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Integrated approach to sediment pollution: A case study in the Gulf of Trieste

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The Gulf of Trieste is located at the northernmost part of the Adriatic Sea (Italy), and is a shallow embayment with an average depth of 17 m. The gulf is an interesting site for studies on anthropic impacts because it has been populated for at least the last 2000 years and activities such as marble quarrying and oyster culture have been practised for centuries; today, it houses several ports and an industrial zone which generate noticeable anthropogenic pressures. The gulf's peculiar geomorphologic and hydrologic conditions make it prone to the accumulation of pollutants, since it is an elongated, sheltered bay with reduced hydrodynamism (Solis-Weiss et al., 2004).

The Servola sewage disposal plant is the most important sewage plant of the city, serving up to 270,000 inhabitants. Its primary treatment has been based on chemical precipitation since 1992. The treated water is discharged via two adjacent submarine ducts (6.5 and 7.5 km) ending at 23 m depth with several diffusers (Novelli, 1996).

In many marine environments, benthic communities constitute the most effective tool for assessing environmental variations of any given habitat. This is because benthos, in contrast to nekton and plankton, remain in place and are thus subjected to different environmental stresses, and react to these synergetically without any escape possibility. The resulting composition of species, replacements, eliminations, diversity or abundance changes, can indicate the recent history of events affecting the area (Solis-Weiss et al., 2001).

The aim of this study was to assess the sediment quality near the outlet of the main wastewater pipeline of the city, applying an integrated approach which considered both the concentration of organic compounds, nutrients and pollutants, and the resulting biological responses.

Sediment samples were collected on 21st June 2004 ca. 1.5 m from the Servola underwater sewage duct (45°38.648′ N, 13°40.962′ E; Fig. 1) using an automatic a KC haps bottom corer (KC-Denmark) with a polycarbonate sample tube (13.3 cm ID; sample area 127 cm^2) and a 0.1 m^2 van Veen grab. At the time of sampling, photosynthetically available radiation (PAR) and sea water temperature were recorded *in situ* using a profiling natural fluorometer PNF-300A (Biospherical Instruments Inc.), 50 cm above the bottom. Macroalgae were totally absent at the sampling station. Sediment cores were sectioned into three layers (0–2 cm; 2–4 cm and 12–14 cm) to determine the following parameters: sediment texture, total organic carbon (TOC), total nitrogen (TN), total phosphorous (TP), labile organic matter (LOM), chl *a* and pheopigments, heavy metals (Al, Fe, Co, Cr, Cu, Hg, Ni, V, Pb, Zn, Ag and Cd), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides. Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) as well as nutrients were analysed in both the sediment pore water and overlying water. A quali-quantitative analysis of the microphytobenthic and macrobenthic communities was performed. Shannon diversity index (H') and the evenness index (J') were calculated for both benthic communities. Sediment toxicity was also assessed. On 21st June 2004 another sampling was carried out at the sublittoral station C1 (17 m depth) of the gulf. Since C1 is located near the marine reserve of Miramare (45°42.05′ N, 13°42.60′ E; Fig. 1) with minimal anthropogenic disturbance, it was used as a reference site for the following parameters: TOC, TN, TP, DOC, DIC, LOM, nutrients, chl *a* and pheopigments, primary production,

