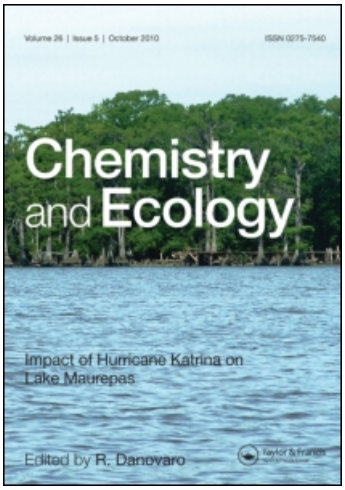

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INFLUENCE OF LARGE-SPECTRUM ENVIRONMENTAL CONTAMINATION ON THE MICRO–MEIOBENTHIC ASSEMBLAGES IN HARBOUR SEDIMENTS OF THE LIGURIAN SEA (W MEDITERRANEAN)

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The concentration of a large spectrum of environmental contaminants (PCBs, PAHs, pesticides and metals) was assessed in surface sediments of two Ligurian harbours (Sanremo and Alassio, NW Mediterranean Sea, Italy) and their relative impact on micro–meiobenthic assemblages was analysed. Concentration, distribution and relative importance of the different contaminants varied considerably between harbours in relation to the different anthropic activities and contamination sources. Results from Principal Component Analysis indicated that high levels of contaminants were typically correlated with low micro–meiobenthic abundance in the sediment. Heavy metals and the organic enrichment were the main factors affecting the distribution and abundance of the bacterial and meiofaunal assemblages in Alassio harbour, whereas hydrocarbons and pesticides played a major role in Sanremo sediments. Neither the bacteria density nor the meiofauna abundance were dependent on sediment grain size, suggesting that micro–meiobenthic parameters may be under the influence of other variables. Our results suggest that high concentrations of contaminants independently from their source or typology are responsible for the impact observed on micro–meiobenthic assemblages in these harbours.

Keywords: Bacteria; Meiofauna; Harbours; Environmental Contaminants

1 INTRODUCTION

Micro and meiobenthic communities belong to the detritus community and have been recently proposed as tools for the monitoring of environmental impact in coastal sediments (Boyd *et al.*, 2000; Lee *et al.*, 2001; Blakely *et al.*, 2002). Bacteria play a key role in the early diagenesis of organic material and the structure of microbial assemblages is sensitive to changes in environmental conditions and trophic state (Yung *et al.*, 1999; Danovaro, 2000; Dahllöf *et al.*, 2001; Danulat *et al.*, 2002; Mirto *et al.*, 2002). On the other hand, meiofaunal assemblages, due to their small size, high turnover rates and lack of larval dispersion respond rapidly to changes in environmental conditions and are currently employed in

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environmental monitoring (Warwick *et al.*, 1990; Warwick, 1993; Danovaro, 2000; Danovaro *et al.*, 2000).

Several studies focusing on the structure of micro and meiobenthos in polluted environments have dealt with organic enrichment and have been largely employed to assess the impact of high organic loads in eutrophicated environments (Rajendran *et al.*, 1997; Moodley *et al.*, 1998; Meyer-Reil and Köster, 2000; Lebaron *et al.*, 2001; Vezzulli *et al.*, 2002; Vezzulli *et al.*, in press).

However little is known on the influence of other sources of contamination such as organic and inorganic chemicals that may also play a role in affecting the structure of micro–meiobenthic assemblages. Harbour sediments are known to be contaminated with a large number of pollutants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides (DDTs) and metals.

Previous studies on the microbial community at both the laboratory and environmental scale mainly target on the effect of a single contaminant and/or a limited number of contaminants (Sunda and Gillespie, 1979; Zavenhuizen *et al.*, 1979; Fabiano *et al.*, 1994; Ellis *et al.*, 2002) but the influence of large spectra of chemicals on the micro–meiobenthic community has thus far not been assessed. This issue is of primary importance since the various environmental parameters may act in antagonistic or synergistic way and may have a complex impact on the living community.

The aim of the present study was to investigate the influence of a large spectrum of environmental contaminants (PCBs, PAHs, pesticides and metals) on the micro–meiobenthic assemblages in surface harbour sediments by mean of comparative analysis.

2 MATERIALS AND METHODS

2.1 Study Sites and Sampling

The study was carried out in two harbours located in the Ligurian Sea (W Mediterranean) (Fig. 1). The Portosole-Sanremo harbour has an area of 167,500 m^2, is 4020 m long and 4–7 m deep, water enters the harbour from the south and has a low renewal time. Ship traffic is very heavy mainly in the central part of the harbour close to the entrance. The Portosole marina can accommodate more than 800 vessels, principal sources of pollution are represented by the various shipping activities (ship traffic, filling stations, etc.) and the plume of the S Francesco River entering the harbour in the NW.

The Alassio harbour covers a smaller area (39,000 m^2), has an average depth of 3–5 m and can accommodate around 400 vessels. The harbour mouth opens NE and is directly intersected by the long shore Ligurian current flowing SW thus improving water renewal and flushing. The main sources of pollution include the shipping activities, the presence of two shipyards and filling stations in the middle area of the harbour.

