Biogeosciences

Bioerosion by microbial euendoliths in benthic foraminifera from heavy metal-polluted coastal environments of Portovesme (south-western Sardinia, Italy)

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Abstract. A monitoring survey of the coastal area facing the industrial area of Portoscuso-Portovesme (south-western Sardinia, Italy) revealed intense bioerosional processes. Benthic foraminifera collected at the same depth (about 2 m) but at different distances from the pollution source show extensive microbial infestation, anomalous Mg/Ca molar ratios and high levels of heavy metals in the shell associated with a decrease in foraminifera richness, population density and biodiversity with the presence of morphologically abnormal specimens. We found that carbonate dissolution induced by euendoliths is selective, depending on the Mg content and morpho-structural types of foraminiferal taxa. This study provides evidences for a connection between heavy metal dispersion, decrease in pH of the sea-water and bioerosional processes on foraminifera.

1 Introduction

Boring microflora, constituted by cyanobacteria, algae and fungi may colonise carbonate substrates of both dead and living tissues of carbonate organisms (Tribollet, 2008). Boring is evident in carbonate organisms from the Mesoproterozoic (Zhang and Golubic, 1987) to the present day as a consequence of environmental conditions (Hallock, 2005). Microbial euendoliths have been described in coral reefs, molluscs, thalli of red algae (e.g. Golubic et al., 1975; Perkins and Tsentas, 1976; Budd and Perkins, 1980; Glaub, 1994, 2004; Chazottes et al., 1995, 2002; Le Campion-Alsumard et al., 1995; Vogel et al., 2000; Tribollet and Payri, 2001; Ghirardelli, 2002; Golubic and Schneider, 2003; Tribollet and Golubic, 2005; Tribollet, 2008; Tribollet et al., 2009), in coastal eroded limestones (Schneider and Torunski, 1983; Radtke et al., 1996), in carbonate grains (Tudhope and Risk, 1985; Al-Thukair and Golubic, 1991; Al-Thukair, 1999), as well in oil polluted marine environments (Campbell, 1983; Al-Thukair, 2002; Al-Thukair et al. 2007) and heavy-metal polluted lagoons (Succi et al., 2010).

Microborings are major agents of bioerosion dissolving large quantities of calcium carbonate with a potential in buffering seawater pH, which leads to new questions on the effects of environmental factors such as eutrophication and atmospheric CO_2 increase on ocean composition evolution (Tribollet, 2008). On the other hand, present-day geochemical models predict that the saturation state of surface ocean water with respect to carbonate minerals should decline during the twenty-first century. As a result calcareous organisms may have difficulties to calcify, leading to production of weaker skeletons and greater vulnerability to bioerosion. Organisms utilizing the more soluble form of $CaCO_3$ (aragonite or high-Mg calcite) would be more adversely affected by elevated pCO_2 than those utilizing the less soluble low-Mg calcite (Morse, 1983; Morse et al., 2007).

Investigations on microborings in foraminifera are documented in a small body of literature (Perkins and Halsey, 1971; Alexanderson, 1972; Golubic et al., 1984; Peebles and Lewis, 1988; Shroba, 1993; Freiwald, 1995; Perry, 1998; Nielsen et al., 2003; Crevison and Hallock, 2007) and open a

debate that needs to be developed. Benthic foraminifera se-creting calcium carbonate shells are considered to be very sensitive to the environmental conditions of the host sea wa-ter (e.g. Boyle, 1981; Wefer et al., 1999; Lea, 2004; Munsel et al., 2010). Species distribution and population densities of foraminifera are in fact used for pollution monitoring (Alve, 1991, 1995; Yanko et al., 1994, 1999; Debenay et al., 2001, 2005; Coccioni et al., 2003, 2005; Armynot du Châtelet et al., 2004; Cherchi et al., 2009; Frontalini et al., 2009; Ro-mano et al., 2009; Frontalini and Coccioni, 2011; Denoyelle et al., 2012; Foster et al., 2012). Hallock (2000a, b) suggested that climatic change including increasing atmospheric CO_2 and stratospheric ozone depletion may be the cause of bor-ings developing on carbonate organisms. Indeed, effects of elevated pCO_2 on coral reef bio-erosion have been observed and explained through a mechanism of carbonate dissolution in a more acidic ocean (Tribollet et al., 2009). The activity of boring microflora can result in differential bioerosion de-pending on both pCO_2 partial pressure (Tribollet et al., 2009) and Mg/Ca ratio of the foraminifera tests (Peebles and Lewis, 1988; Bentov and Erez, 2005).

The aim of our study is to investigate the effects of mi-crobioerosion on benthic foraminifera and trace element dy-namics in a known coastal industrial site polluted by heavy metals and industrial release of CO_2 (Schintu and Degetto, 1999). Field studies in the coastal area neighbouring the in-dustrial complex of Portoscuso-Portovesme (Southern Sar-dinia, Italy, Fig. 1) show that the presence of high levels of heavy metals results in an increase in bioerosional processes on foraminifera by boring microflora. The stressed environ-mental conditions in this area have been highlighted in Cher-chi et al. (2009) through an analysis of the biotic indices of foraminifera tests (Dominance, Shannon–Weaver, Simp-son, Evenness, Menhinick, Margalef, Equitability, Fisher-α, Berger-Parker and Q-mode Cluster Analyses – Ward Method) in this polluted environment.

2   Environmental setting

The study area is characterised by a relatively confined shal-low shelf, which slopes gently to deeper water environments (Fig. 1). Bottom sediments comprise sandy submarine beach (Pleistocene-Holocene in age), while Oligo-Miocene calc-alkaline volcanics crop out north of the sampling area. This south-western coast of Sardinia has been under major an-thropogenic pressure since the 1960s, when the Portoscuso-Portovesme district began to develop and expand its activ-ities. The Portovesme Harbour, built in 1870 to ship ores (zinc blend, galena and pyrite) from the historical mines of the Sulcis-Iglesiente district, received major inputs from in-dustrial discharge until a few decades ago. Chemical analy-sis of marine sediments from 4 cores collected in the harbour of Portovesme facing the industrial complex revealed in the upper 2 cm high concentrations of Hg (up to $50\,mg\,kg^{-1}$), Cd (up to $120\,mg\,kg^{-1}$), Pb (up to $20\,mg\,kg^{-1}$) and Zn (up
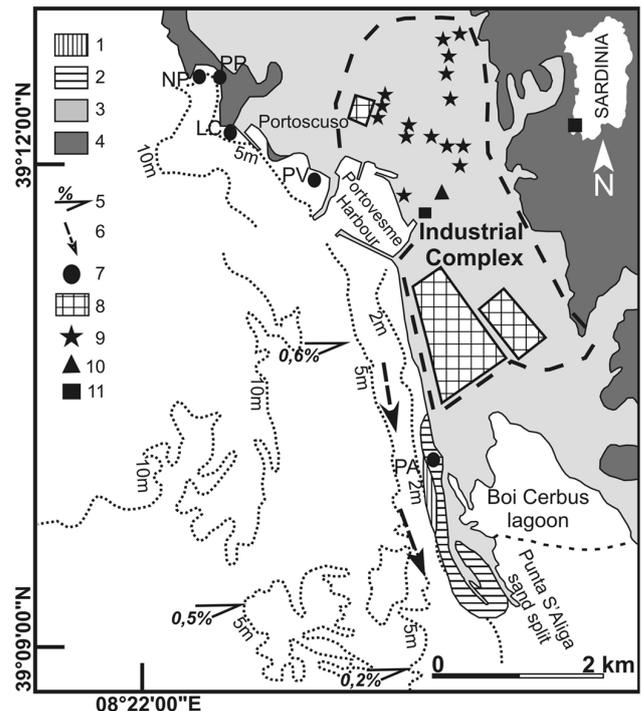

