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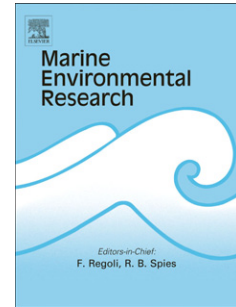
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**DIATOM QUANTIFICATION AND THEIR DISTRIBUTION WITH SALINITY  
BRINES IN COASTAL SEDIMENTS OF TERRA NOVA BAY (ANTARCTICA)**

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## 1 Abstract

2 Benthic diatoms represent an important element of global nutritional productivity; to  
3 raise attention on their role, which is often neglected due to analytical difficulties, surface  
4 (1 cm top layer) coastal sediments from Gerlache inlet to Penguin Bay at Terra Nova Bay  
5 were collected and stored at -20°C. DNA amplification by real-time-PCR, based on diatom-  
6 specific oligonucleotide primers designed on small-subunit rRNA (SSU rRNA), was  
7 performed in addition to diatom conventional cell counting and spectrophotometric  
8 determination of photo-pigments. Moreover, cations and anions were determined in  
9 sediments with the aim to identify factors involved in the control of diatom abundance.  
10 Diatom distribution was found quite heterogeneous displaying significant differences from  
11 site to site. The salinity in sediments ranged from 45.1 at Gerlache inlet to 76.2 at Penguin  
12 Bay and it was correlated with cell abundance, biodiversity, amount of pigments and  
13 amplified DNA. The dominant species, *Fragilariopsis curta*, was associated to sediment  
14 salinity brines.

15  
16 Keywords: diatoms, real-time PCR, coastal sediments, SEM-EDX analyses, salinity brines,  
17 Terra Nova Bay.

## 19 1. Introduction

20 Diatoms are responsible for a substantial part of the carbon fixation (Arrigo et al.,  
21 1999), especially at Ross Sea (Antarctica), which is characterized by relatively high  
22 primary productivity due to the Polynya phenomenon. Pennate diatoms can be found in  
23 water column and in sediments and, at high latitudes they are the most conspicuous  
24 organisms in the pack ice, where they could reach such high concentrations that their  
25 photosynthetic pigments discolor the ice brown (Thomas and Dieckman, 2002). Several  
26 diatom species have been found in this harsh habitat, where low ice temperatures are

27 linked to high levels of salinity brine (Zhang et al., 1999). These unicellular photosynthetic  
28 microalgae are mainly found on the underside of the ice where the light is more intense  
29 than in the rest of the water column. However, their abundance can be significant also in  
30 the surface sediments of the coastal zone where the light reaches the bottom and in some  
31 areas they were found to be more productive than phytoplankton (Brotas and Catarino,  
32 1995; Cabeçadas et al., 2000). Several pennate diatoms manage to migrate through  
33 sediment layers but 80% of the living community is concentrated in the top few millimeters  
34 (MacIntyre et al., 1996).

35 Diatom colonization of sediments is a poorly studied topic due to sampling and  
36 analytical difficulties. Microscopy observations are complicated by the presence of  
37 sediment grains and organic matter, hence, frustule cleaning with acids or hydrogen  
38 peroxide is essential. In such conditions the distinction between live cells, dead cells still  
39 carrying intact biomolecules and empty frustules is very difficult to achieve. However, as it  
40 is still unclear which may be the role of benthic diatoms in the sediment organic carbon  
41 enrichment, it is of primary interest to improve the investigation methods and to obtain  
42 coherent correlation among data. Moreover, recent *in situ* productivity studies based on  
43 detection of biopolimeric carbon (BPC; the sum of carbon equivalents of proteins,  
44 carbohydrates and lipids) in coastal sediments at Terra Nova Bay during the Austral  
45 summer 2004-05 suggest a key role for diatoms (Baldi et al., 2010).

46 The few information available indicate that the dominant species in the settled  
47 material (hence coming from the water column) of the southwestern Ross Sea, as well as  
48 in the surface sediments North of Drygalski Ice Tongue (Western Ross Sea, South of  
49 Terra Nova Bay), is *Fragilaropsis curta*, which is commonly associated with both sea ice  
50 and the retreating ice edge, suggesting that it is probably seeded by melting ice (Leventer  
51 and Dunbar, 1996, Cunningham and Leventer, 1998). *F. curta* was found to characterize  
52 the surface sediments also in other regions of the Antarctic shelf, such as Prydz Bay,

53 representing up to the 60% of total diatoms (Rathburn et al., 1997).

54 The aim of this study was to provide information on diatom abundance and  
55 distribution in poorly studied coastal sediments of Terra Nova Bay (Ross Sea, Antarctica).  
56 Such organisms may represent an essential food source and it is important to understand  
57 their role in the trophic chain mainly in a region characterized by extreme environmental  
58 conditions but also able to guarantee the survival of high trophic level animals (e.g.  
59 penguins; Sweeney et al., 2000). In order to reduce the analytical difficulties linked to the  
60 determination of benthic community different techniques were used. Conventional cell  
61 counting was related to quantitative determination of photopigments, nutrients and PCR  
62 amplified DNA in the attempt to distinguish between DNA containing diatoms and empty  
63 frustules settled in the sediments. Moreover, the correlation between biological and  
64 chemical data may furnish indication on the factor controlling diatom distribution in such an  
65 extreme habitat.

## 67 **2. Materials and methods**

### 68 *2.1. Sampling area*

69 The study area and sampling protocol are described in Baldi et al. (2010). Coastal  
70 sediment samples (1 cm surface layer) were collected at Terra Nova Bay (Ross Sea,  
71 Antarctica) during the Italian Antarctic Expedition of January 2005. During the ice melt-out,  
72 samples were taken in seven different areas (Fig. 1). Stations A and B are located in  
73 Tethys Bay, station C is in Penguin Bay near a rookery, station D at Malippo Pier in the  
74 vicinity of the Italian Base; stations E, F and G are at Gerlache Inlet. The first centimeter of  
75 sediments was cored at water depths ranging between 19 m and 66 m along the coast  
76 (Baldi et al., 2010). Sediment samples were stored at -20°C and freeze-dried depending  
77 on the analytical protocol. Sediment grain size varied from coarse-sand to sand (Dunbar et

78 al., 1985). Sediment water content was estimated as the difference between wet and dry  
79 weights by drying at 60°C to constant weight.

