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Baseline

A multidisciplinary approach for the characterization of the coastal marine ecosystems of Monte Di Procida (Campania, Italy)

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ABSTRACT

A multidisciplinary survey was carried out on the quality of water and sediments of a coastal protected marine area, embedded between the inputs from Bagnoli steel plant to the south and a sewage plant, Volturno River and Regi Lagni channel to the north. The study integrated chemical-sedimentological data with biological and ecotoxicological analyses to assess anthropogenic pressures and natural variability. Data reveal marked differences in anthropogenic pollution between southeastern and northwestern zone, with the north affected by both inorganic and organic flows and the south influenced by levels of As, Pb and Zn in the sediments above law limits, deriving from inputs of the Bagnoli brownfield site. Meiobenthic data revealed at south higher relative abundance of sensitive species to pollution and environmental stress to the south, i.e. *Lobatula lobatula* and *Rosalina bradyi*, whereas to the north relative abundance of stress tolerant *Quinqueloculina lata*, *Quinqueloculina pygmaea* and *Cribroelphidium cuvilleri* were determined.

The European seas are affected by intense human activities, which constitute sources of chemical contamination that represents a risk of serious damage in coastal and marine zones (European Environment Agency, 1999). Therefore, it becomes imperative for countries to find out options of environmental protection in a sustainable and environmentally friendly way. A holistic and integrated ecosystem-based approach is needed to manage human activities and to reinforce our understanding of marine ecosystems, and to evaluate what needs to be done to protect them.

In Italy, as in other parts of the world, protected areas and marine parks are established for the main purposes of protecting biodiversity, as well as for the promotion of tourism. The control of sea health status has been mainly performed by the evaluation of chemical contaminants loads in environmental matrices, i.e. water, sediment, and biota. With the publication of the European Marine Strategy Framework Directive (2008/56/EC, hereafter MSFD), the biological components have become important in assessing the ecological status within offshore waters (e.g. plankton and benthos communities) (Borja et al., 2010). The interest of marine ecologists for the bio-assessment of human impact on littoral ecosystems has largely strengthened (Borja, 2005; Borja and Heinrich, 2005; Dauvin, 2005). As a consequence, numerous bio-assessment tools have been developed, or adapted, to the MSFD requirements in recent years. Several reviews have dealt with different components of the systems, spanning from a monofactorial, to an integrative approach, taking into account both environmental typologies and managerial objectives (Simboura and Reizopoulou, 2007; Mangoni et al., 2013).

Up to now, no multi-disciplinary approach exists concerning the environmental characterization of the marine environment facing the promontory of MDP. Thus, with the aim to fill these temporal and spatial gaps, specific studies are indispensable. Based on comparisons of the data of station each other, the chemical data alone were not always reliable indicators (and, therefore, predictors) of biological effects. The importance and usefulness of a multi-disciplinary approach to characterize marine environments, such that of MDP, in this case seems indisputable.

The current work reports physico-chemical and biological parameters of the marine water and sediments at 19 representative stations of Monte di Procida during June 2014 through a combined fieldwork and multidisciplinary approach.

Surface waters were analysed for inorganic nutrients (NO_2, NO_3, NH_4, PO_4, SiO_4), chlorophyll-*a* (Chl*a*) and pigments of the phytoplankton community. Vertical profiles of pH, temperature (°C), salinity (S), dissolved oxygen (DO, mg L^{-1}), percentage of oxygen saturation (%), turbidity (T, nephelometric turbidity units or NTUs) and fluorescence (UF) were also determined. Sediments were analysed for: i) benthic foraminifers (Protista) and ostracods (Crustacea), both parts of

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meiobenthos, to test the impact of anthropic activities on organisms living in/on bottom sediments; ii) the levels of Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sn, Sr, Ti, V, Zn; iii) the microbiological quality of sediments by the presence of total coliforms, and of intestinal *Enterococci*, *Fecal coliforms*, *Escherichia coli*. In addition, *Clostridium difficile* was isolated. Grain size analysis and eco-toxicological test in samples with the algal growth inhibition test (GI) with *Phaeodactylum tricornutum* were also performed.

Data collection and analysis consisted of acquiring spatial data and performing statistical analyses between conventional chemical assays, biological and eco-toxicological assessments together with the presence and composition of biological indicators of pollution.

One of the most panoramic sites of the Italian peninsula is represented by the marine environment facing the promontory of Monte di Procida (MDP) in southwestern Italy, capturing stupendous seas views and well-preserved ancient buildings, located in the extended area of the Regional Park of Phlegrean Fields, which was established in 2002 also as a national protected marine area. The area extends for 7350 ha in the suburbs of Naples and, besides MDP, it includes approximately the area of municipalities of Pozzuoli, Bacoli, and the Phlegraean islands (Ischia, Procida, Vivara) as well as the western areas of the municipality of Naples. The territory of Phlegrean Fields represents one of the most important areas in the region for its environmental and historical-archaeological value. MDP represents a beautiful landscape on the tip of the promontory, that plunges into the Gulf of Gaeta and is connected to the islet of San Martino, a squat volcanic island facing a wide bay connected to the mainland by a bridge and a tunnel, which once was a base for tuna fishing and scuba excursions.

In the recent past, several marine ecological emergencies in areas very close to MDP occurred. In particular, environmental crisis hit the northern sector, where the Cuma wastewater treatment plant is located, due to improper treatment of sewage effluents, and the southern side, where the former and dismantling metallurgical plant of ILVA at Bagnoli is present, for improper disposal of toxic industrial wastes.

The Cuma plant has especially experienced several problems of malfunctioning in the last few years. Another serious environmental threat for the MDP area is represented by the network of artificial canals of Regi Lagni, which crosses the Campanian Plain along the provinces of Naples and Caserta, and flowing from the *Ager Nolanus* discharges to the south of Castel Volturno. This drainage system was built as early as the XVII century by the Bourbons and covers a catchment of about 1100 km^2. These channels, together with the Volturno River, have been devastated for a long time by discharges and riverbed overmining, and have greatly affected the enjoyment of the waterscape.

Several bathing places at Napoli, Giugliano and Pozzuoli among which MDP, have been often banned and this fact is a further indication of the potential environmental risk for the studied marine area. In the same way, the Bagnoli industrial plant, which was dismissed in 1991 and actually included in Italian national legislation for environmental reclamation of disused and heavily polluted coastal sites, represents another environmental threat for the marine environment of MDP. Thus, there is a serious risk of marine pollution of MDP from north to south, resulting from the lack of waste water purification and delay of the planned remedial plan.

Water and sediments of the sea facing the MDP promontory, aboard an equipped boat identified as M/B "Oceanix", were collected on June 2014 in 15 sites (Fig. 1 and Table 1), between -0.5 and -9 m depth with a 0.5 m increment. Sediments were sampled by a Van Veen grab.

