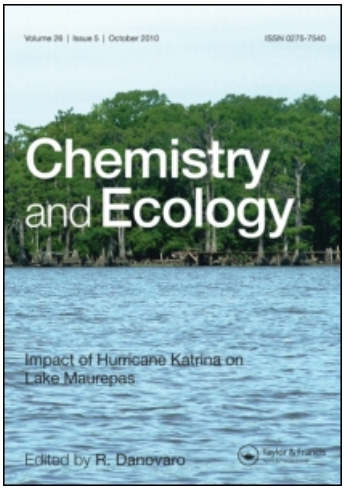

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Influence of the mineralogical composition on microbial activities in marine sediments: an experimental approach

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INFLUENCE OF THE MINERALOGICAL COMPOSITION ON MICROBIAL ACTIVITIES IN MARINE SEDIMENTS: AN EXPERIMENTAL APPROACH

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We investigated the influence of the mineralogical composition of marine sediments on bacterial activity in experimental microcosms. Calcite and quartz were added to natural marine sediments and microbial response in terms of total bacterial abundance and biomass, β-D-glucosidase exo-enzymatic activity and bacterial incorporation of a radio-labelled (^{3}H-leucine) substrate were investigated for a period of one month. We report here that after 15 days the mineralogical composition of the sediment (calcite *vs.* quartz) had an impact on bacterial abundance and activity (reduced for ca 15% and 56%, respectively). However, such impact was mitigated or even disappeared in high organic nutrient conditions.

Keywords: Bacterial activity; Mineralogical composition; Sediment

1 INTRODUCTION

In the marine environment, bacteria play a key role in biogeochemical cycles, early diagenesis of organic matter and nutrient regeneration (Azam, 1998). These processes largely occur on surface sediments, which can be assumed to be hot spots of microbial activity. Bacterial abundance and distribution in the sediment are controlled by a complex array of biotic and abiotic factors. Among these, grain size and the micro-topography of the sediment have long been assumed to play a major role in marine environments (De Flaun and Mayer, 1983). Previous studies have shown the presence of inverse relationship between sediment grain size and bacterial abundance (Dale, 1974; Hargrave, 1972; Yamamoto and Lopez, 1985), and it has been hypothesized that sediment grain surface, available for bacterial colonization, was a potential limiting factor for bacterial growth (Levinton, 1977). On the other hand, micro-topography of sediment grains might affect bacterial colonization, as bacteria generally concentrate in cracks and crevices, which offer protection from abrasion and mechanical cell damage (Batoosingh and Anthony, 1971; Meadows and Anderson, 1966).

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In benthic environments, most bacteria live attached to sediment surfaces and only a minor fraction lives free in the interstitial waters (Meadows and Anderson, 1966). Bacterial attachment to sediment surface has been reported to depend on several factors, such as ionic strength, bacterial species and mineralogical composition (Mills and DeJesus, 1994; Yee *et al.*, 2000). Bacteria-mineral interactions seem to be crucial, as bacterial biofilms can only be formed if bacteria can first adsorb onto the mineral surfaces (Yee *et al.*, 2000). The initial step of microbial colonization of the sediment surface is the bacterial adsorption onto mineral surface, which allows subsequent enhancement of bacterial metabolic processes, such as exo-polymer and exudate production (Costerton *et al.*, 1995). On the basis of these findings, it appears possible that the mineralogical composition of the sediments can influence the carbon cycling and the early diagenesis of organic matter. Moreover, since the presence of bacterial biofilms is a prerequisite for the colonization of the benthic metazoan, the mineralogical composition of the sediment may induce a potential cascade effect on the succession of benthic metazoans (Johnson *et al.*, 1997; Unabia and Hadfield, 1999; Bavestrello *et al.*, 2000).

In the present study, we tested the effect of different mineralogical composition of the sediment on the activity of marine bacteria in experimental conditions. To do this, we added calcite and quartz to natural marine sediments and we investigated (in both food-limiting conditions and after organic enrichment) the effect on several bacterial parameters, such as total bacterial abundance, biomass, exo-enzymatic activity and *C* production. The minerals quartz and calcite were chosen because they represent the most abundant minerals in marine sediments (Hazen *et al.*, 2001).

