± 20	28 ± 4	12 ± 2
Fe	ppm	$84 \pm 8 \times 10^3$	$27 \pm 2 \times 10^3$	$22 \pm 2 \times 10^3$

Although with differences among sample, sediments investigated here were contaminated by metals. In particular, Zn, Cd, As and Pb contents in sediment A were higher than in sediments B and C (in some cases, 10-fold higher), while the Ni content was lower.

Metal partitioning among the geochemical fractions of the sediment samples is described in FONTI *et al.* (2013a) and FONTI *et al.* (2015). Briefly, Zn and As showed differentiated partitioning among the three sediments studied here; in sediment A, about Zn 60 % was partitioned among the non-residual fractions, while in sediments B and C just 30-35 % of the total Zn was in non-residual fractions; As in the non-residual fractions was about 5 %, 20 % and 60 % in sediments A, B and C, respectively. Conversely, the partitioning of Pb, Cd, Ni and Cr did not vary among the sediments: about 50 % and 70 % of Pb and Cd, respectively, and >80 and >90 % of Ni and Cr, respectively, were in the residual fraction of the three sediments investigated in this study.

Data about the sample characterization suggest that sediments B and C were relatively similar from the geochemical point of view, despite the geographical location (Fig. 1). In particular, no statistically significant differences between sediments B and C were observed for TOM (least square mean = 3.886, standard error = 3.111; t = 2.179; $\alpha = 0.05$), carbonate content (least square mean = 35.807, standard error = 16.716; t = 2.179; $\alpha = 0.05$), Zn partitioning (least square mean = 0.067, standard error = 0.054; t = 2.447; $\alpha = 0.05$) and Cr partitioning (least square mean = 0.030, standard error = 0.027; t = 2.447; $\alpha = 0.05$). On the contrary, As partitioning varied among the three sediments (t = 2.447; $\alpha = 0.05$); Ni partitioning was similar in Sediment A and C (least square mean = 0.012, standard error = 0.007; t = 2.447; $\alpha = 0.05$) and statistically different in sediment B; Pb and Cd in Sediment A and B were partitioned in a similar way (for Pb: least square mean = -0.234, standard error = 0.237; for Cd: least square mean = -0.024, standard error = 0.012; t = 2.776; $\alpha = 0.05$). A distance matrix confirmed the similarity between sediments B and C (Tab. 4).

Table 4. Distance matrix for sediment samples (standardized variables; method: Ward; distance: euclidean).

Sediment Sample	**A**	**B**	**C**
A	0	5.383399	5.301284
B	5.383399	0	2.985866
C	5.301284	2.985866	0

3.2 Metal mobilization from marine contaminated sediment by bioaugmentation with acidophilic consortia (bioleaching)

Our bioleaching experiments with sediment samples coming from different commercial seaports have demonstrated that metal and semi-metal solubilisation efficiencies are highly site-specific and metal-specific (Fig. 2A-B). A comparison with the scientific literature shows that such an effect is stronger when a high sediment content is present during the treatment (BEOLCHINI *et al.*, 2009; CHEN and LIN, 2001; ZHAO *et al.*, 2009; CHEN and LIN, 2000; BEOLCHINI *et al.*, 2013).