80

## 81 *2.2. Diatom counting by light microscopy*

82 The frozen sediments were weighed and treated with 10 ml of 10% (v/v) hydrogen  
83 peroxide for 32 hours (Cunningham et al., 2005). The slurry was then diluted with 8 ml of  
84 distilled water and centrifuged for 5 min at 3000 rpm. The supernatant was decanted and  
85 the sediment was suspended again in 10 ml of distilled water. This process was repeated  
86 twice and then the sediment was resuspended in 10 ml of water. The solution was gently  
87 homogenized and 200 µl were allowed to settle on a 2 ml microscope chamber for  
88 analysis on an inverted light microscope. At least 230 cells were counted and identified in  
89 random fields. The diatoms were taxonomically identified according to Peragallo and  
90 Peragallo (1897-1908), Tomas (1996), Vanlandingham (1967-79) and Round et al. (1990)  
91 and cell sizes were measured to estimate the carbon content (biomass) according to  
92 Edler's formulation (1979).

93

## 94 *2.3. Scanning Electron Microscopy and X-ray dispersive microanalyses*

95 An aliquot (100 µg) of homogenized freeze-dried sediment for each sample was  
96 prepared to determine diatom frustules, sediment structure and elemental composition of  
97 particles by scanning electron microscopy energy dispersive X-ray microanalysis  
98 (SEM/EDX). Specimens were glued to standard vacuum-clean stubs and coated with  
99 graphite (Edwards, carbon scancoat, S150A) (Milanesi et al., 2009). Three specimens of  
100 each sediment sample were observed by SEM (Philips XL20) with an instrument equipped  
101 with an EDX DX4 probe, which was used at 20 kV acceleration voltages to identify  
102 elements on the sediment surface by scanning different areas of each specimen. Mean

103 concentrations and standard deviations of each detected element were calculated from ten  
104 random determinations at different spots on each specimen.

105

#### 106 *2.4. Photopigment analysis*

107 Sediment samples for characterization of biochemical composition were freeze-dried and  
108 homogenized, paying attention to remove gravel and shell debris. Chlorophyll-a (Chl-a)  
109 and phaeophytine-a (Phaeo-a) were extracted from 2 g of sediment with 5 ml acetone for  
110 12 h at 4°C; supernatants containing the extracted pigments were recovered by decanting  
111 the supernatant after centrifugation (Lorenzen, 1967; Pusceddu et al., 2003). Chl-a and  
112 Phaeo-a concentrations were determined spectrophotometrically at 750 and 665 nm  
113 before and after acidification with 0.1 N HCl. These data provided an indication of the  
114 pigment concentrations in the sediments without information on the taxonomic group that  
115 produce them. However, it was observed that diatoms are often the dominant taxon  
116 (MacIntyre et al., 1996; reference in Facca and Sfriso, 2007).

117

#### 118 *2.5. Quantification of water-washed cations and anions*

119 Cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{NH}_4^+$ ) and anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ) were quantified to  
120 correlate the presence of diatoms with nutrients in salts precipitated on sediment particles.  
121 Nutrients were washed out from 1 g of frozen sample with 50 ml of milliRo water after 3  
122 min vortexing at 5°C. The supernatant was filtered through Whatman black ribbon 589/1.  
123 An aliquot of 20  $\mu\text{l}$  was injected into a pre-loop filter with 0.45  $\mu\text{m}$  cartridge ionic  
124 chromatograph (METROHM 761 Compact IC) with a conductimeter as detector. For anion  
125 determination the chromatograph was equipped with a column METROSEP ASUPP5 150  
126 mm x 4 mm with a mobile phase consisting of  $\text{NaHCO}_3$  1mM/ $\text{Na}_2\text{CO}_3$  3.2 mM running at a  
127 flow rate of 0.7  $\text{ml}\cdot\text{min}^{-1}$ ; for cation determination the chromatograph was equipped with a



128 METROSEP C3 250 x 4 mm column with a mobile phase consisting of HNO<sub>3</sub> 3mM  
129 working at 1 ml·min<sup>-1</sup>. Salinity was calculated as S=1.80655 Cl<sup>-</sup>.

130

### 131 2.6. Real-time PCR

132 Benthic diatom cells were prepared for the polymerase chain reaction (PCR) analysis by  
133 diluting one gram of each sediment sample in 30 ml of buffer S (880 µM NaNO<sub>3</sub>, 36 µM  
134 NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, and 105 µM Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O pH 7.5). 600 µl of diluted sediment sample  
135 were filtered through nitrocellulose membrane (Millipore 0.45µm, 25mm Ø) to collect and  
136 concentrate diatoms that were extensively washed with 50 ml of distilled water and then  
137 recovered using one ml of resuspension solution (5 mM Tris-HCl, 0.8% (v/v) Tween-20; pH  
138 7.4) as described (Nguyen et al., 2010). 10 µl aliquots of the cell suspension were dried in  
139 a speed-Vac, and frozen at -80°C. Immediately before use they were resuspended in 10 µl  
140 of water, and 2 µl were used for PCR. To inactivate nucleases which are commonly found  
141 in sediment samples, the concentrated cells were incubated at 95°C for 2', before they  
142 were used as source of template for PCR. As shown in Fig 1S (Supplementary materials)  
143 the presence of different amounts of nucleases in the seven sediment samples did not  
144 interfere with the molecular analysis. DNA amplification by PCR was carried out essentially  
145 as described (Nguyen et al., 2010) using genomic DNA released from diatom cells upon  
146 the initial denaturation step in the thermal cycler. Primers used in this study (528F 5'  
147 GCGGTAATTCCAGCTCCAA and 650R 5' AACACTCTAATTTTTTTCACAG) target the  
148 small subunit ribosomal DNA gene (SSU rRNA) and produce an amplicon of  
149 approximately 200 bp suitable for real-time PCR. These primers have already been tested  
150 using environmental samples and proved to correlate with diatom abundance measured  
151 by light microscopy counting (Nguyen et al., 2010). This primer set, though originally  
152 developed for detection of freshwater diatoms, exhibits 100% similarity with most of the  
153 sequences of marine diatoms available. The relative amount of SSU rRNA of various