2. MATERIAL AND METHODS

2.1 Sediment Samples and Mineralogical Analysis

Surface natural marine sediments (top 8 cm) were collected manually using Plexiglas tubes at 1-m depth from a sandy bottom in the Adriatic Sea (Mediterranean Sea). Quartz and calcite grains were obtained in laboratory from breaking of quartzite rock and pure marble (Carrara). Quartz and calcite sediments were sieved and selected for their grain size composition, in order to produce a sediment texture identical to that observed in natural sediments.

Natural sediments texture (analyzed using the mesh-sieving technique) was constituted by 80% of fine-sand (range: 125–250 μm) and 20% of very fine-sand (range: 63–125 μm). The morphology of the sediment grains (natural, quartz and calcite) was determined by Scanning Electron Microscopy (SEM, Philips XL20) to investigate the distinctive shapes and morphological structure of the sediment grains. Mineralogical determinations were conducted under X-ray diffraction analysis.

2.2 Experimental Design

Three different experimental conditions were set in 3-replicate microcosms: (a) 100% natural marine sediments (hereafter defined "natural microcosms"); (b) 45% natural marine sediments and 55% (v:v) quartz sediments (hereafter defined "mixed quartz"); (c) 45% natural marine sediments and 55% (v:v) calcite sediments (hereafter defined "mixed calcite"). Each microcosm (sterile glass beaker of 600 ml) contained about 200 ml of sediment (previously mixed) and 200 ml of seawater (1:1 v:v) collected at the sediment-water interface and filtered through a 20 μm-mesh sieve to eliminate larger grazers. At the beginning of the experiment ($T = 0$), the pure mineral sediment (quartz and calcite) and the seawater contained natural bacterial densities of about 0.4 and 1.2×10^6 cells ml^{-1}, respectively (2–3 orders of

magnitude lower compared to natural sediment). Natural sediments contained 124.5 μg of proteins and 174.4 μg of carbohydrates g^{-1} of sediment dry-weight, while in mixed systems were present 50.8–56.6 μg of proteins and 53.5–67.6 μg of carbohydrates g^{-1} of sediment dry-weight, in calcite and quartz systems respectively. Microcosms were transferred in an incubation chamber (in the dark, at *in situ* temperature of 15 °C) and left 1 day until the first sub-sampling. Sub-samples were collected at the beginning of the experiment (day 1) and after 2, 3, 7, 10, 15, 20 and 30 days. At each sampling time, sediment slurries of 1 ml for each bacterial analysis were withdrawn after gentle agitation of each experimental microcosm.

Measurements of pH were carried out in all microcosms at each sampling time (by using a AMEL-Instruments pHmeter mod.334-B).

In order to investigate the effects of the mineral composition also in different trophic conditions, after 10 days from the beginning of the experiment, glucose (0.59 mgC cm^{-3} of sediment) and albumin (0.16 mgN cm^{-3} of sediment) were added at two of the three replicates of each system. The third replicate without organic input served as control and sub-samples ($n = 3$) were collected. This supply was selected for mimicking a natural organic matter input and for providing a C/N ratio suitable for bacterial growth (*i.e.* 4).

2.3 Bacterial Parameters

For bacterial number and biomass determination, samples of sediment slurry (1 ml) were fixed in 5 ml of 0.2 μm pore size filtered borax-buffered formalin (2% final concentration) and stored at 4 °C for later analyses. Enzymatic activities and bacterial production were measured immediately after sampling and all the analyses were carried out within 2 weeks from sampling.

For bacterial counting, sediments were treated with ultrasounds three times (Branson Sonifier 2200; 60 W for 1 min) and diluted 100–500 times with sterile and 0.2 μm prefiltered formalin (2% final concentration). Samples were stained for 5 min with Acridine Orange (final concentration 5 mg l^{-1}), filtered on black 0.2 μm pore-size Nuclepore Polycarbonate filters, and analyzed as described by epifluorescence microscopy (Zeiss Universal Microscope; magnification 1000*x*). Bacterial biovolume was estimated assigning bacteria into three different size classes (Danovaro and Fabiano, 1995) and then converted into carbon content assuming 310 fgC μm^{-3} (Fry, 1988). Data were normalised to ml of sediment slurry.