Fig. 1. Study area, location of sampling stations (Porto Pauleddu – NP, Porto Paglietto – PP, La Caletta – LC, Portovesme – PV, Punta S'Aliga – PA) and industrial complex of Portoscuso (SW Sar-dinia). 1. Submarine beach (grain size mean 0.13–0.1 mm). 2. Sub-marine beach (grain size mean 0.18–2.00 mm). 3. Alluvial deposits (Pleistocene–Holocene). 4. Volcanics (Oligo–Miocene). 5. Seabot-tom slope (%) from shoreline to 5 m isobath. 6. Longshore drift. 7. Sampling stations. 8. Red mud dumps. 9. Chimneys. 10. Lead-zinc smelter. 11. Electric power plant (1, 2, 5, 6 from Di Gregorio et al., 1996; modified).

to $70\,mg\,kg^{-1}$) were found. Metal concentration decreases with an increase in distance between the sampling station and the industrial effluent discharge point (Schintu and Degetto, 1999). This large industrial development had a considerable environmental impact, such that this industrial district has been declared an environmental hazard (D.P.C.M. – Prime Ministerial decree, 23 April 1993) because of the several "danger centres" recognised (Agenzia Regionale per la Pro-tezione dell'ambiente della Sardegna – ARPAS, 2007).

Emissions into the atmosphere and all of the surrounding environments have been estimated annually at 65 000 tons of SO_x, 4000 tons of dust, 10 tons of Pb and 100 tons of Fe (Gazzetta Ufficiale Italiana, 1993). The large electric power stations and the numerous chimneys produce also significant amounts of CO_2 (Schintu and Degetto, 1999; Bettini and Zanin, 2002). The industrial complex, developed on an al-luvial plain near the sea, includes factories producing alu-minium from bauxite and a lead-zinc smelter producing Pb, Zn, Cd, H_2SO_4 and Hg from Pb and Zn mixed sulphides. A
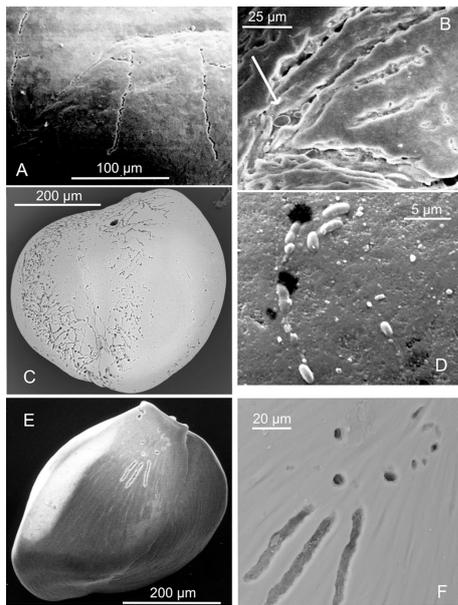
Fig. 2. SEM images of surface bioerosion of porcelanaceous imperforate foraminifera. **(A)** Tubular wavy tunnels parallel to substrate in *Pseudotriloculina rotunda* (d'Orbigny) exhibiting a rectangular ramification belonging to *Orthogonum* (cf. Form 1 in Wisshak et al., 2005). **(B)** Invasive infestation of microborings concentrated along the sutures of the chamber walls producing large galleries frequently occupied by pennate diatoms (arrow) in *Quinqueloculina* sp. **(C)** Dense boring pattern made up of fungal filaments developed in *Triloculina* cf. *marioni* Schlumberger. **(D)** Colony of rod–shaped cryptoendolithic bacteria made into previous holes of larger diameter in the wall surface of *Adelosina* sp. **(E)** Tubular borings, 4–5 μm in diameter, running parallel to surface of the test and penetrating into the wall of *Quinqueloculina ungeriana* d'Orbigny. **(F)** Enlargement of **(E)**.

large dump of red muds (seawater-neutralised bauxite refinery residues) is located near the coast.

3 Material and methods

Sixteen surface sediment samples, coming from an area about 12 km^2, were collected in the inner shelf facing the industrial complex of Portoscuso-Portovesme where seawater is less than 2 m deep. Four stations (PP – Porto Paglietto, LC – La Caletta, PV – Portovesme, PA – Punta S'Aliga, Fig. 1) were sampled in summer. Sampled sediments came from the upper 1–2 cm below the water column. The water temperature ranged between 27.7 °C and 30.6 °C. Temperature, pH, and Eh were measured for both the seawater and pore water of the sediments in every sampling station. Blank samples were also collected for comparison purposes at the same bathymetry and temperature from an unpolluted coastal area (NP – Portopauleddu, Fig. 1). Benthic foraminifera assemblages and their biodiversity indices, as well the geochemical characterisation of the investigated area, was the aim of our
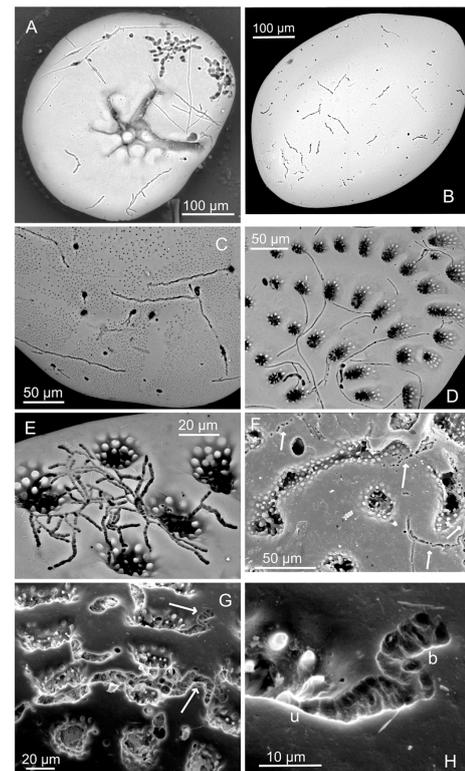

