

Benthic foraminiferal response to trace element pollution—the case study of the Gulf of Milazzo, NE Sicily (Central Mediterranean Sea)

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Abstract The response of benthic foraminiferal assemblages to trace element pollution in the marine sediments of the Gulf of Milazzo (north-eastern Sicily) was investigated. Since the 1960s, this coastal area has been a preferred site for the development of two small marinas and a commercial harbour as well as for heavy industry. Forty samples collected in the uppermost 3–4 cm of an undisturbed layer of sediment in the littoral environment were used for this benthic foraminiferal analysis. The enrichment factors (EFs) of selected trace elements (As, Co, Cr, Cu, Mn, Ni, Pb and Zn) were also calculated. Changes both in benthic foraminiferal assemblages and in some trace elements concentrations have provided evidence that the gulf's littoral zone can be subdivided into three sectors characterised by environmental changes in the

marine ecosystem. In the sector unpolluted, close to the Milazzo Cape, foraminiferal assemblages exhibit high values of species richness and foraminiferal density while trace element concentrations and their EFs are very low. Here, the highest densities of Miliolids and epiphytic species are present. On the contrary, in the sector polluted, from the marinas to the crude oil refinery, foraminiferal density and species diversity are low, and assemblages are dominated, albeit with very low densities, by species that tolerate stressed environmental conditions, such as LOFAs, agglutinants and *Ammonia* spp. Here, the highest trace elements concentrations of Pb, Zn and Cu and related EFs were detected. Eastwards, in the sector moderately polluted, foraminiferal populations are quite poor. They are characterised by low values of species richness and foraminiferal densities, nevertheless trace element concentrations become lower than in the other sectors and their EFs are often below 1. Deformed foraminifera, with percentages up to 7.14 %, were found in all three of the sectors. Differences in benthic foraminiferal assemblages, coupled with results from statistical analysis, indicate that anthropogenic trace element pollution could be considered as one of the most important causes of the modifications of foraminiferal assemblages in the study area.

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Introduction

Background and aims of the work

Benthic foraminifera are among the most abundant microorganisms in the marine ecosystem (Lee and Anderson 1991). They are easy to collect, and owing to their small size, they provide a very reliable database that is useful in the statistical analysis of foraminifera assemblages (Armynot du Châtelet et al. 2004). Benthic foraminifera are microorganisms that readily respond to environmental changes in marine ecosystems; in fact small variations in salinity, temperature, pH, oxygen concentration, food supply and anthropogenic pollution may modify populations and their spatial distribution noticeably (Sen Gupta 1999a and reference therein).

Coastal marine areas are subjected to both natural (i.e. volcanic ash, erosion of igneous and metamorphic rocks and hot spring) and anthropogenic pollutants (i.e. trace elements, sewage waste, and hydrocarbons) that have a negative impact on the marine ecosystem (Alve 1991; Di Leonardo et al. 2007; Frontalini and Coccioni 2008; Tranchina et al. 2008 and reference therein). During the last few decades, a large number of publications have shown that high levels of heavy metals and trace elements in marine sediments can produce local extinctions, and modifications in foraminiferal assemblages, which include pathological processes in the foraminifera cell (Alve 1991; Sharifi et al. 1991; Le Cadre and Debenay 2006), strong variations in population density, changes in the specific abundance, diversity and test size (Alve 1991; Yanko et al. 1994, 1999; Coccioni 2000; Ferraro et al. 2009; Frontalini et al. 2009). Laboratory experiments also show that benthic foraminifera are sensitive to organic pollutants (i.e. Yanko and Flexer 1991; Gustafsson et al. 2000; Ernst et al. 2006). Furthermore, benthic foraminifera living in highly polluted sites can display aberrant morphologies in percentages higher than 1 % of the total assemblages (Alve 1991; Yanko et al. 1994; Frontalini and Coccioni 2008 and reference therein). Thus, the qualitative and quantitative analysis of benthic foraminiferal assemblages and aberrant test morphologies represent a useful tool in pollution assessment and in the environmental monitoring of coastal marine areas (Yanko et al. 1998; Cearreta et al. 2000; Armynot du Châtelet et al. 2004; Saraswat et al. 2004; Ferraro et al. 2006; Thibodeau et al. 2006; Di Leonardo et al. 2007; Frontalini and Coccioni 2008; Caruso et al. 2011).

The Gulf of Milazzo is a natural bay located offshore of north-eastern Sicily (Fig. 1a). Since the 1960s, the coastal area of the gulf was a preferred site for the development of industries (i.e. a crude oil refinery and thermal power plant) as well as for the marina and commercial harbour. All of these activities are potential sources for anthropogenic trace element contamination. Thus, the Gulf of Milazzo represents a natural laboratory for the investigation of the relationships between human pollution sources, distribution and enrichments of trace elements and their effects on benthic foraminifera.

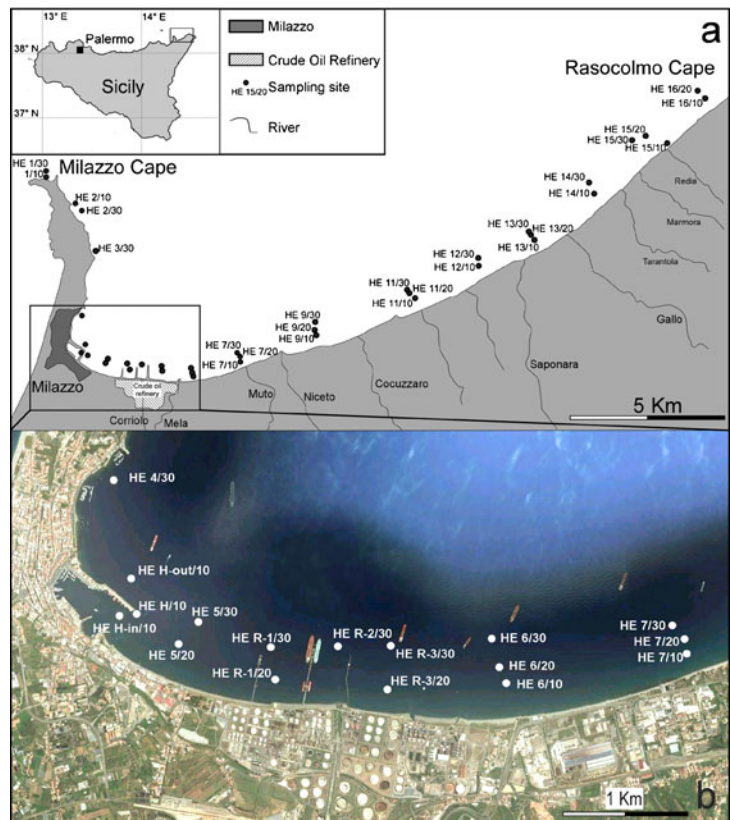
The goal of this study is to observe the benthic foraminiferal response (assemblage variations and the development of aberrant test morphologies) to trace element pollution in the marine ecosystem of the gulf by using a multidisciplinary (micropaleontological, geochemical, sedimentological and statistical) approach.

Environmental settings of the Gulf of Milazzo and geology of the adjacent Peloritani Mountains

The Gulf of Milazzo extends ~26 km along the shore from the Milazzo Cape to the Rasocolmo Cape (Fig. 1a). The continental shelf is ~200 m wide off the Milazzo Cape and increases its size to ~2 km wide at the centre of the gulf. The continental shelf edge lies mostly between 120 and 135 m of water depth. Hydrodynamics of the superficial water layer are complex: a general cyclonic circulation flows eastward from Capo Milazzo to Capo Rasocolmo, whereas an anticyclonic circulation, locally influenced by morphology and winds, characterises the inner sector of the bay (Decembrini et al. 2004).

Twelve small N-S oriented rivers and several streams flow from the Peloritani Mountains into the Gulf of Milazzo, among these the Corriolo river mouth flows quite close to a crude oil refinery (Fig. 1). During periods of high stream discharge, continental waters carry a significant amount of fresh water and sediments produced from the erosion of sedimentary rocks, gneiss and granitoides with subordinate amphibolites and marbles from the extensively exposed Aspromonte Unit of the Peloritani range on the shore of the Gulf of Milazzo (Amodio-Morelli et al. 1976; Bonardi et al. 2001; Messina et al. 2004). The Aspromonte Unit is locally unconformably covered by Middle-Upper Miocene to Pleistocene sedimentary sequences (Bonardi et al. 2001 and references therein). Plio-Quaternary extensional basins formed in the Milazzo onshore are filled with a variety of sediments which mostly consist of gravels,

Fig. 1 **a** The Milazzo Gulf with the positions of the sampling sites; **b** detail of the harbour area and the crude oil refinery (modified from Google Earth)



calcarenites and bioclastic sands, clayey, marls and marly limestone.

Material and methods

Sampling

Forty surface marine sediment samples were collected in the Gulf of Milazzo submerged littoral zone in August 2008. The names of the sampling stations are reported in detail in Fig. 1. Each sampling site (labelled HE) was marked by two numbers: the first one indicates the name of the transect and the second one indicates its bathymetry. Sediment sampling was carried out at water depths of ~10, ~20 and ~30 m, taking the top 3–4 cm (~100 cm³) from the undisturbed layer of sediment by using a 4 kg Van Veen grab. Sediments were scraped using a plastic blade to avoid trace element contamination, immediately sealed in polyethylene flasks and stored at -20 °C until analysis (see Pepe et al. 2010 for

further details). A complete list of the coordinates and bathymetries for each sampling site is given in Table 1.

The age of sediments is approximately constrained by assuming an average sedimentation rate of ~0.8–1 mm/years. These values have been calculated in the Pozzuoli Bay (Eastern Tyrrhenian Margin). Here, two tephras, dated at 5,090±140 years BP (Insinga et al. 2006) and 79 AD, respectively, have provided a calibrated time interval for the bounded marine stratigraphic units (Sacchi et al. 2013). A similar value of 0.85±0.15 mm/years was also derived from marine sediments collected from the coast of south-eastern Sicily (Gerardi et al. 2012). According to this data, the resulting age we determined for the studied sediments is ~40–50 years.

Granulometry

One hundred grams of sediment for each sample was oven-dried at 55 °C for 48 h, and subsequently weighed and washed through a 63-µm sieve. The residual fraction was oven-dried and weighed again. In this way, we

Table 1 Geographic coordinates and bathymetries of the sampling sites

Sample	Latitude (°N)	Longitude (°E)	Depth (m)
HE 1/10	38.2219483	15.2500650	10
HE 1/30	38.2733100	15.2349517	30
HE 2/10	38.2221150	15.2500167	6
HE 2/30	38.2603650	15.2489883	30
HE 3/30	38.2478000	15.2541617	30
HE 4/30	38.2143467	15.2579817	30
HE H-out/10	38.2189467	15.2492150	30
HE H/10	38.2155533	15.2498150	20
HE H-in/10	38.2159983	15.2482267	11
HE 5/20	38.2129167	15.2568583	20
HE 5/30	38.2143800	15.2581367	30
HE R1/20	38.2108067	15.2665667	20
HE R1/30	38.2116483	15.2800850	30
HE R2/30	38.2122750	15.2715150	30
HE R3/20	38.2101217	15.2802800	20
HE R3/30	38.2116483	15.2800850	30
HE 6/10	38.2085300	15.2919133	10
HE 6/20	38.2093350	15.2916217	20
HE 6/30	38.2107200	15.2911867	30
HE 7/10	38.2122117	15.3114750	10
HE 7/20	38.2138850	15.3110850	20
HE 7/30	38.2151517	15.3099000	30
HE 9/10	38.2199417	15.3418367	10
HE 9/20	38.2216600	15.3409667	20
HE 9/30	38.2241767	15.3414650	30
HE 11/10	38.2306450	15.3818550	10
HE 11/20	38.2331233	15.3794917	20
HE 11/30	38.2331233	15.3788250	30
HE 12/10	38.2399733	15.4076550	10
HE 12/30	38.2425083	15.4076383	30
HE 13/10	38.2474583	15.4303583	11
HE 13/20	38.2493017	15.4287917	20
HE 13/30	38.2502133	15.4281467	30
HE 14/10	38.2614467	15.4546367	10
HE 14/30	38.2648400	15.4525983	30
HE 15/10	38.2763617	15.4845883	8
HE 15/20	38.2787867	15.4761683	20
HE 15/30	38.2776900	15.4704250	30
HE 16/10	38.2899100	15.5003383	16
HE 16/20	38.2922683	15.4972800	34

obtained the percentage of mud (silt and clay) by taking the difference between the unwashed and washed sample

(Caruso et al. 2011). Afterwards, the sediment was sieved to obtain separate fractions of the size 0.063–0.5, 0.5–1, 1–2 and >2 mm. Each fraction was weighed and expressed as a percentage of the total sediment's weight (Shepard 1954).