β-D-glucosidase activity was selected among bacterial exo-enzymatic activities because natural sediments displayed the dominance of carbohydrates among biochemical components (as determined immediately after sampling). Sediment slurries were prepared according to Poremba (1995) and incubations were performed in the dark at *in situ* temperature (Hoppe, 1993) for 1 h (exo-enzymatic activity increased linearly with time) in triplicate. Exo-enzymatic activity was measured by adding 150 μl of 4-Methylumbelliferone β-glucoside (final concentration 200 μM, after determination of saturating concentration; Poremba, 1995). After incubation, samples were centrifuged (3000 rpm, 5 min.) and the supernatant analyzed fluorometrically (at 365 excitation, 455 emission; Hoppe, 1993). Solutions of 4-Methylumbelliferone (MUF; 0.1 to 1.0 μM) were used as standards. Blanks were made using seawater 0.2 μm prefiltered used for preparing the slurry. Immediately after substrate inoculation, sample fluorescence (at $t = 0$) was measured and subtracted from fluorescence after 1 h of incubation. Data were normalised to ml of sediment slurry and reported as nmol of MUF released per ml of slurry h^{-1} (Danovaro *et al.*, 2001).

Bacterial secondary production was measured by the incorporation of ^{3}H-leucine following the procedure described by van Duyl and Kop (1994) for sediments. Sediment subsamples (200 μl) added with an aqueous solution of ^{3}H-leucine (6 μCi final concentration, specific activity 51 Ci $mmol^{-1}$), were incubated for 1 hour in the dark at *in situ* temperature. After incubation, samples were added with ethanol (80%) before scintillation counting.

Sediment blanks were made adding ethanol immediately before ^{3}H-leucine addition. Data were normalised to sediment slurry.

2.4 Statistical Analyses

In order to identify differences among sediments with/and without nutrient addition, all benthic bacterial parameters (bacterial abundance and biomass, bacterial production and exo-enzymatic activity) were tested with one-way ANOVA test and significance expressed at $p \leq 0.05$.

3 RESULTS

3.1 X-ray Diffraction Analyses and SEM Observations of the Sediment Grains

X-ray diffraction analysis of natural marine sediments showed fraction of minerals such as: anhydrite, ankerite, dolomite, aluminium sulphate, with a high percentage (>80%) of silica minerals (*e.g.*, alpha quartz, SiO_2) and calcite ($CaCO_3$) (Tab. I). Additional X-ray diffraction analyses were performed on calcite and quartz confirming the expected mineralogical composition.

Scanning electron microphotographs (SEM) of the different sediment types showed heterogeneous shapes and surface morphology (Fig. 1). The morphology of quartz grains was irregular, with highly abraded surfaces and a high number of hollows and cracks (Fig. 1A), whereas calcite particles displayed a smoother surface (Fig. 1B).

3.2 pH Measurements

pH was measured during the whole course of the experiment, to assess changes due to the addition of the minerals. pH was measured both in the water overlying the sediments and within the sediment (Tab. II). In all microcosms, pH did not show marked temporal variations neither significant differences between water and sediments during the entire experiment. Values ranged between 8.0 and 8.3 in the seawater and between 7.7 and 8.0 in the sediments.

TABLE I Mineralogical Composition of Natural Marine Sediments.

Minerals	*Relative importance (%)*
Alpha quartz (SiO_2)	47.0
Calcite ($CaCO_3$)	36.0
Ankerite (Ferroan Dolomite)	3.0
Iron sulfate hydroxide ($Fe2O_3*2SO_3*H_2O$)	2.0
Dolomite ($CaMg(CO_3)_2$)	1.0
Tetrammine Cu sulfate ($Cu(NH_3)_4SO_4$)	1.0
Anhydrite ($CaSO_4$)	0.5
Aluminium sulfate ($Al_2(SO_4)_3$)	0.5
Nickel (Ni)	0.5
Aragonite ($CaCO_3$)	0.5
Others	8.0