154 diatoms found in coastal sediments was determined by the standard curve-based method.  
155 Standard DNA corresponding to a portion of SSU rRNA was prepared as an amplified  
156 1040 bp long DNA fragment purified by a DNA clean-up kit; its concentration was  
157 spectrophotometrically determined. For the calibration curve this DNA was used as a  
158 series of four 10-fold dilutions. Three replicates of each sediment sample and four  
159 replicates of each point of the standard curve were analysed. The efficiency of real-time  
160 PCR amplification was 95.6%. Real-time PCRs were carried out, according to the  
161 manufacturer's protocol (Qiagen Quantitech SYBR Green Kit), in a 20  $\mu$ l volume  
162 containing 0.3  $\mu$ M each primer and 2  $\mu$ l of biomass corresponding to 40  $\mu$ g of sediment  
163 sample. The DNA amplification regime was 15 min at 95°C followed by 40 cycles of 94°C  
164 for 15 s, 50°C for 1 min, and 72°C for 30 s. Cycle threshold (Ct) data were collected from  
165 an MxPro-Mx3000P instrument (Stratagene).

166

### 167 3. Results and discussion

168 Diatoms, which attain such high concentrations as to discolor the ice brown,  
169 generally dominate the ice-edge blooms; their high primary productivity ( $1.3 \text{ g C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ )  
170 could explain the high amount of biogenic siliceous sediments and the large population of  
171 high trophic level animals (Sweeney et al., 2000 and references therein). For example, in  
172 Terra Nova bay in 1998, the phytoplankton bloom, which reached approximately  $8 \times 10^8$   
173  $\text{cells}\cdot\text{l}^{-1}$  and  $32.4 \text{ mg C}\cdot\text{l}^{-1}$ , was dominated by the diatom *Fragilariopsis curta* (mainly  
174 planktonic species), whereas in other areas of the Ross Sea abundances  $>86 \times 10^3 \text{ cells}\cdot\text{l}^{-1}$   
175  $^1$  and  $11.7 \mu\text{g C}\cdot\text{l}^{-1}$  were mainly due to the Prymnesiophyceae *Phaeocystis antarctica*  
176 (Fonda Umani et al., 2005).

177 In this study, 23 diatom species were identified in the seven studied sites (Table 1).  
178 The genera *Fragilariopsis* (Fig. 2 A, B, E), *Navicula* and *Thalassiosira* (Fig. 2, C) were

179 recorded in all stations. The occurrence and abundance of other genera varied in relation  
180 to the site: *Amphora* (Fig. 2 D, E) was abundant (~ 4%) at rookery station (C) but absent  
181 at stations A, E, G; *Luticola* reached up to 5% at the less deep station (D) but was not  
182 recorded in the other sites; *Nitzschia* exceeded 10% at station A (Tethys Bay) but was not  
183 observed at stations B, in the same basin and with similar depth and salinity.

184 All the observations highlighted that the communities were markedly dominated by  
185 *F. curta* (>70%; Table 2) (Fig. 2-A), whose pelagic and benthic distribution in the Antarctic  
186 area was already reported (Rathburn et al., 1997; Fonda Umani et al., 2005). Benthic  
187 diatom biodiversity index tended to decrease with increasing water column depth, from  
188 3,02 H' at 19 m to 1,04 H' at 48 m, where the solar radiation is lower and few diatom  
189 species are sciaphilous (adapted to low light intensity).

190 The lowest cell abundance was found at the stations with salinity below 50 (D and  
191 F), and the highest number of species was detected at station D (Table 2) with a major  
192 contribution of *Fragilariopsis rhombica* and *Navicula* spp. whose relative abundance  
193 altogether was close to 45%. In addition, the sediments of stations D and F contain a  
194 higher percentage of sand than those of other five stations, and it is known that grain size  
195 and sediment density can significantly influence abundance and diversity of benthic  
196 diatoms (Facca and Sfriso, 2007 and references therein). The genera *Amphora*,  
197 *Cocconeis*, *Luticola*, *Navicula* and *Nitzschia*, which are typical of benthic habitats (Round  
198 et al., 1990), contributed significantly to the abundance and diversity of stations  
199 characterized by low salinity (D and F), whereas they were rare at the deepest stations (G  
200 and E; Table 1). Chl-*a*, ranging from 0.44  $\mu\text{g}\cdot\text{g}^{-1}$  at the station (F) with the lowest salinity to  
201 11.0  $\mu\text{g}\cdot\text{g}^{-1}$  at the station (C) with the highest salt content (Table 2), provides general  
202 information concerning all primary producers. The Chl-*a*/Pheo-*a* ratio gives also an  
203 indication of the metabolic state of the diatom community, and the values, ranging from

204 0.22 at station D (19 m) to 0.08 at station E (69 m), suggest a condition of progressive  
205 degradation of the photoautotrophic community.

206 Real-time PCR based on diatom-specific oligonucleotide primers designed on the  
207 SSU rRNA was used to identify the DNA-containing diatoms (Nguyen et al., 2011). Thus,  
208 the chromosomal DNA of diatoms was used as molecular target with the aim to count and  
209 distinguish DNA-containing cells from empty frustules.