Trace elements proxy

The partial manganese concentration was determined in the grain size fraction <63 μm by using an inductively coupled plasma-mass spectrometry (PerkinElmer model ELAN-DRC-e) after digesting 0.5 g of sediment sample with a mixture of HNO_3 – H_2O_2 (2:1) in bombs using a microwave oven (CEM MSD 2000). Partial trace element digestion is widely used in environmental geochemical studies to remove non lattice-bound elements, giving an estimate of the maximum amounts of elements that are potentially modifiable with changing environmental conditions (Chatterjee and Banerjee 1999; Yawar et al. 2010). Precision, reported as a relative standard deviation) percentage, was better than 5 %.

The enrichment factors (EFs) for manganese and other trace elements determined in the same samples (As, Co, Cr, Cu, Ni, Pb and Zn; Pepe et al. 2010) were calculated as “Element_{sample}/ Element_{background}.” To select background level values as close to the local pre-industrial ones as possible, the results obtained for the Sicily Channel from Di Leonardo et al. (2013) and Tranchida et al. (2010) were considered.

The normalisation of trace elements in respect to aluminium, or other aluminosilicate elements, generally used to correct for differences in sediment grain size and mineralogy (e.g. Carral et al. 1995; Daskalakis and O'Connor 1995; Morford et al. 2001; Alagarsamy and Zhang 2010), is not necessary here considering that the same granulometric fraction (<63 μm) was used for the digestion of both the samples collected in the Gulf of Milazzo and in the sediments analysed in the work of Di Leonardo et al. (2013) and Tranchida et al. (2010).

Trace element measurements on samples HE 1/10, HE 2/10, HE 13/20 and HE 15/10 have not been performed. Thus, these samples were not taken into account in the statistical analyses (see below).

Foraminiferal analysis

A review of the relevant literature raises several questions concerning the thickness of the undisturbed layer of sediment sufficient to obtain a reliable database of

benthic foraminiferal assemblages that can be used as bio-indicators of pollution in a marine ecosystem. In this study, the choice was made to analyse the uppermost 3–4 cm of the top-soft sediment because the highest percentages of benthic foraminifera (>90 %) usually live in this portion (Jorissen 1988).

Presently, an intense debate exists around the use of living or dead benthic foraminifera in the assessment of anthropogenic pollution in a marine ecosystem (i.e. Bernhard 2000; Murray 2000; Morvan et al. 2006). Because of the impossibility of carrying out seasonal sampling, it was chosen to study the total (living+non-living specimens) benthic foraminiferal assemblages. The latter provides integrated information about the general conditions of a marine ecosystem over a long period, being mostly comparable to assemblages in core samples (Alve and Nagy 1986; Debenay et al. 2009; Martinez-Colon et al. 2009).

The qualitative and quantitative analyses of the benthic foraminifera were carried out on the fraction >63 μm , which was subsequently subdivided into smaller statistically representative aliquots of the total sample by using an Otto microsplitter. The split fraction was weighed and all specimens were counted, classifying normal and deformed ones for each species. The classifications proposed by Loeblich and Tappan (1987), Cimerman and Langer (1991), Sgarrella and Moncharmont Zei (1993) and Sen Gupta (1999b) were used to recognise the species. In all samples, benthic foraminifera tests seem to be well preserved, without mechanical damages or re-crystallisation; furthermore, no displaced or re-worked species were recognised.

Since many samples are characterised by a low number of individuals (Table 2), it was chosen to plot each species or group as a foraminiferal density (foram g^{-1} of dry sediment) rather than as a percentage. In spite of some authors' (e.g. Goineau et al. 2011) assertions that percentages provide more reliable data on replicated cores, the use of percentages in samples characterised by variable granulometry and by a very low number of specimens might give rise to an overestimation of certain species. On this basis, and taking into account that the size of recognised foraminifera is lower than 0.5 mm, two types of foraminiferal density (FD-1 and FD-2) were calculated. FD-1 corresponds to the number of individuals per gram of total dry sediment (foram per gram of dry sediment), while FD-2 corresponds to the number of individuals per gram of dry sediment, excluding the fraction greater than 0.5 mm. FD-2 has been

applied to calculate densities of each species and/or group.

Five foraminiferal parameters were calculated using the Paleontological Statistics Data Analysis (PAST) software (Hammer et al. 2001): (1) species richness (S), as the number of total taxa and (2) Shannon–Weaver (H), (3) Dominance (D) and (4) Fisher- α index, i.e. the relationship between number of species and number of individuals in an assemblage (Fisher et al. 1943; Murray 1973), and (5) Margalef's richness index.

Some species and/or genera were grouped together on the basis of ecological considerations. Four species of *Ammonia* were recognised (*Ammonia beccarii*, *A. gaimardii*, *A. tepida*, and *A. parkinsoniana*; Hayward et al. 2004) and also plotted together as *Ammonia* spp. because they are generally considered as pollution-tolerant taxa (Samir and El-Din 2001; Caruso et al. 2011).

Bolivina spp., *Bulimina* spp., *Cassidulina carinata*, *Cassidulina crassa*, *Fursenkoina acuta*, *Globobulimina affinis*, *Globocassidulina subglobosa* and *Uvigerina peregrina* were grouped together because they are considered as low-oxygen foraminiferal assemblages (LOFAs; Smart 2002).

The *Elphidium* group contains three species: *Elphidium aculeatum*, *Elphidium crispum* and *Elphidium macellum*.

Asterigerinata planorbis, *Buccella granulata*, *Elphidium* spp., *Gavelinopsis praegeri*, *Lobatula lobatula*, *Peneroplis* spp., *Planorbulina acervalis*, *Rosalina bradyi*, *Rosalina obtusa* and *Sorites orbiculus* were grouped together because they are epiphytic foraminifera (Langer 1993).

The order of "Miliolida" includes the following genera: *Adelosina*, *Quinqueloculina*, *Sigmoilopsis*, *Sorites*, *Spiroloculina*, *Triloculina* and *Vertebralina*. Miliolids were chosen because they are known to be sensitive to pollution (Samir and El-Din 2001; Di Leonardo et al. 2007; Valenti et al. 2008; Ferraro et al. 2009).

The genera *Cribrostomoides*, *Lagenammina*, *Saccammina* and *Textularia* were grouped together as "agglutinants".

Deformed specimens belonging to *L. lobatula*, *A. parkinsoniana*, *Quinqueloculina* spp. and *R. obtusa* were also recognised, separately counted and reported as total deformed foraminifera (TDF).

Carbon-coated selected specimens were observed and documented by a scanning electron microscope (SEM) using a LEO 440 with EDS System OXFORD ISIS 300 Link and Si (Li) PENTAFET detector.

Table 2 Concentrations in parts per million of investigated metals (modified from Pepe et al. 2010), number of foraminifera counted, and benthic foraminiferal densities (FD-1 and FD-2) reported in terms of foram per gram of dry sediment

Samples	Mn	Co	Ni	Cu	Zn	Pb	Cr	As	number of counted foraminifera	FD-1 (foram/g of total sediment)	FD-2 (foram/g of sediment, 0.063-0.5 mm)
HE 1/10	–	–	–	–	–	–	–	–	149	18.64	116.26
HE 1/30	52.14	2.20	0.00	5.90	12.56	4.79	8.18	0.00	186	28.22	526.32
HE 2/10	–	–	–	–	–	–	–	–	20	1.80	9.27
HE 2/30	148.54	3.95	8.79	9.20	33.84	14.36	15.86	0.78	513	2,045.50	2,488.95
HE 3/30	149.58	4.83	10.87	15.92	45.13	14.89	21.97	1.28	287	209.26	278.85
HE 4/30	446.74	14.03	36.83	40.57	123.25	42.72	58.83	5.70	247	201.27	1,092.06
HE H out/10	407.35	12.94	34.08	35.08	134.04	49.03	76.20	4.37	154	161.26	241.51
HE H/10	345.19	10.18	24.63	27.83	182.03	32.42	40.92	3.27	130	117.68	250.02
HE H-in/10	417.24	12.65	31.77	39.23	122.14	29.17	50.81	4.21	35	17.65	20.31
HE 5/20	652.35	20.60	45.56	51.09	167.35	49.19	86.64	5.43	0	0.00	0.00
HE 5/30	350.44	12.84	30.39	22.14	105.51	18.01	77.96	2.74	2	0.00	0.00
HE R-1/20	498.90	14.09	34.89	35.54	113.58	20.27	54.20	6.04	60	28.62	36.88
HE R-1/30	428.51	13.87	33.05	34.04	120.34	18.78	77.02	3.06	22	12.16	20.83
HE R-2/30	473.17	16.52	44.21	44.49	137.77	21.34	88.13	3.15	28	13.71	25.93
HE R-3/20	208.19	6.37	13.74	14.02	53.49	9.29	21.99	2.22	45	16.55	21.81
HE R-3/30	386.46	13.67	34.85	34.23	112.57	18.74	76.32	2.72	35	7.32	16.82
HE 6/10	545.16	11.77	29.80	30.34	115.11	23.72	45.39	5.01	2	0.00	0.00
HE 6/20	312.30	8.76	20.87	23.89	73.82	17.14	37.71	3.76	5	6.97	8.17
HE 6/30	488.21	14.85	37.48	36.72	114.56	20.36	54.44	6.06	14	12.51	23.53
HE 7/10	658.72	13.96	32.29	40.02	130.39	29.62	45.73	5.52	2	0.00	0.00
HE 7/20	229.93	6.54	13.71	13.02	49.03	10.74	26.08	1.96	14	23.21	29.06
HE 7/30	413.48	12.81	29.64	31.05	110.80	19.31	61.41	3.29	8	10.82	22.87
HE 9/10	232.41	6.32	15.08	18.16	69.09	11.45	23.82	1.95	1	0.00	0.00
HE 9/20	469.07	13.17	38.20	32.73	123.77	20.99	62.72	5.64	1	0.00	0.00
HE 9/30	376.96	11.73	26.93	27.53	105.95	15.56	63.16	2.56	46	39.64	80.89
HE 11/10	103.10	2.89	2.48	8.28	23.27	4.75	12.03	0.00	1	0.00	0.00
HE 11/20	297.44	8.31	18.33	18.09	85.61	15.51	55.52	2.67	11	14.62	16.06
HE 11/30	411.77	10.48	22.27	25.24	99.55	18.34	41.99	4.15	36	68.69	86.61
HE 12/10	126.66	3.23	5.36	11.50	31.87	6.82	20.48	0.44	0	0.00	0.00
HE 12/30	425.97	11.01	24.76	28.16	108.24	23.06	65.70	3.61	14	20.58	22.69
HE 13/10	158.86	3.90	3.60	11.10	28.58	7.37	13.85	0.14	1	0.00	0.00
HE 13/20	–	–	–	–	–	–	–	–	8	8.84	9.61
HE 13/30	519.20	11.91	28.33	32.35	106.36	21.97	47.51	6.14	22	41.40	47.44
HE 14/10	448.38	9.70	21.74	24.63	91.61	19.10	49.32	3.65	6	0.00	0.00
HE 14/30	428.41	10.45	23.43	26.07	102.28	19.37	47.06	4.47	14	17.98	19.16
HE 15/10	–	–	–	–	–	–	–	–	1	0.00	0.00
HE 15/20	388.51	8.73	22.08	25.72	111.11	20.45	52.36	3.88	2	0.00	0.00
HE 15/30	527.10	14.40	34.63	29.17	136.42	24.02	64.00	7.34	9	10.94	12.23
HE 16/10	519.90	12.56	84.50	32.13	109.76	30.21	201.33	4.61	1	0.00	0.00
HE 16/20	446.19	12.49	34.30	30.82	139.78	22.00	83.62	4.13	8	12.10	12.58

Statistical analysis

A data matrix including foraminiferal densities (FD-1 and FD-2), trace element concentrations and their EFs, relative abundance of grain-size fractions, some foraminiferal groups and abundance of TDF was created to investigate the relationships between biotic and abiotic features. Data were treated both with bivariate (Pearson correlation matrix) and multivariate techniques of cluster analysis (CA) and principal component analysis (PCA) using the PAST software.