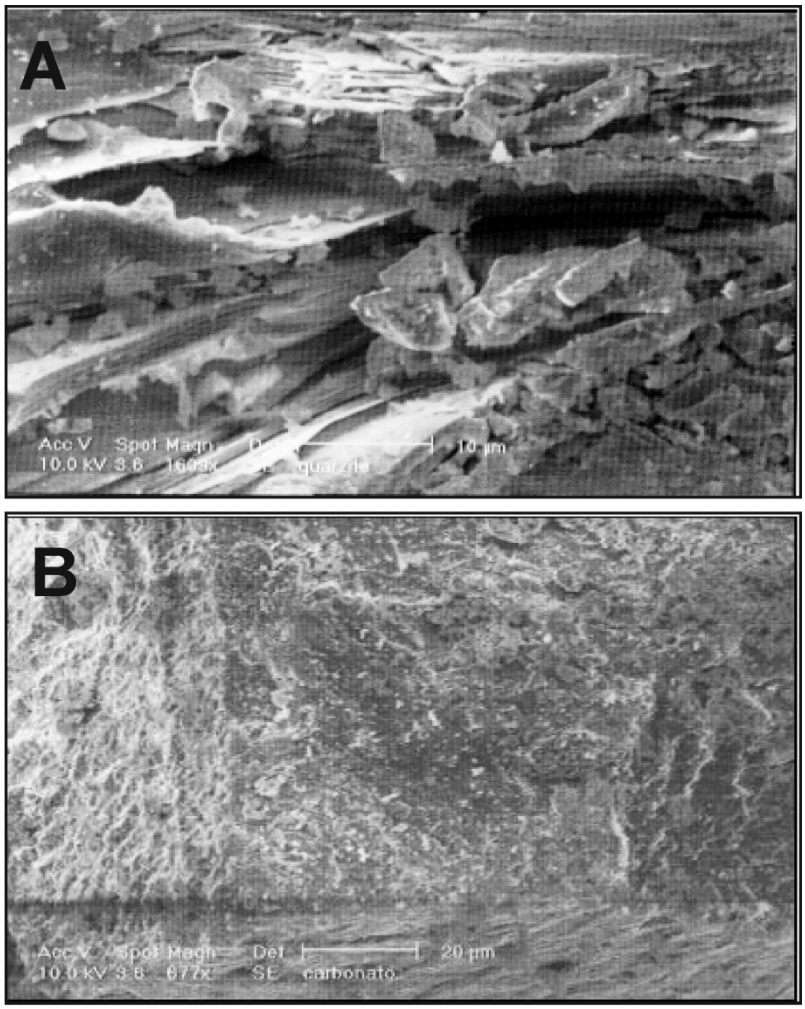

FIGURE 1 Scanning electron microphotographs (SEM) of the mineral grains. Reported are: (A) quartz grain (magnification 1609×) and (B) calcite grain (magnification 677×).

3.3 Bacterial Parameters

Table III shows the results of the bacterial parameters "observed" in the different sets of microcosms (natural, mixed quartz and mixed calcite) and those "expected". The "expected" values are calculated dividing values of natural microcosms by a factor 1.8 (dilution natural sediment to minerals of 45 to 55%; v/v), ignoring the low contribution of seawater and pure mineral additional (about 1%).

At the beginning of the experiment (*i.e.* 1 day after the start of the experiment), natural microcosms displayed a bacterial abundance of $1.02 \pm 0.34 \times 10^8$ cells ml^{-1}. Bacterial abundance increased during the course of the experiment, reaching a maximum at day 15 ($2.7 \pm 0.2 \times 10^8$ cells ml^{-1}), and then decreased to recover gradually the initial values. According to the dilution caused by the addition of new sediment, mixed calcite and quartz microcosms displayed initially a number of bacteria half of the natural sediment

TABLE II Values of pH in Seawater and in the Sediments in Each Microcosm.