The individuation of the sampling sites was confirmed by a preliminary ship survey through a serpentine path with real time determinations of the superficial water temperature and salinity to find out any possible anthropic pressure. A peristaltic pump provided water for continuous recording by means Sea-Bird Elec.-SBE 45 and interfaced with a GPS (Garmin Map 78 S). Based on the results of this survey, 6 transects were positioned on an onshore-coast line, perpendicular to the isobaths. Along each transect three stations, 1, 2 and 3 were identified at 100, 200 and 300 m from the coast as much closer to the coast line to detect eventual inputs of pollutants. According to the specific morphobathymetric features of each site: stations A1, A2, A3, B1, B2 B3, C1, C2, C3 were placed along the southeastern sector of the promontory, whereas stations D1, D2, D3, E1, E2, E3, F1, F2, F3 were situated along the northwestern sector of the study area. Sediment samples could not have been taken at B1, B2, C3 because of the presence of bedrock and at C3 due to dense matter of *Posidonia oceanica* prairie. An additional station P was placed inside the port of Acquamorta.

Vertical profiles of pH, temperature (°C), salinity (S), dissolved oxygen (DO, mg L^{-1}), percentage of oxygen saturation (%), turbidity (T, nephelometric turbidity units or NTUs) and fluorescence (UF), from surface to bottom were performed using a Sea Bird Electronic, SBE 19 Plus CTD probe, equipped with a SBE Oxygen sensor and a submersible fluorometer, Scufa Turner Designs Inc. (Sunnyvale, CA).

Surface water samples for the determination of inorganic nutrients (NO_2, NO_3, NH_4, PO_4, SiO4) concentrations were collected in vial of 20 mL from the Niskin bottle and stored at 4 °C until they were analysed, following the procedure described by Hansen and Grasshoff (1983).

Phytoplankton biomass and composition, in terms of larger taxonomical groups, were performed in surface water. Three litres of sea water was then drawn from the Niskin bottle and was filtered on GF/F Whatman filters (47-mm diameter). These were stored in liquid nitrogen until HPLC analyses for pigment spectra determinations according to Vidussi et al. (1996) by a Hewlett Packard (mod. 1100 Series). The photosynthetic pigments analysed were: alloxanthin (AX), β-carotene (β-car), chlorophyll *a* (Chl*a*), chlorophyll *c*2 (Chl*c*2), chlorophyll *c*3 (CC3), diadinoxanthin (Dd), diatoxanthin (Dt), 19′ butanoxyloxyfucoxanthin (BU), fucoxanthin (FU), 19′ hexanoyloxyfucoxanthin (Hex), zeaxanthin (ZX).

The amount of Chl*a* was used to indicate the total phytoplankton biomass. The contribution of the main phytoplankton groups to the total Chl*a* was estimated on the basis of the concentrations of biomarker pigments, using the chemical taxonomy software CHEMTAX (Mackey et al., 1996).

Sediment samples were transported to the laboratory and after careful preparation and washing with vacuum pump, all the samples were dried in an oven at 110 °C for 24 h, then weighed with an analytical balance and subjected to dry sieving through a series of stacked sieves, with 1/4 Φ class interval, up to 31 μm, in a Ro-Tap mechanical sieve shaker for 15′.

The ≤2000 μm fraction was used for analyses of the total pool of Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sn, Sr, Ti, V, Zn by digesting about 0.5 g of sediment in 12 mL of H_2O_2–HNO_3, in Teflon vessels in an Ethos Plus Microwave Lab Station (Milestone) for 15 min; the obtained solution was taken to a final volume of 100 mL with 5% HCl and then filtered by 0.45 μm (Cicchella et al., 2008). The concentrations of the elements were determined by ICP-AES by a Thermo Electron Corporation IRIS Intrepid II spectrometer.

For the determination of the organic matter (C), samples of 0.5 g of ≤2000 μm sediment were sequentially treated at 105, 180 and 600 °C in a furnace in ceramic vessels up to constant weight for the determination of residual humidity, crystallization water and organic matter (Byers et al., 1978).

Sediments were also analysed for the 16 polycyclic aromatic hydrocarbons (PAHs) indicated by the Environmental Protection Agency (EPA) as important toxicological contaminants. Polycyclic aromatic hydrocarbons (PAHs) analyses were performed by a preliminary extraction and successive purification on silica gel treatment. Successively, the determination of PAHs was carried out by HPLC using a spectrofluorimetric detector (Ausili et al., 1998).

Total hydrocarbons (THCs) were determined by a slightly modified U.S. EPA standard methods (US EPA, 1997).

The marine algal growth of *Phaeodactylum tricornutum* was performed by the International Standard ISO 10253 modified according to Lukavsky (1992).

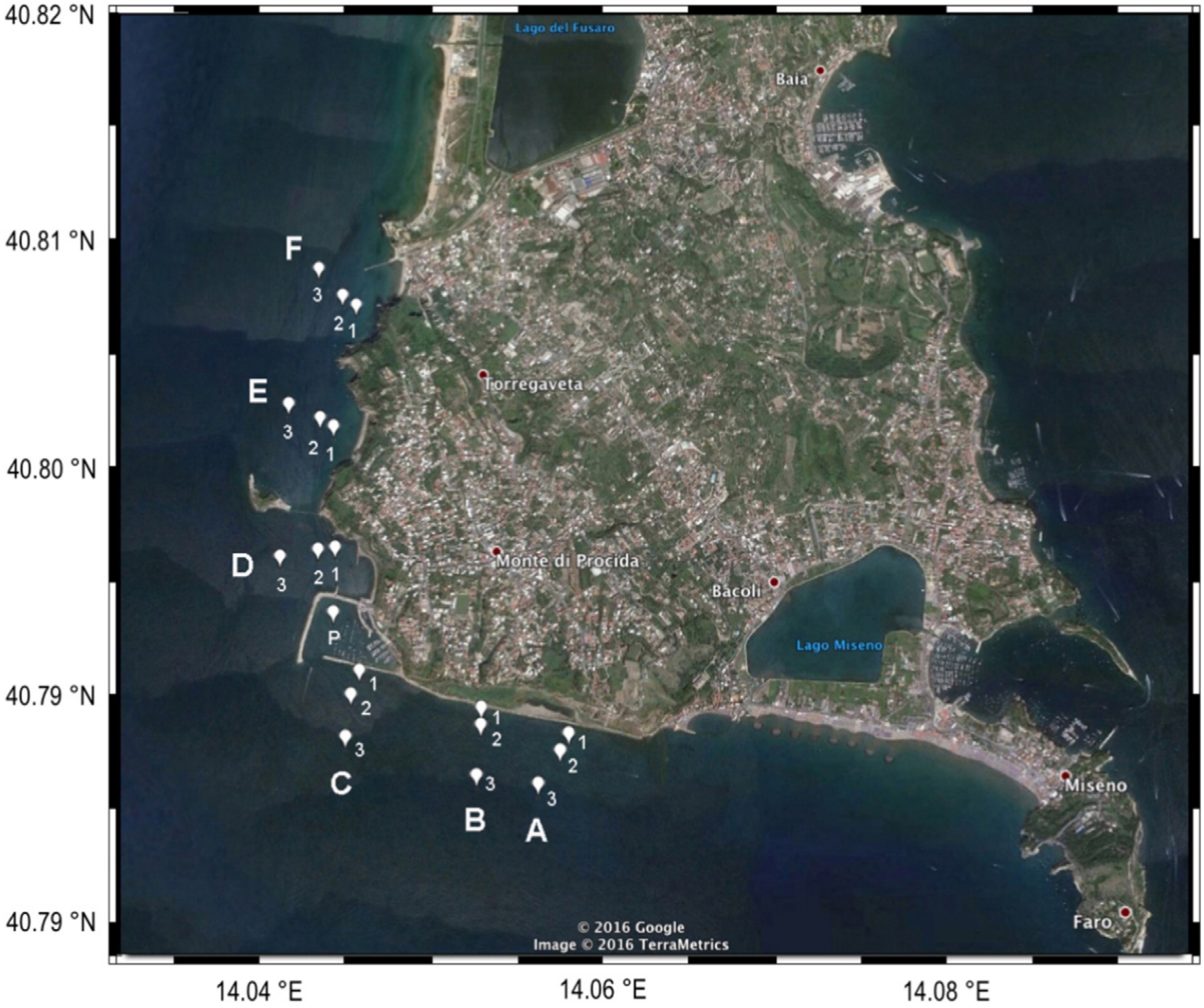

Fig. 1. Study area and location of sampling stations.