Q-mode CA using the Ward's linkage method based on Euclidean distance, which produces dendrograms with exceptionally well defined clusters (Parker and Arnold 1999), was performed in order to group samples with similar: (a) foraminiferal distribution, (b) trace element concentrations and (c) grain-size fraction, trace element concentrations, FD-1 and FD-2, percentages of TDF and density of agglutinants, Miliolids, Epiphytes, *Ammonia* spp. and LOFAs.

PCA was applied to highlight the relationships among trace element concentrations, FD-1 and FD-2, TDF, agglutinants, LOFAs, *Ammonia* spp., Miliolids, and Epiphytes.

Results

Grain-size distribution patterns

Sediments consist of muddy gravel, gravelly muddy sand, muddy sand or sandy mud. The fraction >1 mm (Fig. 2a) is composed of fragments of molluscs, bryozoans and minerals pertaining to the Aspromonte unit. This fraction is abundant in the westernmost part of the gulf, decreases up to 0.86 % in HE H-in/10 and it is absent in HE 5/20. Peaks of abundance from 10 to 72 % were observed in samples HE 1/10, HE 1/30, HE 2/10, HE 4/30 and HE H/10.

The fraction 0.5–1 mm is abundant in samples HE 1/10, HE 1/30, HE 2/10 and HE 12/10 (Fig. 2a). The fraction 0.063–0.5 mm is abundant in almost all samples, showing an increasing trend towards the easternmost part of the gulf. The mud fraction (<0.063 mm) is higher than 40 % in samples HE 5/30, HE R-2/30, HE R-3/30, HE 7/30 and HE 9/30 collected in front of the harbour and close to the crude oil refinery (Fig. 2b).

Trace elements

Concentrations of trace elements As, Co, Cr, Cu, Mn, Ni, Pb and Zn are listed in Table 2 and their EFs are shown in Fig. 2c–f. Concentrations of trace elements sharply increase from HE 4/30 eastwards. For further details, also see Pepe et al. (2010).

Strong EFs in Cr and Ni are evident at the Rasocolmo Cape (HE 16/10) with EFs of 2.53 and 2.20, respectively. Enrichments just above 1 characterise samples HE 5/20 and HE R-2/30. Also, they are moderately enriched in Co (1.35 for HE 5/20 and 1.08 for HE R2/30). Regarding Zn, Pb and Cu, many samples are characterised by enrichments up to 2.49, 3.21 and 1.66 respectively. As is never enriched.

Benthic foraminiferal assemblages

Diversity of benthic foraminifera

The number of counted foraminifera and diversity indexes is shown in Fig. 3. The highest number of individuals was counted in the westernmost part of the gulf, from sample HE 1/10 to HE H/10, with the exception of the sample HE 2/10 which was collected at the water depth of ~6 m.

The species richness (S) ranges from 1 in samples HE 9/10, HE 9/20, HE 11/10, HE 13/10, HE 15/10, HE 16/10 to 27 in sample HE H-out/10. Samples HE 5/20 and HE 12/10 are barren.

Dominance shows the lowest values in the westernmost part of the gulf and close to the crude oil refinery. D values range between 0.092 (HE R-3/30) and 1 in samples where only one species was recognised (HE 9/10, HE 9/20, HE 11/10, HE 13/10, HE 15/10). These samples are also characterised by a Shannon-Weaver index of zero, while the highest Shannon-Weaver index (2.52) was found at site HE 4/30.

The Fisher- α index varies from zero (HE 5/30, HE 6/10, HE 6/20, HE 7/10, HE 9/10, HE 9/20, HE 11/10, HE 13/10, HE 15/10, HE 15/20, HE 16/10) to 23.69 (HE 12/30).

The Margalef index shows the highest values in the westernmost part of the gulf, up to 5.16 in site HE H out/10.

Benthic foraminiferal distribution

A total of 60 benthic foraminiferal species, pertaining to 42 genera, were recognised. They included 4

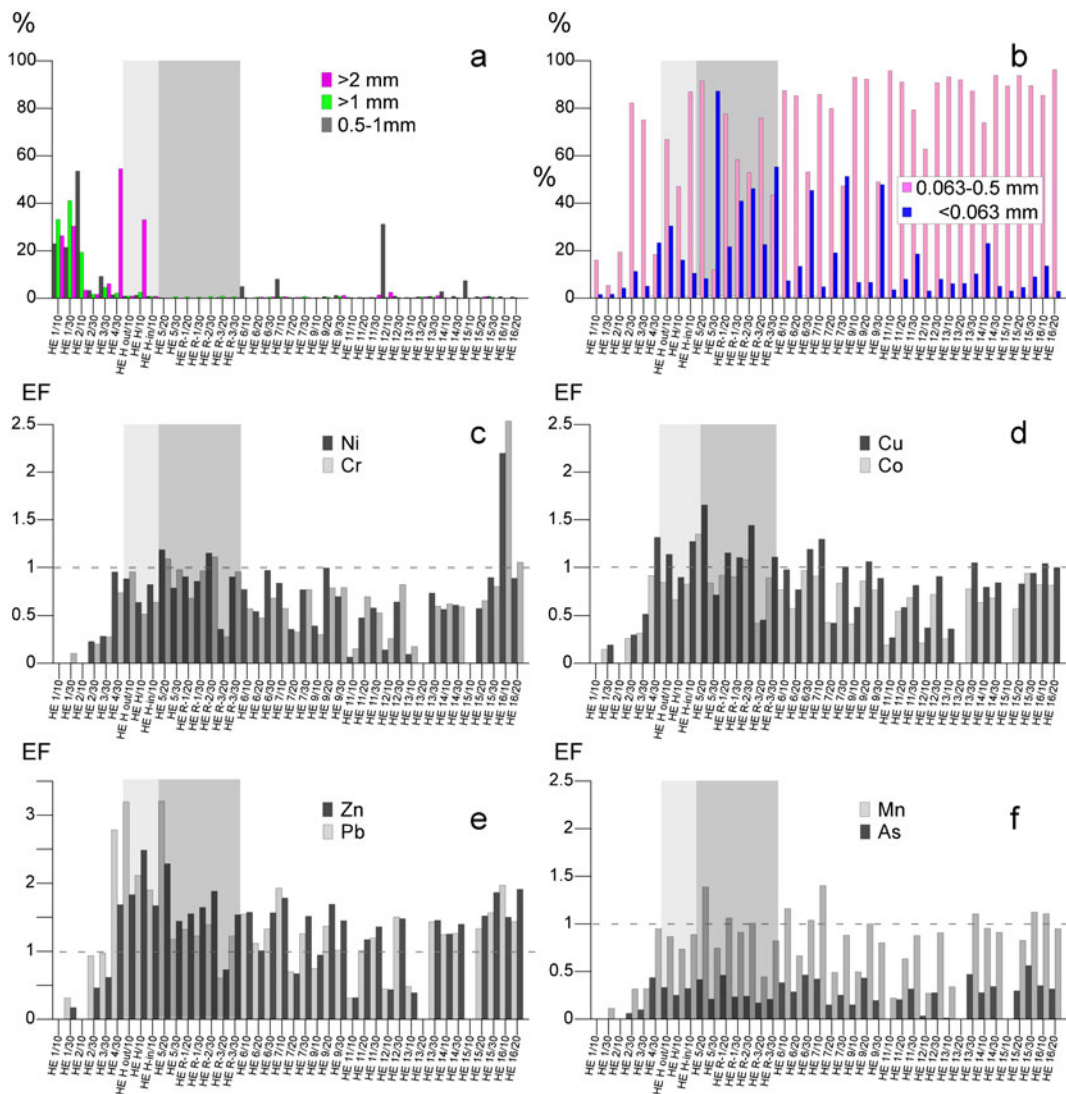


Fig. 2 a, b Grain-sized fractions reported as percentages; c–f EFs of trace elements. The *light gray area* indicates the harbour area while the *dark gray area* indicates the crude oil refinery

agglutinated, 40 calcareous hyaline and 16 calcareous porcelanaceous species (Appendix 1 in Supplementary Material and Plates 1, 2, 3).

The highest values of FD-2 were found in the area close to the Milazzo Cape (samples from HE 1/10 to HE 4/30; Fig. 3). Benthic foraminifera are abundant in samples HE 1/30, HE 3/30 and HE 4/30 but FD-1 shows a low value owing to the high percentages of the coarser and sandy fractions (see also Fig. 2a). On the contrary, FD-2 shows values that reach 526, 278 and 1092 foram g^{-1} of dry sediment, respectively (Fig. 3). Besides sample HE 2/30 which shows both a high number of counted foraminifera and FD-1/2,

samples HE 1/30, HE 3/30 and HE 4/30 show low values of FD-1, even though a relatively high number of benthic foraminifera is present due to the high percentages of the coarser and sandy fraction (Fig. 2a). On the contrary, FD-2 which don't take into account the granulometric fraction >0.5 mm, shows values that in the above three samples reach respectively 526, 278 and 1092 foram g^{-1} of dry sediment. Among the samples collected close to the Milazzo Cape, the sample HE 2/10 taken at a water depth of c.a. 6 m shows a low value of FD-2 (9 foram g^{-1} of dry sediment) and the assemblage is characterised by rare specimens of *A. beccarii*, *Elphidium* spp., *L.*

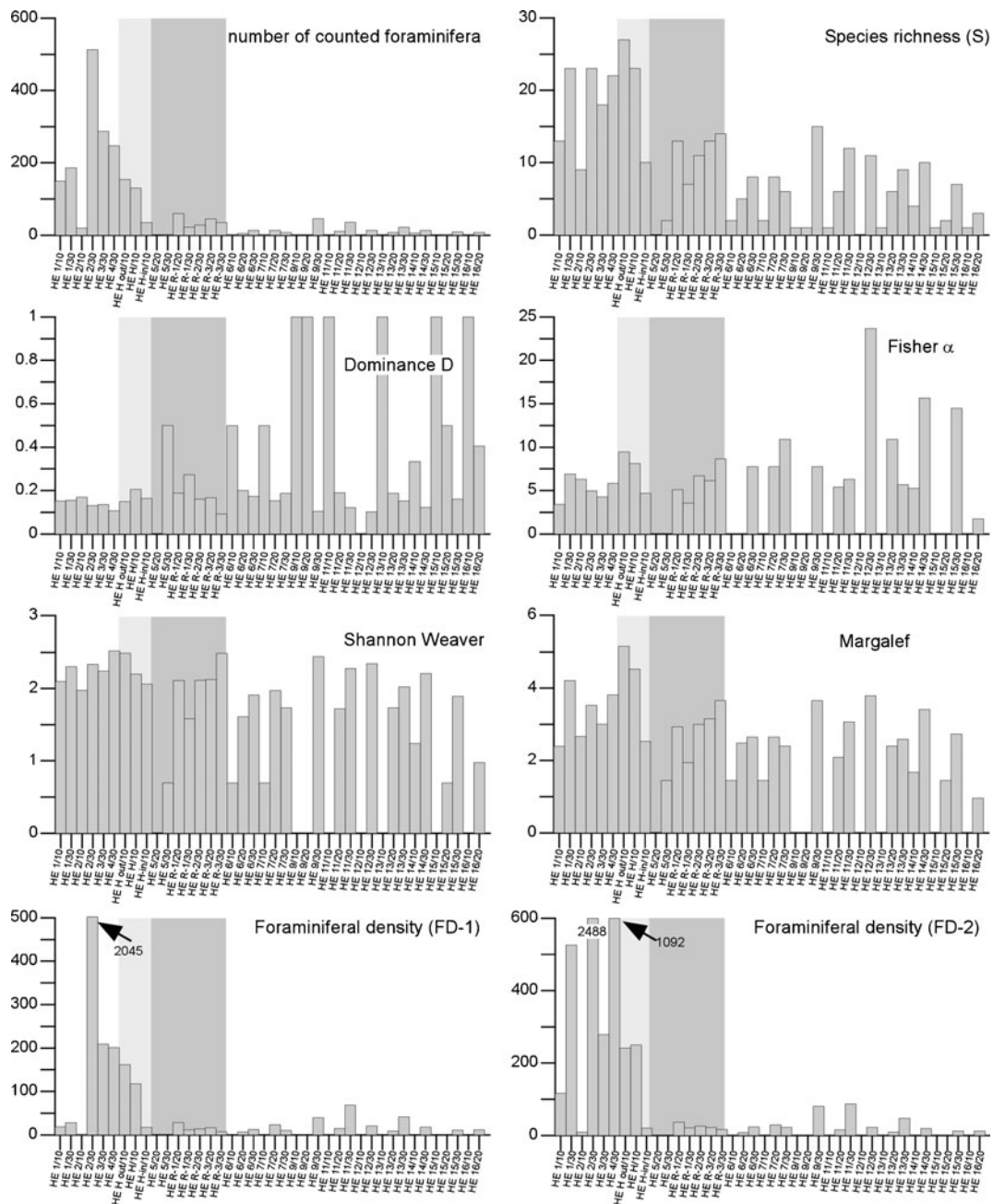


Fig. 3 Biodiversity indexes calculated for foraminiferal assemblages of the studied samples and values of foraminiferal densities (FD-1 is reported as number of individuals per gram of total dry

lobatula and *R. obtusa*, all species that typically live in shallow water (Sen Gupta 1999a).