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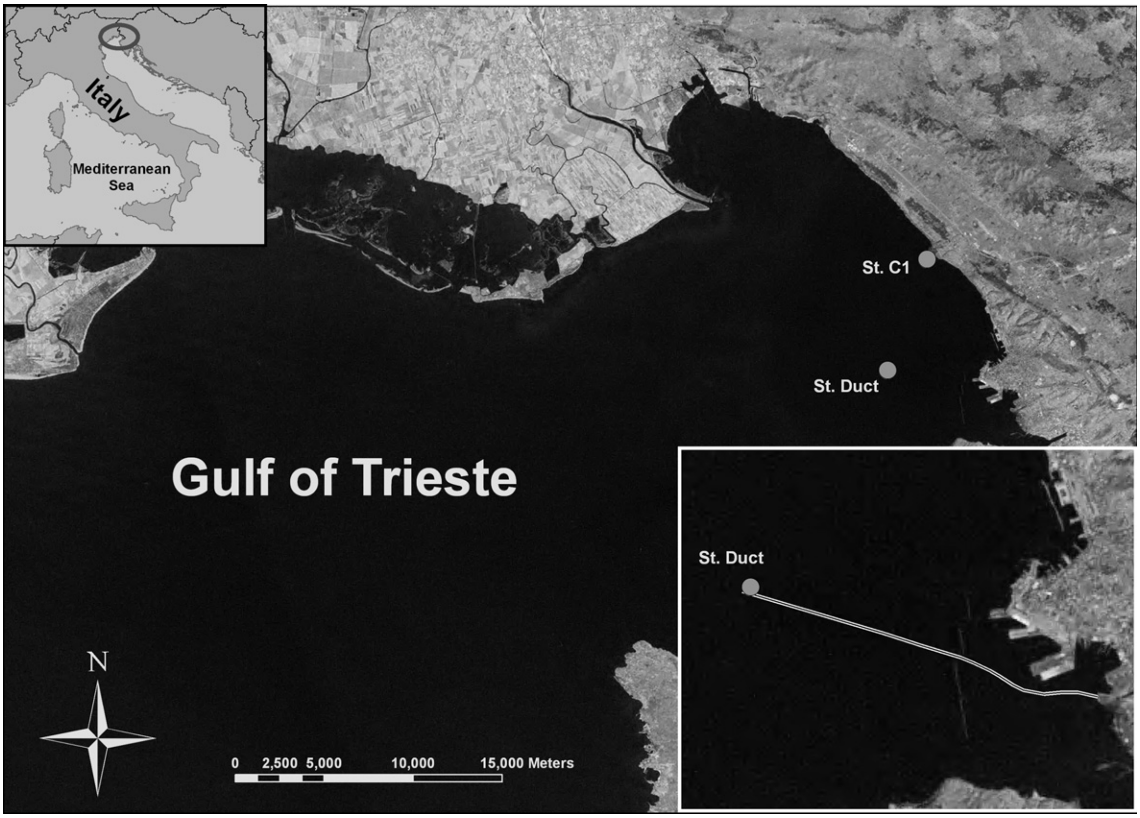

Fig. 1. Location of the sampling stations in the Gulf of Trieste.

microphyto- and macrobenthos. For data comparison, only the uppermost layer (0–1 cm) at station C1 was considered.

Samples for grain size analysis were pre-treated with 10% hydrogen peroxide and fractionated by wet sieving. The pelitic fraction (<0.63 μm) was filtered through Whatman50 paper filter; the filter was dried at 60 °C and weighed. The sand fraction (>0.63 μm) was dried at 105 °C and weighed.

TOC and TN were determined on freeze-dried and homogenized samples after acidification with 1 N HCl (Hedges and Stern, 1984) using a Perkin Elmer 2400 CHNS/O at combustion temperature of 950 °C calibrated with acetanilide. TP was extracted from dry sediment by ignition at high temperature (550 °C) and acidification with 1 N HCl for 24 h (Aspila et al., 1976). Analyses were conducted at room temperature on a five-channel Bran+Luebbe autoanalyzer 3 continuous flow analyzer (Bran+Luebbe, Norderstedt, Germany), using standard procedures (Bran+Luebbe, 2003 and references therein). Performance of the system was checked before and after sample analysis by running replicates of internal standards.

Lipids were analysed following the method proposed by Bligh and Dyer (1959) and modified for sediments. Proteins were extracted with NaOH (0.5 M) for 4 h and determined according to Hartree (1972). Water and EDTA extractable carbohydrates were analysed following the method proposed by Blasutto et al. (2005). Carbohydrates, proteins and lipids were converted into $\mu g\ C\ g^{-1}$ of dry sediment (Fichez, 1991). All determinations were carried out in triplicate, with standard deviation lower than 5%.

Sediment cores for DIC, DOC and nutrient analyses were extruded and sectioned in slices in a N_2-filled chamber after supernatant water collection. DIC and DOC analyses were performed using a Shimadzu TOC 5000A analyzer. For DIC, samples were injected into the IC instrument port and directly acidified with phosphoric acid (25%). For DOC analysis, water samples were previously acidified (6 M HCl, 200 μl) and after CO_2 elimination, concentration was determined using a high temperature catalytic method (Covelli et al., 2008). Analysis showed the variation coefficient <2%. The reproducibility of the method was between 1.5% and 3%.

Overlying and pore water for the determination of dissolved inorganic nutrient concentrations ($N-NO_2^-$, $N-NO_3^-$, $N-NH_4^+$, $P-HPO_4^{2-}$ and Si–Si$(OH)_4$) was sampled and filtered on Millipore HA filters (0.45 μm pore size). Analyses were performed at room temperature on a five-channel Bran+Luebbe autoanalyzer 3 continuous flow analyzer (Bran+Luebbe, Norderstedt, Germany), using standard procedures as described by Cibic et al. (2007a). Performance of the system was checked before and after sample analysis by running replicates of internal standards.