Qualitative and quantitative analyses of benthic foraminifer and ostracod assemblages were carried out. Sediments, 200 g dried weight, were washed through 230 and 120 mesh sieves (63 μm and 125 μm respectively) and splitted; both foraminifer and ostracod shells were picked up from the coarser fraction, classified and counted for quantitative analysis. Ostracods were counted both as minimum number of individuals, (MNI: it is calculated by adding the greater number between right and left adult valves to the number of adult carapaces; when only juveniles occur MNI = 1) and total number of valves (TNV, the number of all the valves, including juveniles). Assemblage indexes (abundance, i.e. individuals per 100 g of sediment (I); dominance (D); specific diversity (S), i.e. Shannon index (H); equitability (E)) were performed using the free software Past version 3.01 (Hammer et al., 2001). The comparison of foraminifer and ostracod assemblage structure (taxonomic composition) and indexes reflect the conditions of bottom sediment and water.

Table 1
Sampling stations, geographic coordinates, water depths and grain-size data.

Stations	Latitude	Longitude	Water depth (m)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)
A1	40.787162°	14.055203°	4.10	0.33	98.94	0.73	–
A2	40.786373°	14.055196°	5.22	0.21	99.16	0.63	–
A3	40.784764°	14.053597°	7.47	0.04	99.74	0.22	–
B3	40.785279°	14.049995°	7.88	19.32	80.67	0.01	–
C1	40.790046°	14.042855°	5.33	0.09	98.88	1.02	0.01
D1	40.795907°	14.040849°	4.14	0.39	93.56	6.05	–
D2	40.795868°	14.039736°	5.41	0.52	94.14	5.34	–
D3	40.795580°	14.037335°	8.91	0.20	80.58	19.22	–
E1	40.801885°	14.040586°	3.64	0.06	96.76	3.18	–
E2	40.802253°	14.039922°	5.26	0.28	96.75	2.97	–
E3	40.802191°	14.037610°	6.98	4.36	95.32	0.31	0.01
F1	40.808258°	14.042227°	3.31	0.60	97.52	1.88	–
F2	40.808560°	14.040782°	5.40	0.64	97.74	1.61	0.01
F3	40.809974°	14.039160°	6.70	0.16	88.81	11.03	–
P	40.792811°	14.041144°	4.40	0.43	98.51	1.06	–

The total viable bacterial population of Coliforms was determined as described by APHA AWWA, AEF (1998). The enumeration of intestinal *Enterococci*, *Fecal coliforms*, *Escherichia coli* was carried out by the methods ISO 7899-2, 2000; ISO/DIS 16649-1, 1999; UNI EN 12780, 2002; UNI EN 10980 2002 (APAT IRSA CNR, 2003). Spores of sulphite-reducing clostridia (SP) were determined by the method of APAT CNR IRSA 7060 B Man 29 2003. *Clostridium difficile* was isolated according to the method outlined by Pasquale (2012). Experiments were performed in triplicates; the numbers of bacterial colonies were expressed in CFU mL^{-1} of sea water and CFU g^{-1} of sediment.

Five sediment samples were chosen randomly among those withdrawn from the sites and spiked with a mixture of the elements and standards of PAHs in such a quantity to nearly double the original concentration of elements in the sample, as well as the concentration sum of PAHs from all stations, and then extracted as above described for quality control determinations. Mean recoveries ranged from a minimum of 85% to a maximum of 97%. Statistical analysis of data was performed using STATISTICA 5 (StatSoft Inc., Tulsa, OK, USA).

Table 2

Total biomass [in term of chlorophyll *a* (Chl*a*)] and functional groups of the phytoplankton communities (percentage value) and data from microbiological (UFC/g) and ecotoxicological (%) assays. The acronyms were reported only for species processed for statistical analysis.

	Chlorophyll *a* (Chl*a*)	% Prasinophyceae (P)	% dinoflagellates (D)	% Cryptophyceae (CP)	% Haptophyceae	% Pelagophyceae (PP)	% Cyanophyceae (CY)	% diatoms	Enterococci (EC)	Spores of sulphite-reducing clostridia (SP)	Algal growth inhibition *P. tricornutum*
A1	1.367	0.07	0.06	0.43	23.58	17.86	13.83	44.18	<10	<10	−23.7
A2	1.110	0.10	0.13	0.76	22.96	17.41	7.23	51.40			
A3	0.945	0.07	0.07	0.46	23.78	15.73	7.92	51.97	<10	<10	−42.3
B3	1.210	0.15	0.21	0.98	18.72	8.93	12.94	58.07	<10	50	−26.0
C1	0.976	0.02	0.10	0.37	18.59	10.21	14.42	56.29	<10	64	−11.0
D1	1.013	0.09	0.11	0.68	12.06	6.09	6.77	74.20	<10	13	−49.3
D2	0.710	0.08	0.09	0.41	13.99	5.68	5.87	73.88			
D3	0.908	0.06	0.06	0.27	15.25	8.19	12.06	64.11	<10	1567	−30.0
E1	1.188	0.09	0.08	0.38	9.61	<0.02	4.50	85.33	13	769	−42.7
E2	1.137	0.04	0.08	0.06	10.80	2.68	5.53	80.81	127	3308	−20.3
E3	1.473	0.14	0.17	0.81	11.07	2.19	5.55	80.08	<10	598	−31.0
F1	1.830	0.03	0.03	0.07	5.09	<0.02	6.74	88.04	<10	548	−21.3
F2	1.251	0.12	0.02	0.31	9.14	1.23	5.28	83.90	26	1627	−65.3
F3	1.187	0.05	<0.02	<0.02	16.80	7.27	15.30	60.58	297	4057	−8.01
P	1.038	0.23	0.18	0.90	20.58	13.23	6.06	58.82	30	720	−57.3
Mean	1.160	0.09	0.09	0.46	15.47	7.78	8.67	67.44	98	1211	−32.9
SD	0.266	0.05	0.06	0.31	5.83	6.11	3.84	14.20	119	1347	17.4
RSD%	22.97	61	64	67	37.70	78.60	44.30	21.05	122	111	−52.8

Data were subjected to analysis of variance (ANOVA) to determine the significance of the spatial distribution of analytes and separation of means was performed by LSD test at $p < 0.05$ level of significance. A principal component analysis (PCA), based on a Pearson's correlation matrix, was conducted in order to investigate the relationship between the investigated variables at the sampled stations. All analyses were performed using the SPAD software package (Système Portable d'Analyse des Données, 2002) and the differences were deemed statistically significant at $p < 0.01$.