FD-2 sharply decreases in sample HE H-out/10, collected outside the harbour, and it continues to decrease eastwards. In particular, FD-2 shows low values in sample HE H-in/10 (20 foram g⁻¹ of

sediment and FD-2 is reported as the number of individuals per gram of dry sediment, excluding the fraction greater than 0.5 mm; see text). Light and dark gray areas as in Fig. 2

dry sediment), collected inside the harbour, and in the area between the harbour and the crude oil refinery. Benthic foraminifera are rare in samples HE R-3/30, HE 7/30, HE 9/10, HE 9/30, HE 14/10 and HE 14/30, while they are absent in samples HE 5/20 and HE 12/10.

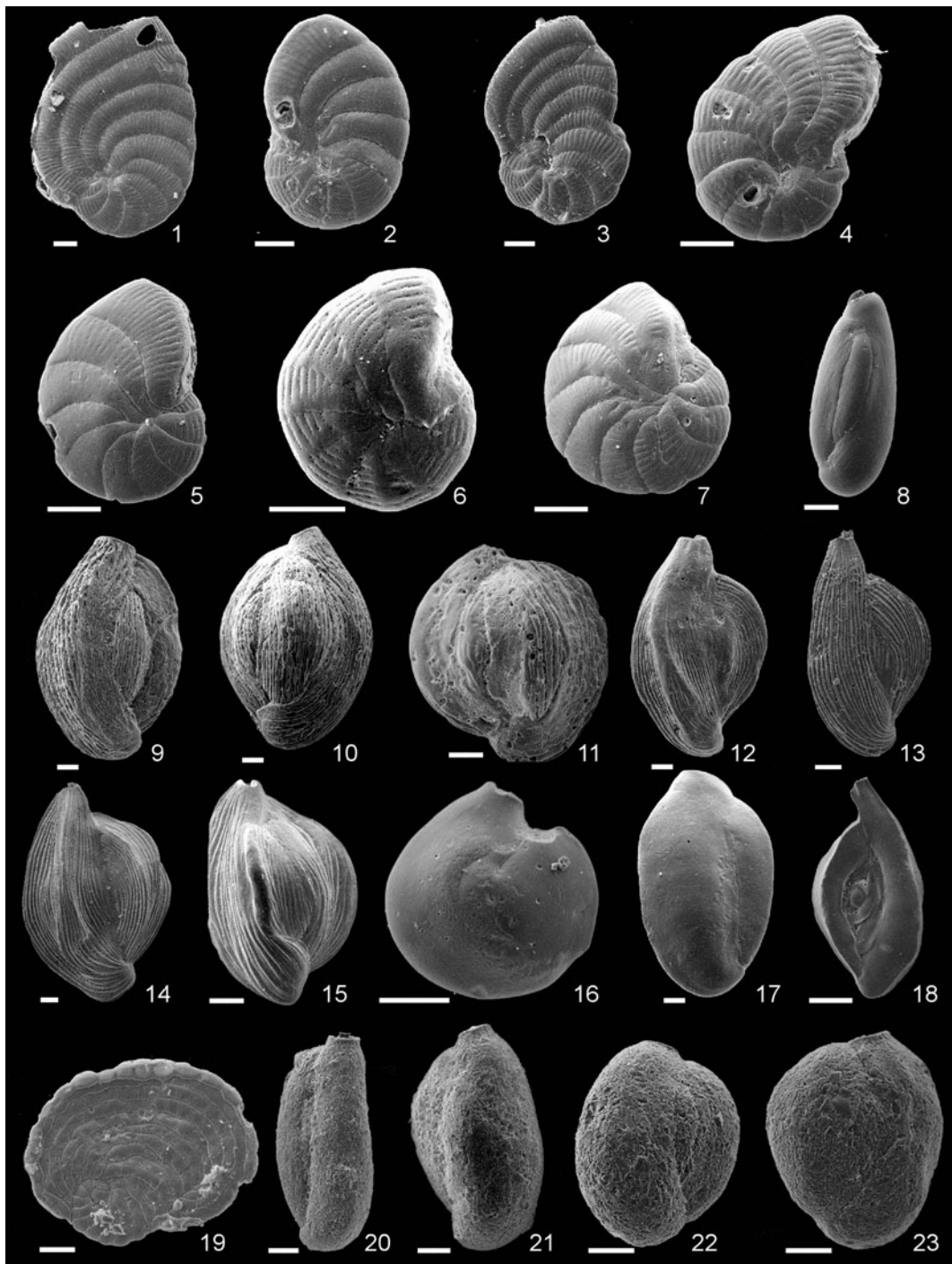


Plate 1 1–3 *Peneroplis planatus* (HE 1/10). 4–7 *P. pertusus* (HE 1/10). 8 *Quinqueloculina boschiana* (HE 1/10). 9–11 *Q. disparilis* (HE 1/10). 12–15 *Adelosina mediterraneensis* (HE 1/10). 16 *Adelosina* sp. (HE 1/10). 17 *Quinqueloculina lata* (HE

1/10). 18 *Spiroloculina angulata* (HE 1/10). 19 *Laevipeneroplis* sp. (HE 1–10). 20–21 *Siphonaperta aspera* (HE 1/30). 22–23 *Sigmoilopsis asperula* (HE 1/10). Scale bar=100 microns

The highest densities of Miliolids and epiphytes were found in the area between the Milazzo Cape

and the harbour (from HE 1/10 to HE H/10) with the highest values in sample HE 2/30 (1197 and 1126

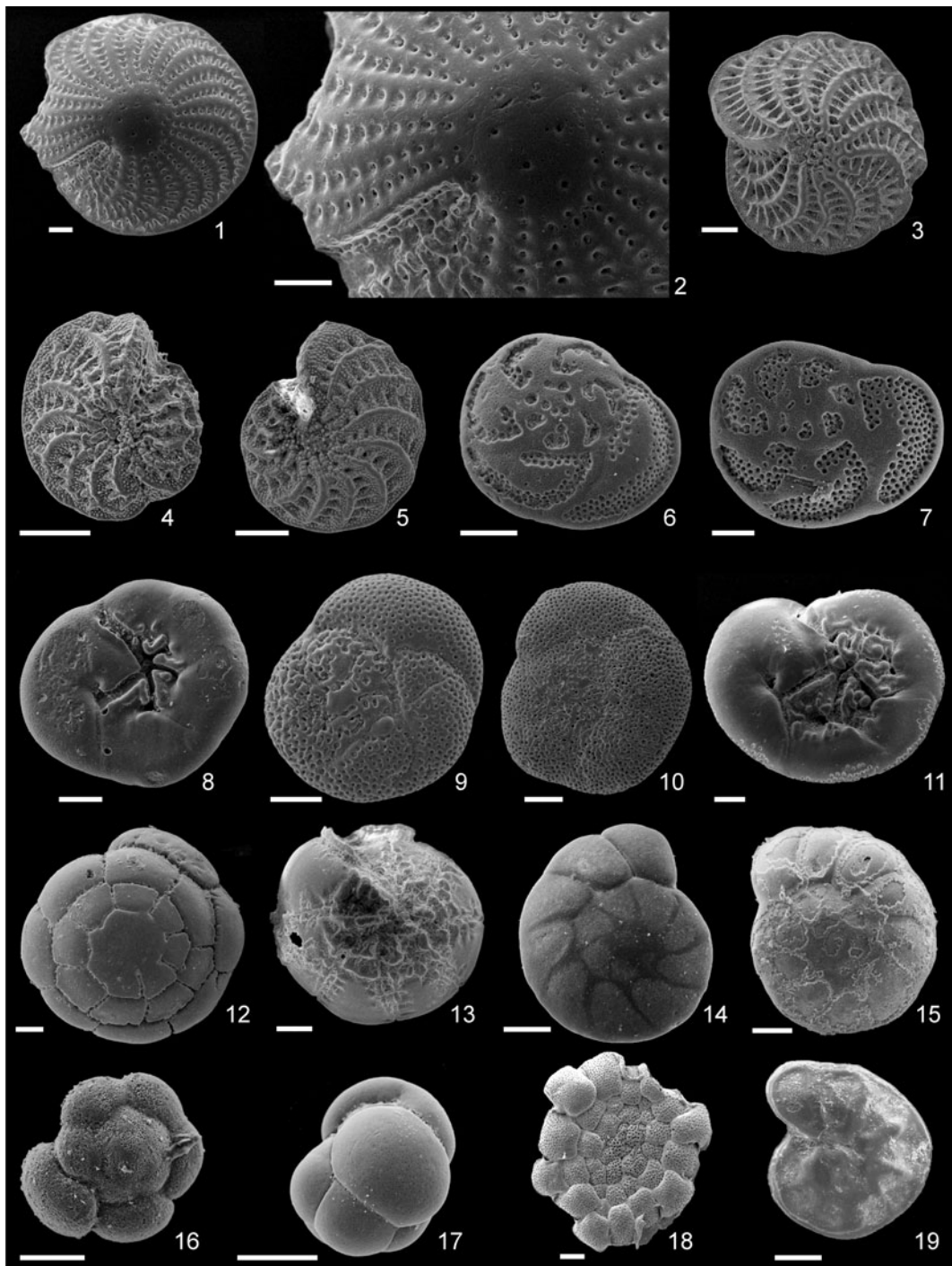


Plate 2 1–2 *Ephidium crispum* (HE 1/10). 3–5 *Ephidium macellum* (HE 1/10). 6–7 *Rosalina bradyi*, spiral side (HE 1/10). 8 *Rosalina bradyi*, ventral side (HE 1/10). 9–10 *Rosalina obtusa*, spiral side (HE 1/10). 11 *R. obtusa*, ventral side (HE 1/10). 12 *Ammonia gaimardii*, spiral side (HE 1/30). 13 *Ammonia*

gaimardii, ventral side (HE 1/30). 14 *Ammonia parkinsoniana*, spiral side (HE 12/30). 15 *A. parkinsoniana* (HE 12/30). 16 *Ammonia tepida*, spiral side (HE 12/30). 17 *Cassidulina crassa* (HE 12/30). 18 *Planorbulina acervalis* (HE 1/30). 19 *Hyalinea balthica* (HE 13/30). Scale bar=100 microns