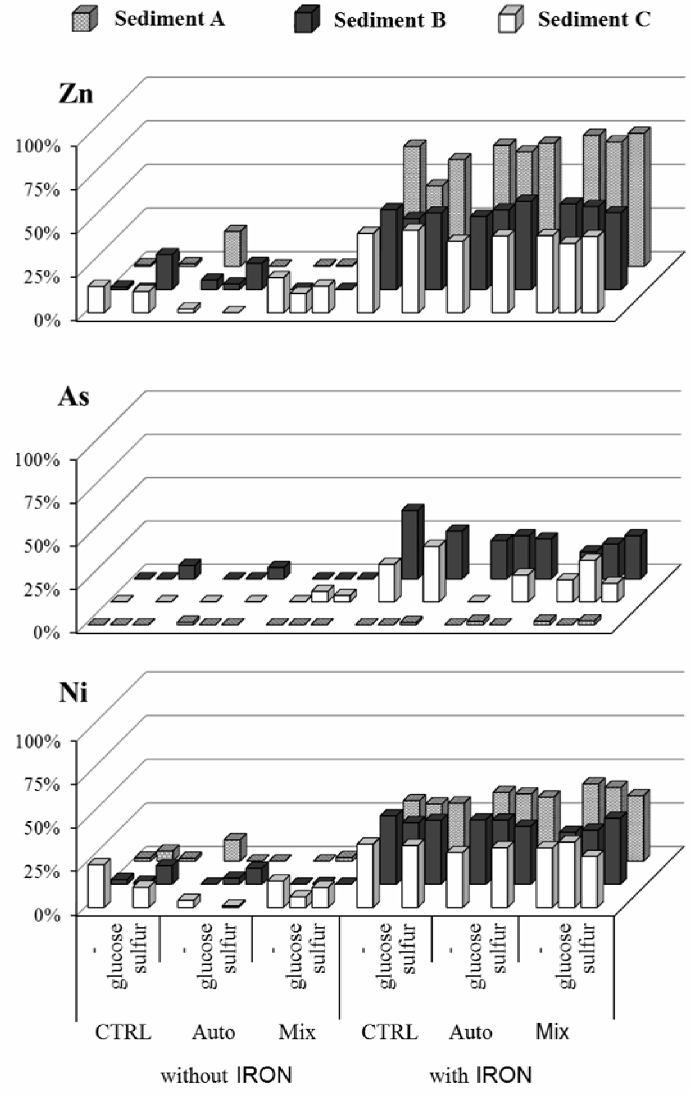

Fig. 2A. Zn, As, Ni solubilisation efficiencies from marine sediment samples after a 14 day bioleaching treatment. Sediment A= samples coming from the port of Piombino (Tyrrhenian Sea, Italy); Sediment B= samples coming from the port of Livorno (Tyrrhenian Sea, Italy); Sediment C= samples coming from the port of Ancona (Adriatic Sea, Italy). CTRL= abiotic control (no inoculum); AUTO= autotrophic strains (*Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans*); MIX= autotrophic and heterotrophic strains together (*At. ferrooxidans*, *At. thiooxidans*, *L. ferrooxidans* and *Acidiphilium cryptum*). See also Fig.1 and Tab.1.