	Natural 100%		*Natural 50% + calcite 50%*		*Natural 50% + quartz 50%*	
Days	*pH water*	*pH sediment*	*pH water*	*pH sediment*	*pH water*	*pH sediment*
1	8.13	nd	8.07	na	8.15	nd
7	8.02	7.94	8.03	7.88	8.16	8.00
10	8.07	7.89	8.05	7.86	8.15	7.90
15	8.29	7.90	8.10	7.99	8.24	8.00
20	8.20	7.83	8.01	7.92	8.10	7.80
30	8.13	7.73	8.02	7.94	8.12	7.93
Average ± se	8.1 ± 0.04	7.9 ± 0.03	8.0 ± 0.01	7.9 ± 0.02	8.2 ± 0.02	7.9 ± 0.03

($0.50 \pm 0.14 \times 10^8$ cells ml^{-1} and $0.45 \pm 0.09 \times 10^8$cells ml^{-1}, respectively); also in these systems bacterial density increased by 2–3 fold in the following 10–15 days and then decreased with values generally lower than the "expected". In all microcosms, organic substrate addition determined a significant increase of the bacterial number (ANOVA $p < 0.01$), which reached mean values about 2 times higher than in the relative non enriched samples.

In all microcosms, bacterial biomass followed a similar temporal pattern. In natural and mixed quartz, bacterial biomass increased up to day 15 (30.2 ± 0.1 and 15.2 ± 0.5 μgC ml^{-1} sed., respectively for natural and mixed quartz, respectively), while mixed calcite increased up to day 10 (18.3 ± 0.3 μgC ml^{-1} sed.); then all the samples gradually decreased untill the end of the experiment with values, in mixed microcosms, generally lower than the "expected". At the end of the experiment ($T = 30$), the effect of organic enrichment on bacterial biomass was particularly evident in mixed calcite and quartz, with values about 2 times higher than in the relative non enriched samples and higher (by 30–60%, respectively) than "expected" values.

Temporal patterns of β-glucosidase activity in all experimental microcosms are reported in Table III. In natural microcosms, β-glucosidase activity ranged from 0.4 to 0.7 nmol ml^{-1} sed. h^{-1} but in both mixed microcosms, β-glucosidase activity was significantly lower than expected values (ANOVA, $p < 0.001$). In all microcosms, after organic enrichment, β-glucosidase activity displayed values significantly higher (by 2 to ca 5 times in natural and mixed microcosms, respectively; ANOVA, $p < 0.05$) and in this effect was particularly marked in mixed quartz microcosm.

In the first 20 days of the experiment, bacterial secondary production in natural microcosms was rather constant (on average, 143.3 ± 5.4 ngC ml^{-1} sed. h^{-1}), but a peak was measured at the end of the experiment, up to 258.5 ± 64.3 ngC ml^{-1} sed. h^{-1} (ANOVA, ns). Bacterial production in mixed calcite was always higher than "expected" while in quartz microcosms such enhancement was clear only for the first 7 days. In natural sediment and in mixed calcite, the addition of glucose and albumin at day 10 determined an increase of bacterial production (day 15) which then strongly decreased till day 30. In contrast, mixed quartz always displayed higher values compared to relative non enriched samples (ANOVA, $p < 0.01$).

4 DISCUSSION

The aim of this study was investigating the influence of the mineralogical composition of the sediments (*e.g.* quartz and calcite) on several marine bacterial parameters. In aquatic environments, sediment surfaces are usually highly colonized by microbes (De Flaun and Mayer,

TABLE III Bacterial Abundance, Bacterial Biomass, β-Glucosidase Activity, Bacterial Carbon Production, in All Investigated Microcosms. Reported are Standard Deviation and Averages of the Observed and Expected Values at Each Sampling Time.