Sampling stations were selected along an inside–outside harbour gradient (inside, at the entrance, outside). Ten stations were selected in Sanremo-Portosole: six stations were located inside the harbour (SR1, SR2, SR3, SR4, SR5, SR6), one station was located at the entrance (SR7) and the three remaining stations were located outside (SR8, SR9, SR10). Six station were selected in Alassio: three were inside the harbour (AL1, AL2, AL3), one at the entrance (AL4) and two were placed outside (AL5, AL6) (Fig. 1).

Three samplings were carried out in the two harbours in December 2000 to provide adequate replicates. Surface sediment samples were collected by means of a Van Veen grab. For chemical parameters the top 3 cm were collected in plastic jars, transported to the laboratory and stored at $-20\,^{\circ}C$ until characterisation. For meiofaunal analysis the top 3 cm were added

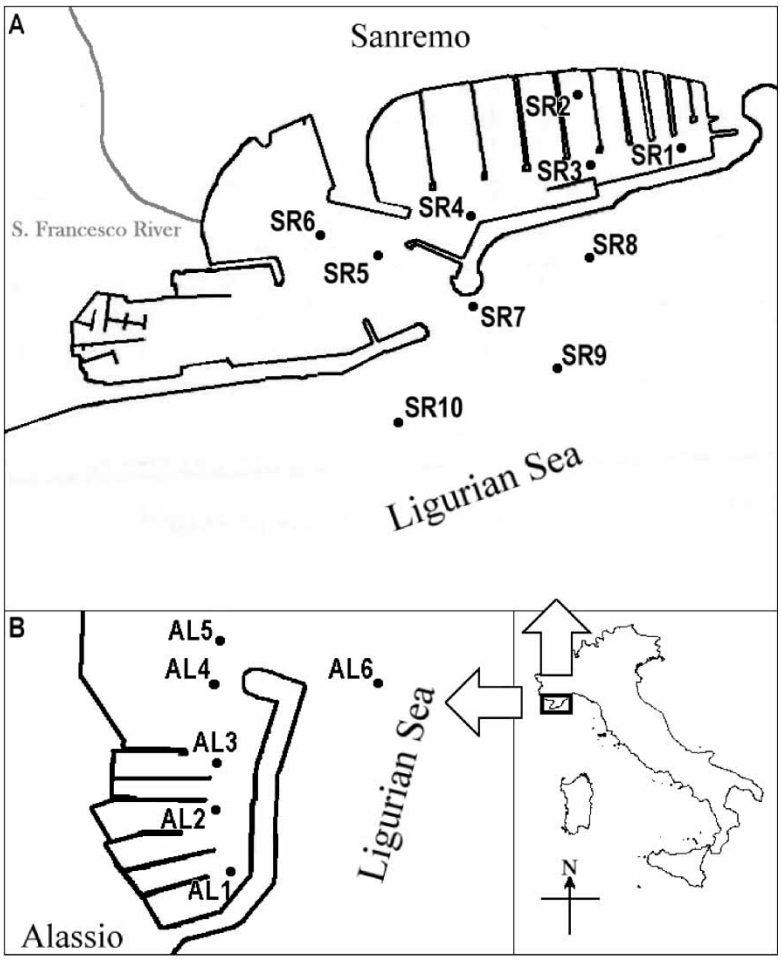

FIGURE 1 Maps of (A) Sanremo and (B) Alassio harbours (Ligurian Sea, NW Mediterranean) and locations of the 16 sampling stations.

with formalin (4%) and stored at room temperature. Finally for total bacterial counts the top 3 cm of sediment were transferred into sterile test-tubes, 5 ml of formalin (2%) was added, and samples were stored at −20 °C.

2.2 Organic Matter and Particle Size Analysis

Total organic matter (TOM) was calculated as the difference between the dry weight (60 °C) of the sediment and weight of the residue left after combustion at 450 °C (Parker, 1983).

Proteins (PRT) were determined according to Hartree (1972). The absorbance was evaluated at 650 nm. Bovine albumin solutions were used as standards. Carbohydrates (CHO) were analysed according to Dubois *et al.* (1956). The absorbance was measured at 490 nm. D(+) Glucose solutions were used as standards. Lipids (LIP) were extracted by direct elution with chloroform and methanol according to Bligh and Dyer (1959) and measured following Marsh and Weinstein (1959). Absorbance was measured at 375 nm. Tripalmitine solutions were used as standards.

Sediment samples for particle size analysis were pre-treated with a H_2O_2 solution in order to oxidise the organic fraction and were dried in a thermostat at 70 °C. Particle size profiles were determined by dry-sieving and provided as percentage composition of the following size categories (μm): >2000 (gravel), 2000–200 (coarse sand), 200–63 (fine sand), 63–4 (silt) and <4 (clay).

2.3 Heavy Metals

For metals (Cd, Cr, Hg, Pb, Cu and Zn) determination 0.5 g of sediment was digested in 9 ml of concentrated nitric acid, 2 ml of hydrochloric acid and 3 ml of hydrofluoric acid for 15 minutes using microwave heating by a suitable laboratory microwave system. The sample and acid were placed in suitably inert polymeric microwave vessels. The vessel was sealed and heated in the microwave. The temperature profile was specified to allow specific reactions reaching 180 ± 5 °C in approximately 5.5 minutes and kept at 180 ± 5 °C for 9.5 minutes (Kingston and Haswell, 1997). After cooling, the vessel content was filtered, centrifuged, diluted to volume, and analysed by the appropriate SW-846 method (SW-846 USEPA method 3052, 1995).