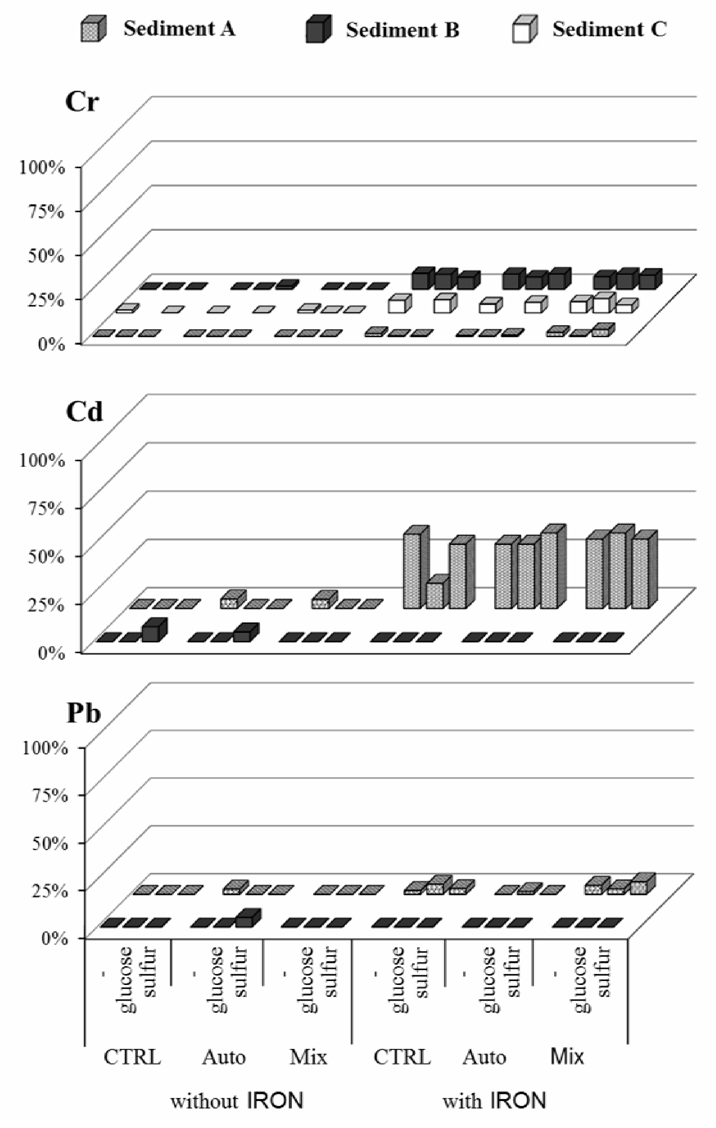

Fig. 2B. Cr, Cd, Pb solubilisation efficiencies from marine sediment samples after a 14 day bioleaching treatment. Sediment A= samples coming from the port of Piombino (Tyrrhenian Sea, Italy); Sediment B= samples coming from the port of Livorno (Tyrrhenian Sea, Italy); Sediment C= samples coming from the port of Ancona (Adriatic Sea, Italy). CTRL= abiotic control (no inoculum); AUTO= autotrophic strains (*Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans*); MIX= autotrophic and heterotrophic strains together (*At. ferrooxidans*, *At. thiooxidans*, *L. ferrooxidans* and *Acidiphilium cryptum*). See also Fig.1 and Tab.1.

The highest solubilisation efficiencies were observed for Zn (up to 76 % in sediment A, up to 50 % in sediments B and C), Ni (up to 44 % with a common pattern in the three sediments). Both As and Cd hardly solubilized from sediment A, while mobilized from sediment B and C with solubilisation yield up to 40 %. Cr and Pb solubilisation yields were very low (i.e. <10 %).

We found that the maintenance of low pH values is a key condition for remediation strategies based on the bioleaching, since only with acidic conditions metals that solubilize are stable in the aqueous phase (FONTI *et al.*, 2013a). Among the experimental factors investigated, the presence of Fe(II) was the only one to have a significant effect on metal solubilisation efficiencies. The sole element to be not affected by the presence of Fe(II) was Pb. Re-precipitation events were very likely responsible for the scarce Pb solubilisation: insoluble salts (i.e. $PbSO_4$; $Pb_5(PO_4)_3Cl$; $Pb_5(PO_4)_3OH$) are the main leaching products for Pb under our experimental conditions (FONTI *et al.*, 2013a). Fe/S oxidizing bacteria didn't affect directly the metal solubilisation but they accelerated the oxidation of Fe and the decrease of pH values. Moreover, considering the mineralogical compositions of the three sediments, and of marine sediment in general (SCHIPPERS and JØRGENSEN, 2002), carbonate chemistry was very likely one of the main factors responsible for controlling pH; in the presence of Fe(III), which is generated by Fe/S oxidizing bacteria metabolism, the buffering effect due to carbonates decreases and this allow decreasing pH conditions (BEOLCHINI *et al.*, 2013; FONTI *et al.*, 2013b; FONTI *et al.*, 2013a).

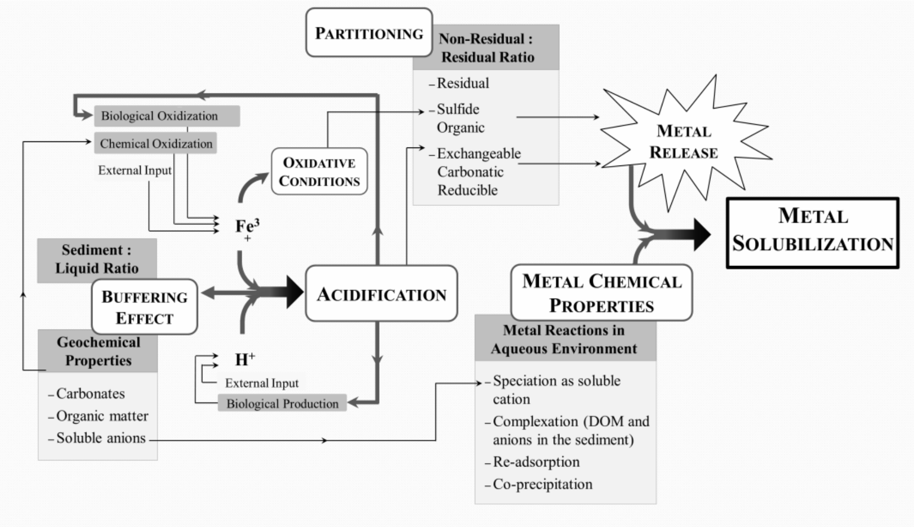

Fig. 3. Conceptual model of the main biogeochemical processes that occur during a sediment bioleaching treatment. Arrows show the main interactions among processes and their effects on metal solubilisation efficiencies. Sea details in (FONTI *et al.,* 2013a).