210 Amplified DNA ranged between  $12 \text{ ng}\cdot\text{g}^{-1}$  at Penguin Bay (station C) to  $0.18 \text{ ng}\cdot\text{g}^{-1}$   
211 at Gerlache inlet (station F). The amount of SSU rRNA detected by real-time PCR was  
212 consistently higher in sample C, characterized by the highest salinity, compared to all  
213 other stations examined (Fig. 3). We can conclude that sediments at station C present the  
214 highest concentration of photo-pigments and amplified SSU rRNA, and these features  
215 correlate well with the abundance of frustules. Of the 23 diatom species described in this  
216 study, the SSU rRNA gene sequence of only three species is available in public  
217 databases. A thorough DNA sequencing study of the remaining diatom species would be  
218 interesting, as it would corroborate the microscopy data with molecular data, and would  
219 provide a more refined phylogenetic view.

220 Why are sediments at Penguin Bay (station C) so rich in diatoms? To answer this  
221 question, we focused our attention on the chemical composition of superficial sediment  
222 grains in all stations; each sediment specimen was examined by SEM/EDX and a semi-  
223 quantitative analysis of major elements was carried out by means of X ray emission. The  
224 sediment particles were patched with copious diatom assemblages with some siliceous  
225 spicules belonging to Porifera (Fig. 4). The sediment particles were constituted mainly by  
226 silica ( $15.5\% \pm 2.77$ ,  $n = 10$ ), oxygen ( $54.1\% \pm 2.97$ ,  $n = 10$ ), aluminum ( $3.44 \pm 1.9$ ,  $n = 10$ )  
227 and other less represented elements ( $< 1\%$ ), while microbial assemblages (Fig. 4) were  
228 enriched in K ( $3.67\% \pm 1.21$ ), Na ( $2.75\% \pm 1.01$ ), and Cl ( $1.57\% \pm 0.87$ ). These data  
229 suggest the presence of salinity brines (Frank et al., 2010) associated with diatom deposits

230 on sediment particles. In water leached sediments the presence of salinity brines was  
231 confirmed by cation and anion determination (Table 3). The sum of the concentrations  
232 ( $\text{mg}\cdot\text{g}^{-1}$  d.w.) of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{SO}_4^{2-}$  are consistent with the amounts of  $\text{Cl}^-$  and the  
233 calculated salinity ranged between 45.1 and 75.2.  $\text{NO}_3^-$  was almost constant in all stations,  
234 whereas some soluble nutrients such as  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  with acetate traces were more  
235 concentrated in stations A and B confirming the high fermenting activity in semi-enclosed  
236 Tethys Bay (Baldi et al., 2010). Finally, the low content of water in all samples (range 29%-  
237 18%) confirmed the prevalent sandy composition of sediments.

238 The highest salinity (76.2) was found at station C, concomitant with the highest  
239 diatom concentration. This high value of salinity is not surprising since associations of  
240 diatoms with salinity brines displaying values higher than 200 were previously reported  
241 (Cox and Weeks, 1983).

242 All the analyses clearly indicated a marked difference between the community at  
243 station C and the other stations. In fact, at station C most of *Fragilariopsis* spp. cells were  
244 presumably either live cells or, if dead diatoms, they were still containing active  
245 biomolecules, as confirmed by high chlorophyll concentrations and high amount of  
246 amplified SSU rRNA gene. Although the number of samples available was relatively low,  
247 high correlation factors ( $r^2 > 0.9$ ) were found between salinity and biodiversity index (Fig.  
248 5A) and photopigment ratios (Fig. 5B), with the exception of stations D and F. These  
249 sediments were characterized by the lowest salinity (<50), water content (18%) and  $\text{Na}^+$ ,  
250  $\text{Mg}^{2+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$  concentrations. On the other hand biodiversity indices were the highest  
251 and few *F. curta* frustules were recorded. Salinity was also correlated with the amount of  
252 SSU rRNA (Fig. 5C) and the number of frustules belonging to *F. curta* (Fig. 5D) except for  
253 sediments at stations A, B and E, where diatoms contained low amounts of detectable  
254 DNA compared with total frustule abundance, and showed fewer *F. curta* frustules than in  
255 stations F, D, G and C. At stations A and B (Tethys Bay) sediments were prevalently

256 anoxic (Baldi et al., 2010) and probably diatoms had settled from ice pack and water  
257 column and were predated by heterotrophic bacteria. At station E, sediments were those  
258 collected at the deepest level (66 m) and the lowest photo-pigments ratio detected reflects  
259 the scarce solar irradiation. Therefore, we hypothesize that most of the diatoms found at  
260 station E were probably not viable cells and the frustules probably were transported from  
261 shallow to deeper waters by pycnoclinic currents (Frignani et al., 2003).

262 In station C, we found a strong correlation between the abundance of DNA-  
263 containing diatoms (among which *Fragilariopsis* cells were the most represented) and the  
264 level of salinity which is the highest in seawaters of that region (Fonda Umani et al., 2005).  
265 (Fig. 5C). This result is consistent with other reports where *Fragilariopsis* was found to be  
266 able to cope with high salt concentration and low temperatures, which are the necessary  
267 conditions for the expression of ice antifreeze proteins (AFPs) (Bayer-Giraldi et al., 2010).  
268 AFP production is involved in cold adaptation contributing to the successful reproduction of  
269 *Fragilariopsis* and perhaps of other polar diatom species.

270

#### 271 **4. Conclusion**

272 In this study, the integration of data derived from standard ecological parameters  
273 with results obtained from quantitative detection of diatom DNA using molecular tools,  
274 allowed us to monitor the physiological status of diatoms within a complex community. The  
275 determination of biological, biochemical and chemical parameters in coastal sediments of  
276 Terra Nova Bay focus the attention on salinity as the major parameter influencing diatom  
277 distribution and growth. Variations in salt concentration, rather than the amounts of  
278 nutrients available, appeared to be the most important environmental factor influencing the  
279 growth of *F. curta*. Biodiversity index instead, shows that high salinity levels represent a  
280 discrimination factor that might limit the development of a panel of different diatom  
281 species. This is true for sediments at Penguin Bay, where *F. curta*, an organism adapted to

282 living in hypersaline conditions, markedly dominates over all the other species. This finding  
283 confirms that only few diatom species, compared with the million of existing diatom  
284 species, are adapted to thrive at high salinity.