2.4 Policyclic Aromatic Hydrocarbons (PAHs)

For PAHs determination 10 g of dried sediment samples was screened through a 2-mm sieve and then placed into a 150 ml tube with 20 ml of acetone. The mixture was shaken for 30 min in order to extract the sample. After centrifugation at 1000*g* for 5 min, the extract was diluted with Milli-Q water and cleaned on a SPE C_{18} column (Waters, Milford MA, USA) under vacuum. The column was washed with dichloromethane. The eluate was injected into a HPLC column equipped with a fluorescence detector coupled with a UV-Vis variable-wavelength spectrophotometer (Kootstra *et al.*, 1995).

2.5 Organo-Chlorine Pesticides and Polychlorinated Biphenyls (PCBs)

For organo-chlorine and polychlorinated biphenyl analysis, 10 g of sediments were extracted with 50 ml of methylene chloride/isooctane/acetone (50/50/50) and soaked for 45 minutes by ultrasounds. The sample was then evaporated under vacuum at 45 °C and dried under an inert gas stream. The remainder was treated with 3 ml of isooctane.

To eliminate sulphur, isooctane solution was added to 0.5 ml of mercury and stirred till sulphur completely transformed into mercury sulphide. After centrifugation, surnatant was added with 5 ml of concentrated sulphide acid (96%), stirred and centrifuged. Finally, the organic solution was chromatographically detected (HKGL, 1990; HOKLAS, 1994). Seven organo-chlorine pesticides were assessed in the sediment: *o*,*p*′-DDT; *p*,*p*′-DDT; *o*,*p*′-DDD; *p*,*p*′-DDD; *o*,*p*′-DDE; *p*,*p*′-DDE; Endosulfan. Fourteen single PCBs (31, 28, 52, 101, 81, 77, 118, 153, 105, 138, 128, 156, 180, 169) were dosed by internal calibration with S.I. Aldrin or by external calibration and expressed as total PCBs in the sediments (PCBtot). PCBs, PAHs, pesticides and metals analyses have been carried out by 'Agenzia Regionale per la Protezione del l'Ambiente Ligure' (ARPAL).

2.6 Micro–Meiobenthic Parameters

2.6.1 Bacterial Parameters

For bacterial analysis 0.5–1 g of each sediment replicate was added to 5 ml of freshly-prepared prefiltered and sterilised seawater with prefiltered formaldehyde (2%). Samples were

homogenised three times for 1 min (Meyer-Reil, 1983). Sub-samples were diluted in relation to cellular density. Portions of subsamples were withdrawn and stained for 5 min with Acridine Orange and filtered on Black Nucleopore 0.2 µm. Ten to twenty randomly chosen grids on each duplicate filter per sample were counted with epifluorescence microscopy (Hobbie *et al.*, 1977). Bacterial size was determined by using a micrometric ocular and assigning bacteria to different size classes (small $<0.065\,\mu m^3$, medium $0.065–0.320\,\mu m^3$ and large $0.320–0.780\,\mu m^3$). The number of dividing cells (NDC) defined as cells with a clearly visible invagination, was determined according to Newell and Christian (1981).

2.6.2 Meiofaunal Analysis

After a preliminary assessment of the percentage of recovery (PR) of total meiofaunal density in the top 10 cm layer (data not shown) only the top 3 cm were analysed (PR > 90%). Each sediment sample was washed in tap water through 1000 and 37 µm mesh sieves to retain the smallest meiofaunal organisms (Danovaro *et al.*, 2000). The fraction remaining on the 37 µm sieve was centrifuged three times with Ludox HS 40 colloidal silica (density $1.24\,g\,cm^{-3}$) as described by Heip *et al.* (1985). All meiobenthic animals were counted and classified per taxon under a stereomicroscope after staining with Rose Bengal ($0.5\,g\,L^{-1}$).

2.7 Data Analysis

Differences in investigated parameters between stations and correlations among variables were tested using parametric analyses. The *T*-test and Hotelling T^2 test were used for univariate and multivariate two samples comparison, respectively. The Pearson correlation analysis was carried out to test correlation among chemical and environmental parameters in the 16 stations during the sampling period. Finally Principal Component Analysis (PCA) was employed to investigate quantitative differences and similarity among sites and in order to identify the main factors affecting micro–meiobenthic density and distribution. All statistical tests and correlation analyses were performed using the Statistics Toolbox, R12, of MATLAB.

3 RESULTS AND DISCUSSION

3.1 Organic Matter, PCBs, PAHs, Pesticides and Heavy Metals in Harbour Sediments

Results from total organic matter and particles size analysis are reported in Table I. The concentrations of PCBs, PAHs, pesticides and heavy metals in sediments of Alassio and Sanremo harbours in December 2000 are reported in Table II. Figure 2 shows the standardised values (zero mean and unit variance) for each class of contaminants in the 16 sampling stations.

Total organic matter (TOM) concentrations displayed very high values ranging from 0.5% to 10.8% and from 0.6% to 5.4% for Alassio and Sanremo respectively. The highest TOM, PRT (range $14.1–1127.7\,\mu g\,g^{-1}$), CHO (range $89.9–5592.2\,\mu g\,g^{-1}$) and LIP (range $21.9–1361.1\,\mu g\,g^{-1}$) concentrations were found in Alassio (Hotelling T^2 test, $p < 0.05$) and were inversely correlated with the grain size of the sediment ($n = 14$, $p < 0.05$). The sum of PRT, CHO and LIP in the sediment is a measure of the trophic state of the benthic ecosystem and provides a measure of organic enrichment in eutrophicated environments (Dell'Anno *et al.*, 2002).