Fig. 3. SEM images of surface bioerosion of hyaline perforate foraminifera. **(A)** *Rosalina bradyi* Cushman (umbilical side) exhibiting indeterminable filaments and, at right, two bunches of radiating tunnels (cf. *Fascichnus*). **(B)** *Polymorphina* sp. (oblique side view) showing filaments of indeterminable borings in the whole test. **(C)** Detail of *Ammonia tepida* (Cushman) spiral side intensively bored by long filaments of fungal hyphae with reproductive organs (cf. *Saccomorpha clava*). **(D)** *Elphidium crispum* (Linnaeus) showing in dorsal view a boring pattern of curved thin tunnels (1–2 μm in diameter) belonging to cf. *Scolecia filosa*, goings towards the primary pores of the foraminifer. **(E)** Densely ramified boring pattern of *Ichnoreticulina elegans*, characterised by dichotomous branching goings into the primary pores of *E. crispum*. **(F)** Invasive colonies of indeterminable microborings (most likely bacteria, possibly cyanobacteria) around and into primary pores of *E. crispum* (arrows) producing a lateral enlargement of these until two–three contiguous pores join (cf. Freiwald, 1995; Fig. 4). **(G)** Boring system developed among contiguous pores of *E. crispum*, characterised by large tunnels (arrows). **(H)** Enlargement of **(G)** showing large microbial overprint going into the pore and its transition from uniseriate **(u)** to biseriate **(b)** cell arrangements, belonging to *Fascichnus*.

earlier paper, in order to assess the foraminiferal response to heavy metal pollution (Cherchi et al., 2009).

A cluster of four subsamples of constant volume (50 cm^3) from every station (PP, LC, PV, PA) was collected to study foraminifera assemblages used as environmental bioindicators. In laboratory, 16 subsamples were stained with Rose Bengal (Walton, 1952) to differentiate living from dead specimens. Samples were then washed through a set of nested

sieves (63, 125, 180, 250 μm). Foraminifera from 5 cc of the > 125 μm size fraction (a – medium grained) and 5 cc of the < 125 μm size fraction (b – fine-grained) were picked, counted and classified.

Considering the very low number (1–4 %) of living individuals in the polluted environments (mainly at the PV and PA stations), total benthic foraminifera assemblages (dead and living) were used for statistical purposes, providing a larger database (Scott and Medioli, 1980; Samir and El-Din, 2001; Fatela and Taborda, 2002; Armynot du Châtelet et al., 2004; Debenay and Guiral, 2006; Frezza and Carboni, 2009). Total assemblages are almost identical statistically and only diverge when the living/total becomes large (Murray, 1976, 1991) and biotic indices do not show significant differences between living and dead populations (Yanko et al., 1994). Occurrence of iron-oxy-hydroxides in bottom sediments, pyritized infaunal benthic foraminfera and framboidal pyrite in the tests from the polluted sampling stations were occasionally found indicating reducing conditions.

Environmental scanning electron microscopy (ESEM) images allow recognition of an extensive infestation by microbial communities in the calcareous foraminiferal tests. The bioerosional process on foraminiferal tests was analysed via ESEM (QUANTA 200, FEI, Hillsboro, Oregon, and partly with EVOLS15, ZEISS). The epoxy resin casts of foraminifera were made at Erlangen University (Germany). Samples were gold coated before imaging, for analysis of both external surface and on epoxy-resin cast (Golubic et al., 1970, 1983; Wisshak et al., 2008). From ESEM images, microbial filaments and boreholes on the external surfaces have been compared with positive epoxy resin casts, and measured. To estimate quantitatively the impact of the microbial borings on the foraminifera and the selective bioerosion on high-Mg and low-Mg foraminiferal tests, 300 individuals were picked randomly from the fraction > 63 μm of each sample and were observed with a scanning electron microscope. A total of 1200 foraminifera was examined. The bioerosional features on several morpho-structural taxa have been quantitatively calculated. The percentage of calcareous dissolution in foraminifera, distinguishing between high-Mg and low-Mg tests, has been compared with heavy metal values both in foraminiferal tests and the sediments, the pH of pore waters and richness of specimens.

Seawater was collected at the water-sediment interface of the 4 polluted stations and at the reference pollution-free station. After sampling seawater was rapidly filtered in situ through a 0.4 μm pore-size polycarbonate filter with an all-plastic filtration assembly. Samples for cation analysis were acidified with HNO_3 suprapure acid to pH around 3. At each sampling site, temperature, pH, and Eh were measured for both seawater and sediment porewater. The Eh was measured by platinum electrode and the value was corrected against Zobell's solution (Nordstrom, 1977). The electrode used for pH potentiometric determination was calibrated against three NIST-traceable buffer solutions (pH = 4.01, 7.00, 9.00 at 298 K). Reproducibility of pH calibrations, carried out before and after measurements of a single solution, was better than 0.005 pH unit. However because of problems inherent to the use of glass electrodes calibrated using NIST buffers in strong electrolyte solutions (see Dickson and Goyet, 1994), this measurement was only used to verify the solution electrochemical difference between the different sampled stations.

Anions were determined by ion chromatography (IC Dionex DX-120) and cations by ICP-AES (ARL-3520B) or ICP-MS (Perkin Elmer DRC-e). Because of the complex seawater matrix, the standard addition method, was employed for the trace element determination (Cd, Pb and Zn) (Danzer and Currie, 1998; Cidu, 1999). The method validation was verified by applying the same conditions to the CASS-3 coastal seawater reference material. Samples and CASS-3 were diluted five times with a 1 % ultrapure HNO_3 solution before sample spiking. Accuracy and precision were estimated at 10 % or better using the standard reference solution and random duplicate samples. The limit of quantification (10σ value of blank solution response over time) was, respectively 0.01 μg l^{-1} for Cd, 0.6 μg l^{-1} for Pb and 0.1 for Zn. The ionic balance was always in the order of ±8 %.

For digestion of samples with porcelanaceous foraminiferal tests, two portions of each sample were washed with ultrapure water (MilliQ®) by mechanical agitation in order to remove detrital grains. The samples were dried, accurately weighed and digested by slow heating in a temperature bath at 30 °C in a Teflon beaker with 3 ml of ultrapure HNO_3 (67 %). Solutions were diluted to 10 ml with ultrapure water in volumetric flasks, and transferred to new HD-polyethylene bottles for storage (Jarvis, 1992). Metals were determined by ICP-AES and ICP-MS.

Field Emission Gun SEM (FEG-SEM) is a scanning electron microscope with a high-energy beam of electrons in a raster scan pattern producing information about surface topography and composition. The Quanta 200 FEG Environmental Scanning Electron Microscope (ESEM) uses a field-emission gun (FEG) electron source in an exceptionally high chamber pressure environment. It combines the advantages of nanometer resolution to high signal to noise ratio in both regular high vacuum and environmental (wet) modes. EDX Analysis stands for energy-dispersive X-ray analysis; it is sometimes referred to as EDS or EDAX. EDX analysis allows identification of the elemental composition of the specimen, or an area of interest thereof. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM), and can not operate on its own without the latter.