Moreover, we have observed that the site-specific geochemical properties of the sediment affected significantly the metal removal efficiencies from marine contaminated sediments. Main geochemical properties that appeared to be mainly relevant were the initial partitioning of each metal , the carbonate content and TOM content (FONTI *et al.*, 2013a). In this regard, we proposed a conceptual model that relates and summarizes the main biogeochemical processes that occur during a sediment bioleaching treatment (Fig. 3). Although such model was outlined on the basis of results obtained from experimentations with marine sediments, it can be extended to aquatic sediments with a different nature, since the same biogeochemical processes are involved.

3.3 Changes in metal partitioning due to biostimulation strategies

Bioremediation strategies applied to contaminated sediments can determine significant changes in the metal partitioning with potential consequences on their bioavailability and toxicity. A 60 day biostimulation experiment in hypoxic conditions was carried out with the aim of assessing changes in metal mobility to be caused by sediment bioremediation actions (i.e. biostimulation of sediment intrinsic biodegradative functions). We observed that, under the experimental investigated conditions, the mobility of Zn, Pb, Cd and As increased after the bio-treatment (i.e. a significant increase in the exchangeable/carbonatic, at the detriment of the oxidizable fraction or the residual one), while Cr increased its relative concentration in the residual fraction. Anyway, in congruence with their high association to the residual fraction, Cr and As displayed very small variations, (Fig. 4A-B). Changes in partitioning were not associated with metal solubilization processes as the concentrations of all metals and As in the solution phase were below their detection limits (i.e. Cd <1 μg/L; Cr <5 μg/L; Zn, Pb and As <10 μg/L).

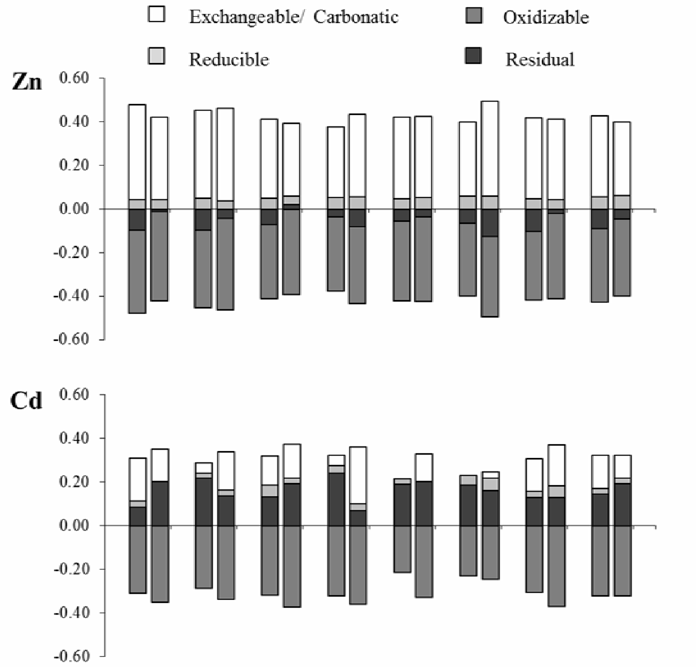

Fig. 4A. Continue on next page.

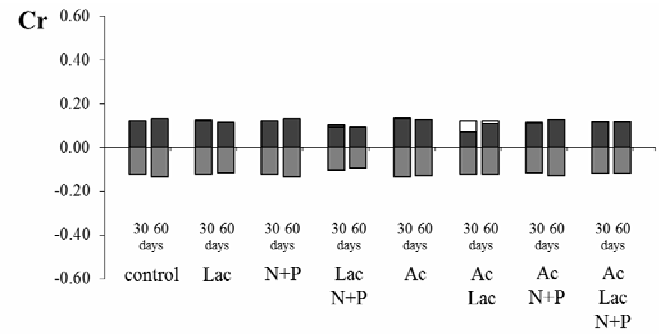

Fig. 4A. Variation in Zn, Cd and Cr partitioning in the sediment after biostimulation of the autochthonous microbial community. Experimental design is described in Table 2.