Metals concentrations were also higher in sediments of the Alassio harbour (Hotelling T^2 test, $p < 0.05$). According to Fabiano *et al.* (1994) metals contamination is strongly related with

TABLE I Average Concentrations of Biochemical Parameters and Percentage Composition of Sediment Particles in the Top-3 cm Sediment Layer of the Sanremo and Alassio Harbours in December 2000.

Station	*TOM (%)*	*PRT ($\mu g\,g^{-1}$)*	*CHO ($\mu g\,g^{-1}$)*	*LIP ($\mu g\,g^{-1}$)*	*Gravel (%)*	*Corse sand (%)*	*Fine sand (%)*	*Silt (%)*	*Clay (%)*
SR1	2.3	43.8	682.3	527.1	1	33	31	34	1
SR2	1.8	72.3	441.8	223.7	1	15	76	7	1
SR3	3	102.1	916.3	270.6	0	19	65	14	2
SR4	5.4	493.6	635.1	667.2	0	21	30	44	5
SR5	2.3	268.4	2012.8	634.1	0	15	62	21	2
SR6	2.4	45.8	184.5	189.9	0	7	44	44	5
SR7	0.6	5.8	48.1	34.6	0	11	84	4	1
SR8	0.6	16.1	97.8	39.7	0	9	85	5	1
SR9	0.9	13.2	415.5	203.7	0	5	84	9	2
SR10	1.1	10.2	733.1	367.7	0	31	63	5	1
AL1	10.8	1127.7	4055.0	986.1	0	32	40	27	1
AL2	6.4	506.7	5592.2	904.1	1	30	39	29	1
AL3	4.1	587.8	5114.6	1132.6	0	25	39	36	0
AL4	1.7	668.9	4637.0	1361.1	0	6	62	30	2
AL5	0.8	14.1	190.1	255.0	0	16	77	6	1
AL6	0.5	35.9	89.9	21.9	0	62	37	1	0

Note: TOM, total organic matter; PRT, proteins; CHO, carbohydrates; LIP, lipids. Size categories (μm): >2000 (gravel), 2000–200 (coarse sand), 200–63 (fine sand), 63–4 (silt) and <4 (clay). Coefficient of variation (CV) <30.

TOM concentration in marine sediments. This was observed for all the analysed metals in our study ($n = 14$, $p < 0.05$). Zinc and copper displayed the highest concentrations and showed a range of 22–263 $mg\,kg^{-1}$ for zinc and of 4.2–177.2 $mg\,kg^{-1}$ for copper respectively. Criteria proposed by NOAA (National Oceanic and Atmospheric Administration) suggested ERL (Effect-Range Low) (*e.g.* Cu 34 $mg\,kg^{-1}$; Zn 150 $mg\,kg^{-1}$) and ERM (Effect-Range Median) (*e.g.* Cu 270 $mg\,kg^{-1}$; Zn 410 $mg\,kg^{-1}$) values for these contaminants (Long *et al.*, 1995). Based on our data none of the studied stations exceeded the ERM values and in the Sanremo harbour 70% of the stations did not exceed the ERL values either for Cu or Zn. Also chromium (range 10.5–62.9 $mg\,kg^{-1}$) concentrations displayed values less than ERL while cadmium (range 0.4–1.8 $mg\,kg^{-1}$), mercury (range below detection (bd) $-0.4\,mg\,kg^{-1}$) and lead (range 23.5–92.2 $mg\,kg^{-1}$) displayed concentrations between ERL–ERM values.

Total PCBs concentrations ranged from 0.5 to 67.1 $\mu g\,kg^{-1}$ in the top 3 cm of the sediment but no significant differences were found between the two harbours (*T*-test, ns). The highest concentrations were recorded at Station 2 in Alassio. In contrast DDTs (*o*,*p*′-DDT; *p*,*p*′-DDT) and DDTs derived compounds (*o*,*p*′-DDE; *p*,*p*′-DDE; *o*,*p*′-DDD; *p*,*p*′-DDD) displayed significantly higher concentrations in sediment of Sanremo harbour (Hotelling T^2 test, $p < 0.05$) and showed a range of 0.4–39.6 $\mu g\,kg^{-1}$ and 1.1–193.4 $\mu g\,kg^{-1}$ for *o*,*p*′-DDT and *p*,*p*′-DDT respectively. The occurrence of high DDT concentrations in this harbour is most probably caused by the discharge of pesticides from the S. Francesco River coming from floriculture and agricultural practices.

Sediments of the Sanremo harbour were also characterised by significantly higher concentrations of PAHs in the sediments (Hotelling T^2 test, $p < 0.05$). Phenanthrene (range 19.5–322.9 $\mu g\,kg^{-1}$), fluoranthene (range 23.7–729.6 $\mu g\,kg^{-1}$), pyrene (range 16.3–835.3 $\mu g\,kg^{-1}$) and benzo a pyrene (range 14.3–671.9 $\mu g\,kg^{-1}$) displayed the highest concentrations and showed an accumulation in stations 9 and 10 located in front of the harbour entrance in relation to the intense ship traffic of this area. Lower concentrations of hydrocarbons were found in Alassio sediments (Fig. 2).