Table 1. Distribution on microborings at different sampling stations (PP, LC, PV and PA, see Fig. 1), taken from porcelanaceous (Po) and hyaline (Hy) foraminiferal substrates and their relative abundance (++ very common, + common, = rare).

Foraminifera substrata		PP Po	PP Hy	LC Po	LC Hy	PV Po	PV Hy	PA Po	PA Hy
Borings	**Trace-makers**								
Eurigonum nodosum Schmidt	*Mastigocoleus testarum* Lagerhein (cyanophyte)	X		X		X	X	XX	X
Fascichnus cf *dactylus* (Radtke)	*Hyella caespitosa* Bornet and Flahault (cyanophyte)			X		XX	X	XX	X
Scolecia filosa Radtke	*Plectonema terebrans* Bornet and Flahault (cyanophyte)	X	X		X	XX	X	X	X
Scolecia meandria Radtke	"vermicular borings" (Zeff and Perkins, 1979) (cyanophyte)							X	
cf. *Planobola macrogota* Schmidt	cyanobacterium cf. alga cf.					X	X	X	
cf. *Cavernula pediculata* Radtke	*Gomontia polyrhiza* (Lagerhein) Bornet and Flahault (chlorophyte)				X	X			X
Ichnoreticulina elegans (Radtke)	*Ostreobium quekettii* Bornet and Flahault (chlorophyte)		XX		XX	X	X		X
Rhopalia catenata Radtke	*Phaeophila dendroides* Cronan (chlorophyte)		=		X		X		
Orthogonum fusiferum Radtke	*Ostracoblabe implexa* Bornet and Flahault (fungus)			X		X		X	
Orthogonum lineare Glaub *Orthogonum* isp. (Form 1 in Wisshak et al., 2005)	cf. fungus				=	XX X	X	X	X
Saccomorpha clava Radtke	*Dodgella priscus* Zebrowski (fungus)	=		X	=	XX	X	XX	X
	Coccoid bacteria					XX	X	X	

4 Results and discussion

4.1 Bioerosion features

The presence of microboring traces and cavities on sampled benthic foraminifera has been recognised using ESEM images of both surface tests and epoxy resin casts. Identified taxa, reported in Table 1, provide evidence of traces and cavities produced by phototrophic (cyanobacteria, chlorophyta) and heterotrophic (fungi) organisms. In Figs. 2 to 6, we illustrate evidence of several endolithic traces of microbial borings with morphological differences. Details of borings will be described according to the different observational techniques: wall surfaces and epoxy resin casts.

Test surfaces of the porcelanaceous miliolids present the heaviest bioerosion features under the form of microbial clusters. Endolithic traces have morphological differences related to biodiversity and to mode of life of the boring microbial organisms. Infestation is characterised by thin individual tunnels, occasionally bifurcated, bag-shaped cavities and branching patterns composed by rhizoidal and short galleries radiating laterally from the central area. This branch-

ing pattern shows similarities with *Fascichnus* isp. (Fig. 7a, b). From ESEM images of the traces on external surfaces of foraminiferal tests, our observations show that the taxa affected by higher bioerosion belong to the high-Mg porcelanaceous group (Suborder Miliolina) and, among these, to the genus *Quinqueloculina* which exhibits heavy microbial bioerosion. Figure 8 shows that in *Quinqueloculina* sp. the Mg content at the bottom of a boring is one order of magnitude lower compared to the unaltered shell surface, suggesting chemical reorganisation of the carbonate mineral composition through a dissolution-precipitation process.

In several specimens the infestation is concentrated along the sutures of the chamber walls providing more easily a nutrient source (Fig. 2b). A heavy infestation by diversified microborings in the test of *Triloculina* has been recorded (Fig. 2c). Radiating traces are developed parallel to the whole surface of foraminifers, in both porcelanaceous and hyaline types (Fig. 3 a,g,h and Fig. 7b), as previously observed by Glaub (2004) for "*Fasciculus* isp. 2". Lined rod-shaped bacteria are sometimes visible on the later chambers of *Adelosina* among their weakly developed striae. These
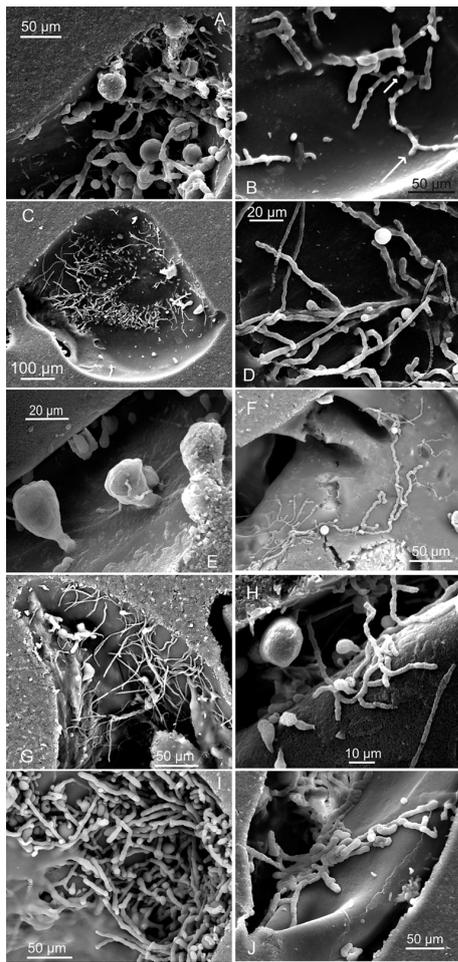
Fig. 4. SEM images of porcelanaceous foraminifera resin casts. **(A)** Chamber-wall of miliolid densely bored by *Eurigonum nodosum* and globular *Saccomorpha clava.* **(B)** *E. nodosum* with diagnostic lateral heterocysts (arrows) inside the thick chamber wall of miliolid. **(C)** Dense assemblage of euendolithic borings in miliolid test. **(D)** Enlargement of **(C)** showing *E. nodosum* with diagnostic heterocysts. **(E)** Pavement of *Saccomorpha clava* into miliolid chamber wall. **(F)** Colony of *Scolecia meandria* in miliolid chamber wall. **(G)** Microbial pavement in miliolid chamber wall exhibiting *Scolecia filosa*, *E. nodosum* and fungal sack-shaped cavities. **(H)** Diversified ichnotraces belonging to *S. clava*, cf. *Planobola macrogota* and *Ichnoreticulina elegans* in miliolid test. **(I)** Dense colonies of *E. nodosum* in miliolid test. **(J)** Colonies of *E. nodosum* inside the thick chamber wall of miliolid.