Heavy metals were determined on the pelitic fraction (<0.63 μm) obtained by wet sieving following the method proposed by Loring and Rantala (1992). Briefly, 0.2–0.3 g of dry sediment, obtained via liophylization (Lyphlock, Labconco) was digested with a mixture of HF and *aqua regia* in a closed microwave system (Multiwave 3000, Anton Paar). The obtained solutions were analysed by means of an atomic absorption spectrophotometer device (AAS, 5100PC Perkin Elmer). Quality control procedure was tested using a certified reference material (PACS-2, Marine Sediment, NRCC). The results were in good agreement with the certified values and within their uncertainties. Single analyses showed a variation coefficient lower than 4%. The degree of anthropogenic input was estimated with respect to natural values of the earth's crust. Al, which is considered a conservative element, was employed for geochemical normalization (Din, 1992). The metal enrichment factor (EF) was calculated as follows:

$$EF = \frac{(Me/Al)_{sample}}{(Me/Al)_{baseline}}$$

where $(\mathrm{Me/Al})_{\mathrm{sample}}$ is the ratio between the potentially enriched metal and Al of the sample and $(\mathrm{Me/Al})_{\mathrm{baseline}}$ is the ratio obtained applying natural background values.

Zhang and Liu (2002) hypothesized that if the EF value lies between 0.5 and 1.5, the trace metal may be derived from the crust materials, while a value greater than 1.5 could be correlated to other sources (point and non-point or biotic). Due to the lack of metal background values for our study area, we used those reported by Wedepohl (1995). The index of geoaccumulation (I_{geo}) defined by Müller (1981) was also considered and calculated as follows:

$$I_{\mathrm{geo}} = \frac{\log_2 C_n}{1.5 B_n}$$

where C_n is the measured concentration of the n element in the sediment (or size fraction) and B_n is the background concentration of the n element, which could be directly measured in texturally equivalent uncontaminated sediments (or size fraction) or taken from the literature.

The factor 1.5 is introduced to take into consideration possible differences in the background values due to lithological variation. I_{geo} assesses the degree of metal pollution defining seven classes of sediment quality (Müller, 1981; Table 1).

PCBs, organochlorine pesticides and PAHs were extracted from ca. 10 g of freeze-dried sediment by pressurized solvent extraction (PSE one, applied separations), using dichloromethane and acetone (1:1 v/v) following the EPA Method 3545 (1995). After extraction, the solvent was evaporated and sulfur was eliminated using tetrabutylammonium sulfite (EPA Method 3660, 1996). The extracts for PCBs and organochlorine pesticides determination were purified by passage through a Florisil column, sequentially eluted with *n*-hexane, *n*-hexane:toluene (80:20) and *n*-hexane:toluene:ethyl acetate (80:19:1). The determination was performed using a Perkin Elmer autosystem XL gas chromatograph equipped with an ECD detector and an HT8 capillary column (SGE) (30 m, 0.25 mm ID, 0.25 μm film thickness).

The extracts for PAH determination were purified by passing them through a silica gel column with cyclohexane (EPA Method 3630, 1996) and then analysed using a Hewlett–Packard 6890 gas chromatograph equipped with a Hewlett–Packard 5973 mass selective detector. The separation was performed using a HP5-MS crosslinked 5% phenyl-methyl silicone capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness). The quantification was achieved working in selected ion monitoring (SIM) mode. PAHs were identified and quantified by comparison with known standards injected under the same conditions. The recoveries, calculated by analysing blank samples spiked with known amounts of PAHs, ranged from 58% to 91%.

Table 1
Müller's geoaccumulation index defining seven classes of sediment quality (Müller, 1981)

I_{geo} value	Class	Quality of sediment
⩽0	0	Unpolluted
0–1	1	From unpolluted to moderately polluted
1–2	2	Moderately polluted
2–3	3	From moderately to strongly polluted
3–4	4	Strongly polluted
4–5	5	From strongly to extremely polluted
>5	6	Extremely polluted

To assess sediment toxicity two bioassays were used as follows: the decrease of bacterial bioluminescence in *Vibrio fischeri* (Microtox®) on solid phase, pore water and elutriate (Azur Environmental, 1994); and the inhibition of growth of *Dunaliella tertiolecta*, a microalgal flagellate, using both pore water and elutriate (Walsh and Merril, 1984 modified by ARPAT-CEDIF, 1998). For the Microtox® assay pore water was obtained by centrifugation (4000*g* for 30 min at 4 °C) and then filtered using 0.45 μm nitrate cellulose. As the solid-phase test is subject to several interferences related to matrix variability, the results of this test were normalized to the pelitic fraction of samples and expressed as sediment toxicity index (STI) (Onorati et al., 1999). STI was calculated as follows:

$$\mathrm{STI} = \frac{\text{TU of the sample}}{\text{natural TU}}$$

where TU is the toxic unit of the sample, expressed as dry mass, calculated dividing the EC_{50} (dry mass) by 100 (i.e., TU = $\mathrm{EC}_{50}/100$) and natural TU is the threshold of the natural toxicity estimated by the equation: $0.28 + 3.49x$ (where x is the percentage of pelite in the sample).