Among the analysed chemicals, metals, PCBs and pesticides showed a significant positive correlation with the silt and clay content of the sediment ($n = 14$, $p < 0.05$) while no correlation was found for hydrocarbon concentrations or grain size ($n = 14$, $p > 0.05$).

TABLE II Average Concentrations of Environmental Contaminants in the Top-3-cm Sediment Layer of Sanremo and Alassio Harbours in December 2000.

	Station															
	SR1	*SR2*	*SR3*	*SR4*	*SR5*	*SR6*	*SR7*	*SR8*	*SR9*	*SR10*	*AL1*	*AL2*	*AL3*	*AL4*	*AL5*	*AL6*
PCBtot ($\mu g\,kg^{-1}$)	33.8	8.8	13.1	33.8	18.0	25.0	3.9	6.7	8.1	27.7	25.8	67.1	12.0	1.5	0.8	0.5
Pesticides ($\mu g\,kg^{-1}$)																
o,p′-DDT	4.0	2.3	5.9	6.4	4.3	39.6	0.4	0.4	2.6	1.8	0.2	1.1	1.4	bd	bd	bd
p,p′-DDT	17.6	7.0	34.1	193.4	85.4	168.2	1.1	3.1	43.8	4.3	1.0	1.7	6.0	0.5	bd	bd
o,p′-DDE	1.8	1.1	1.4	2.4	0.8	4.6	0.2	0.4	0.9	bd	0.3	1.2	0.3	bd	bd	bd
p,p′-DDE	43.2	29.4	41.6	179.6	35.6	129	4.5	3.9	18.2	11.6	3.8	7.6	10.4	1.2	0.4	0.4
o,p′-DDD	18.4	3.9	7.1	24.1	13.9	14.6	1.3	1.2	2.5	5.8	3.5	13.7	1.5	0.5	bd	0.2
p,p′-DDD	21.4	6.7	15.2	34.6	18.3	14.6	2.0	1.5	5.8	5.4	1.6	3.2	2.5	0.7	bd	0.4
Endosulfan	11.2	2.7	1.6	bd	4.6	3.2	0.6	bd	2.5	0.2	1.4	0.5	3.9	0.2	bd	0.2
Hydrocarbons ($\mu g\,kg^{-1}$)																
Phenanthrene	72.7	19.5	43.0	48.4	145.6	36.5	26.8	47.1	122.6	322.9	26.0	12.5	61.0	56.1	6.4	8.6
Anthracene	9.5	3.4	6.9	6.7	34.5	3.4	6.3	10.7	43.8	71.2	12.3	11.8	14.4	16.7	3.6	2.1
Fluoranthene	117.5	23.7	50.1	52.8	403	31.8	55.2	80.4	282.8	729.6	78.8	72.5	110.9	125.9	12.4	10.9
Pyrene	121.6	16.3	21.6	30.6	417.5	18.3	59.5	49.2	336	835.3	22.8	20.8	34.4	41.5	2.1	3.4
Benzo(a) anthracene	43.0	8.5	18.8	11.9	148.9	7.0	29.2	46.1	138.9	329.5	19.9	10.9	21.1	46.4	2.6	bd
Chrysene	75.6	15.0	40.3	24.1	180.7	21.7	42.8	67.4	180.6	473.4	58.1	56.6	54.1	55.3	6.5	4.4
Benzo(a) pyrene	91.3	19.8	41.2	41.2	412.6	14.3	53.9	78.7	302.8	671.9	62.2	61.4	44.2	61.6	7.0	3.6
Heavy metals ($mg\,kg^{-1}$)																
Cd	1.2	0.9	1.1	1.2	1.1	1.0	1.1	0.7	0.8	0.9	1.8	0.7	1.1	1.1	0.4	0.5
Cr	25.6	15.8	27.7	56.1	17.4	36.9	10.5	12.0	16.9	16.9	42.4	62.9	55.8	36.1	26.5	11.7
Hg	0.05	bd	bd	0.03	0.05	0.02	bd	bd	bd	0.03	0.42	0.15	0.13	0.08	0.2	0.12
Pb	45.3	40.1	38.9	56.1	44.8	43.5	23.5	25.1	31.8	33.8	72.4	92.2	60.9	42.3	33.2	29.5
Cu	113.1	46.4	32.7	42.7	27.4	24.6	5.3	4.2	8.0	7.8	114.2	177.2	64.7	14.4	7.5	4.7
Zn	128	94	88	144	72	79	24	24	36	38	174	263	132	56	38	22

Note: bd, below detection; Coefficient of variation (CV) <30.