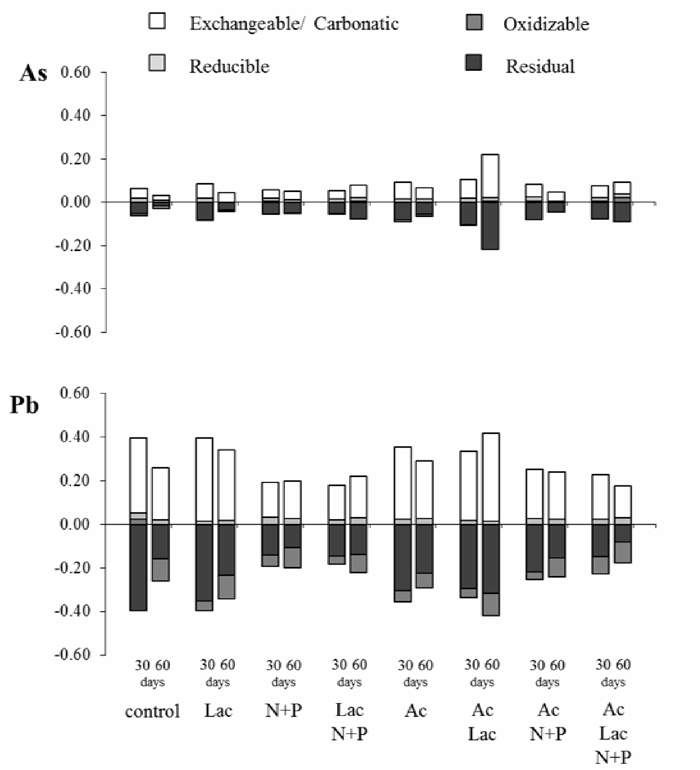

Fig. 4B. Variation in As and Pb partitioning in the sediment after biostimulation of the autochthonous microbial community. Experimental design is described in Table 2.

Our results have highlighted that metal mobility changes were depending upon their initial partitioning in the sediment (site-specific) and their intrinsic (metal-specific) physical-chemical characteristics; microorganisms can influence strongly the direction of the mobility changes (mobilization vs immobilization), so that alterations in bacterial assemblage composition (a direct consequence of the selection of different bacterial taxa due to biostimulation with different amendants.

The ecological approach of our study allowed to obtain a better understanding of the interactions among the main abiotic and biotic processes that affect metal mobility during a sediment bioremediation/biostimulation action, with operational conditions similar to those investigated here. Fig. 5 shows a new conceptual model in which the interactions among the main abiotic and biotic processes are showed for the first time in explicit form.

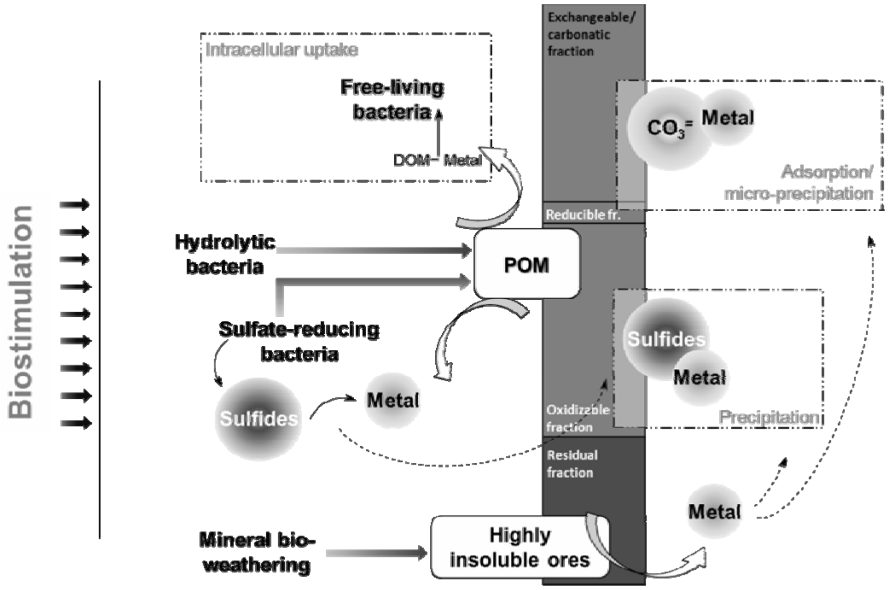

Fig. 5. Conceptual model of the main biotic and abiotic processes that occur during a sediment bioremediation action (biostimulation of intrinsic biodegradative functions) and of the effects of such processes on metal partitioning. Sea details in (FONTI *et al.*, 2015).