285

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291

292

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386 **Figure Captions**

387 **Fig. 1.** Sampling sites of coastal sediments at Terra Nova Bay from Gerlache Inlet to  
 388 Penguin Bay adjacent to the Italian Base.

389

390 **Fig. 2** Photographs of six diatom species identified in the superficial sediments.

391 A: *Fragilariopsis curta* small size one 30  $\mu\text{m}$  x 8  $\mu\text{m}$ ; large size one 40  $\mu\text{m}$  x 9  $\mu\text{m}$

392 B: *Fragilariopsis rhombica* size 23  $\mu\text{m}$  x 12  $\mu\text{m}$

393 C: *Thalassiosira lentiginosa* diameter 60  $\mu\text{m}$

394 D: *Amphora ovalis* size 55  $\mu\text{m}$  x 25  $\mu\text{m}$

395 E: *Fragilariopsis* spp., *Amphora* sp.

396 F: *Cocconeis costata* size 45  $\mu\text{m}$  x 25  $\mu\text{m}$

397 *Amphora* and *Cocconeis* (together with *Navicula*, *Nitzschia*) are typical of benthic habitat.

398

399 **Fig. 3.** The real-time PCR histogram showing the relative amount of diatom DNA found in  
 400 the seven stations. The calibration curve consisted of serial 10-fold dilutions of the 1040  
 401 bp fragment described in the materials and methods section. The standard curve showed  
 402 a linear response from 3.5 fg to 35 pg of DNA ( $r^2 = 0.981$ ). The error bars represent  
 403 standard deviations.

404

405 **Fig.4** (A) SEM micrographs of sediments from station C showing diatom aggregates.

406 Diatom frustules and debris are closely associated with some Porifera spicula. (B) X-ray

407 dispersive microanalyses of a spot (square) indicating peaks of Cl and Na (arrows).

408

409 **Fig. 5** Significant correlation of salinity versus the amount of amplified SSU rRNA

410 (AmpDNA; graph A), chlorophyll *a*/phaeopigment *a* ratio (Chl-*a*/Phaeo *a*; graph B),

411 Shannon diversity index (graph C) and *Fragilariopsis curta* (graph D).

Table 1. Floristic list of taxa identified at the light microscope analysing frozen samples. The presence in each station is marked by a X.

Taxa	Species Authors	Station						
		A	B	C	D	E	F	G
<i>Achnanthes brevipes</i>	C. Agardh			X	X			X
<i>Amphora ovalis</i>	(Kützing) Kützing			X	X			X
<i>Amphora</i> sp.			X	X				X
<i>Cocconeis costata</i>	Gregory			X	X			
<i>Cocconeis</i> cfr. <i>molesta</i>	Kützing				X	X		
<i>Cocconeis</i> cfr. <i>scutellum</i>	Ehrenberg		X		X			X
<i>Fragilariopsis curta</i>	(Van Heurck) Hustedt	X	X	X	X	X	X	X
<i>Fragilariopsis kerguelensis</i>	(O'Meara) Hustedt	X	X	X	X	X		X
<i>Fragilariopsis rhombica</i>	(O'Meara) Hustedt	X	X	X	X	X	X	X
<i>Fragilariopsis</i> sp.		X						
<i>Luticola</i> sp. A					X			X
<i>Luticola</i> sp. B					X			
<i>Navicula directa</i>	(W. Smith) Ralfs	X	X	X	X	X	X	X
<i>Navicula</i> sp. A (25 µm)				X	X			X
<i>Navicula</i> sp. B (10 µm)		X	X	X	X			X
<i>Nitzschia</i> sp. A (25 µm)		X		X				X
<i>Nitzschia</i> sp. B (10 µm)					X			X
<i>Pseudonitzschia delicatissima</i> complex	(Cleve) Heiden							X
<i>Pseudostaurosira brevistriata</i>	(Grunow) D.M. Williams & Round		X					X
<i>Thalassionema nitzschioides</i>	(Grunow) Mereschkowsky	X		X	X	X	X	X
<i>Thalassiosira antarctica</i>	Comber	X	X	X	X	X	X	X
<i>Thalassiosira lentiginosa</i>	(Janisch) G. Fryxell		X	X	X	X		X
<i>Thalassiosira</i> sp.		X	X	X	X	X	X	X
Centricae not identified		X						
Pennatae not identified						X		X

\*In the elaboration (Table 3) the term Other indicates all the taxa excluding the genera *Fragilariopsis* and *Thalassiosira* which were the most abundant.

**Table 2.** Data on diatom abundance, biomass, diversity and main composition measured by light microscopic analysis on frozen sediment samples.

Station	Depth (m)	Diatom density in sediments										
		Abundance (10 <sup>6</sup> cells/g)	Biomass (µg C/g)	Chl a (µg/g)	Phaeo a (µg/g)	Chl/Phaeo	Species (number)	Diversity (H', log <sub>2</sub> ) <sup>1</sup>	<i>Fragilariopsis</i> <i>curta</i> (%)	<i>Fragilariopsis</i> spp. (%)	<i>Thalassiosira</i> spp. (%)	Other (%)
<i>Tethys Bay</i>												
A	20	1.90	170	2.30	17.3	0.13	11	1.66	71.5	3.83	4.68	20.0
B	20	4.17	522	1.13	13.8	0.10	11	1.43	77.9	7.08	4.42	10.6
<i>Penguin Bay</i>												
C	27	14.1	1646	11.0	57.5	0.19	15	1.80	71.5	2.53	2.53	23.5
<i>Malippo pier</i>												
D	19	0.24	22	2.14	9.56	0.22	18	3.02	34.0	17.9	4.26	43.8
<i>Gerlache inlet</i>												
E	66	3.65	521	0.96	12.4	0.08	10	1.31	79.4	4.76	9.92	5.95
F	31	0.27	25	0.44	3.10	0.14	16	1.89	71.3	0.70	4.20	23.8
G	48	2.83	399	0.87	15.6	0.06	10	1.04	84.8	2.80	7.60	3.60

\* The term Other includes the taxa listed in Table 1 with the exception of the genera *Fragilariopsis* and *Thalassiosira*.