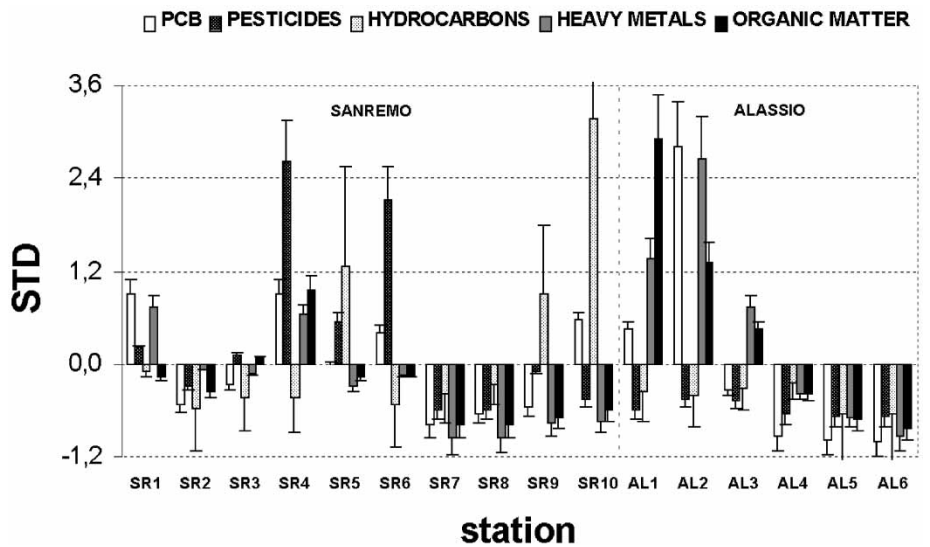

FIGURE 2 Standardised total concentration values (zero mean and unit variance) for each class of contaminants in the Sanremo and Alassio harbours in December 2000.

3.2 Influence of Contamination on Micro–Meiobenthic Assemblages

The bacterial and meiofaunal parameters in sediments of Alassio and Sanremo harbours in December 2000 are reported in Table III. Figure 3 shows the standardised values (zero mean and unit variance) for total bacterial and meiofaunal abundance in the 16 sampling stations.

In order to investigate the influence of chemical parameters on the distribution and structure of the micro–meiobenthic community we ran a Principal Component Analysis (PCA) based on the correlation matrix calculated from the raw data. Figures 4 and 5 show a plot of the values of the 16 sampling stations for the first two principal components (PC) and the corresponding plot of loadings showing correlation between PC and the investigated variables. The first three PC accounted for ~70% of the variation of the data. Ordination for variables (Fig. 5) against PC1 (30% of variance explained) displayed two main groups: bacterial and meiofaunal variables here referred as the 'biological group' (BG) that showed negative correlation with PC1 and PCBs, PAHs, pesticides and metals variables here referred as the 'chemical group' (CG) that showed positive correlation with PC1. Ordination for PC2 (25% of variance explained) concurred to a further division occurring within the two groups identified by PC1. The 'biological group' fractionated into two main sub-groups: the 'meiofauna sub-group' (MSG) that showed lower negative correlation with PC2 and the 'bacterial sub-group' (BSG) that showed higher negative correlation with PC2. On the other hand the 'chemical group' was fractionated into three sub-groups: the hydrocarbons (HSG), pesticides (PSG) and OM-metals (OSG) sub-groups showing positive, none and negative correlation with PC2 respectively. The plot of scores displayed similar groups of stations (SBG, SCG) (Fig. 4) and suggested a close association with the corresponding grouped variables.

Interpretation of the PCA output provided a powerful tool in assessing the relationship occurring between stations and variables and between biological and chemical parameters in the two harbours.

Stations AL4, AL5 and AL6 located at the entrance and outside the Alassio harbour and stations SR1 and SR2 located inside the Sanremo harbour were characterised by the highest bacterial (range $11.7–16.6 \times 10^8$ cells g^{-1}) and meiofaunal (range 851–2303 ind./10 cm^2)

TABLE III Micro–Meiobenthic Parameters in the Top-3-cm Sediment Layer of Sanremo and Alassio Harbours in December 2000.

	BACTERIA (no. 10^8 cell g^{-1})					*MEIOFAUNA (ind. 10 cm^{-2})*										
Station	*TBN1*	*TBN2*	*TBN3*	*TBNtot*	*NDC*	*NEM*	*COP*	*NAU*	*POLY*	*BIV*	*TURB*	*OLIGO*	*TARD*	*ISO*	*NEM*	*MEIOtot*
SR1	2.0	10.0	0.2	12.1	0.6	1934	147	41	15	23	121	10	4	0	8	2303
SR2	2.0	11.0	0.2	13.2	0.6	1521	123	22	6	18	74	8	2	0	6	1780
SR3	0.1	2.1	0.03	2.3	0.2	1548	168	29	6	13	47	5	0	0	6	1822
SR4	1.4	8.1	0.1	9.6	0.6	743	94	9	5	11	43	4	1	0	4	914
SR5	0.4	4.1	0.4	4.8	0.4	521	65	16	0	9	29	4	0	0	4	648
SR6	0.2	2.1	0.1	2.4	0.3	354	43	11	0	6	36	6	2	0	5	463
SR7	1.1	4.5	0.1	5.7	0.3	199	31	9	4	5	16	7	0	0	4	275
SR8	0.2	2.6	0.2	3.0	0.4	534	101	16	9	10	24	14	0	0	4	712
SR9	0.6	4.9	0.1	5.7	0.3	468	109	19	11	12	61	10	1	0	7	698
SR10	0.3	1.9	0.03	2.2	0.2	389	62	21	8	14	44	8	2	0	5	553
AL1	0.4	2.1	0.2	2.7	0.2	503	31	8	6	12	25	3	0	0	4	592
AL2	1.2	3.3	0.1	4.7	0.4	389	24	9	5	16	23	3	0	1	2	472
AL3	1.1	7.3	0.2	8.6	0.6	631	54	14	5	10	47	5	0	0	1	767
AL4	1.8	14.1	0.7	16.6	1.7	856	102	18	11	14	84	5	2	0	6	1098
AL5	1.4	14.4	0.2	16.0	1.0	976	204	32	10	19	119	6	5	6	9	1386
AL6	1.2	9.9	0.5	11.7	0.9	534	167	11	6	13	96	12	1	4	7	851

Note: TBN1, small-size bacterial density; TBN2, medium-size bacterial density; TBN3, large-size bacterial density; TBNtot, total bacterial density; NDC, number of dividing cells; NEM, nematodes; COP, copepods; NAU, nauplii; POLY, polychaetes; BIV, bivalves; TURB, turbellarians; OLIGO, oligochaetes; TARD, tardigrades; ISO, isopods; NEM, nemertines; MEIOtot, total meiofauna. Coefficient of variation (CV) <30.