Fig. 5. SEM images of hyaline foraminifera resin casts. **(A)** Euendoliths (arrows) inside the skeletal structure of *Elphidium crispum* (Linnaeus). **(B)** Enlargement of **(A)** showing indeterminable filaments. **(C)** and **(D)** *Saccomorpha clava* and indeterminable cyanobacteria boring skeletal structure of *E. crispum*. **(E)** Enlargement of **(D)** showing spherical cavities of cf. *Planobola macrogota* and sack-shaped cavity of cf. *Cavernula*. **(F)** *E. crispum* (vertical section) showing indeterminable filamentous (arrows) in the complex skeletal pattern. **(G)** Enlargement of **(F)** showing filaments inside the skeletal of the foraminifera. **(H)** *E. crispum* (oblique section). **(I)** Enlargement of **(H)** showing tunnels (arrows) of borings in skeletal structures.

cryptoendoliths bacteria (sensu Golubic et al., 1981) can go into pre-existing holes, characterised by a larger diameter, clearly bored previously by another larger boring organism (Fig. 2d). Hyaline perforated tests (e.g. *Elphidium crispum*) frequently exhibit well-organised systems of microborings belonging to *Ichnoreticulina elegans* surrounding and directed to pores (Fig. 3e). Bacteria infestation can produce teratological modifications as observed in *E. crispum* (Fig. 3f) where bacteria colonies induce lateral enlargement of pri-
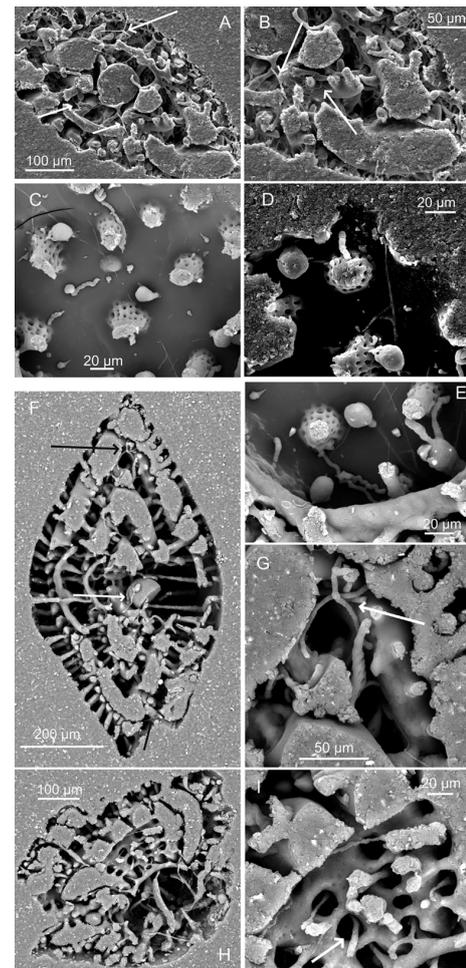
mary pores with enlargement of contiguous pores (cf. Freiwald, 1995, in *Cibicides lobatulus*). Superficial traces made by endolithic boring communities can completely cover the walls (Fig. 3a–c).

Several specimens of *E. crispum* show a boring pattern made of curved tunnels of 1–2 μm in diameter. Tunnels, after long runs, go towards the primary pores showing a comparable behaviour to *I. elegans* and they are tentatively attributed to *Scolecia filosa*. Infestation producing the primary pore connection may be related to possible CO_2 bioavailability
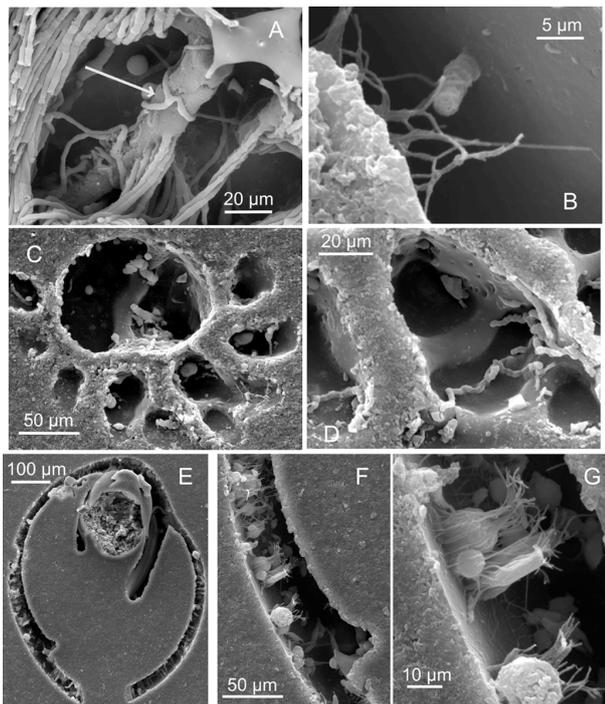
Fig. 6. SEM images of hyaline foraminifera resin casts. **(A)** Tripartite gallery of underteminable boring (cf. *Orthogonum* sp.) and globular-shape cavities in skeletal structure of *Ammonia beccarii* (Linnaeus). **(B)** *Ichnoreticulina elegans* in *Ammonia* sp. **(C)** Euendoliths inside the peripherical wall chambers of *Ammonia tepida* (Cushman). **(D)** Colony of *Eurigonum nodosum* in *Ammonia* sp. **(E)** Pavement of spherical cavities in the finely perforate test of *Polymorphina* sp. **(F)** and **(G)** Enlargement of **(D)** showing spherical cavities of cf. *Planobola macrogota* and sack-shaped cavities of cf. *Cavernula* surrounded by foraminiferal tubules.

for phototrophic organisms from the respiratory activity of the heterotrophic host.

Epoxy resin casts reveal rich microbial communities, fairly diversified, both in porcelanaceous and hyaline tests (Table 1). Analysis of resin casts reveals euendoliths belonging to phototrophic (chlorophyta, cyanobacteria) and heterotrophic (fungi) organisms boring both porcelanaceous and hyaline foraminifera. The wider and thicker walls of miliolids provide a protected niche for the growth of euendoliths, as shown in Fig. 4. In porcelanaceous tests, dense pavements of well-developed colonies of cyanobacteria and fungal sporangial cavities are very common. The presence of organic films inside the skeleton structure of these foraminifera provides a source of food.

The ichnological interpretation of the euendoliths inside the imperforate porcelanaceous tests is easier than for those inside the perforate hyaline hosts. The very complex internal architecture of the taxa belonging to the families Elphidiidae and Rotaliidae sometimes makes it difficult to distinguish euendoliths from foraminifera ultrastructures (Figs. 5 and 6). Even if the ichnological attribution of the euendoliths

Table 2. Seawater composition at the four sampling stations.