The analysis of the water column revealed some differences among the sites. Salinity mean data of the water column up to 3.0 m of the sites A, B and F were quite low, 37.067, 37.091, 37.015 g/L, respectively, whereas at site C a peak mean value of salinity of 37.208 g/L was observed. This trend was likely due to different input of freshwater; in fact, sites A and B, in the south, were affected by underground hydrothermal flows, which are typical of the Phlegrean Fields, whereas site F, to the north was affected by superficial inputs from Volturno River, Regi Lagni and Cuma wastewater treatment plant. Mean SiO_4 surface level in northern waters was about twofold that of the southern part, 2.503 vs. 1.297 μM, likely due to freshwater inputs. It was also interesting to note a significant increase of the mean values of nutrient loads from south to north, with increases of NO_2 up to twentyfold, from 0.011 to 0.195 μM and of NO_3 up to one hundred fold, from 0.019 to 1.847 μM. Higher peaks of PO_4 levels were also found to the north respect to the south, 0.230 vs. 0.103 μM. On the overall, the ratio between the total dissolved pool of nitrogen relative to that of phosphorus (Redfield ratio) was ~10, the half of that reported for the Gulf of Naples as well as the Mediterranean Sea (Ribera d'Alcalà et al., 2003; Pujo-Pay et al., 2011).

The highest values of total biomass (Chl*a*), Table 2, were recorded in the northern area, station F1, with a value of 1.830 μg/L. Diatoms were the major contributors to the biomass, accounting for 67%, making the Haptoficeae the most important group. In a few stations, the diatoms make up about 80%. The southern coastal area is characterised by more diversified phytoplankton communities, in relation to the northern sector, which are made up of Diatoms, Haptophyceae, Pelagophyceae and Cyanophyceae.

Results of grain size and chemical analyses are given in Tables 1 and 3, respectively. Most of the specimens were mainly made of sand with a range of 88.81% in F3 and of 99.74% in A3. Lower levels of sand of about 80.6% were detected only at two sites, B3 and D3, where significant contents of gravel in one case of 19.32% and silt in the other of 19.22% were found. It is also interesting to note that whereas in the southern eastern site of the promontory, A, the content of sand remains unvaried along the transect, in the northern site the sand content decreases with the distance from the coast line, from 93.56 to 80.58% at stations D, 96.76–95.32% at stations E and from 97.52 to 88.81% at stations F, with a parallel significant increment of the finest fraction of silt at D, 19.22%, and F, 11.03%. The northern and the southern sectors also seem to differ for a more marked presence of finer sediments (more silty fraction) to the north. The contribution of silt is probably due to the additions from the northern coastal areas, as a result of currents along the coast with strong direction from northwest to southeast and that draw their origin from river dispersion processes. Therefore, the distal deposits are partly influenced by a dispersion of the sediments affected by river dynamics (entry of pelitic fluvial sediments from the Rivers Volturno and Garigliano). While sediment sedimentation more proximal to the coast is controlled by selective actions generated by waves, De Pippo et al. (2004) speculated that the small island of San Martino acts as morphological barrier to the transit of sediments along the coast from the Domitian coast. For the south sector, the same above cited diagrams (data not shown) revealed that the sediments are of beach environment and were later influenced by coastal processes. Clay is present only at few sites and in any case at very low level, ~ 0.01%.

Mean data of Cd, Cr, Hg, Ni, Table 3, were generally largely below, from three to six fold, the national regulatory guidelines (D.M. 367/

Table 3
Results of chemical analyses (mg/kg d.w.).

Transect	As	B	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Se	Sn	V	Zn	C%	THCs
A1	**12.1**	12.0	0.1	7.6	13.7	4.0	**39,711**	0.08	**1275**	10.6	15.0	0.2	<0.1	3.2	188.6	65.1	0.1	17.5
A2	11.8	10.6	0.1	6.9	8.9	4.1	**44,290**	<0.01	**1109**	13.9	13.8	0.2	0.3	3.0	164.7	63.9	0.2	21.7
A3	9.7	9.4	0.2	11.5	10.2	5.0	**43,671**	<0.01	**2198**	11.0	16.4	0.2	0.2	4.9	430.1	**124.8**	0.2	17.0
Mean	11.2 B	10.7 C	0.1	8.7 A	10.9 A	4.4 B	**42,557 A**		**1527 A**	11.8 A	15.1 C	0.2		3.7	261 A	84.6 A	0.2	18.7 C
B3	9.0 B	8.7 C	0.1	6.4 B	5.2 B	4.1 B	**40,662 A**	<0.01	**840 C**	14.3 A	14.9 C	0.2	<0.1	2.6	177.5 B	54.9 B	0.2	6.4 D
C1	**15.8** A	16.6 B	0.1	5.7 B	9.7 A	4.2 B	23,778 B	<0.01	**994 C**	11.3 A	18.5 B	0.0	<0.1	1.3	66.1 C	44.8 B	0.3	18.1 C
D1	10.4	13.6	0.1	4.6	7.5	3.7	19,109	<0.01	**1207**	9.1	15.1	0.3	0.3	2.5	65.8	36.3	0.2	18.3
D2	10.3	15.1	0.1	5.0	9.3	14.8	22,333	<0.01	**1167**	9.8	17.6	0.3	0.2	3.1	70.0	44.3	0.2	17.2
D3	11.7	25.9	0.2	5.6	16.0	10.9	**28,006**	0.04	**1112**	11.2	**30.6**	0.5	0.5	4.9	98.2	54.7	0.9	38.6
Mean	10.8 B	18.2 B	0.1	5.1 B	10.9 A	9.8 A	23,149 B		**1162 B**	10.0 B	21.1 B	0.4	0.3	3.5	78.0 C	45.1 B	0.4	24.7 B
E1	10.9	17.4	0.2	4.8	8.1	5.3	24,914	<0.01	**1115**	7.7	26.5	0.4	0.2	4.3	88.9	45.2	0.3	3.1
E2	10.8	30.6	0.2	4.5	13.6	14.4	**26,036**	0.02	**915**	8.3	**40.6**	0.9	0.7	4.5	98.7	57.3	2.1	5.2
E3	9.4	11.8	0.1	3.3	2.8	3.5	19,411	<0.01	642	3.7	20.5	0.3	0.5	2.7	81.6	32.9	0.3	19.8
Mean	10.3 B	19.9 B	0.2	4.2 B	8.2 B	7.7 A	23,453 B		**890 C**	6.6 C	29.2 A	0.5	0.5	3.8	89.7 C	45.1 B	0.9	9.4 D
F1	11.9	12.4	0.1	5.0	7.4	3.2	21,052	<0.01	**1315**	9.7	18.0	0.3	0.3	2.6	82.4	30.1	0.2	15.8
F2	10.0	16.5	0.1	6.4	13.3	5.6	**25,633**	<0.01	**1357**	13.1	19.3	0.4	0.2	3.0	97.2	38.4	0.5	19.6
F3	11.4	27.8	0.2	7.8	20.0	15.3	**25,829**	<0.01	**1056**	17.4	28.3	0.0	0.4	2.7	58.9	61.3	2.2	21.8
Mean	11.1 B	18.9 B	0.1	6.4 B	13.6 A	8.0 A	**24,171 B**		**1242 B**	13.4 A	**21.9 B**	0.2	0.3	2.8	79.5 C	43.3 B	1.0	19.1 C
P	10.6 B	25.6 A	0.2	6.4 B	17.2 A	10.3 A	**28,107 B**	<0.01	**993 C**	13.8 A	**30.5 A**	0.4	0.4	4.6	93.3 C	65.5 B	0.8	38.0 A
D.M. 367/03	12		0.3		50			0.3		30	30							
Damiani et al. (1987)					30	20	25,000	0.25	700	20	60					80		
UNEP (1996)	40–1400		0.02–64			0.6–1890		0.05–0.1			3–3300					1.7–6200		