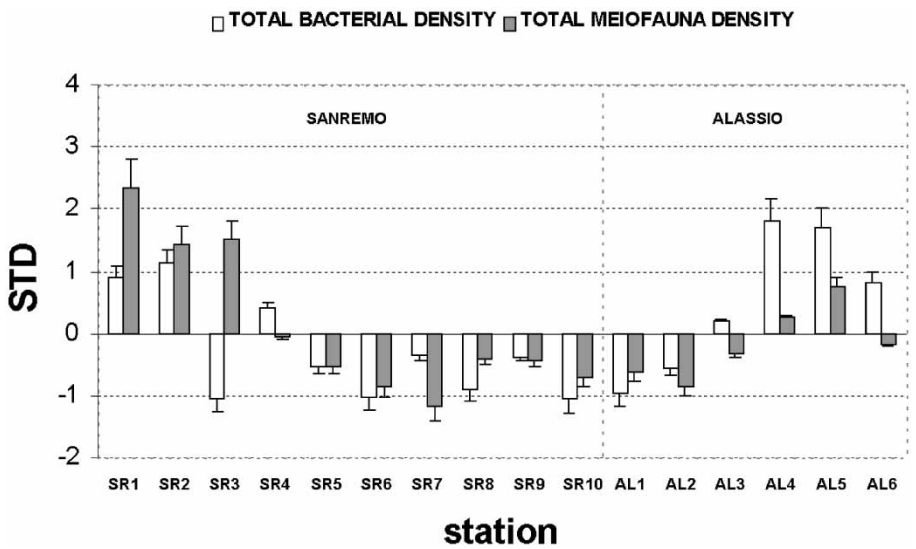

FIGURE 3 Standardised values (zero mean and unit variance) for total bacterial and meiofaunal abundance in the Sanremo and Alassio harbours in December 2000.

abundance together with the lowest contamination of chemicals. In contrast stations AL1, AL2, AL3 in Alassio and SR4, SR5, SR6, SR10 in Sanremo were characterised by the highest concentration of chemicals and showed the lower abundance of micro (range 2.2–9.6×10^8 cells g^{-1}) and meiobenthic (range 463–914 ind./10 cm^2) communities. Stations SR3, SR7, SR8, SR9 displayed intermediate concentrations of chemical parameters and showed bacterial and meiofaunal density ranging from 2.3 to 5.7×10^8 cells g^{-1} and from 275 to 1822 ind./10 cm^2 respectively.

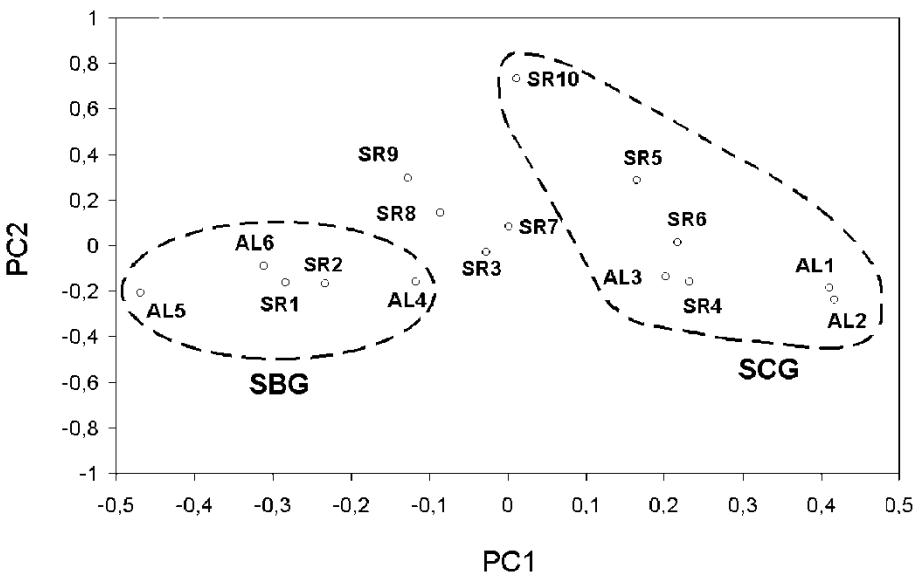

FIGURE 4 Plot of 16 sampling stations against their values for the two principal component (plot of scores) (reported are SBG = biological group, SCG = chemical group).