		PP	LC	PV	PA
pH		8.23	8.21	8.13	7.91
Eh	V	0.35	0.36	0.34	0.37
T	°C	27.7	27.9	29.6	30.6
Ca	mol l^{-1}	0.011	0.012	0.011	0.011
Mg	mol l^{-1}	0.058	0.059	0.057	0.058
K	mol l^{-1}	0.012	0.012	0.012	0.012
Na	mol l^{-1}	0.480	0.482	0.472	0.483
SO$_4$	mol l^{-1}	0.037	0.040	0.043	0.037
CI	mol l^{-1}	0.604	0.592	0.564	0.564
HCO$_3$	mol l^{-1}	0.002	0.002	0.002	0.002
Br	mol l^{-1}	0.001	0.001	0.001	0.001
Ba	mol l^{-1}	0.053	0.052	0.058	0.076
Cd	mol l^{-1}	0.002	0.002	0.002	0.004
Li	mol l^{-1}	19.882	20.602	19.882	20.314
Mo	mol l^{-1}	0.125	0.136	0.125	0.125
Pb	mol l^{-1}	0.010	0.014	0.016	0.016
Sr	mol l^{-1}	91.589	93.460	93.232	94.122
Zn	mol l^{-1}	0.061	0.076	0.061	0.076

in the hyaline perforate taxa is generally uncertain, their presence in the fine skeletal structure indicates that microbial colonies can use foraminiferal canal-systems to penetrate inside the test, and subsequently to develop boring activity in the foraminiferal skeleton.

Our study shows that microbioerosion affects porcelanaceous (high-Mg) imperforate miliolids (*Adelosina*, *Pseudotriloculina*, *Quinqueloculina* and *Triloculina*) and peneroplids as well as hyaline (low-Mg) perforate foraminifera (*Ammonia*, *Elphidium*, *Lobatula*, *Rosalina*) (Fig. 9). Porcelanaceous miliolids (*Quinqueloculina* and *Triloculina* spp.) exhibit higher percentages of bioerosional features in PA station (28.72 %) and in PV station (21.75 %) while hyaline *Lobatula lobatula* and *Elphidium crispum* reveal higher values of microbial infestation in PA (16.89 %) and LC stations (15.02 %), respectively. In particular, *Elphidium* tests show well-developed colonies of *Ichnoreticulina elegans* surrounding and directed to primary pores. The clorophyceans in our samples seem to be more frequent in less polluted sediments (LC). Figure 10a and b show that the number of infested tests is 2 times higher in porcelanaceous compared to hyalines foraminifera and that the proportion of infested tests is 2–3 times higher in the lowest pH conditions. Bioerosion in hyaline specimens increases from 19.9 % (PP) to 59.7 % (PA), whereas in a porcelanaceous forms it increases from 35.1 % (PP) to 80.3 % (PA). Our observations demonstrate that taxa affected by higher bioerosion belong to the high-Mg porcelanaceous group (Suborder Miliolina), especially *Quinqueloculina*. Infestation is often concentrated along the chamber wall sutures where nutrient material is high (Fig. 2b). Diversified microborings on an infested test of *Triloculina* are illustrated in Fig. 2c.
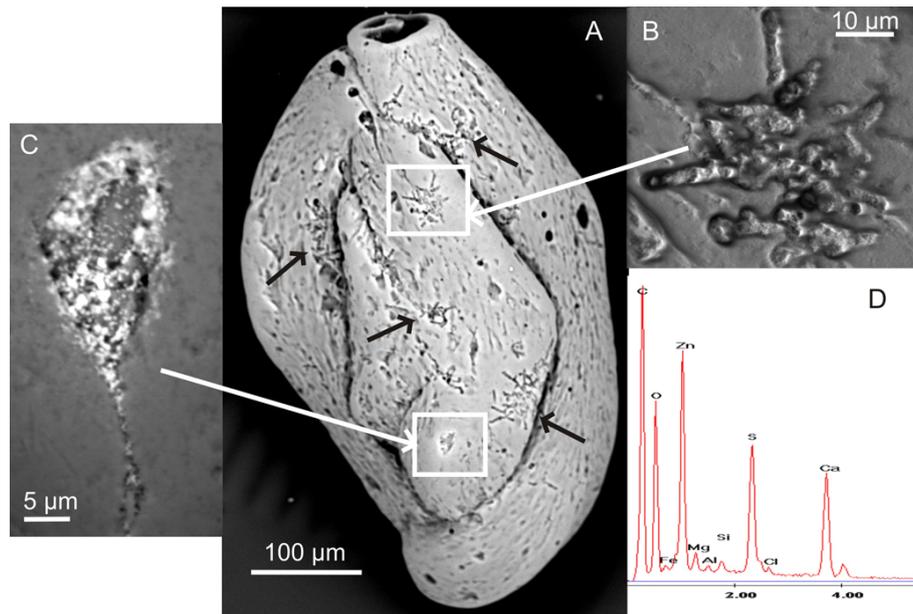
Fig. 7. SEM images of porcelanaceous foraminifera. **(A)** *Quinqueloculina* cf. *berthelotiana* d'Orbigny intensively bored by euendoliths (black arrows) among which are colonies of *Fascichnus* cf. *dactylus*. **(B)** Enlargement of **(A)** exhibiting a well-developed colony of *F.* cf. *dactylus* characterised by tunnels radiating from a central area. **(C)** Enlargement of **(A)** showing dome-shaped cavity of cf. *Cavernula pediculata* with rhizoidal appendages, Zn biomineralised. **(D)** EDX analysis of dome-shaped cavity of cf. *Cavernula pediculata*.

4.2　Environmental conditions and bioerosion

Seawater composition from four sampled stations is given in Table 2. As previously reported porcelanaceous foraminiferal tests, known to be high-Mg foraminifera, are more infested by bioerosion than hyaline forms. We found that Mg/Ca molar ratio of porcelanaceous infested tests is between 0.09 and 0.13, while in foraminiferal tests collected from an unpolluted site (NP) without an apparent sign of bioerosion have a Mg/Ca value of 0.01 (Fig. 10c). Since Mg/Ca molar ratio of benthic foraminifera is normally assumed to be between 0.0005 and $0.01 \, \mathrm{mol \, mol^{-1}}$ (Lea, 1999; Toler et al., 2001; Toyofuku and Kitazato, 2005), we propose that conditions of environmental stress may influence the amount of Mg incorporated during the foraminifera's growth. Our results are in agreement with the experimental investigation on Mg intrashell reactions of Bentov and Erez (2005) and which are attributed to a kinetic mechanism played by the carbonate ions in the calcite growth by Lopez et al. (2009). Porcelanaceous foraminiferal tests from the anthropogenically polluted lagoon of Santa Gilla (Frontalini et al., 2009) have a similar Mg/Ca ratio $(0.11 \, \mathrm{mol \, mol^{-1}})$ which is greater than that collected at station NP, confirming the influence of the stressed environment on the Mg/Ca ratio of these biominerals.