The concentrations of national regulatory guidelines (D.M. 367/03 – Official Bulletin of the Italian Republic, 2004), the background concentrations for the study area (Damiani et al., 1987), and the ranges recorded by UNEP (1996) for the Mediterranean area are given at the bottom of the table. Values exceeding the reference values are evidenced in bold. Capital letters represent significant differences among transects (LSD test; $\alpha = 0.05$). Letters are not shown in cases where no differences were obtained at this level of significance.

03), whereas that of As, 11.1 mg/kg d.w., was very close to the law limit of 12.0 mg/kg d.w. of the D.M. 367/03. Peaks of As of 15.8 and 12.1 mg/kg d.w. were determined at C1 and A1 sampling stations and with the exception of the transect D, there was a general decreasing concentration trend from coast to offshore direction. Mean level of Pb was 21.7 mg/kg d.w. and hence below the D.M. 367/03 limit of 30 mg/kg d.w. However three illegal values were detected at D3, 30.6 mg/kg d.w., where there was also an evident increasing offshore enrichment of the sediments from 15.1 to 30.6 mg/kg d.w., and at E2, 40.6 mg/kg d.w. and at P, 30.5 mg/kg d.w. The levels of Cr, Cu, Hg, Ni, Pb and Zn fall below, from 1.6 as for Zn to 6 fold as for Hg, the background concentrations for the study area (Damiani et al., 1987). Even though below the background level of 80 mg/kg d.w., Zn concentrations appeared to be particularly high at the transect A and nearby the coast, A1 and A2 of about 65.1 mg/kg d.w., and doubled offshore, 124.8 mg/kg d.w. Respect to the same limits, outstanding high mean levels of both Fe, 28,836 vs. 25,000 mg/kg d.w., and Mn, 1152 vs. 700 mg/kg d.w., were determined. The loads of Fe were particularly high for the transect A (42,557 mg/kg d.w.) and the station B3 (40,662 mg/kg d.w.), whereas those of Mn exceeded the background values throughout all the sampled sites and likewise for Fe the levels were higher at the transect A with a peak of 2198 mg/kg d.w. at A3. Regarding the spatial distribution of metals along each transect it was interesting to note an offshore increasing trend of the concentrations with the exception of Mn at D, E and F sites where an opposite trend was determined. Even the loads of V were quite high at transect A, with mean values six fold higher than at transect F, 261 vs. 43.3 mg/kg d.w. For most of the analysed elements it was also noted a Gaussian accumulation trend at transect E with higher presences at the middle site E2. Among the different transects the analysis of variance, Table 3, evidenced a more significant accumulation, $p < 0.005$, of Co, Fe, Mn, V and Zn in the transect A, which is likely affected by the polluting effect of the Bagnoli brownfield site, placed little further south respect to this site. In fact, the accumulation of Fe and Zn in transect A was almost twofold higher with respect to the northern transect F, 42,557 vs. 24,171 mg/kg d.w. and 84.6 vs. 43.3 mg/kg d.w. The lower values that characterize the northern sector of the studied area (transect D, E, F) can also be due to the minor importance of metal inputs to this area, e.g., freshwater discharge from the Volturno River, run-off, irrigation channels, and from uncontrolled sewage discharge including those from the nearby main sewer of Cuma. It is likely that important amounts of sediments are carried by the Volturno River and Regi Lagni channel network which tend to sink to the bottom within the northern area, thus most likely causing the dilution of metal contents in the surficial sediments of this sector. By contrast, Pb and especially Cu, concentrated more significantly up twofold from 4.4 to 8.0 mg/kg d.w. in the central and northern transects of the studied area and Ni at the northern and south eastern transects, being likely affected also by the polluting flows from the Cuma plant, Regi Lagni and Volturno River. In the case of the harbour, site P, beside the illegal already described level of Pb, only Fe and Mn slightly exceeded the background levels, 28,107 vs. 25,000 and 993 vs. 700 mg/kg d.w. Sediment mean organic matter (C) content along each transect was low and varied from 0.1 to 2.2% with an evident increasing trend from site A, to the south, to site F, to the north. The concentration of PAHs, data not shown, was very low, <0.01 mg/kg, and below the imposed rules. In the same way the presence of THCs, Table 3, was below the regulatory levels Official Bulletin of the Italian Republic (2006).

Table 2 also displays the richness of enterococci (EC) and spores of sulphides reducing clostridia (SP). According to the European rules for sea-costal water (Official Journal of the European Union, 2008) a low levels of EC was generally determined with the exception of the sites E2 and F3 (127 and 297 UFC/g) which are closer to the polluting flows coming from the north of the studied area. The load of SP tends to be higher than that of EC and to increase by moving toward the northwest side, sites E and F. The data had a similar trend as that observed for EC,

Table 4
Specific diversity (number of species – S, Shannon index – H), number of individuals – I, dominance – D, equitability – E for foraminifers (F) and ostracods (O).

	A1	A2	A3	B3	C1	D1	D2	D3	E1	E2	E3	F1	F2	F3	P
Foraminifers															
S	53	59	56	47	54	39	44	46	38	39	35	33	38	46	52
I	2312	2856	6080	490	21,952	1840	4352	8672	3424	1100	888	1872	9888	5280	42,240
D	0.05	0.04	0.04	0.06	0.05	0.10	0.08	0.11	0.11	0.11	0.13	0.10	0.14	0.11	0.12
H	3.43	3.58	3.53	3.23	3.36	2.81	3.07	2.97	2.73	2.77	2.50	2.75	2.65	2.92	2.96
E	0.86	0.88	0.88	0.84	0.84	0.77	0.81	0.77	0.75	0.75	0.70	0.79	0.73	0.76	0.75
Ostracods MNI															
S	18	21	23	10	29	17	22	47	13	16	15	16	28	33	41
I	113	183	183	16	714	234	326	353	117	62	63	104	774	578	8519
D	0.13	0.18	0.11	0.16	0.12	0.12	0.14	0.06	0.22	0.20	0.15	0.16	0.07	0.09	0.08
H	2.39	2.29	2.61	2.10	2.62	2.45	2.38	3.25	2.00	2.19	2.25	2.24	2.92	2.76	2.92
E	0.83	0.75	0.83	0.91	0.78	0.86	0.77	0.84	0.78	0.79	0.83	0.81	0.87	0.79	0.79
Ostracods TNV															
S	18	21	23	10	29	17	23	47	13	16	15	16	27	33	40
I	380	416	380	28	2112	504	1104	1264	292	90	110	232	4112	2672	26,112
D	0.28	0.22	0.16	0.20	0.20	0.28	0.20	0.10	0.31	0.26	0.18	0.22	0.11	0.11	0.10
H	1.83	2.16	2.41	1.93	2.22	1.99	2.19	2.88	1.74	2.01	2.20	2.10	2.66	2.55	2.83
E	0.63	0.71	0.77	0.84	0.66	0.70	0.70	0.75	0.68	0.72	0.81	0.76	0.81	0.73	0.77

Table 5
Bivariate correlation (BC) with the Pearson correlation coefficient. Only couples of variables with significant correlation are shown. Values in bold: correlation is significant at 0.01 level. Values in italics: correlation is significant at 0.05 level.