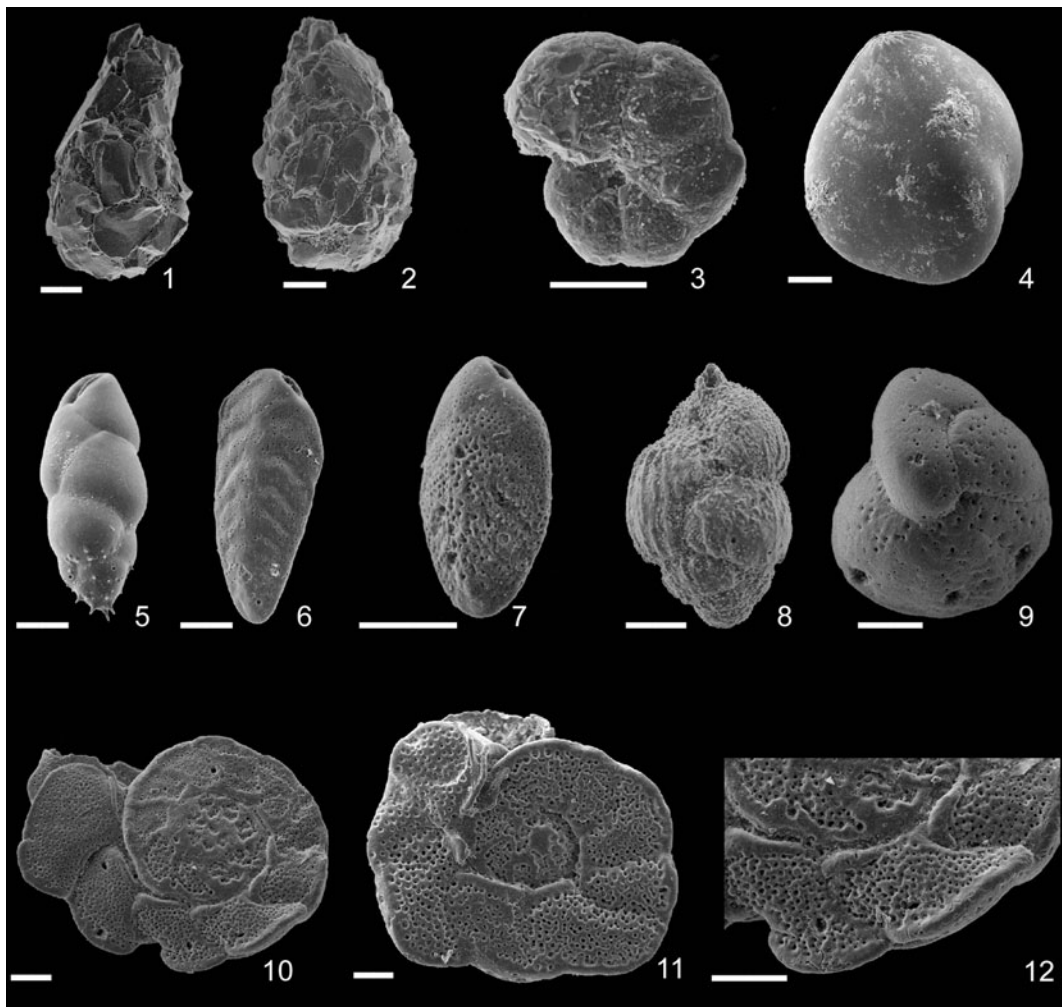


Plate 3 1–2 *Lagenammina atlantica* (HE 13/30). 3 *Cribrostomoides jeffreysi* (HE 13/30). 4 *Globobulimina* sp. (HE 1/10). 5 *Bulimina aculeata* (HE 13/30). 6 *Bolivina spathulata* (HE 12–30). *Bolivina catanensis* (HE 12/30). 8 *U.*

peregrina (HE 13/30). 9 *L. lobatula*, umbilical side (HE 1/10). 10–11 *L. lobatula*, spiral side (HE 1/10). 12 Detail of 11. Scale bar=100 μm

foram g^{-1} of dry sediment respectively, Fig. 4). Both foraminiferal groups are rare or absent towards the east. *Lobatula lobatula*, *Rosalina* spp., *G. praegeri* and *P. acervalis* are the most abundant species among epi-phytes. *Quinqueloculina*, *Adelosina* and *Triloculina* are the most abundant genera among Miliolids. The density of *Adelosina* spp., *Quinqueloculina* spp. and *Vertebralina* spp. is characterised by a trend which is quite similar with the exception of samples HE 4/30 and HE H-in/10 where the genus *Vertebralina* was not recognised. Rare specimens of *Quinqueloculina* spp. were recognised in a few samples collected between the crude oil refinery and the Rasocolmo Cape. Specimens of the genus *Triloculina*

were found in samples HE 1/30, HE 2/30, HE 4/30, HE H out/10 and HE H/10.

Peneroplis spp. were found in the samples from HE 1/10 to HE 3/30 with density values up to 32 foram g^{-1} of dry sediment (Fig. 4) while they are absent in samples collected from the harbour to the Rasocolmo Cape (HE 4/30–HE 16/20).

Rare specimens of *Laevipeneroplis* sp. (up to 5 foram g^{-1} of dry sediment) were found in samples HE 1/10 and HE H/10. *S. orbiculus* is present only at sample HE 1/10 (1.6 5 foram g^{-1} of dry sediment).

Densities of *Nonion* spp. and *Elphidium* spp. are characterised by a similar trend (Fig. 5). In particular,

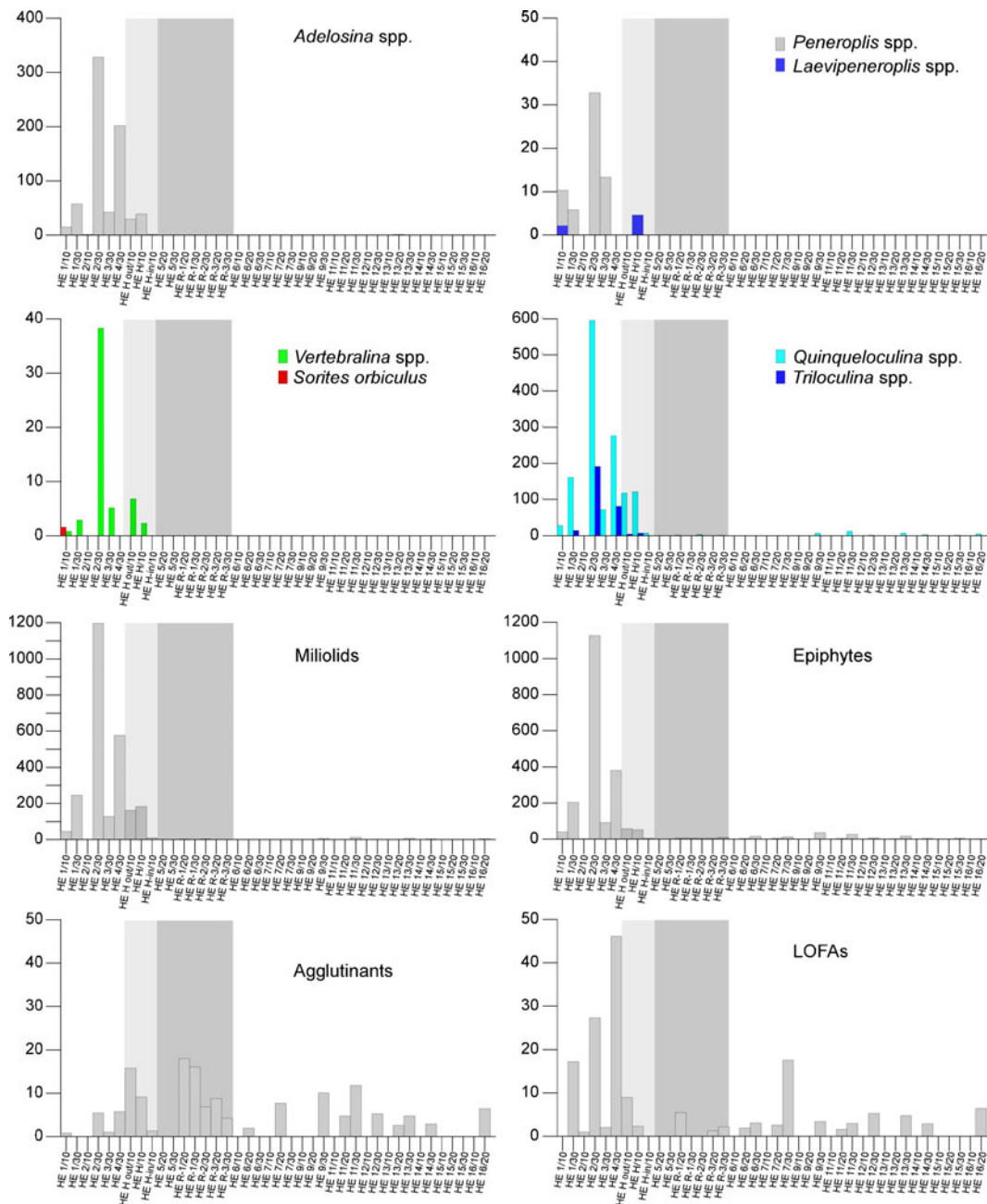


Fig. 4 Values of foraminiferal density of some selected benthic foraminifera species and groups (reported as number of individuals per gram of dry sediment normalised with respect to the

percentage of fraction comprised between 0.063 and 0.5 mm). Light and dark gray areas as in Fig. 2

these genera are abundant in samples HE 2/30 and HE 4/30 while they become rare or absent elsewhere.

The density of *Ammonia* spp. reaches the highest values in samples HE 1/30, HE 2/30, HE 4/30 and HE 9/30. *Ammonia parkinsoniana* and *A. beccarii* are the

dominant species. The latter one is more abundant than other species in samples collected at a water depth of ~30 m (Fig. 5).

Ammonia spp. and agglutinants are the most abundant foraminifera in front of the crude oil refinery

(Figs. 4 and 5). Agglutinants were also recognised in some samples collected close to the Milazzo Cape; their density reaches high values in samples HE H out/10 and HE H/10 (15 and 9 foram g^{-1} of dry sediment respectively) and in the central sector of the studied area where several rivers flow into the gulf (see also Fig. 1).

LOFAs are generally rare in all samples (Fig. 4); four peaks of abundance were observed in samples HE 1/30, HE 2/30, HE 4/30 and HE 7/30, with the highest value (46 foram g^{-1} of dry sediment) in sample HE 4/30.

Deformed specimens of benthic foraminifera were only recognised in three samples. TDF reaches the value 0.39 % of the total assemblage in sample HE 2/30 while it reaches the values of 1.53 and 7.14 % in samples HE H/10 and HE 6/30, respectively.

Statistical analysis

Q-mode cluster analysis

A Q-mode cluster analysis that groups samples with similar foraminiferal assemblages, applying a cut-off of 32, produces 5 clusters (labelled from A to E, Figs. 6a and 7a) with a number of samples ranging from 2 (cluster A and E) to 19 (cluster B). Samples collected close to the Milazzo Cape (HE 1/10, HE 1/30, HE 2/30, HE 3/30 and HE 4/30) were not grouped into any cluster because each one of them was characterised by a particular foraminiferal assemblage. For instance, *S. orbiculus* is only recognised in sample HE 1/10. Also, the highest densities of epiphytes and Miliolids (1,126 and 1,197 foram g^{-1} of dry sediment, respectively) and foraminiferal density (FD-2, 2488 foram g^{-1} of dry sediment) were found in sample HE 2/30.

- Cluster A contains two samples (HE H out/10 and HE H/10) collected close to the harbour's wharf. Their assemblages are characterised by moderate values of FD-2 (241 and 250 foram g^{-1} dry sediment, respectively; Fig. 3). The density of epiphytes and Miliolids (Fig. 4) shows intermediate values respect to the samples collected elsewhere.
- Cluster B contains samples collected mainly at a water depth of ~10 m. A sub-cluster is composed of samples HE H-in/10 and HE 16/20 collected inside the harbour and at the Rasocolmo Cape.

They are characterised by the lowest values of FD-2 and their assemblages are constituted by a very low number of species (Fig. 3).

- Cluster C is composed by three samples (HE 6/30, HE 7/30 and HE 13/30) collected at a water depth of ~30 m. FD-2 shows values ranging from 22 to 47 foram g^{-1} dry sediment. The assemblages were characterised by the presence of few species (Fig. 3). LOFAs are the dominant foraminifera in sample HE 7/30 (Fig. 4).
- Cluster D is constituted by nine samples (HE 5/30, HE 7/20, HE 11/20, HE 12/30, HE R-1/20, HE R-1/30, HE R-2/30, HE R-3/30 and HE R-3/20). FD-2 is characterised by very low values (Fig. 3). The foraminiferal assemblages are made up of a few species that are dominated by agglutinants, *Ammonia* spp. and LOFAs (Figs. 4 and 5). Miliolids and epiphytes are rare or absent (Fig. 4).
- Cluster E is made up of two samples (HE 9/30 and HE 11/30) collected at a water depth of ~30 m. *A. parkinsoniana*, *A. beccarii* and *A. tepida* were the dominant species. Miliolids and epiphytes are rare (Fig. 4).