Our results suggest that *in-situ* biostimulation actions can increase the probability of adverse biological effects due to metal contamination, even when the total metal concentrations in the sediments are below the threshold levels assumed to induce such detrimental effects: naturally occurring processes (e.g. sediment resuspension and bioturbation) can favour the release of (semi-)metals from the more mobile fractions of the sediment to the solution phase and/or increase bioaccumulation processes within the benthic food webs, with potential detrimental effects on ecosystem and human health (BAUMANN and FISHER, 2011; CALMANO *et al.*, 1993).

Our results have highlighted that the biodegradation of organic matter was the main (but not the sole) biological mechanisms to affect metal mobility changes. Next generation pyrosequencing analysis showed that the biostimulation favoured heterotrophic bacterial taxa able to degrade complex organic substrates (*Bacteroidetes*, *Planctomycetes* and *Firmicutes*; BERNARDET and YASUYOSHI, 2006; TADONLÉKÉ, 2007; FENCHEL *et al.*, 2012; FERNÁNDEZ-GÓMEZ *et al.*, 2013).

However, the relative importance of the different microbial metabolisms varied on the basis of the amendants used in biostimulation. The presence of inorganic nutrients (N+P) may be unfavourable to sulphate reducing bacteria (*Desulfobacteraceae* and *Desulfobulbaceae*) and favourable *Flavobacteriaceae* and *Rhodobacteraceae* (FONTI *et al.*, 2015).

3.4 Geochemical properties of the sediments and effect on metal mobility in marine sediment

Results of the studies described above showed that metal partitioning among fractions of the sediment, organic matter (TOM) and carbonate content are very important factors that exert much influence on the mobility (i.e. variation in partitioning, potential solubilisation and removal from sedimentary matrix). The effect of such factors is highly metal specific, since it is related to element-specific chemistry of the metal species to be present. Table 5 show linear regressions among metal mobilization potential and sediment geochemical properties. In our studies we observed that the initial partitioning influenced metal behaviour both in terms of solubilization (during an *ex-situ* treatment) and in terms of variation in metal partitioning. Nevertheless, As, Cd, Ni and Pb bioleaching from marine sediment appeared to be less affected by their initial partitioning; partitioning was more important for Pb at neutral pH values (FONTI *et al.*, 2015; FONTI *et al.*, 2013a). A regression analysis has confirmed a positive correlation between partitioning and metal solubilisation potential for Zn and Cr, but a linear univariate model is not sufficient for describing the relationship. On the contrary, we have found a positive linear correlation between Zn mobilization and TOM (Fig. 6). Similarly, we have found a positive correlation between TOM and As mobilization and a negative correlation with Cr, but a lack of fit test revealed a need for higher-order effects for both. Particulate organic matter represents a very important scavenger of metals and semi-metals in the sediment (WANG and MULLIGAN, 2006; WARREN and HAACK, 2001).

Table 5. Summary output table of the analyses of linear regression among metal solubilization potential and geochemical properties of the sediment.

Metal	**Predictive variables**[1]	**Goodness of Fit**	
		Coef. of determination[2]	**Lack of fit**[3]
Zn	**M_Zn**	r^2= 0.658 (+)	F ratio= 14.427, p < F 0.0009*
	TOM	r^2= 0.789 (+)	F ratio= 0.781, p < F 0.0788 n.s
	Carbonates	r^2= 0.658 (-)	F ratio= 18.532, p < F 0.0003*
Cd	**M_Cd**	r^2= 0.907 (-)	n.d.
	TOM	r^2= 0.907 (+)	n.d.
	Carbonates	r^2= 0.907 (-)	n.d.

Table 5. continue on next page.

Table 5 continuation.