The test with *D. tertiolecta* was based on five concentrations and three replicates. The end point algal biomass was estimated after 72 h by measuring the abundance in each test and control. Although the end point was estimated after only 72 h, this test is to be considered a subchronic test (Walsh and Merril, 1984) since during this period *D. tertiolecta* reproduces several times. Cell growth was measured directly by means of an electronic particle counter (Coulter Multisizer II). All algal tests were conducted at 20 ± 1 °C under continuous illumination (3600 lux).

Chl *a* and pheopigments were estimated in the uppermost layer (Lorenzen and Jeffrey, 1980). In the surface sediment (0–1 cm), microphytobenthic abundance (ABU) and biomass (BIOM) were estimated following the method proposed by Cibic et al. (2007a). BIOM, expressed as $\mu\mathrm{g\,C\,cm^{-3}}$, was obtained multiplying ABU (cell $\mathrm{cm^{-3}}$) by the carbon content of each counted cell. The carbon content, in turn, was obtained from biovolume estimates. Benthic primary production (PP) was estimated *in situ* by the $^{14}\mathrm{C}$ tracer method (Cibic et al., 2008).

Macrobenthos was sampled with a 0.1 $\mathrm{m^2}$ van Veen grab; three grabs were taken, sieved through a 0.5 mm sieve and preserved in a 4% buffered formaldehyde solution containing Rose Bengal stain. Macrobenthic organisms were later sorted and identified to species or higher taxon (Rees et al., 1990).

Univariate diversity analysis was performed on the microphytobenthic and macrobenthic communities using PRIMER-5 software (PRIMER-E Ltd, Plymouth, UK), considering diversity index (H') (Shannon and Weaver, 1949) and equitability index (J') (Pielou, 1966).

The sediment, prevalently pelitic (<6% of sand), was characterised by large amounts of organic matter. TOC, TN, TP and LOM values are reported in Table 2. TOC and TN (24.1 and 3.6 $\mathrm{mg\,g^{-1}}$, respectively) were almost double compared to the reference station (13.6 and 1.9 $\mathrm{mg\,g^{-1}}$, respectively) and also higher than that reported by Covelli et al. (1999). TP was 1.5 times higher than that measured at a similar depth of the Gulf of Trieste (Falconi, personal communication). At the surface layers LOM was largely composed of proteins (65%). These were twice higher at the impacted site (3175 $\mu\mathrm{g\,C\,g^{-1}}$) than at the reference station (1162 $\mu\mathrm{g\,C\,g^{-1}}$) while the lipid content was even three times higher.

In pore water, mineralization of the organic matter was reflected in high DOC concentration (21.93 $\mathrm{mg\,l^{-1}}$), much higher than that at station C1 (4.53 $\mathrm{mg\,l^{-1}}$). High nutrient concentrations were obtained in both the overlying and the pore water (Table 3). In particular, $\mathrm{P-HPO_4^{2-}}$ in the overlying water was one order of magnitude higher than at the reference station. Also $\mathrm{N-NH_4^+}$ and $\mathrm{Si–Si(HO)_4}$ were almost four times and twice higher, respectively, than those measured at station C1.

Heavy metal values are reported in Table 4. Co, Cr and Ni showed relatively uniform content within the three layers. On

Table 2
Total organic carbon, total nitrogen, total phosphorous and labile organic matter analysed in the sediment nearby the sewage duct

Station	Sediment layer (cm)	TOC		TN		C/N	TP	CHO_{H_2O}		CHO_{EDTA}		Lipids		Proteins		LOM	Proteins (%)
		Mean	SD	Mean	SD			Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Duct	0–2	24.1	0.4	3.6	0.1	7.8	757	106.4	2.4	524.8	2.3	1274.8	12.2	3175.1	10.3	5081.1	62.5
	2–4	15.3	0.9	2.0	0.2	8.9	505	29.3	1.6	434.6	10.8	771.0	7.7	2513.3	25.2	3748.2	67.1
	12–14	11.0	0.2	1.0	0.1	12.8	430	3.4	0.0	476.5	4.3	469.8	4.1	525.1	12.2	1474.8	35.6
C1	0–1	13.6	0.3	1.9	0.1	8.2	NA	42.2	0.2	327.1	10.5	404.2	1.6	1162.0	16.6	1935.5	60.0