A Q-mode cluster analysis that groups samples by similar trace element content, applying a cut-off of 130, produces 6 clusters (labelled from A' to F', Figs. 6b and 7b) with a number of samples ranging from 2 (Cluster A') to 9 (Cluster C'). High concentrations of Ni and Cr (84.50 and 201.33 mg kg^{-1} , respectively) are detected in sample HE 16/10. The latter one has not been grouped into any cluster.

The concentration of trace elements shows a decreasing trend from cluster A' to E'.

- Cluster A' is constituted by two samples (HE 5/20 and HE 7/10) characterised by a high concentrations of trace elements. With the exception of Mn and As which are similar, the concentration of trace elements are higher in the HE 5/20 sample than in the HE 7/10 sample. In respect to all of the samples, the highest concentrations of Co (20.60 mg kg^{-1}), Cu (51.09 mg kg^{-1}) and Pb (49.19 mg kg^{-1}) were detected in sample HE 5/20 (Table 2).
- Cluster B' contains eight samples (HE 11/30, HE 14/30, HE 14/10, HE H out /10, HE H in/10, HE R-1/30, HE 7/30, HE 12/30) characterised by high concentrations in trace elements (Table 2).

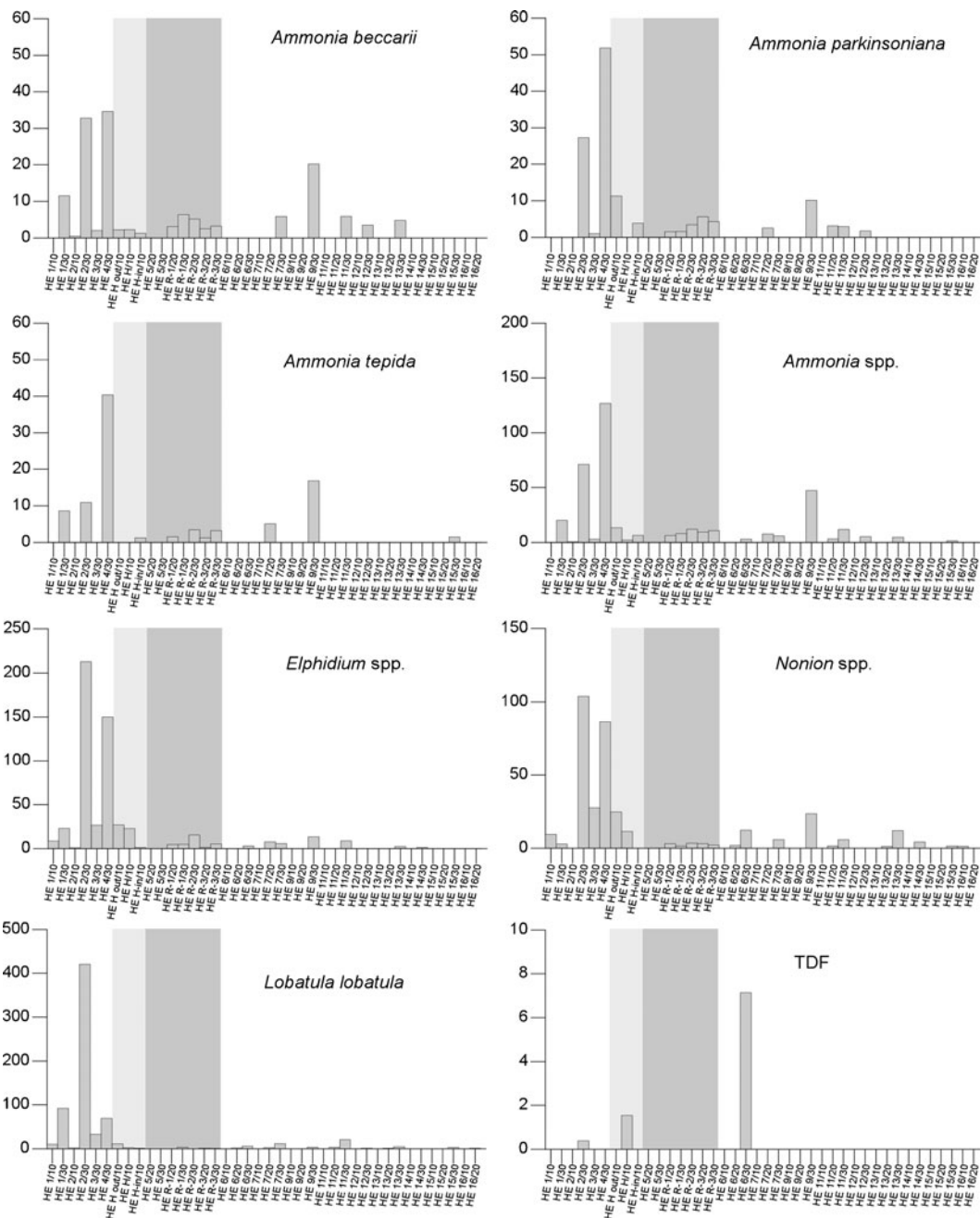


Fig. 5 Values of foraminiferal density of some selected benthic foraminifera species and groups (reported as number of individuals per gram of dry sediment normalised with respect to the

percentage of fraction comprise between 0.063 and 0.5 mm). TDF values are reported as percentages. *Light and dark gray areas* as in Fig. 2

- Cluster C' includes nine samples (HE 4/30, HE 16/20, HE 9/20, HE R-2/30, HE R-1/20, HE 6/30, HE 15/30, HE 13/30 and HE 6/10). This cluster includes the sample HE 15/30, in which the

highest concentration of As (7.34 mg kg⁻¹) was detected (Table 2).

- Cluster D' is composed of five samples (HE H/10, HE 5/30, HE R-3/30, HE 9/30, HE 15/20). This

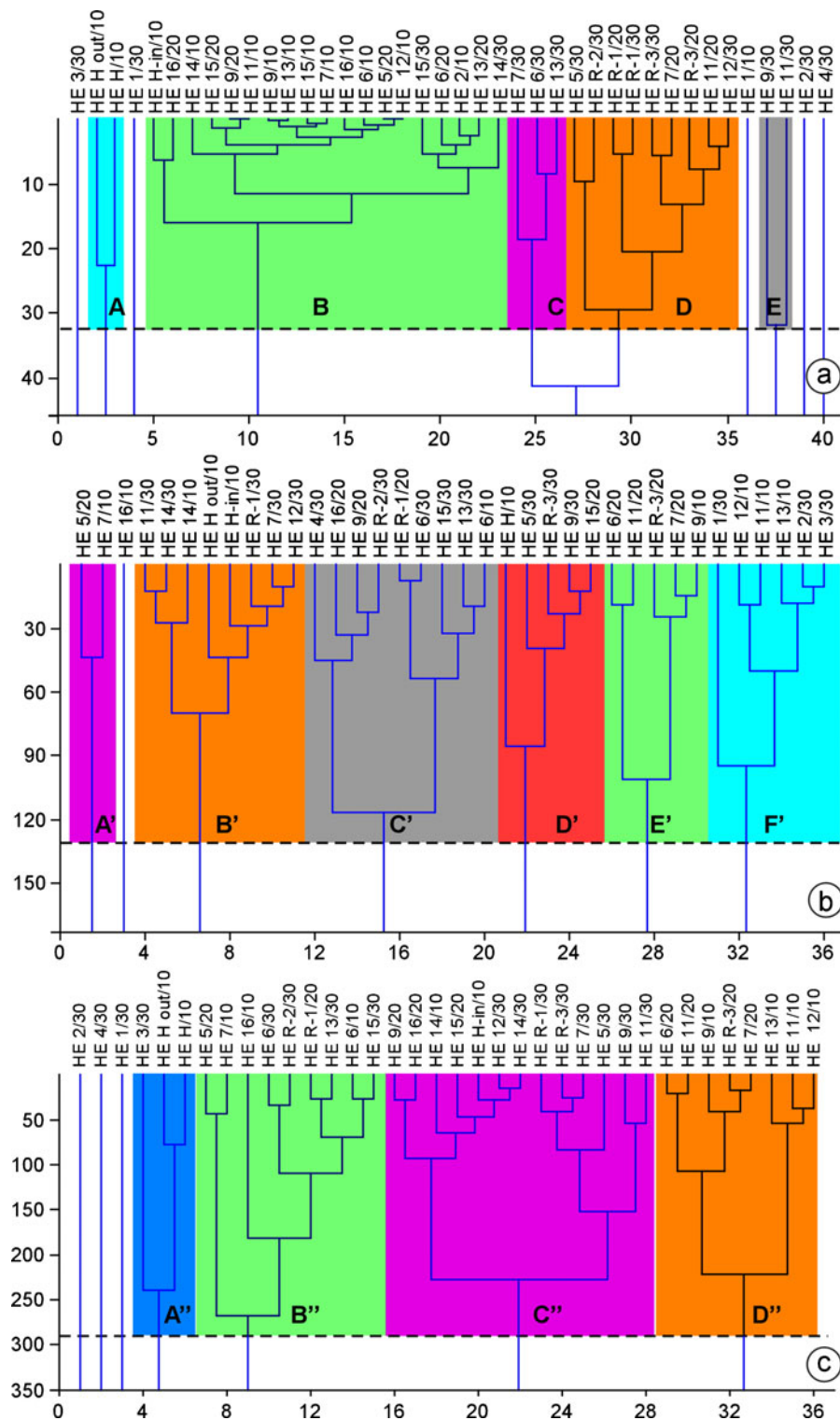


Fig. 6 Dendrogram classification resulting from a Q-Mode Cluster Analysis based on: **a** foraminiferal assemblages (in terms of densities of the single species), **b** trace element

concentrations and **c** grain-size percentages, trace element concentrations and foraminiferal assemblages. *Colours and capital letters* indicate different clusters