		Bacterial density (cells × 10^8 cell ml^{-1})				*Bacterial biomass ($\mu gC\, ml^{-1}$)*			
		Observed value		*Expected value*		*Observed value*		*Expected value*	
Sediment type	*Days*	*Control*	*Enriched*	*Control*	*Enriched*	*Control*	*Enriched*	*Control*	*Enriched*
Natural 100%	1	1.02 ± 0.34				8.5 ± 0.04			
	2	1.49 ± 0.48				12.8 ± 0.77			
	3	1.65 ± 0.04				16.8 ± 0.53			
	7	1.37 ± 0.07				14.6 ± 0.10			
	10	2.51 ± 0.40	Supply			27.5 ± 0.46	Supply		
	15	2.74 ± 0.16	2.8 ± 0.13			30.2 ± 0.12	23.7 ± 0.46		
	20	2.41 ± 0.13	3.0 ± 0.02			27.4 ± 3.03	22.7 ± 0.52		
	30	1.38 ± 0.08	4.0 ± 1.38			16.6 ± 0.25	22.5 ± 1.23		
	Avg ± se	1.87 ± 0.19	3.5 ± 0.27			19.8 ± 2.41	22.6 ± 0.07		
Mixed calcite	1	0.50 ± 0.14		0.57		3.8 ± 0.07		4.73	
	2	1.01 ± 0.06		0.83		9.4 ± 0.03		7.12	
	3	1.46 ± 0.15		0.91		14.5 ± 0.88		9.32	
	7	1.06 ± 0.22		0.76		12.0 ± 0.10		8.12	
	10	1.53 ± 0.13	Supply	1.40	Supply	18.3 ± 0.33	Supply	15.30	Supply
	15	1.17 ± 0.00	1.3 ± 0.13	1.52	1.57	11.8 ± 0.88	12.0 ± 2.1	16.79	13.16
	20	1.13 ± 0.07	1.9 ± 0.07	1.34	1.68	10.5 ± 0.33	16.2 ± 2.7	15.22	12.62
	30	0.68 ± 2.57	2.6 ± 0.29	0.77	2.21	8.0 ± 0.00	16.5 ± 0.00	9.24	12.48
	Avg ± se	1.07 ± 0.12	1.9 ± 0.30	1.01	1.82	11.0 ± 1.43	14.9 ± 1.18	10.73	12.76
Mixed quartz	1	0.45 ± 0.09		0.57		4.1 ± 0.20		4.73	
	2	0.90 ± 0.12		0.83		6.9 ± 0.04		7.12	
	3	1.29 ± 0.29		0.91		12.3 ± 0.17		9.32	
	7	1.19 ± 0.05		0.76		13.1 ± 0.43		8.12	
	10	1.25 ± 0.06	Supply	1.40	Supply	13.1 ± 0.58	Supply	15.30	Supply
	15	1.27 ± 0.05	1.1 ± 0.02	1.52	1.57	15.2 ± 0.50	10.1 ± 0.00	16.79	13.16
	20	1.24 ± 0.06	2.1 ± 0.06	1.34	1.68	13.2 ± 0.65	15.4 ± 5.60	15.22	12.62
	30	0.93 ± 0.03	2.7 ± 0.20	0.77	2.21	10.3 ± 0.41	19.8 ± 2.30	9.24	12.48
	Avg ± se	1.06 ± 0.10	2.0 ± 0.38	1.01	1.82	11.0 ± 1.24	15.1 ± 2.29	10.73	12.76

(Continued)