Metal	Predictive variables[1]	Goodness of Fit	
		Coef. of determination[2]	Lack of fit[3]
Cr	**M_Cr**	r^2= 0.695 (-)	F ratio= 29.937, p < F <0.0001*
	TOM	r^2= 0.777 (-)	F ratio= 15.674, p < F 0.0006*
	Carbonates	r^2= 0.839 (+)	F ratio= 4.953, p < F 0.0361*
As	**M_As**	r^2= 0.209	F ratio= 36.956, p < F <0.0001*
	TOM	r^2= 0.580 (+)	F ratio= 8.797, p < F 0.0069*
	Carbonates	r^2= 0.691 (+)	F ratio= 0.433, p < F 0.5170 n.s
Ni	**M_Ni**	r^2= 0.004	F ratio= 4.706, p < F 0.0406*
	TOM	r^2= 0.166	F ratio= 0.203, p < F 0.6566 n.s.
	Carbonates	r^2= 0.098	F ratio= 2.090, p < F 0.1618 n.s
Pb	**M_Pb**	r^2 = 0.469	n.d.
	TOM	r^2 = 0.469	n.d.
	Carbonates	r^2 = 0.469	n.d.

[1] M_= index of mobility for a specific metal (calculated on the basis of partitioning among sediment geochemical fractions); TOM= content of total organic matter in the sediment; Carbonates = total carbonate content in the sediment.

[2] signs (+) or (-) are given only when $r^2>0.50$.

[3] The null hypothesis is that the model does not need higher-order effects. n.s. = non-significant lack of fit; * = significant lack-of-fit (even if r^2 is high, the correlation is not significant; the model needs more effects); n.d. = not determined (saturated model; Pb and Cd were not investigated in sediment C because of their very low concentrations).

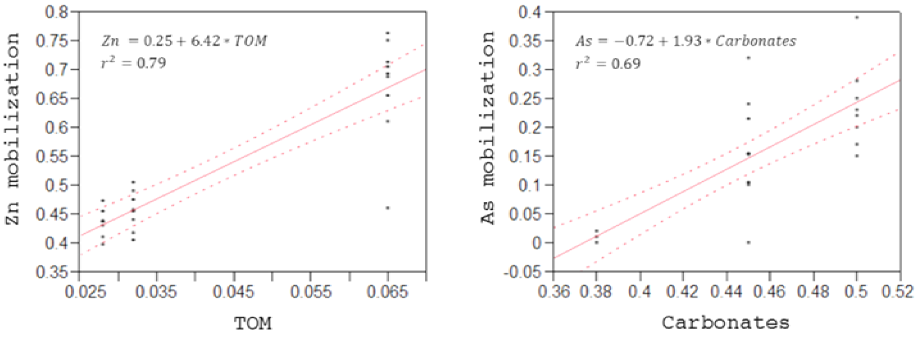

Fig. 6. Examples of linear regression between metal solubilization potential and geochemical properties of the sediment. In this figure we show the correlation between Zn mobilization and TOM in the sediment and the correlation between As and carbonate content. Other correlations and further details are listed in Tab. 5.

Carbonates correlate in positive way with a non-significant lack of fit only with As (Fig. 6). Cr was correlated positively to sediment carbonate content, too; on the contrary, Zn was negatively related, but a lack of fit was found for both of them. We expected a Zn negative correlation with carbonates because Zn is highly affected by pH (CHEN and LIN, 2001; MCBRIDE *et al.*, 1997) and carbonates are the main compounds to be responsible for the acid-neutralizing capacity of the sediment (i.e. buffering capacity); the positive correlation between carbonates and As either Cr mobilization needs further understanding efforts.

Nickel was not correlated with any factors (Tab. 5); however, our bioleaching experiment suggested that pH could be the most important parameter to affect Ni mobilization (FONTI *et al.*, 2013a).

4. Conclusions

The prediction of metal fate in sediment environment, both in *ex-situ* bioleaching treatments and in *in-situ* biostimulation strategies, appears to require metal-specific and site-specific tools. Those two very different bioremediation strategies were applied on similar sediment samples; a comparison of the results of the two experimentations has provided new evidences about the key-constraints to study in depth, towards the definition of sediment bioremediation know-how, as well as of predictive tools to apply to several types of sediment. In particular, TOM and pH could be the main variables in describing and predicting Zn behaviour. Cd, Pb and Ni could require multivariate and/or non-linear approaches. As solubilisation was positively correlated with sediment carbonate content and, although As solubilized hardly, the variations in the microbial community could increase significantly its concentration in the most mobile sediment fractions, with a potential increase in bio-availability and toxicity. A similar effect was observed also for Pb but other investigations are needed. On the contrary, we have gained few insights about Cr behaviour and further studies should be performed on sediment with high Cr concentrations in non-residual fractions.

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