Three sediment layers were analysed at station Duct. Data from the uppermost layer at station C1 were considered for comparison. TOC and TN are expressed as percentage, TP is expressed in mg kg^{-1}, carbohydrates (water and EDTA extractable), lipids, proteins and total LOM are expressed in μg C $g^{-1}$of dry sediment. NA denotes not available.

Table 3
Dissolved organic and inorganic carbon and nutrients, measured both at the sewage duct and at the reference station

Station	Sediment layer	DOC		DIC	$N - NH_4^+$	$N - NO_2^-$	$N - NO_3^-$	$P - HPO_4^{2-}$	$Si–Si(HO)_4$
		Mean	SD						
Duct	Overlying water	4.56	0.06	33.31	31.92	0.12	1.20	2.50	9.15
	0–2 cm	21.93	0.15	34.61	65.47	0.10	1.93	6.64	14.92
	2–4 cm	9.73	0.16	32.85	56.46	0.06	2.13	8.49	35.83
	12–14 cm	4.66	0.04	43.22	125.09	0.15	3.06	10.62	72.91
C1	Overlying water	4.53	0.02	30.80	8.42	0.24	4.23	0.25	5.07

Data from the overlying water at station C1 were considered for comparison. DOC and DIC are expressed in mg l^{-1}, nutrients are expressed in μM.

Table 4
Analysed metals at the sewage duct. Al and Fe are expressed as percentage; heavy metals are expressed in μg g^{-1}

Sediment layer (cm)	Al	Fe	Co	Cr	Cu	Hg	Ni	V	Pb	Zn	Ag	Cd
0–2	6.84	3.44	14.0	133.8	40.0	0.8	75.9	175.0	79.7	208.0	2.8	0.5
2–4	6.94	3.57	15.0	132.6	27.6	0.6	89.0	161.4	63.2	145.0	0.6	0.3
12–14	7.37	3.66	16.7	153.4	14.1	0.2	89.4	136.5	18.2	109.0	<0.1	0.2

the contrary, Cu, Hg, V, Pb, Zn, Ag and Cd exhibited a clear decreasing trend with depth. Our EF values suggest that only Co and Cu could be considered of natural origin. On the other hand, all the other metals showed high EF values (ranging from 3.3 for Zn to 57.6 for Ag; Table 5) suggesting their anthropogenic origin. However, Cr, Pb and Ni obtained from EF calculations are probably overestimated due to the high background values of these metals in our study site. In fact, previous studies performed on sediment cores collected from different sites in the Gulf of Trieste showed similar values (Covelli and Fontolan, 1997). If high contents of both Pb and Ni have never been justified, it is likely that high Cr content originates from the heavy mineral fraction which in turn derives from flysh (chromite and Cr-bearing spinels) (Lenaz et al., 1996). In Table 5, are shown the classes as distinguished from I_{geo} values proposed by Müller (1981). The results suggest that among all heavy metals only Hg ($I_{geo} = 2.87$) and Ag ($I_{geo} = 10.22$) may be classified as "moderately to strongly polluted" and "extremely polluted", respectively, whereas the other elements are classified as "unpolluted to moderately polluted". The Gulf of Trieste is influenced by the Hg polluted river Soča/Isonzo which drains the cinnabar-rich deposits of the Idrija mining district in the northwestern part of Slovenia (Horvat et al., 1999). This feature explains the high Hg values, whereas the massive presence of Ag is probably due to the continuous discharge of photographic materials through local urban runoff.

Table 5
The metal enrichment factor (EF) values and the geoaccumulation (I_{geo}) index values with the corresponding classes of metal pollution

Metal	EF	I_{geo}	Class
Al	1.0	0.18	0
Fe	1.3	0.22	0
Co	1.6	0.28	0
Cr	4.3	0.77	1
Cu	1.8	0.32	0
Hg	16.2	2.87	2
Ni	4.3	0.76	0
V	3.7	0.66	0
Pb	5.3	0.94	0
Zn	3.3	0.59	0
Ag	57.6	10.22	6
Cd	5.5	0.98	0

Both indices are calculated from surface values.