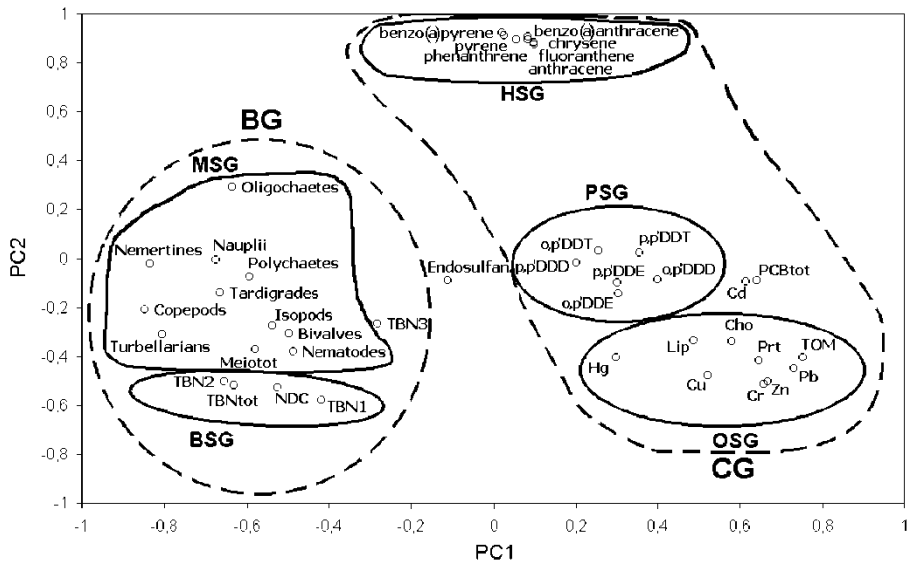

FIGURE 5 Plot of 41 investigated variables against their values for the two principal component (plot of loadings). Reported are BG = biological group, CG = chemical group, MSG = meiofaunal sub-group, BSG = bacterial sub-group, HSG = hydrocarbon sub-group, PSG = pesticide sub-group, OSG = organic matter-metal sub-group.

Distribution of pollutants and biological variables displayed an inverse putative gradient in the two harbours. Increasing 'chemical content' coupled with a decrease in the 'biological content' was observed moving from inside to outside stations in the Alassio harbour while the opposite gradient was observed in Sanremo. This can be explained by the different loads and type of contaminants due to the different magnitude/location of activities and sources of contamination (*e.g.* organic-metal enrichment in the inner part of Alassio and hydrocarbon-pesticides contamination in the middle part of Sanremo). From ordination against PC1 we can thus summarise that as generally expected a high 'chemical content' was correlated with a low 'micro–meiobenthic' content in the sediment, and this helped to explain 30% of variation in our data.

PC1 helped also to explain much of the relationships occurring between the chemical and biological variables in the Alassio harbour. Metals and the organic enrichment were the main factors affecting the distribution and abundance of the micro–meiobenthic community in these sediments. Generally an increase in benthic bacterial density results from an increase of organic carbon and nitrogen in the sediment (Köster *et al.*, 1997). However, in our study, an opposite trend was observed, and this is plausibly explained by the strong association found between organic matter and metals concentrations. Among metals, Cd displayed the least correlation with the bacterial parameters according to Fabiano *et al.* (1994) who found bacterial populations to be significantly affected by Cd concentrations in the sediment. The meiofaunal community is known to be sensitive to organic enrichment if compared to bacteria (Mirto *et al.*, 2000). Nemertines, turbellarians and copepods were the more sensitive taxa within the meiofaunal community. Harpacticoid copepods are considered sensitive to environmental perturbation and their densities are themselves indicators of pollution stress (Van Damme *et al.*, 1984; Hicks and Coull, 1984). This is less evident for other taxa such as nematodes that are generally considered resistant to pollution (Murrel and Fleeger, 1989; Hendelberg and Jensen, 1993) and accounted for 80% of total meiofaunal density in these sites.

PC2 further explained the relationship occurring between the chemical and biological variables in the Sanremo harbour. Hydrocarbons and pesticides contamination appeared to play the major effect in influencing the micro–meiobenthic community structure in these sediments and displayed the highest negative correlation with the bacterial and meiofaunal parameters. Significant toxicological effects of PAHs on benthic organisms have been observed at concentrations of $\geq$1 mg-PAH kg dry sediment (*e.g.*, Long, 1992). Field and laboratory studies have documented that the meiofaunal component of the benthos is sensitive to petroleum contaminants (Coull and Chandler, 1992). Oligochaetes resulted the more PAHs tolerant taxa within the meiofaunal community. In contrast all the bacterial parameters as well as nematodes appeared more sensitive to hydrocarbon contaminations (Montagna and Li, 1997).

Finally, neither the bacteria density nor the meiofauna abundance correlate with the grain size of the sediment ($n = 14$, $p > 0.05$) in both Sanremo and Alassio harbour. This is an important observation as in non-impacted environments the sediment structure is regarded as the 'super factor' in determining bacterial and meiofaunal community structure (Hargrave, 1972; Higgins and Thiel, 1988). The absence of a correlation of either measures with the sediment structure indicates that both parameters are under the influence of other variables.

4 CONCLUSIONS

Micro and meiobenthic communities respond similarly to large spectra contamination in Sanremo and Alassio sediments and showed a decrease in density. Different types of contaminants had the marked effects on the micro–meiobenthic assemblages in the two harbours (*i.e.* organic enrichment and metals in Alassio; hydrocarbons and pesticides in Sanremo). Generally, absolute concentrations of contaminants (quantity) more than their types (quality) had a greater role in affecting the density and distribution of micro–meiobenthic communities and this should be taken into consideration when using bacterial and meiofaunal parameters to monitor the environmental impact of coastal marine sediments.

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