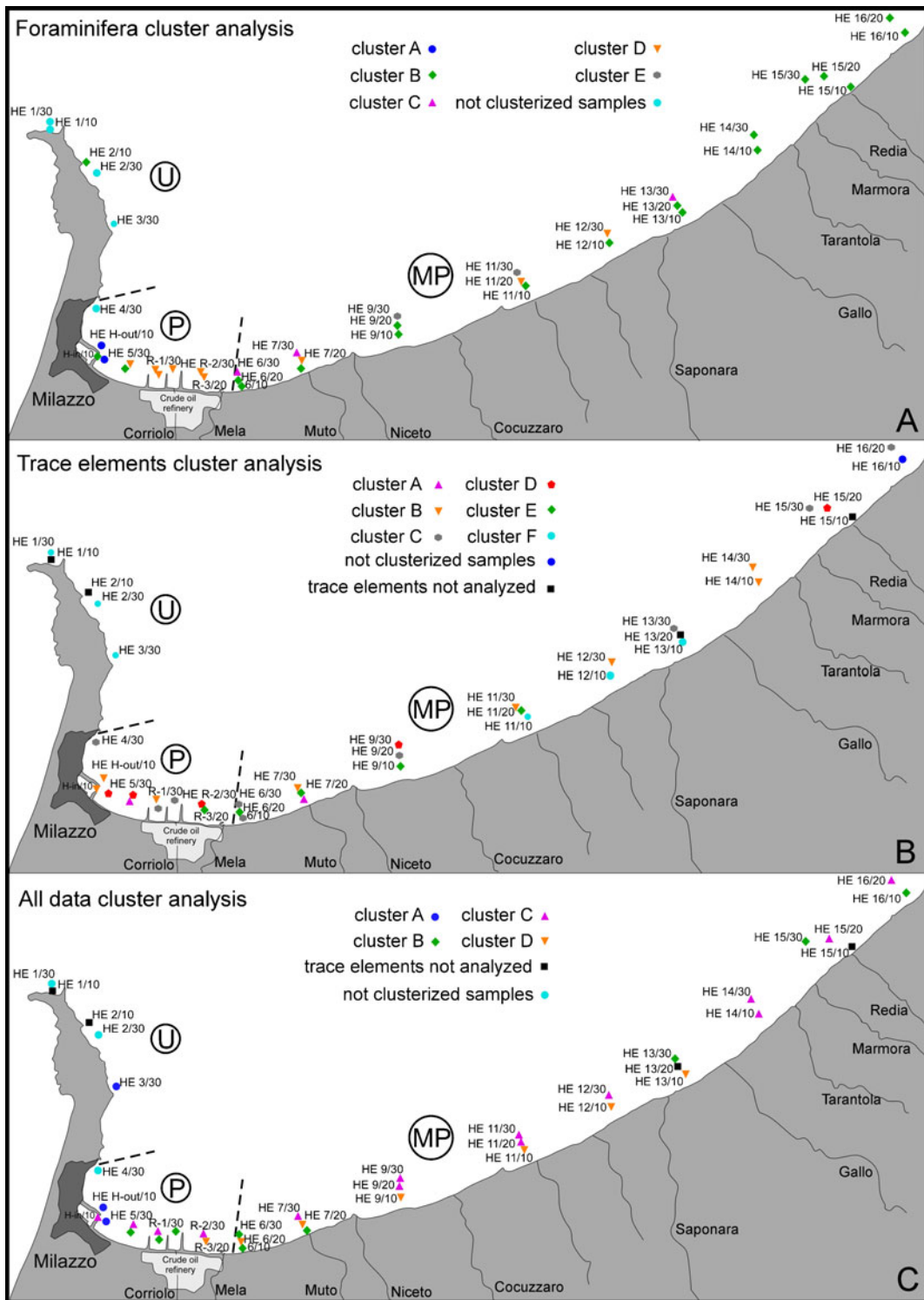


Fig. 7 Geographical distribution of sampling sites grouped on the basis of a cluster analysis. *U* unpolluted sector, *MP* moderately polluted sector, *P* polluted sector described in the “Discussion”

cluster includes the sample HE H/10, in which the highest concentration of Zn (182.03 mg kg⁻¹; Table 2) was detected.

- Cluster E' includes five samples (HE 6/20, HE 11/20, HE R-3/20, HE 7/20, HE 9/10), all collected in the area between the crude oil refinery (HE R-3/20) and the central sector of the gulf.
- Cluster F' includes six samples (HE 1/30, HE 12/10, HE 11/10, HE 13/10, HE 2/30, HE 3/30) and has the lowest concentrations of trace elements (Table 2); these samples were collected close to the Milazzo Cape (HE 1/30, HE 2/30 and HE 3/30) and in the central sector of the gulf (HE 11/10, HE 12/10 and HE 13/10) at 10 m of water depth.

A Q-mode cluster analysis that groups samples with similar trace element concentrations, foraminiferal densities and grain size fractions, applying a cut-off of 300, produces 4 clusters (labelled from A" to D", Figs. 6c and 7c) with a number of samples ranging from 3 (cluster A") to 13 (cluster C"). Three samples (HE 1/30, HE 2/30 and HE 4/30) collected close to the Milazzo Cape were not grouped into any cluster.

- Cluster A" is constituted by samples HE 3/30, HE H out/10 and HE H/10. They are characterised by intermediate values of trace metals and FD-2 values (between 241 and 278 foram g⁻¹ dry sediment; Fig. 3). Miliolids are the dominant foraminifera (Fig. 3).
- Cluster B" includes nine samples (HE 5/20, HE 7/10, HE 16/10, HE 6/30, HE R-2/30, HE R-1/20, HE 13/30, HE 6/10 and HE 15/30). This cluster contains the samples HE 5/20 and HE 7/10 characterised by high concentrations of trace elements (Table 2) and very low values of FD-2 (Fig. 3). Also, this cluster contains the sample HE 6/30 which has the highest TDF percentage (7.14 %) (Fig. 5).
- Cluster C" contains 13 samples (HE 9/20, HE 16/20, HE 14/10, HE 15/20, HE H in/10, HE 12/30, HE 14/30, HE R-1/30, HE R-3/30, HE 7/30, HE 5/30, HE 9/30 and HE 11/30). These samples are characterised by a concentration of trace elements that varies from low values of FD-2 (Fig. 3) to moderate and high values (Table 2).
- Cluster D" is composed by eight samples (HE 6/20, HE 11/20, HE 9/10, HE R-3/20, HE 7/20, HE 13/10, HE 11/10 and HE 12/10). This cluster

contains the samples characterised by the lowest FD-2 values (Fig. 3) and trace metals concentrations (Table 2).

Principal component analysis and pearson correlation matrix

The PCA ordination diagram shows that trace elements, *Ammonia* spp., LOFAs, agglutinants and TDF are located very close to each other with the exception of Mn (Fig. 8). FD-1, FD-2, Miliolids and epiphytes are, on the contrary, located far from trace elements.

The Pearson correlation matrix shows that trace elements have high correlation coefficients among themselves and are negatively correlated with foraminiferal density (i.e. Mn and FD-2, -0.29; Cu and FD-1, -0.26; Zn and FD-1, -0.25), some foraminiferal groups (i.e. Miliolids and Mn, -0.28; Miliolids and Co, -0.24; epiphytes and Mn, -0.29; epiphytes and Zn, -0.27) are positively correlated with TDF (Table 3). Only the genus *Ammonia* shows a positive correlation coefficient with Pb (0.22).

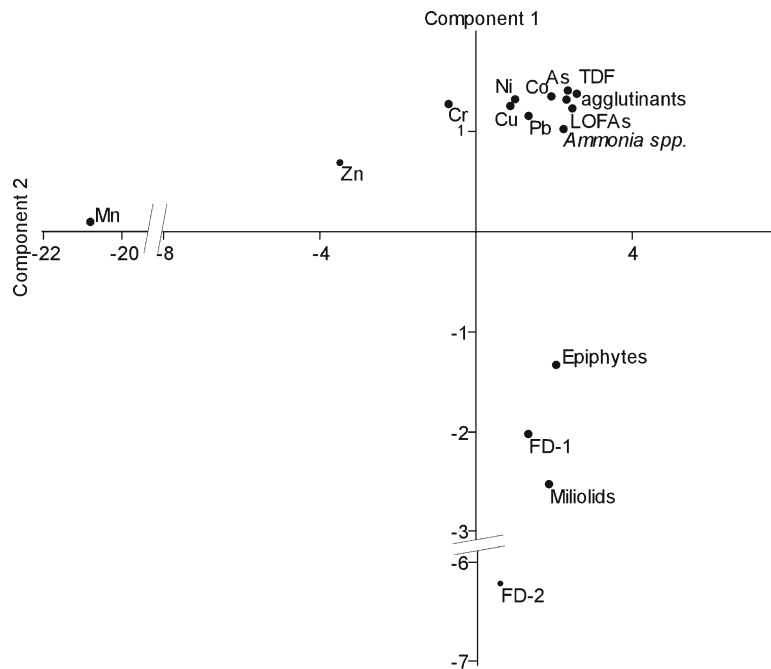
All of the above mentioned correlation coefficients are not high, probably owing to the low foraminiferal density in most samples.

Discussion

Q-mode cluster analysis performed on benthic foraminiferal assemblages permitted the sub-division of the Gulf of Milazzo into three sectors (Fig. 7a), namely unpolluted (U), polluted (P) and moderately polluted (MP). The sector U extends from the promontory of the Milazzo Cape to the north of the harbour (site samples from HE 1/10 to HE 3/30), sector P extends from the two small marinas to the crude oil refinery (site samples from HE 4/30 to HE R-3/30) while the sector MP extends eastwards from the crude oil refinery to the Rasocolmo Cape (site samples from HE 6/10 to HE 16/20).

A Q-mode cluster analysis performed on trace elements shows a notable difference between sectors U and the others ones. Between sectors P and MP the differences in terms of concentrations and EFs are only remarkable for Pb, Cu and Zn, while the other trace elements do not show significant differences (Fig. 7b). A similar pattern is observed in the Q-

Fig. 8 PCA ordination diagram



mode cluster analysis performed taking into account trace element concentrations, foraminiferal densities and grain size fractions (Fig. 7c).

Sector U (HE 1/10–HE 3/30)

Sector U is characterised by the lowest trace element concentrations and EFs as well as the highest values of FD-2 and species richness, with the exception of site HE 2/10 where FD-2 is very low because the sample was collected at a water depth of ~6 m. In particular, the contemporaneous high values of species richness and low values of Dominance indicate that well diversified assemblages occur. This sector is also characterised by the highest densities of Miliolids and epiphytic species. In particular, abundant specimens of *P. pertusus* and *P. planatus* and rare individuals of *S. orbiculus* have been recognised. Peneroplids and Soritids generally proliferate in unpolluted shallow waters and in subtropical-tropical areas; furthermore, it is well known that they host symbionts belonging to the red and green algae and dinoflagellate symbiont group, respectively (Hallock 1999). *Sorites* is a typical genus of the Indo-Pacific area that prefers a habitat with sea-grass where it lives attached to rhizomes of *P. oceanica* or to green and red algae (Langer 1993). Miliolids and epiphytic species live in well-oxygenated and unpolluted sea

water (Sen Gupta 1999a). Therefore, the contemporaneous presence of these species together with the low values of trace element concentrations and their EFs permit us to consider this sector of the Gulf of Milazzo as unpolluted.

The PCA ordination diagram highlights that FD-1, FD-2 Miliolids and epiphytes are located far from trace elements (Fig. 8), signifying that these foraminiferal groups are negatively influenced by trace elements. This is also confirmed by the Pearson correlation matrix in which trace elements and their EFs show an anti-correlative behaviour with FD-1, FD-2 Miliolids and epiphytic species (Table 3).

Sector P (HE 4/30–HE R-3/30)

Sector P is characterised by the highest trace element concentrations of Pb, Cu and Zn and to a lesser extent of Co and As, and their EFs increase sharply from sample HE 4/30 eastwards. Here, the increase in trace element concentrations is supposed to be linked to the activities of the two small marinas located close to the HE 4/30 sampling site since Cu and Zn are generally used in marine paints as anti-fouling agents, while Zn is used as an anode in marine engines. Sector P is characterized by low FD-2 values, remarkably lower than those determined in sector U. FD-2 remain quite high in sample HE 4/30; conversely, its values slightly