Seven PCBs and 13 organochlorine pesticides were analysed (Table 6). The seven monitored PCBs are those requested by the Italian ministry of the environment for the seasonal sediment monitoring plan. Among the analysed PCBs, PCB 153 and PCB 138 were present at low concentrations, whereas the other ones were below the detection limit. While PCB 153 and PCB 138 had comparable concentrations in the uppermost layers, they showed lower concentrations in the bottom layer. Among the thirteen organochlorine pesticides, only p,p′-DDE was not below the detection limit. It showed a decreasing concentration with depth, ranging from 2.1 ng g^{-1} in the surface sediment to 0.2 ng g^{-1} in the bottom layer.

The concentrations of the 16 parent PAHs are presented in Table 7. Total PAHs showed comparable concentrations in both the surface and subsurface layer (661.1 and 553.4 ng g^{-1}, respectively), whereas concentrations considerably decreased in the deepest layer (101.3 ng g^{-1}). The four-ring PAHs (fluoranthene, pyrene, chrysene and benzo[a]anthracene) were the most abundant fraction in all three layers. Total PAH concentration in the surface sediment is comparable with that found by Notar et al. (2001) in a nearby sampling site (station A3, 604.8 ng g^{-1}, considering the

Table 6
Data expressed in ng g^{-1}

Sediment layer (cm)	PCB 52	PCB 81	PCB 77	PCB 153	PCB 138	PCB 128	PCB 169	α-HCH	β-HCH	γ-HCH
0–2	<LOD	<LOD	<LOD	2.6	1.9	<LOD	<LOD	<LOD	<LOD	<LOD
2–4	<LOD	<LOD	<LOD	2.6	1.8	<LOD	<LOD	<LOD	<LOD	<LOD
12–14	<LOD	<LOD	<LOD	0.6	0.4	<LOD	<LOD	<LOD	<LOD	<LOD
	δ-HCH	Aldrin	Dieldrin	HCB	o,p′-DDT	p,p′-DDT	o,p′-DDD	p,p′-DDD	o,p′-DDE	p,p′-DDE
0–2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.1
2–4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.6
12–14	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.2

LOD (limit of detection) = 0.1 ng g^{-1}. Abbreviations: α-HCH, β-HCH, γ-HCH and δ-HCH are α-, β-, γ- and δ-isomers of hexachlorocyclohexane and HCB is hexachlorobenzene.

Table 7
Data expressed in ng g^{-1}

Sediment layer (cm)	Na	Ace	Acen	Fl	Phe	An	Flu	Py	Chry	
0–2	2.6	0.4	3.2	3.0	30.4	8.1	85.3	94.0	59.0	
2–4	0.6	0.2	0.8	1.1	28.2	6.8	80.8	84.7	51.3	
12–14	1.7	0.1	0.1	0.3	5.1	2.4	12.9	12.5	8.9	
	B[a]An	B[b]Flu	B[k]Flu	B[a]Py	IndPy	Db[a,h]An	B[g,h,i]Per	Total	Phe/An	Flu/Py
0–2	69.5	63.0	55.2	65.7	71.5	20.6	29.8	661.1	3.8	0.9
2–4	60.0	64.4	31.4	60.3	49.1	9.9	23.9	553.4	4.2	1.0
12–14	10.3	12.5	7.9	9.3	7.6	7.1	2.5	101.3	2.1	1.0

Phe/An and Flu/Py are adimensional ratios. Abbreviations: Na is naphthalene; Ace is acenaphthylene; Acen is acenaphthene; Fl is fluorene; Phe is phenanthrene; Flu is fluoranthene; Py is pyrene; Chry is chrysene; B[a]An is b[a]anthracene; B[b]Flu is benzo[b]fluoranthene; B[k]Flu is benzo[k]fluoranthene; B[a]Py is benzo[a]pyrene; IndPy is Indeno[1,2,3-c,d]pyrene; Db[a,h]An is dibenzo[a,h]anthracene and B[g,h,i]Per is benzo[g,h,i]perylene.

sum of the same PAHs). According to the classification proposed by those authors, our sediment can be classified as "relatively highly contaminated" (total PAHs > 500 ng g^{-1}). Calculating the ratios between PAHs parent compounds it is possible to estimate the contamination source (Benlahcen et al., 1997; Notar et al., 2001). The combined values of Phe/Ant < 10 and Flu/Py > 1 indicate that the contamination is of pyrolitic origin. Applying these ratios to our data, we found that while Phe/Ant is <10 in all three layers, Flu/Py is >1 only in the deepest layer (1.04), indicating a possible combustion source. On the contrary, in the surface and subsurface layers, our Flu/Py ratios are slightly <1 (Flu/Py = 0.91 in the surface; Flu/Py = 0.95 in the subsurface) indicating an unclear origin.