	B	V	Cr	Fe	Cu	Zn	Se	Sb	Ba	THs	NO_2	NO_3	PO_4	CC3	BU	ZX	EC	P	D	CP	PP	CY	G	S
A_P	0.068	−0.213	−0.216	−0.296	0.167	−0.239	0.109	0.321	0.143	**−0.737**	0.152	0.076	0.113	−0.302	−0.392	−0.153	0.210	*−0.510*	−0.255	−0.400	−0.387	−0.282	−0.223	−0.139
A_T	0.485	−0.255	*0.634*	−0.236	0.423	0.029	0.170	−0.050	0.406	*0.586*	0.435	0.429	0.270	−0.014	0.011	−0.170	0.348	*0.517*	−0.095	0.022	0.019	−0.108	−0.293	0.127
E_A	−0.408	0.321	−0.351	*0.511*	−0.229	0.161	−0.472	−0.206	−0.421	−0.361	*−0.526*	−0.428	−0.371	0.047	0.311	0.351	−0.175	0.247	*0.533*	0.479	0.199	0.348	**0.942**	−0.271
L_L	−0.484	**0.688**	−0.158	**0.722**	−0.333	*0.587*	**−0.632**	−0.469	**−0.677**	−0.107	**−0.706**	*−0.571*	*−0.526*	*0.568*	*0.555*	0.438	−0.286	−0.116	0.248	0.271	**0.651**	*0.573*	0.454	−0.252
Q_L	*0.522*	*−0.526*	0.441	*−0.619*	0.289	−0.385	0.421	0.195	*0.518*	*0.510*	**0.708**	**0.648**	*0.554*	−0.403	−0.492	−0.247	0.221	0.200	−0.385	−0.291	−0.504	−0.250	−0.390	0.493
Q_P	0.450	−0.468	0.420	−0.434	0.376	−0.298	0.160	0.031	0.232	0.433	0.318	0.335	0.223	−0.130	−0.465	−0.153	0.154	−0.180	−0.473	−0.395	−0.299	0.068	−0.425	**0.792**
C_A	−0.174	0.253	−0.216	0.463	−0.096	0.129	−0.199	0.091	−0.185	−0.486	−0.349	−0.361	−0.185	−0.015	0.260	0.433	0.007	0.056	0.372	0.200	0.103	0.284	0.805	−0.306
L_M	0.277	−0.120	*0.545*	0.026	0.296	0.026	−0.201	−0.345	−0.009	0.128	0.436	0.430	*0.532*	0.011	0.008	0.504	*0.547*	0.131	−0.354	−0.251	−0.087	0.431	0.202	0.221
L_A	−0.338	0.421	−0.127	*0.517*	−0.257	0.320	*−0.542*	−0.249	−0.468	0.055	**−0.646**	−0.464	*−0.576*	0.260	0.408	0.204	−0.384	0.301	0.462	*0.517*	0.414	0.411	**0.696**	−0.107
N_M	−0.306	0.280	0.123	0.467	−0.262	0.315	−0.399	*−0.597*	*−0.523*	0.176	−0.164	−0.100	−0.077	**0.737**	*0.518*	0.354	0.009	−0.322	−0.187	−0.068	**0.679**	0.388	−0.370	−0.204
PO_T	−0.328	0.155	**−0.665**	0.109	−0.381	−0.061	0.047	0.285	0.028	**−0.752**	−0.271	−0.402	−0.165	−0.263	−0.157	−0.104	−0.254	−0.233	0.275	0.085	−0.199	−0.306	0.223	−0.416
S_R	0.127	−0.024	0.250	−0.087	−0.051	−0.065	0.032	0.083	0.108	−0.037	0.420	0.251	0.482	0.055	−0.240	0.372	0.080	−0.409	*−0.583*	*−0.611*	−0.171	0.108	−0.282	0.068
S_S	0.457	−0.395	0.337	*−0.539*	*0.590*	−0.153	0.136	0.028	0.441	0.231	0.356	0.227	0.216	−0.266	−0.412	−0.358	0.269	0.066	−0.229	−0.249	−0.313	−0.188	−0.408	0.265
C_A°	−0.032	0.174	−0.051	0.450	0.061	0.158	−0.109	−0.029	−0.145	−0.381	−0.206	−0.180	−0.045	−0.001	0.289	*0.518*	0.276	0.103	0.335	0.171	0.129	0.366	**0.775**	−0.198
L_A°	−0.153	0.188	−0.063	0.357	−0.065	0.129	−0.375	−0.105	−0.296	0.149	*−0.576*	−0.407	*−0.542*	0.144	0.250	0.205	−0.327	0.311	0.466	0.455	0.262	0.423	**0.735**	0.150
N_M°	−0.354	0.358	0.158	0.431	−0.239	0.353	−0.471	*−0.593*	*−0.594*	0.168	−0.134	−0.059	−0.049	**0.771**	0.487	0.368	−0.030	−0.373	−0.336	−0.163	**0.648**	0.397	−0.405	−0.183
P_T°	0.500	−0.400	0.422	−0.392	0.338	−0.196	0.354	0.150	*0.620*	0.465	0.503	0.317	0.411	−0.290	−0.398	−0.319	0.205	0.328	−0.205	−0.160	−0.334	−0.312	−0.391	0.348
S_S°	*0.562*	−0.331	0.453	*−0.529*	*0.606*	−0.062	0.292	0.023	0.472	0.290	0.494	0.351	0.410	−0.270	−0.332	−0.231	0.442	0.012	−0.339	−0.358	−0.321	−0.107	−0.440	0.299
IF	0.363	−0.129	0.443	−0.087	0.153	0.120	−0.014	−0.065	0.368	*0.605*	0.018	−0.020	−0.147	0.157	0.173	−0.148	−0.061	0.478	0.234	0.236	0.271	0.022	−0.209	−0.065
SO	0.584	−0.133	**0.746**	−0.073	0.449	0.176	0.252	−0.029	0.243	**0.875**	0.120	0.177	0.020	0.275	0.095	0.006	0.202	0.102	−0.223	−0.167	0.249	0.275	−0.393	*0.617*
IO	0.366	−0.122	0.424	−0.053	0.209	0.117	0.145	0.082	0.466	*0.577*	0.054	0.040	−0.137	0.102	0.194	−0.216	0.003	**0.694**	0.344	0.356	0.239	−0.155	−0.117	−0.109
SO°	*0.583*	−0.134	**0.742**	−0.077	0.465	0.177	0.252	−0.036	0.233	**0.873**	0.112	0.173	0.010	0.281	0.088	0.008	0.207	0.080	−0.228	−0.177	0.252	0.285	−0.397	*0.634*
IO°	0.384	−0.138	0.458	−0.064	0.227	0.103	0.143	0.077	0.467	*0.587*	0.098	0.077	−0.089	0.088	0.186	−0.208	0.034	**0.696**	0.302	0.328	0.217	−0.149	−0.123	−0.094