Table 3 Pearson correlation matrix

	Mn	Co	Ni	Cu	Zn	Pb	Cr	As	FD-1	FD-2	% <0.063 mm	% 0.063–0.5 mm	% 0.5–1 mm	% >1 mm	% >2 mm
Mn	0.000														
Co	0.913	0.000													
Ni	0.779	0.800	0.000												
Cu	0.899	0.955	0.778	0.000											
Zn	0.853	0.891	0.717	0.880	0.000										
Pb	0.705	0.739	0.650	0.794	0.787	0.000									
Cr	0.611	0.648	0.937	0.597	0.580	0.525	0.000								
As	0.898	0.806	0.683	0.787	0.770	0.664	0.468	0.000							
FD-1	-0.272	-0.266	-0.202	-0.263	-0.252	-0.039	-0.204	-0.237	0.000						
FD-2	-0.294	-0.260	-0.203	-0.231	-0.248	0.043	-0.222	-0.216	0.934	0.000					
% <0.063 mm	0.149	0.399	0.272	0.258	0.245	0.060	0.293	-0.073	-0.078	0.000					
% 0.063–0.5 mm	0.166	-0.065	0.006	-0.016	-0.008	-0.019	-0.014	0.176	0.020	-0.198	-0.644				
% 0.5–1 mm	-0.411	-0.462	-0.388	-0.386	-0.447	-0.301	-0.324	-0.398	0.021	0.101	-0.288	0.000			
% >1 mm	-0.396	-0.353	-0.311	-0.337	-0.361	-0.243	-0.255	-0.333	0.009	0.192	-0.153	0.526	0.000		
% >2 mm	-0.147	-0.076	-0.078	0.009	0.063	0.250	-0.140	-0.018	0.064	0.389	-0.071	0.222	0.458	0.000	
Co EF	0.913	1.000	0.800	0.955	0.891	0.739	0.648	0.806	-0.266	-0.260	0.399	-0.462	-0.353	-0.076	
Ni EF	0.779	0.800	1.000	0.778	0.717	0.650	0.937	0.683	-0.202	-0.203	0.272	-0.388	-0.311	-0.078	
Cu EF	0.899	0.955	0.778	1.000	0.880	0.794	0.597	0.787	-0.263	-0.231	0.258	-0.386	-0.337	0.009	
Pb EF	0.853	0.891	0.717	0.880	1.000	0.787	0.580	0.770	-0.248	-0.248	0.245	-0.447	-0.361	0.063	
Cr EF	0.705	0.739	0.650	0.794	0.787	1.000	0.525	0.664	-0.039	0.043	0.060	-0.301	-0.243	0.250	
Mn EF	0.611	0.648	0.937	0.597	0.580	0.525	1.000	0.468	-0.204	-0.222	0.293	-0.324	-0.255	-0.140	
As EF	1.000	0.913	0.779	0.899	0.853	0.705	0.611	0.898	-0.272	-0.294	0.149	-0.411	-0.396	-0.147	
Miliolids	0.898	0.806	0.683	0.787	0.770	0.664	0.468	1.000	-0.237	-0.216	0.035	-0.398	-0.333	-0.018	
Epiphytes	-0.285	-0.248	-0.189	-0.212	-0.215	0.089	-0.213	-0.202	0.919	0.997	-0.083	-0.212	0.098	0.186	0.432
Agglutinants	-0.296	-0.271	-0.210	-0.253	-0.276	-0.008	-0.223	-0.232	0.959	0.994	-0.077	0.096	0.170	0.298	0.298
Ammonia spp.	0.075	0.184	0.074	0.168	0.227	0.181	0.066	0.096	0.100	0.100	0.229	-0.254	-0.123	0.060	0.060
LOFAS	-0.090	0.005	-0.015	0.052	-0.048	0.222	-0.070	-0.008	0.502	0.732	0.119	-0.006	0.112	0.660	0.660
LOFAS	-0.124	-0.050	-0.064	-0.001	-0.084	0.221	-0.110	-0.002	0.497	0.741	0.041	0.071	0.277	0.700	0.700
TDF	0.106	0.155	0.105	0.141	0.128	0.030	-0.020	0.211	0.019	0.009	0.216	-0.073	-0.028	0.044	0.044

Table 3 (continued)

	Co EF	Ni EF	Cu EF	Zn EF	Pb EF	Cr EF	Mn EF	As EF	Miliolids	Epiphytes	Agglutinants	Ammonia spp.	LOFAs	TDF
As														
FD-1														
FD-2														
% <0.063 mm														
% 0.063–0.5 mm														
% 0.5–1 mm														
% >1 mm														
% >2 mm														
Co EF	0.000													
Ni EF	0.800	0.000												
Cu EF	0.955	0.778	0.000											
Zn EF	0.891	0.717	0.880	0.000										
Pb EF	0.739	0.650	0.794	0.787	0.000									
Cr EF	0.648	0.937	0.597	0.580	0.525	0.000								
Mn EF	0.913	0.779	0.899	0.853	0.705	0.611	0.000							
As EF	0.806	0.683	0.787	0.770	0.664	0.468	0.898	0.000						
Miliolids	-0.248	-0.189	-0.212	-0.215	0.089	-0.213	-0.285	-0.202	0.000					
Epiphytes	-0.271	-0.210	-0.253	-0.276	-0.008	-0.223	-0.296	-0.232	0.984	0.000				
Agglutinants	0.184	0.074	0.168	0.227	0.181	0.066	0.075	0.096	0.103	0.068	0.000			
Ammonia spp.	0.005	-0.015	0.052	-0.048	0.222	-0.070	-0.090	-0.008	0.740	0.682	0.225	0.000		
LOFAs	-0.050	-0.064	-0.001	-0.084	0.221	-0.110	-0.124	-0.002	0.754	0.689	0.131	0.886	0.000	
TDF	0.155	0.105	0.141	0.128	0.030	-0.020	0.106	0.211	0.011	0.013	-0.096	-0.041	-0.014	0.000

decrease in the HE H-out/10 and HE H/10 samples and become very low in HE H in/10 inside the harbour and in all the others samples. Miliolids, *Peneroplis* spp., *Ammonia* spp., *Elphidium* spp., *Nonion* spp. and epiphytes show a similar decreasing trend. Also, a decreasing trend is observed in species richness while Dominance values are slightly higher with respect to the sector U. The above mentioned EF trace element trend is in accordance with the low values of FD-2 determined in the area between the harbour's wharf and the crude oil refinery as well as with the decreasing trend of species richness and the disappearance of several foraminifera (i.e. *Peneroplis* spp. and epiphytes) that live in unpolluted marine sediments. This data demonstrates the fact that the presence of trace elements, together with other possible pollutants produced by the crude oil refinery (i.e. PAHs or PCB) as well as the harbour and marinas, is a factor that strongly influences the foraminiferal assemblages in this sector, depauperating populations in both abundance and diversity. Conversely, some genera like *Elphidium* spp., *Nonion* spp., *Ammonia* spp., LOFAs and agglutinants are the only foraminifera that are able to survive, even if with low density values, in samples located in front of the crude oil refinery.

The PCA ordination diagram (Fig. 8) shows that trace elements are located very close to *Ammonia* spp., LOFAs and agglutinants, signifying that they are not influenced by these elements. Areas polluted by trace elements are generally characterised by a low foraminiferal diversity, and the assemblages are dominated by few genera, typical of stressed environments such as, for instance, *Nonion* and *Ammonia* (Frontalini and Coccioni 2008; Frontalini et al. 2010; Caruso et al. 2011 and reference therein). Therefore, on the basis of the disappearance of Miliolids and epiphytes and the contemporaneous survival of *Ammonia*, agglutinants and LOFAs together with the low values of FD-2 and species richness and the high trace element concentrations and EFs, we consider this sector of the Gulf of Milazzo as polluted.

Sector MP (HE 6/10–HE 16/20)

Sector MP is characterized by trace element concentrations lower than in sector P; in fact, their EFs are often below 1. Nevertheless, benthic foraminiferal populations still remain quite poor, showing very depauperate foraminiferal assemblages (in many cases

fewer than 23 individuals per gram of sediment). Here, the critical environmental conditions of the marine ecosystem are shown by the presence of the lowest values of both FD-2 and species richness as well as by the highest values of Dominance, thus signifying that oligotypic assemblages are present. Miliolids, *Elphidium* spp. and epiphytes are absent, or in some samples rare, while *Ammonia* spp., *Nonion* spp., LOFAs and agglutinants are the most abundant foraminifera. The contemporaneous increase of these genera is probably related to the fresh water influx coming from several rivers and streams that flow into the gulf (Fig. 1). *Nonion*, *Ammonia* and *Elphidium* are generally considered to be pioneer species in paralic environments (Debenay et al. 2009; Frontalini et al. 2010). In particular, *Nonion* and *Ammonia* are tolerant species that are able to survive in environments characterised by salinity variations and high levels of anthropogenic pollutants (e.g. trace elements, crude oil, sewage waste). Furthermore, the presence of agglutinants can be interpreted as the result of changes in pH that prevent the formation of calcitic shells or, possibly, cause their selective dissolution. Therefore, these distribution patterns, coupled with the decrease of trace elements, could be explained by a combination of natural and anthropogenic environmental factors (i.e. other pollutants in addition to the studied trace elements) that can interact together in modifying benthic foraminiferal assemblages.

Deformed foraminifera

Deformed foraminifera have been found in samples collected in all of the three sectors with TDF percentages of 0.39 % (HE 2/30 in sector U) and higher than 1.5 % (HE H/10 and HE 6/30, in sector P and MP, respectively) of the total assemblages, respectively. A review of the relevant literature indicates that up to 1 % of the total assemblages found in a population living in a non-stressed environment can be deformed specimens (Alve 1991). Morphological deformations in benthic foraminiferal tests with percentages higher than 1 % of the total assemblages can be linked to the presence of a high concentration of metals (i.e. Zn, Cu, Cr and Pb; Samir and El-Din 2001; Le Cadre and Debenay 2006; Frontalini et al. 2009 and references therein) as well as Polychlorobiphenyls (Scott et al. 2005). Moreover, the presence of relatively high percentages of deformed specimens can also be due to rapid changes in the chemical and physical

parameters of sea waters, such as salinity and pH (Geslin et al. 2002; Polovodova and Schönfeld 2008). Thus, the low TDF percentage in sample HE 2/30 coupled with all of the data discussed above suggests that a non-stressed environment is present in sector U. The higher TDF percentages (up to 7.14 %) observed in samples HE H/10 and HE 6/30 suggest, on the contrary, that a stressed environment is present both in sector P and MP. It must be underlined that, in spite of the fact that some Authors found positive and significant correlation coefficients between the abundance of deformed tests and metals (Yanko et al. 1999; Frontalini et al. 2009 and reference therein), in the investigated sediment samples only a weak positive correlation between TDF and trace element concentrations has been found. On the contrary, the highest TDF percentages have been found in sector MP, where sediments are less polluted by trace elements than in the other sectors. Furthermore, the fact that correlation coefficients are not very high between TDF and trace element concentrations strengthen the hypothesis that, as for the foraminiferal assemblages in sector MP (see “Sector MP (HE 6/10–HE 16/20)”), an interaction between natural environmental factors and other anthropogenic pollutants, besides the studied trace elements, could have been invoked to better explain the distribution pattern of both benthic foraminifera and TDF. Also, the relatively low percentages of TDF in the most polluted area could be due to the fact that trace element concentrations are so high that they prevent the survival of foraminifera. This hypothesis is in accordance with the sharp decrease shown by foraminiferal density in sector P. However, due to the lack of correlation between the TDF and the trace element concentrations, we cannot exclude the possibility that other contaminants (i.e. organic pollutants) produced by the crude oil refinery, as well as the harbour and marinas, might influence the survival of some species and as a consequence explain the differences in their distributional patterns.

Conclusions

The qualitative and quantitative sedimentological, micropaleontological and geochemical analysis of marine sediment samples collected in the Gulf of Milazzo have provided evidence that the littoral zone of the gulf can be subdivided into three sectors (U, P and MP) characterised by environmental changes in the

marine ecosystem. In sector U (close to the Milazzo Cape), benthic foraminiferal assemblages exhibit high values of foraminiferal density and species richness and Miliolids, epiphytes and *Elphidium* are the most abundant foraminifera. Trace element concentrations and their EFs, on the contrary, are very low. Based on this evidence, we conclude that the anthropogenic pressure on the marine ecosystem in sector U is low, thus favouring the preservation of healthy environmental conditions. In sector P (from the marinas to the crude oil refinery), on the contrary, foraminiferal density and species richness are low; furthermore, benthic foraminifera assemblages are dominated by species that tolerate stressed environmental conditions, such as *Ammonia* spp. and LOFAs while Miliolids and epiphytic species are absent. This sector is also characterised by the highest trace element concentrations of Pb, Zn and Cu and to a lesser extent of Co and As, and by substantial EFs in Pb, Zn and Cu (values up to 3 times higher than the background level) in the marinas and harbour. Based on this evidence, we speculate that the anthropogenic pressure, due to the presence of factories as well as marinas and a harbour, is causing the extreme deterioration of the marine ecosystem. Eastwards (sector MP), benthic foraminiferal populations remain quite poor, showing extremely depauperated foraminiferal assemblages, nevertheless trace element concentrations become lower than in sector P and their EFs are often below 1. Here, the critical environmental conditions of the marine ecosystem are shown by the presence of the lowest values of both foraminiferal density and species richness as well as by the highest values of Dominance. Deformed foraminifera with TDF percentages higher than 1.5 % have been found in sector P and MP.

On the basis of this data coupled with the results from the PCA, we propose that anthropogenic trace element pollution could be considered as one of the most important causes of the modifications of foraminiferal assemblages and of the presence of deformed specimens in both sectors P and MP although it is not possible to exclude that other contaminants (i.e. PAHs or PCB) or other factors (i.e. changes in salinity, temperature, pH, organic pollutants) may contribute or, locally, can be the principal cause for modifications in benthic foraminiferal assemblages and their spatial distribution in sector MP.

Results from this study provide, for the first time, a baseline database for future monitoring programs of the ecological modifications in the marine ecosystem of the Gulf of Milazzo over time.

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