TABLE III *Continued.*

		β-Glucosidase activity (nmol ml^{-1} h^{-1})				Bacterial carbon production (ngC ml^{-1} h^{-1})			
		Observed value		*Expected value*		*Observed value*		*Expected value*	
Sediment type	*Days*	*Control*	*Enriched*	*Control*	*Enriched*	*Control*	*Enriched*	*Control*	*Enriched*
Natural 100%	1	0.6 ± 0.05				119.8 ± 7.57			
	2	0.5 ± 0.06				158.9 ± 12.57			
	3	0.4 ± 0.03				159.7 ± 10.07			
	7	0.5 ± 0.17				155.5 ± 30.40			
	10	0.5 ± 0.08	Supply			137.6 ± 7.53	Supply		
	15	0.7 ± 0.07	0.9 ± 0.07			130.2 ± 0.00	197.9 ± 7.38		
	20	0.5 ± 0.15	1.2 ± 0.24			141.2 ± 1.59	128.0 ± 7.56		
	30	0.5 ± 0.00	1.2 ± 0.06			258.5 ± 64.26	77.1 ± 22.14		
	Avg ± se	0.5 ± 0.03	1.2 ± 0.01			160.97 ± 13.5	102.5 ± 14.7		
Mixed calcite	1	0.20 ± 0.02		0.31		162.8 ± 29.5		66.55	
	2	0.13 ± 0.03		0.27		119.3 ± 0.8		88.30	
	3	0.16 ± 0.02		0.21		113.7 ± 12.2		88.74	
	7	0.07 ± 0.03		0.28		158.5 ± 24.7		86.38	
	10	0.09 ± 0.00	Supply	0.26	Supply	52.7 ± 8.1	Supply	76.44	Supply
	15	0.05 ± 0.02	0.20 ± 0.02	0.38	0.53	159.1 ± 0.00	166.1 ± 36.3	72.35	109.95
	20	0.22 ± 0.04	0.53 ± 0.14	0.31	0.68	163.4 ± 15.7	107.5 ± 21.0	78.42	71.13
	30	0.11 ± 0.00	0.76 ± 0.09	0.27	0.66	190.0 ± 15.6	19.0 ± 1.9	143.60	42.85
	Avg ± se	0.13 ± 0.02	0.50 ± 0.13	0.29	0.62	139.9 ± 14.3	97.5 ± 34.9	87.60	74.64
Mixed quartz	1	0.18 ± 0.03		0.31		115.9 ± 4.4		66.55	
	2	0.13 ± 0.00		0.27		154.3 ± 13.7		88.30	
	3	0.17 ± 0.03		0.21		75.2 ± 10.7		88.74	
	7	0.07 ± 0.03		0.28		119.3 ± 28.1		86.38	
	10	0.11 ± 0.04	Supply	0.26	Supply	66.1 ± 4.4	Supply	76.44	Supply
	15	0.10 ± 0.02	0.56 ± 0.20	0.38	0.53	85.9 ± 0.0	135.5	72.35	109.95
	20	0.32 ± 0.06	1.11 ± 0.04	0.31	0.68	75.4 ± 0.0	101.4	78.42	71.13
	30	0.18 ± 0.06	1.06 ± 0.25	0.27	0.66	78.1 ± 2.1	155.6	143.60	42.85
	Avg ± se	0.16 ± 0.03	0.91 ± 0.14	0.29	0.62	96.3 ± 10.1	130.8 ± 12.9	87.60	74.64

1983), but the rate and the extent of bacterial attachment to mineral surfaces, as well as the factors controlling bacterial adsorption onto mineral surfaces, remain still poorly known (Mayer, 1999; Ransom *et al.*, 1999). Moreover, the possible role of mineralogy in influencing the activities of attached microbes in marine sediments has been till now much neglected.

The results obtained from the different sets of microcosms (mixed quartz and mixed calcite) were compared to "expected" values, calculated according to those measured in natural systems (diving values of natural microcosms by a factor 1.8). As a confirm of such a dilution event at 1 day, the bacterial abundance in mixed systems was about half than in natural systems (Tab. III). However, bacterial parameters displayed a clear resilience and recovered to expected values within the first 10 days, indicating a limited effect of mineral addition.

Comparing the expected values with mixed microcosms from 15 day till the end of the experiment, we found evident discrepancies. Bacterial abundance and biomass in mixed sediments were, on average, 15–18% lower than the expected value (Fig. 2). β-Glucosidase activities were strongly reduced after addition of both minerals (on average, 56% Fig. 2), while bacterial heterotrophic production was enhanced in calcite microcosms and was reduced in quartz microcosms (up to 25%). These results suggest an inhibition on bacterial metabolic activities more than on bacterial number and biomass which can be attributed only to the different mineralogical composition of the sediments since the others main environmental constrains (pH, temperature, light, grain size) remained unvaried.

Explaining the reason of such a negative impact by minerals is a quite difficult task. It could be due either to the oxidant properties of the crystal quartz surface (Marasas and Harington, 1960; Shi *et al.*, 1988; Langer and Nolan, 1986), either to the presence in calcite microcosms of high Ca^{2+} concentrations and/or changes in ionic strength (due to the partial dissociation of carbonates in aqueous solution). Quartz is known to have toxic effects at cellular level (Syed and Hunter, 1997), but literature information on its effects on aquatic bacteria is extremely scant and controversial. Gordon *et al.* (1983) studying the metabolism of *Vibrio alginolyticus*, found that bacterial attachment was not affected by the presence of quartz particles. Moreover, Scholl (1989) reported high numbers of bacterial cells attached to quartz surface. Conversely, Herbold-Paschke *et al.* (1990) found that only a small percentage of the bacteria (*i.e.*, *Escherichia coli*) was adsorbed to quartz grains and Mills and Maubrey