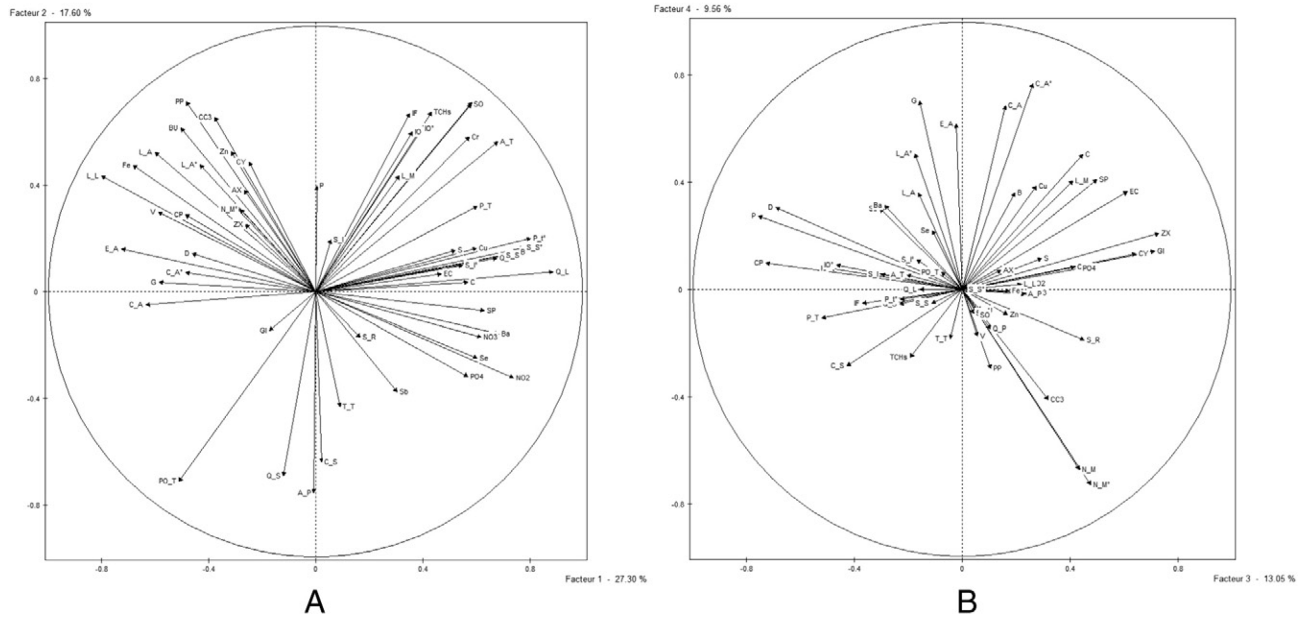

Fig. 2. Output of Principal Component Analysis (PCA) by correlation circles for factors 1, 2 (A) and 3, 4 (B).

with values at E2 and F3 of 3308 and 4057 UFC/g, close to the upper limit of the range of variation reported in literature ($10–10^4$ UFC/g, Technical Manual, ICRAM-MATT 2001). This discrepancy between EC and SP loads could be due to a remote pollution situation as well as to greater resistance of the spores in the marine environment (Skanavis and Yanko, 2001). The algal growth inhibition test with *Phaeodactylum tricornutum* shows just one site, F2, with a peak of toxicity of −65.3%, with an effect which could be considered eutrophying (Balzamo et al., 2003). Other two sites, D1 and E1, were slightly toxic being the reduction of growth of −49.3 and −42.7%. For the rest of the sites a certain biostimulating effect can be detected.

The analysis of the meiobenthic assemblages revealed 138 species of foraminifers (115 species pertaining to 46 genera considered autochthonous) and 95 species of ostracods referred to 45 genera.

In particular data from Table 4 revealed that in transects A, B and C foraminifer assemblages possessed a moderately higher diversity, S: 47–59, respect to that in transect D, E and F, where S range was of 33–46. The former transects also showed significant higher values of abundance, with a peak of I of 21,952 which was more than twofold higher than that detected in D, E and F. In transects A, B and C there was a high relative abundance of *Lobatula lobatula* (4.48–10.20%) and *Rosalina bradyi* (2.04–6.57%), which have been reported as sensitive species to pollution and environmental stress (Dimiza et al., 2016). The same transects also displayed high abundance of *Elphidium aculeatum* (1.04–11.84%). By contrast, transects C, D and F showed high relative abundance of *Quinqueloculina lata* (9.45–31.72%),*Quinqueloculina pygmaea* (1.82–12.55%) and of *Cribroelphidium cuvilleri* (1.48–6.15%) where only the former already described as stress tolerant species (Romano et al., 2013). Ostracod assemblages, data not shown, indicated that transects A, B and C were mainly characterised by the high relative abundance of *Cytheretta* adriatica and *Loxoconcha affinis* whereas transects D, E and F were characterised by *Semycitherura sulcata* and by *Palmoconcha turbida*, known as s tolerant species (Ruiz et al., 2006). In the case of the Port, a particular feature of the meiobenthic assemblage, i.e. maximum foraminifer and ostracod abundance I, was observed.

Only data having an RSD% > 40 were processed by principal component analysis (PCA) and BC. In the PCA, 67.51% of the total variability was explained by components 1, 2, 3 and 4, on the whole, Fig. 2A and B. However, the first factorial plane, Fig. 2A, explains almost the half of the variance, ~45% with a first axes value of 27.3%. It is on the base of these four factors that we will then proceed to the subsequent analysis of the groups.

The interpretation of the factors is strictly linked to the coordinates of the variables. These coordinates represent the linear coefficient factors of the variable with the factor. The typical representation of these relations is the so called correlation circle, with a ray of 1. In the correlation circle relative to the first two components, Fig. 2A, variables split in two main groups with positive and negative values of component 1. The variables with the highest correlation coefficient for factor 1, $|r| \geq 0.8$, were identified to be *L. lobatula* and *Q. lata*. It was interesting to note that most of the chemical parameters, except Zn and especially Fe, had positive values of component 1 and the nutrients NO_2, NO_3 and PO_4 located in the diagram very closely, generating similar positive and slightly negative values for factors 1 and 2. The variables relatively to factor 2 were mainly positive and with a high correlation coefficient, >0.7, determined only for % Pelagophyceae (PP), number of species of ostracods as MNI (SO) and number of species of ostracods as TNV (SO°). The only variables that had a very negative linear correlation coefficient, $r \leq 0.7$, were *P. turbida* and *Ammonia parkinsoniana*. The same variables showed a similar distribution pattern relative to factors 3 and 4, Fig. 2B, with a third axes explaining 13.05% of the total variance. Relatively to this factor, the unique variables assuming the highest positive values were GI and ZX, with an $r > 0.7$, whereas P and CP assumed the highest negative values, ≤0.7.