Microtox® assays on pore water and elutriate are expressed as percentage of inhibition of *V. fischeri* bioluminescence. According to the index proposed by Volpi Ghirardini et al. (1995), based on toxicity classes, our sediment was moderately toxic (Table 8). Pore water and elutriate are important matrixes where hydrophilic contaminants are dissolved and released in the water column becoming bioavailable. In contrast, Microtox® bioassays on solid phase showed low toxicity, probably due to the lower bioavailability of organic contaminants like PAHs and PCBs. In fact, these compounds remain strongly attached to sediment particles because of their hydrophobic characteristics. The *D. tertiolecta* test showed in both matrixes a stimulating effect in respect to the control (Table 8). The toxicity effect was probably hidden by the high nutrient concentration that caused an increase of the algal growth. The results of the sediment toxicity tests were comparable to those obtained in other sites of the gulf (Tamberlich et al., 2007).

In an oligotrophic system such as the Gulf of Trieste (Cibic et al., 2007a), some organic matter input could favour local benthic communities. Chl *a* content, ABU and BIOM were higher at the sewage duct than those obtained at the reference station (Table 9). Although the biomass value at the sewage duct was threefold the value at the reference station, chl *a* content did not show such a difference between the two stations. This could be due to the fact that chl *a* as biomass estimator has some limitations because of the carbon: chl *a* ratio variability, leading to biomass underestimates (Cibic et al., 2007b).

The primary production value confirmed the presence of a photosynthetically active microphytobenthic community at the sewage duct (Table 9). The value estimated at the wastewater pipeline (4.9 mg C m^{-2} h^{-1}) was higher than that estimated at the reference station (2.6 mg C m^{-2} h^{-1}), in spite of the major light availability at station C1 (145 μE m^{-2} s^{-1}) compared to the sewage duct (57 μE m^{-2} s^{-1}). A high degree of autotrophy may function to a certain degree as a buffering mechanism, mitigating the overall response of illuminated sediments (Sundbäck et al., 2007). Yet, in a recent study performed in the Gulf of Trieste at a sublittoral

Table 8
Sediment toxicity bioassays based on the decrease of bacterial bioluminescence in *V. fischeri* and on the inhibition of growth of *D. tertiolecta*

Matrix	Bioassay	Inhibition (%)	EC_{50}	(95%) Confidence limits	TU_{50}	(95%) Confidence limits	R^2	STI	Toxicity class	Designation of toxicity (toxicity classes)
Elutriate	*V. fischeri*	28.36							2	Moderately toxic
Pore water	*V. fischeri*	23.85							2	Moderately toxic
Solid phase	*V. fischeri*	98.47	0.181	0.132–0.25	551.1	415.2–731.7	0.97	2.822	1	Low toxicity
Elutriate	*D. tertiolecta*	−102.26							−1	Stimulation
Pore water	*D. tertiolecta*	−127.16							−1	Stimulation

For details see the text.

Table 9
PAR irradiance and temperature measured in the overlying water at the bottom during sampling nearby the two sampling stations

Station	PAR (μE m^{-2} s^{-1})	Temperature (°C)	Chl *a*		Pheo		ABU		BIOM (mg C cm^{-3})	PP		Macrobenthic abundance (ind. m^{-2})	Number of macrobenthic taxa
			Mean (μg g^{-1})	SD	Mean (μg g^{-1})	SD	Mean (cell cm^{-3})	SD		Mean (mg C m^{-2} h^{-1})	SD		
Duct	56.9	15.7	5.5	0.6	54.5	7.1	121806	4714	39.1	4.9	0.3	3673	57
C1	145.1	16.9	4.7	0.1	8.4	0.3	55954	6385	13.0	2.6	0.1	1091	64

Abbreviations: chl *a* is chlorophyll *a*, pheo is pheopigments, ABU is microphytobenthic abundance, BIOM is microphytobenthic biomass, and PP is primary production. Chl *a*, pheo, ABU, BIOM and PP were estimated at the surface layer.

Table 10
Dominant taxa representing the microphytobenthic and macrobenthic communities at the sewage duct

Microphytobenthos (diatom genera)	RA	CA	Macrobenthos (taxa)	RA	CA
Navicula	69.1	69.1	Polychaetes	93.4	93.4
Diploneis	9.5	78.6	Mollusks	3.5	96.9
Nitzschia	7.7	86.3	Echinoderms	0.8	97.7
Paralia	3.6	89.9	Crustaceans	0.7	98.4

RA is relative abundance (%) and CA is cumulative abundance (%).

station (21 m) we found that the percentage of PAR at the bottom fell below 1% from September to February (unpublished results). For this reason, we infer that at this depth the buffering mechanism induced by the benthic photoautotrophs may be restricted to the spring and summer period.