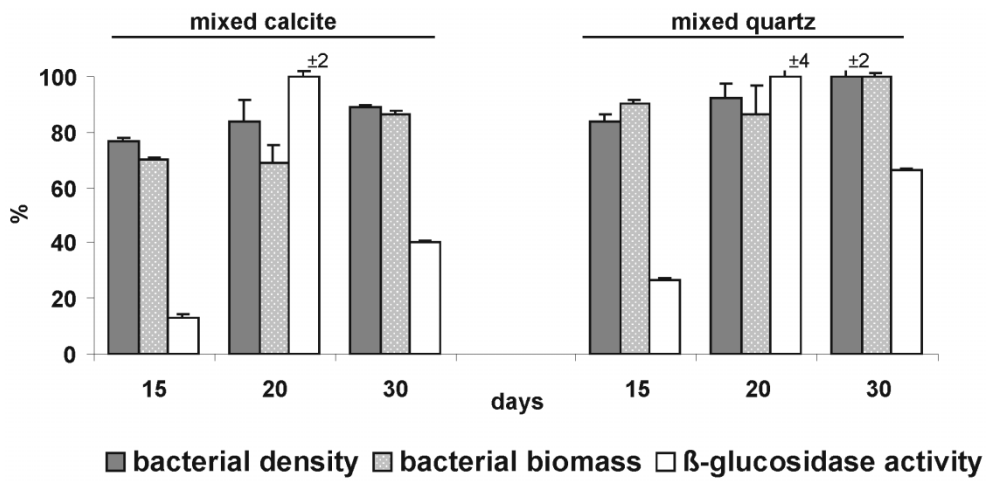

FIGURE 2 Inhibition of bacterial metabolic activity expressed as percentage of the expected value: bacterial abundance, bacterial biomass, β-glucosidase activity in mixed quartz and calcite, measured at each sampling.

(1981), comparing microbial colonization on exposed and submerged hard substrates, reported that bacterial colonization of quartz was always significantly higher than for calcite. Yee *et al.* (2000) found experimentally a very weak adsorption of *Bacillus subtilis* onto quartz surfaces. Ferris *et al.* (1989) investigating heterotrophic bacteria from freshwater systems, found that bacterial abundance on granite, gabbro, rhyolite, basalt and quartz was 10–100 fold lower than on limestone.

Contrary to quartz, calcium carbonate is not known to exert cytotoxic effects. According to this, it could have not direct effects on cell metabolism, but could alter ionic strength in the interstitial waters due to the high solubility of calcite in seawater.

Despite SEM analyses revealed a different morphology of the sediment grains (Fig.1), quartz and calcite apparently had a similar impact on microbial parameters. We know that the micro-topography of sediment grains influences bacterial colonization (Shimp and Pfaender, 1982; Yamamoto and Lopez, 1985). In fact, grains showing irregular morphology seem to favor microbial colonization, since cracks and crevices offer protection from abrasion and mechanical cell damage, shear stress and/or grazing by predators. On the basis of SEM analysis, quartz grains represented more suitable substrates for bacterial colonization than well-rounded grains of carbonate. However, we did not find differences in the degree of inhibition among quartz and calcite systems. This result can indirectly suggest that, in absence of water movement and grazing pressure, micro-topography of sediment grains does not affect bacterial assemblages bulk properties.

In both natural and mixed calcite and quartz microcosms, after nutrient addition, bacterial parameters responded increasing their cell metabolism. The addition of organic substrates into the microcosms greatly reduced the impact of minerals on bacterial activity especially in quartz microcosms, where the organic supply can act as a scavenger of produced oxyradicals (thus reducing their toxicity; DeLange and Glazer, 1989).

In conclusion, the results presented here indicate that the mineralogical composition of the sediment grain might significantly affect benthic bacterial assemblages. A strong presence of quartz and calcite can have a clearly negative impact on bacterial activities reducing the formation of the primary bacterial biofilm with cascade effects on the succession of benthic metazoans which, for their colonization of incoherent and hard substrates, depend on the presence of bacterial films. Such impact can be mitigated and neutralized by the presence of an organic coating of the mineral surface reducing the reactivity of minerals in aqueous solutions and favouring the substratum attachment (Mayer, 1999; Thomas *et al.*, 1993).

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