This feature was also supported by the results of the BC, Table 5, with most of the taxa, except *A. parkinsoniana*, *Q. pygmaea*, *Sahnicythere retroflexa* , *C. adriatica*, *L. affinis*, being significantly correlated, positively or negatively, with trace elements. The highest correlation coefficients, $p < 0.01$, were observed between species such as *L. lobatula* and *Quinqueloculina seminulum* with V and Fe, (positive values), and with Se and Ba (negative values). A very significant positive correlation ($r > 0.7$) was also observed between SO and SO° and Cr.

Respect to THCs, *A. parkinsoniana* and *P. turbida* displayed very significant negative correlation, $r \leq 0.7$, even though SO and SO° appeared positively correlated with THCs, with an $r > 0.8$. *L. lobatula*, *Q. lata* and *L. affinis* were, among the studied taxa, the more sensitive species to NO_2^-, NO_3^-, with a very positive and significant correlation coefficient determined only for *Q. lata*. Chlorophyll *c*3 (CC3) was significantly correlated, $p < 0.01$, merely with *Neocytherideis muelleri* (N_M) and N_M°), with $r > 0.7$, as evidenced also by the first quadrant of the correlation circle

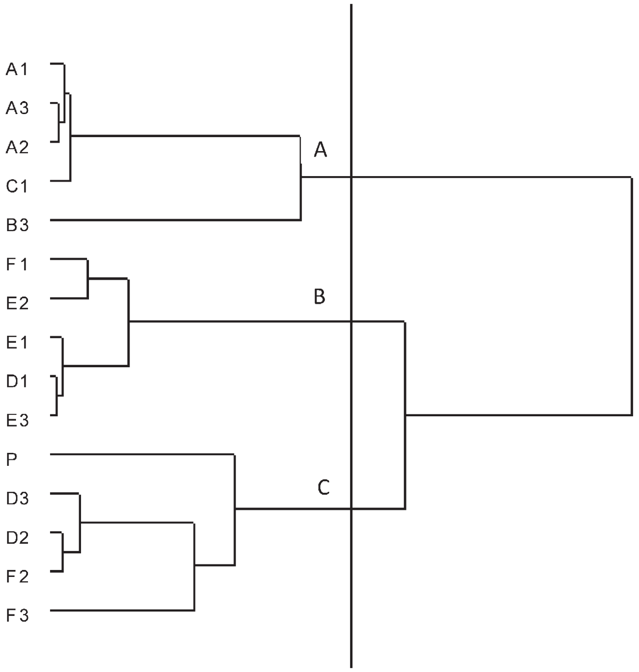

Fig. 3. Output of hierarchical cluster analysis (HCA). Letters indicate clusters.

Table 6
Characterization of the clusters of the dendrogram of ward.

Cluster	V test	Characterising variable
A	3.35	L_L
	3.00	Fe
	2.80	PP
	2.67	BU
	2.51	CC3
	2.38	N_M
	2.36	V
	−2.43	P_T°
	−2.46	Se
	−2.56	Ba
	−2.62	NO_2
	−3.05	Q_L
B	2.58	A_P
	2.53	C_S
	2.33	Q_S
	−2.34	CY
	−2.42	Fe
	−2.46	CC3
	−2.59	BU
	−2.59	PP
C	3.10	SO
	3.04	SO°
	2.96	A_T
	2.91	Q_L
	2.87	Cr
	2.58	TCHs
	2.58	P_T°
	2.52	L_M
	−3.10	PO_T

diagram, Fig. 2A. Among the different phytoplankton communities and taxa existed only very few significant correlations, like those between number of individuals ostracods as MNI (IO) and number of individual ostracods as TNV (IO°) and % Prasinophyceae (P), $r > 0.69$ at $p < 0.01$ and *L. lobatula*, *N. muelleri* (N_M) and (N_M°) and % Pelagophyceae (PP), $r \sim 0.65$ at $p < 0.05$. Five taxa, *E. aculeatum*, *C. adriatica* (C_A and C_A°), *L. affinis* (L_A and L_A°), showed very high positive correlation, $p < 0.05$, with gravel, G, with a peak of $r = 0.94$ for *E. aculeatum* and just one species, the foraminifer *Q. pygmaea*, had a significant correlation with silt, S, $r = 0.79$.

The hierarchical cluster analysis (HCA), Fig. 3, was performed by the criteria of Ward (Lebart et al., 1984). At this scope, test values were calculated for each variable in terms of the difference between its total mean and that inside each cluster, and the result divided by the mean square deviation of the variable in the same cluster.

Only values >2 (in absolute value) were taken in consideration, Table 6 (Lebart et al., 1984). The diagram allows us to clearly recognise three main clusters (A, B and C) that are very distinct at high hierarchical level, Fig. 3. The output of Table 6 helps to illustrate the variables that mainly discriminate the three clusters shown by the HCA, Fig. 3. Cluster A groups samples located in the southeastern part of the studied area. Based on test values, Table 6, cluster A is mainly discriminated by decreasing positive values of the test for *L. lobatula*, Fe, PP, BU, CC3, *N. muelleri* and V, with a peak, for the first formers ones, meaning that these variables were present in great abundance respect to the other two groups. Another set of variables, *P. turbida*, Se, Ba, NO_2^- and *Q. lata* had negative test values meaning lower content than in the other clusters.

Cluster C groups samples of the central-northern part and also the port of the studied area and includes a lower number of characterising variables, belonging mainly to taxa of foraminifera and ostracoda with positive decreasing test values of number of species ostracods (SO, and SO°), *Ammonia tepida*, *Q. lata*, *P. turbida* and *Leptocythere macella*, from 3.10 to 2.52. Only two chemical parameters, Cr and THCs also belong to these cluster and outperformed respect to the other groups. In the end just one variable, the taxa, PO_T, had a very highly negative test value, −3.10. Cluster B includes mainly the stations sampled near the coast of the central-northern sector with taxa assemblage dominated essentially by *A. parkinsoniana*, *Cytheretta subradiosa*, *Q. seminulum* with a test value of about 2.48.

Even though the overall data from the proposed multidisciplinary study regard a very small piece of marine coast, two well defined areas were determined, one to the north and another to the south of the small island of St. Martin, with different degree of pollution. The data revealed that the studied area was affected by two main sources of pollution, one to the north dominated by the inorganic inputs and another to the south affected by the former activities of the brownfield site of Bagnoli, northwest of Naples. The low value of the N/P ratio in the study area could be due to the particular quality of the land waters (more availability of phosphorus than nitrogen), and to the dynamics of utilization of inorganic nutrient by the different functional groups of phytoplankton.

The analysis of the distribution of the meiobenthic assemblages revealed that both foraminifers and ostracods were more sensitive to the organic discharges which affect the northern part of the region respect to the input of heavy metals originated from the metallurgical plant to the south. Data seems to highlight that *E. aculeatum, C. adriatica* and *L. affinis* could be considered as pollution sensitive, while *Q. pygmaea, C. cuvilleri* and *S. sulcata* as stress tolerant. These results are encouraging for the use of benthic foraminifera foraminifers and ostracods in integrated programs for pollution monitoring.

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