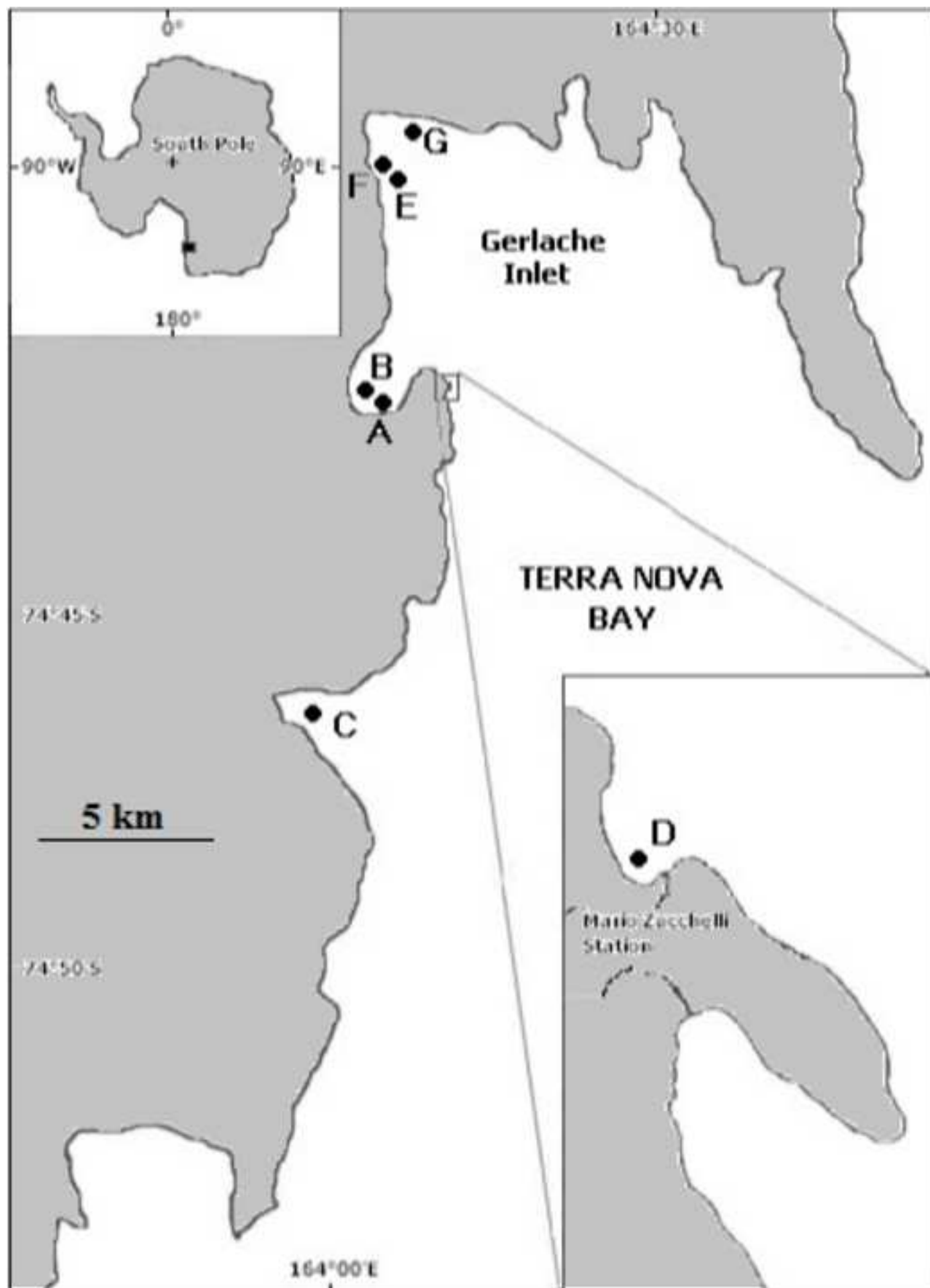
<sup>1</sup> Shannon diversity index.