Figure 10d shows that the amount of Cd, Zn and Pb incorporated in the bioinfested shells in PP, LC, PV and PA is higher compared to the pollution-free NP station. We found that the amount of heavy metals incorporated in the infested tests is higher when the proportion of infested tests is higher.

The process of bioerosion in calcareous skeletal structures can be regarded differently in high-Mg porcelanaceous imperforate tests and low-Mg bilamellar hyaline perforate tests and can also be related to the decrease of seawater pH (Fig. 10b). The porcelanaceous wall consists of a thick layer of high-Mg calcite needles with relatively large interstices filled with organic matter. Needles are randomly arranged and coated inside by an inner organic lining and outside by the outer organic layer (Towe and Cifelli, 1967; Hemleben et al., 1986; Debenay et al., 2000a). The common infestation of euendoliths in porcelanaceous tests rather than in hyaline tests can also be related to the thick calcite layer of the porcelanaceous wall, allowing an adequate erosional space for their growth. The perforate foraminifera are characterised by structures of greater complexity (chamber-partitions, shell cavities, canal systems) compared with those of the porcelanaceous group. In the bilamellar hyaline perforate group, the wall comprises carbonate layers separated by an organic median layer (Towe and Cifelli, 1967; Hansen and Reiss, 1971; Hottinger, 1978, 2000; Hansen, 1999; Debenay et al., 2000b). Our data show that increasing heavy metal concentrations in the sediments corresponds to an increase of microbial infestation, reaching a peak at the more polluted sites (PV and PA; Fig. 10e).
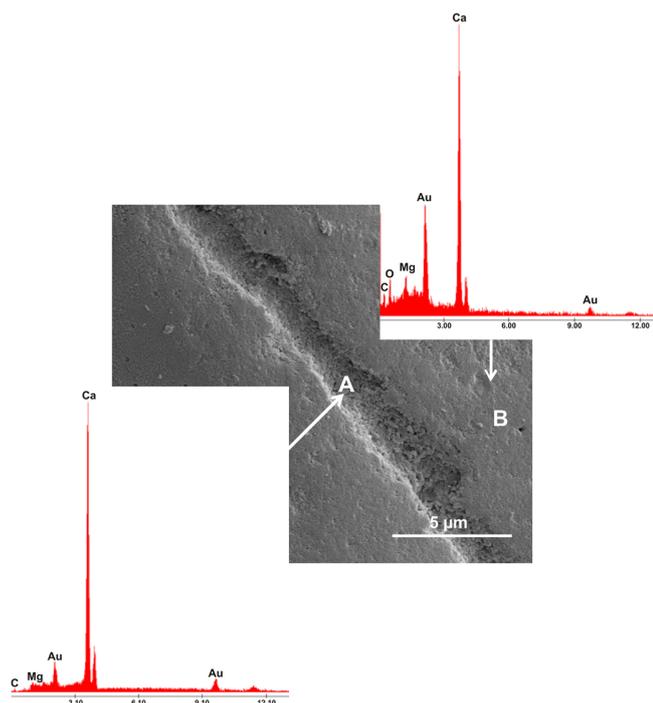
Fig. 8. SEM FEG images of porcelanaceous foraminifera (*Quinque-loculina* sp.). EDX analysis shows that Mg content at the bottom of boring (**A**) is significantly lower than on unaltered shell surface (**B**).

Fig. 9. Occurrence of bioerosion given in percent in selected taxa belonging to porcelanaceous (high-Mg) imperforate miliolids and peneroplids (*) and hyaline (low-Mg) perforate foraminifera (*Ammonia*, *Elphidium*, *Lobatula*, *Rosalina*) from sampling stations (PP, LC, PV, PA).

higher concentrations of Mg and other elements from seawater into their tests (Yanko et al., 1994, 1998).

In the sandy beach part of the study area, limestone outcrops are lacking and bottom sediment is made of quartz grains. Anomalous concentrations of euendolithic communities in shallow polluted waters, question the capacity of intensive industrial activity to produce extreme environmental conditions in restricted local areas only. Mine waste containing toxic concentrations of heavy metals (Fe, Al, Cu, Zn, Cd, Pb, Ni, Co and Cr) discharged into aquatic systems may produce degradation of water quality and aquatic life (Nordstrom, 2011). Our study reveals that peculiar environments affected by heavy metal fluxes from industrial processing are not biologically dead as they are teeming with microbes, including bacteria, archaea, fungi and algae.

Global scale geochemical models that predict the carbonate saturation state of the surface waters in the twenty-first century suggest that calcareous organisms may have difficulty calcifying, leading to production of weaker skeletons and greater vulnerability to erosion. We estimated the $p\mathrm{CO_2}$ partial pressure at equilibrium with the sampled seawater (by pH and alkalinity) using the Millero and Scheiber (1982) ion pairing model to estimate activity coefficients and found values between 3–5 times higher than the average for the open sea nearby. This study of biomineralisation confirms that, at least in shallow waters, high-Mg carbonates are altered faster compared to low-Mg carbonates (Fig. 10b), confirming that organisms using the more soluble forms of $\mathrm{CaCO_3}$ (aragonite and high-Mg calcite) are more adversely affected by higher $p\mathrm{CO_2}$. Our results are in agreement with earlier laboratory experiments (Morse, 1983; Morse et al., 2007) where it was predicted that dissolution of various benthic marine organisms may increase under natural higher $p\mathrm{CO_2}$ partial pressure (Ries et al., 2009).