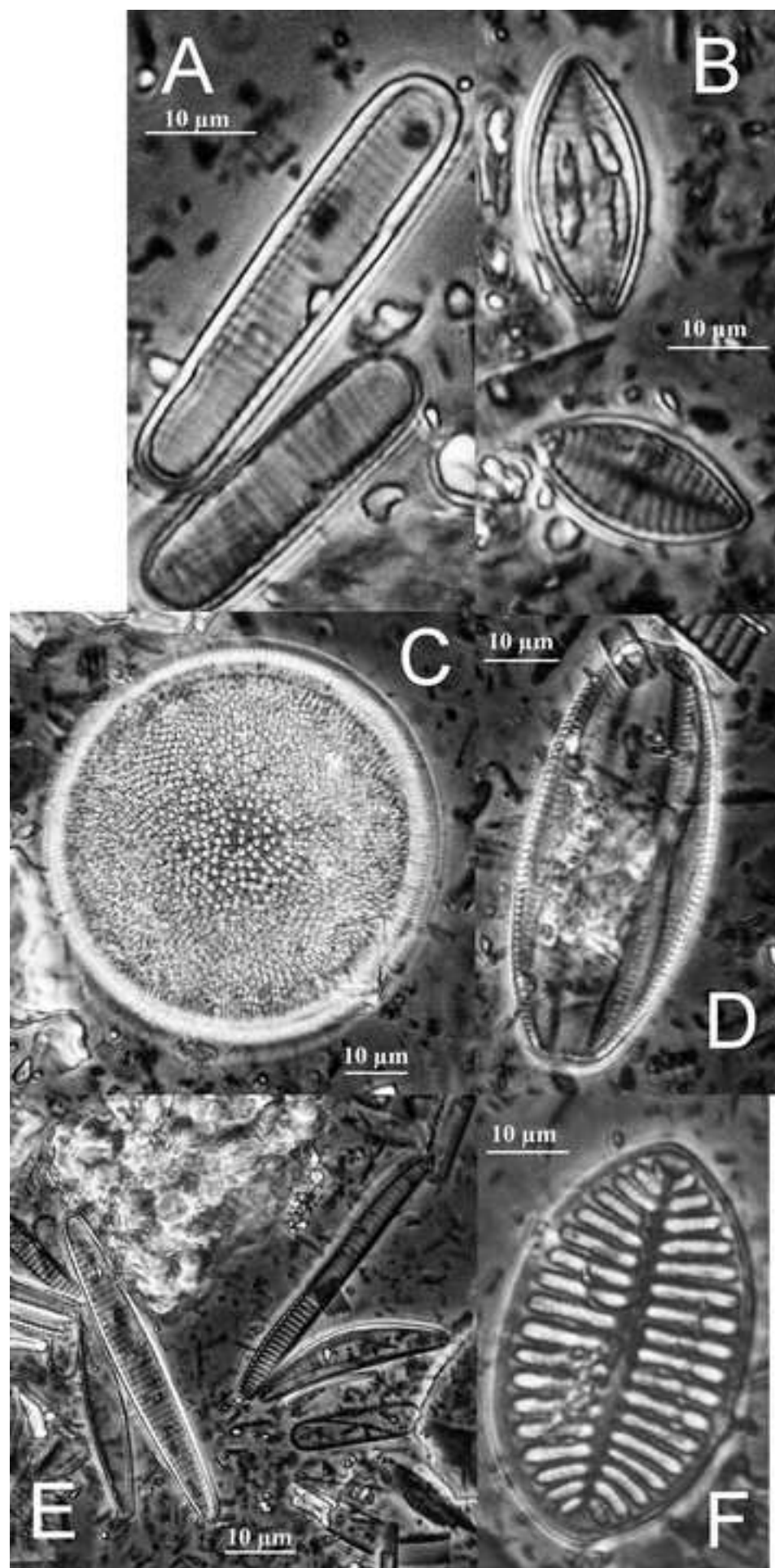
**Table 3.** Data on main composition of elutriate extracted by distilled water from sediments (concentration values are reported on the basis of sediment dry weight). S\*=salinity values.

Station	Quantitative determination of main ionic species of sediment elutriate											
	H <sub>2</sub> O	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	NH <sub>4</sub> <sup>+</sup>	Cl <sup>-</sup>	S*	SO <sub>4</sub> <sup>2-</sup>	PO <sub>4</sub> <sup>3-</sup>	NO <sub>3</sub> <sup>-</sup>	Acetate
	%	(mg /g <sub>sdw</sub> )						(mg /g <sub>sdw</sub> )				
<i>Tethys Bay</i>												
A	23	5.00	0.57	0.57	0.46	0.11	11.1	67.1	0.31	0.46	0.02	0.34
B	25	5.88	0.61	0.65	0.71	0.05	12.3	66.6	0.58	0.39	0.02	0.16
<i>Penguin Bay</i>												
C	25	3.65	0.21	0.43	0.27	0.02	14.1	76.2	0.57	0.06	0.02	0.01
<i>Malippo pier</i>												
D	18	3.31	0.21	0.37	0.22	0.01	5.85	48.2	0.23	n.d.	0.01	n.d.
<i>Gerlache inlet</i>												
E	23	4.37	0.25	0.44	0.80	0.02	10.2	61.5	0.30	0.02	0.02	n.d
F	18	2.64	0.17	0.36	0.62	0.02	5.49	45.1	0.19	n.d.	0.02	n.d
G	29	5.52	0.37	0.67	0.70	0.03	12.4	54.9	0.42	0.03	0.02	n.d.

Figure 1

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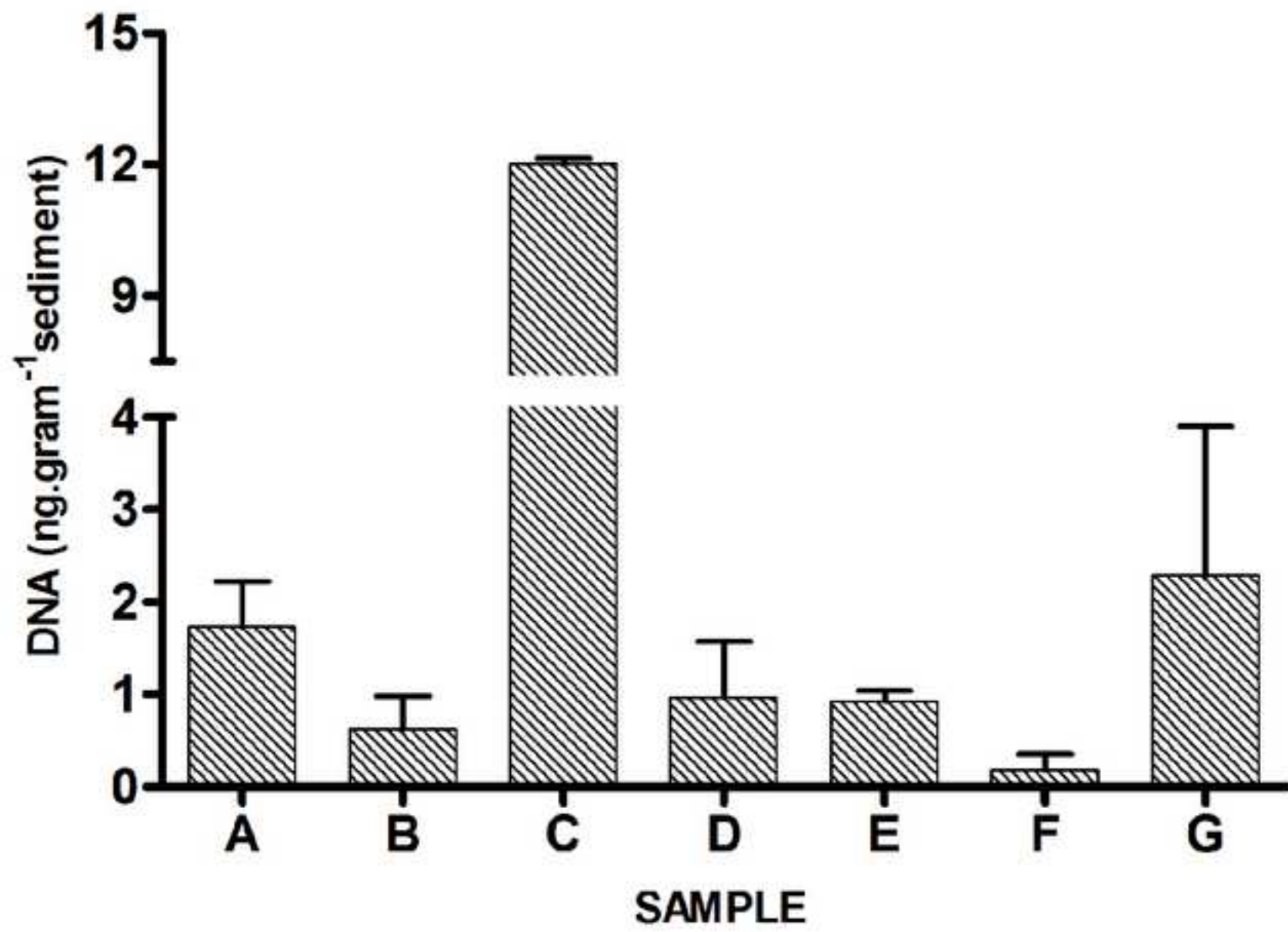
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Figure 4

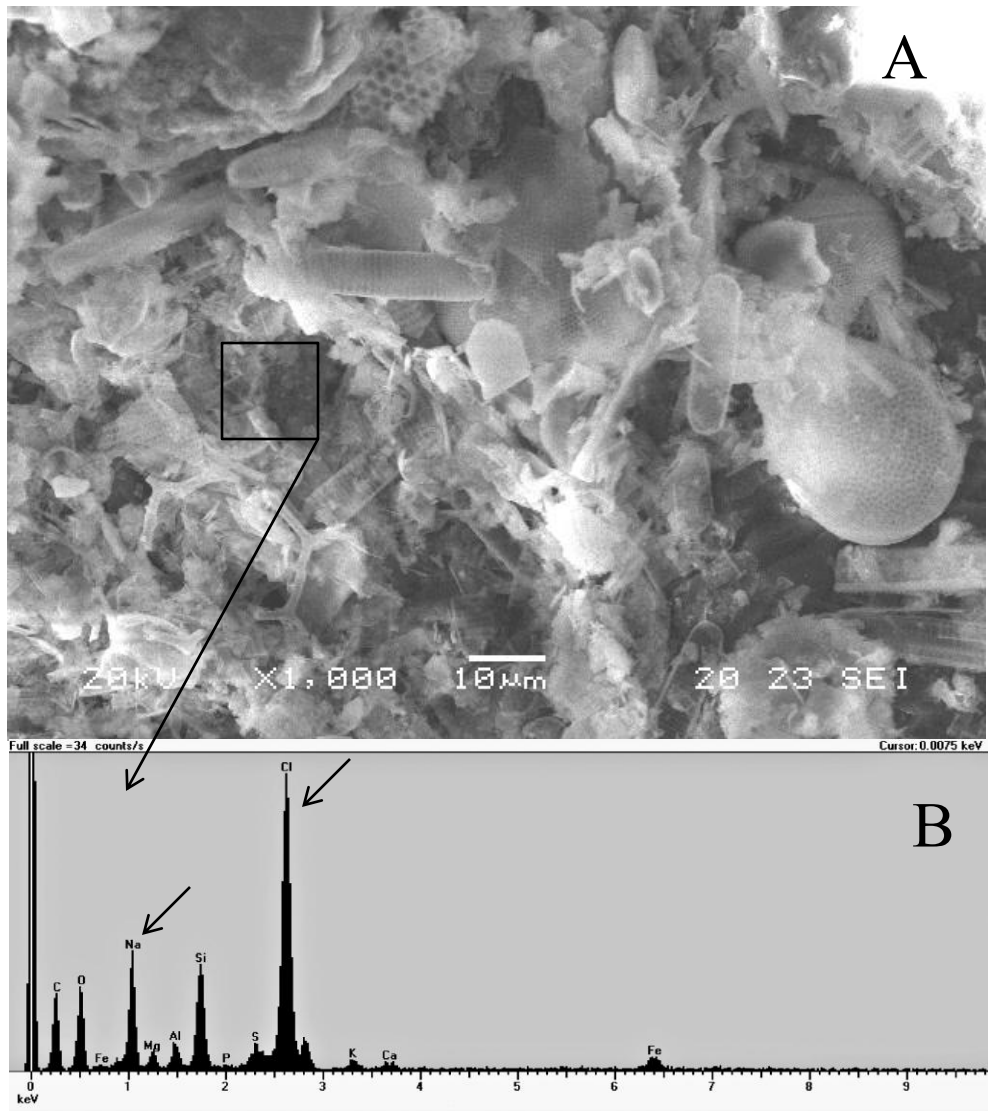


Figure 5