The observed high level of microboring infestation is related to the anomalous mechanism of bio-mineral construction in the presence of a high level of heavy metals. In these heavily polluted environments, euendolithic bioerosion develops in small shells of the biocarbonate substrates such as calcareous foraminifera. Microbial organisms need the development of a trophic strategy related to the decrease of micrograzers (mainly benthic foraminifera). According to Mojtahid et al. (2011) some foraminifera species, such as *Haynesina germanica*, *Ammonia beccarii* and the single-chambered species *Psammophaga* sp., consume and digest large quantities of bacteria. The estimated grazing rates of these three intertidal species were comparable: ~ 3.2 ng C ind^{-1} h^{-1} ($\sim 16\,000$ bacterial cells ind^{-1} h^{-1}) for low bacterial concentration ($\sim 43 \times 10^6$ cells) and ~ 5.7 ng C ind^{-1} h^{-1} ($\sim 28\,000$ bacterial cells ind^{-1} h^{-1}) for higher bacterial concentration ($\sim 86 \times 10^6$ cells). Biotic indices (Faunal Density, Species Richness, Dominance, Shannon-Weaver, Simpson, Evenness, Menhinick, Margalef, Equitability, Fisher-α, Berger-Parker; Cherchi et al., 2009) performed on foraminiferal assemblages show an abrupt decrease with increasing heavy metal fluxes (Fig. 10a, d). Optical observations reveal an high number of abnormal and small specimens in accordance with an increase of industrial pollution. Morphological abnormalities, which are commonly found in tests from pollution-stressed benthic foraminiferal populations, may be related to incorporation of
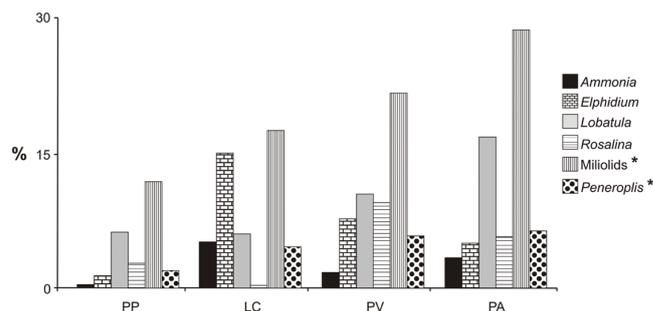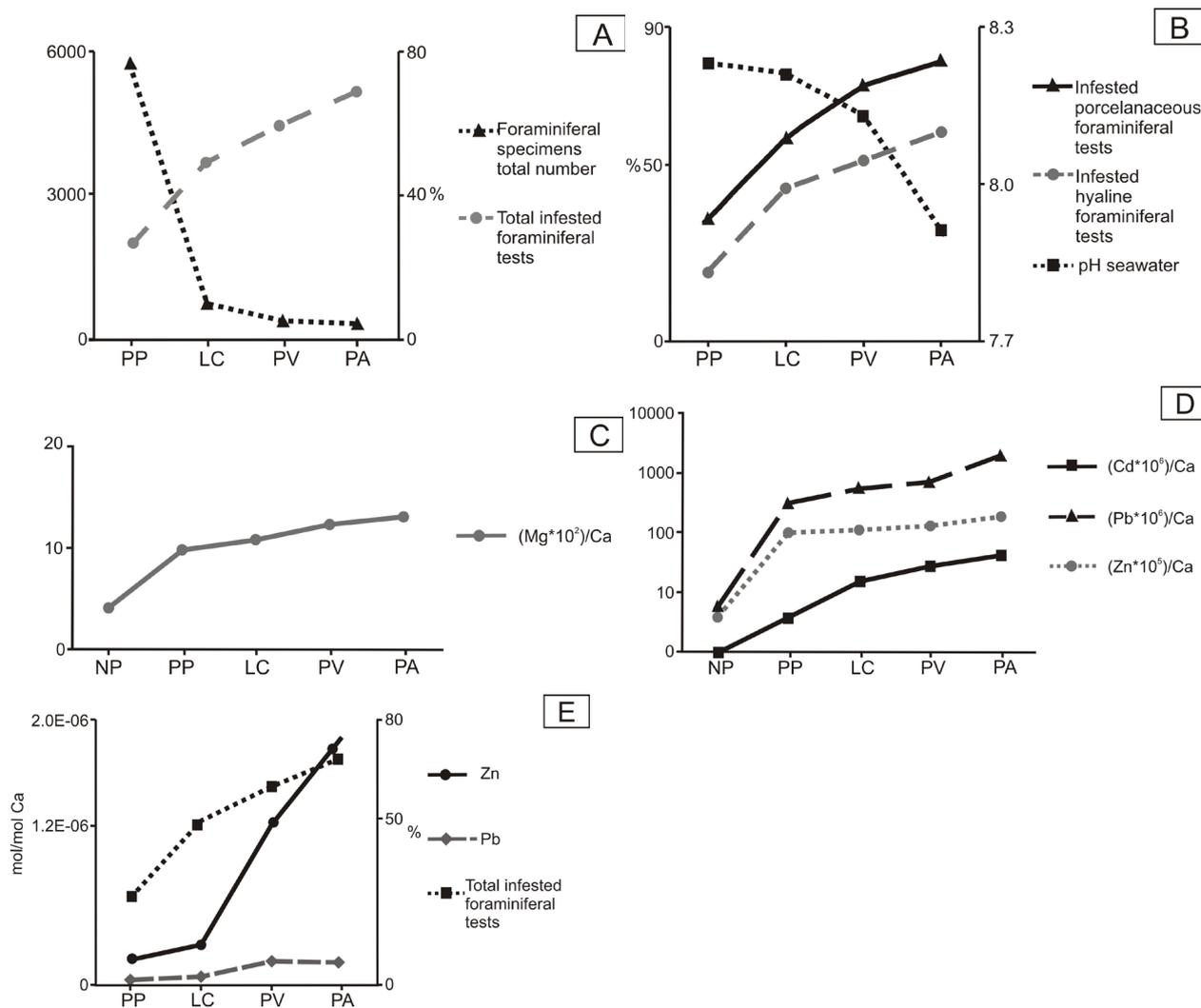
Fig. 10. Occurrence of bioerosion given in percent in microbially infested foraminiferal tests from Portoscuso coastal environments. **(A)** Correlation between infested foraminifera percentages and total number of foraminiferal tests. **(B)** Correlation between porcelanaceous high-Mg and hyaline low-Mg tests and sea water pH. **(C)** Mg/Ca ratio in foraminiferal tests increases in more highly polluted sites. **(D)** Cd/Ca, Pb/Ca and Zn/Ca ratio in foraminiferal tests increase in more highly polluted sites. **(E)** Increase of heavy metal values in sediments corresponds to increase in microbial infestation.

5 Summary and conclusions

In our study, samples were collected at the same depth (about 2 m) from shallow sediments affected by serious industrial contamination in the coastal environments of Portoscuso-Portovesme. In this area, foraminiferal shells have a high content of heavy metals and are actively infested by microborings. As far as we know, these infested foraminiferal communities provide a state-of-the-art dataset for the understanding of bioerosional processes in foraminiferal hosts in polluted environments.

The results show (i) microbioerosion is higher in high-Mg foraminiferal tests compared to the low-Mg tests, (ii) the role of foraminiferal skeletal architecture in the boring process,

(iii) the abundance of euendoliths is favoured by the effect of greater concentrations of heavy metals, especially Zn, as an inorganic nutrient.

We interpret that in the shallow water and low hydrodynamic conditions of the Portoscuso-Portovesme lagoon, the heavy metals leached from mine tailings and industrial discharge are not immediately dispersed in the sea water and, thus, foraminifera can concentrate heavy metals in their shells. This takes place in a complex biomineralisation process. In agreement with geochemical models and previous literature, we found that high-Mg carbonate shells dissolve faster and are deeply infested by microborings. While geochemical models already predict that CO_2 increase is affecting biogenic carbonate reservoirs, this study demonstrates

that the heavy metal dispersion process can contribute to global CO_2 change via a complex ecological process.

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