The microphytobenthic community was mainly composed of Bacillariophyceae (92.1%), with a total of 18 genera and 29 species. Shannon diversity index ($H' = 2.034$) and the evenness index ($J' = 0.567$) showed lower biodiversity and higher dominance when compared to the reference station ($H' = 2.891$ and $J' = 0.808$, respectively). The genus *Navicula* dominated, accounting for 69.1% of the total benthic diatoms (Table 10). *Navicula directa* alone accounted for 51.5% of the total diatom abundance and 60.9% of the total biomass. Benthic diatom biomass is stimulated by anthropogenic N load (Mallin et al., 2007). In a laboratory experiment, Sundbäck and Snoeijs (1991) demonstrated that N and P addition favours larger cells. Agatz et al. (1999) reported that at a strongly eutrophic site the original diatom community had almost been displaced by a monoculture of *Navicula gregaria*. The authors identified a nutrient-loving assemblage comprising *N. gregaria*, *Nitzschia sigma* and *Nitzschia tryblionella*. Also, our floristic list comprised *N. tryblionella* and *N. sigma* with a relative abundance of 1.9 and 0.5%, respectively. As a genus, *Navicula* is considered to be highly tolerant of pollution (Palmer, 1969). Moreno-Garrido et al. (2007) exposed three diatoms to different levels of pollutants and found that *Navicula* sp. was the least sensitive among the species used. Cunningham et al. (2005) previously reported that *N. directa* occurred at higher relative abundances in sites contaminated by heavy metals, suggesting that this species may be metal tolerant. Metal tolerance would enable this species to compete more effectively and capitalize on the reduced presence of pollution sensitive species, thus increasing in abundance. There is still little information concerning the combined effects of pollutants-nutrients on diatoms. The few existing studies on combined nutrient-stressor effects have generally found that nutrient-rich systems are less sensitive to stressors (Sundbäck et al., 2007 and references therein). Increased nutrient load may decrease the toxicant effect due to high nutrient status of organisms and dilution of the toxicant in increasing amounts of biomass (Sundbäck et al., 2007). Previously, Agatz et al. (1999) found that the dependence of diatoms upon nutrient concentrations is predominant. We surmise that the toxic effect that might have been induced by heavy metals and organic pollutants on benthic diatoms in our study site was hidden by the stimulating effect of the nutrient load. Benthic diatom mats are rich in extracellular polymeric substances which may function as a protective barrier against toxic compounds, as well as enhance the uptake of favourable ones. This feature could decrease the vulnerability of microbial mats to toxic compounds (Sundbäck et al., 2007 and references therein).

Nearby the sewage duct, 57 macrofaunal taxa were observed with a total abundance of 3673 ind. m^{-2} (Table 9). Polychaetes were the richest group (27 taxa) followed by molluscs (14 taxa), crustaceans (6 taxa) and echinoderms (4 taxa) (Table 10). The dominant species was the polychaete *Capitella capitata*, known worldwide as an opportunistic species, with 2983 ind. m^{-2} accounting for 81% of the total abundance. The peak of this species pointed out the presence of disturbed conditions, as well as the values of the H' and J' indices (1.156 and 0.286, respectively). Nevertheless, the presence of echinoderms underlined a medium impact of the area, because this taxon is, generally, the first one to disappear with a strong organic enrichment or in the presence of toxic substances (Lenihan et al., 2003). The total macrofaunal abundance at the pipeline was three times higher than that observed on the same sampling date at station C1 (1091 ind. m^{-2}; Table 9). In contrast, the number of taxa nearby the underwater duct was slightly lower than at the reference station (57 against 64 taxa). In conditions of moderate organic enrichment, an increase in the number of species can occur (Solis-Weiss et al., 2007), while in conditions of high organic load a decrease in species diversity is expected (Simonini et al., 2004). Macrobenthic communities often respond to moderate nutrient loading and organic matter content by an increase in abundance (Pearson and Rosenberg, 1978). Under excessive nutrient loads the sedimentation of organic matter may exceed the rate of its degradation; bottom oxygen can be exhausted and alter macrofaunal communities (Taylor et al., 1998). In fact, until 1993, when the Servola sewage treatment plant became operative (Novelli, 1996), the relative abundance of *C. capitata* at the underwater pipeline was negligible (Solis-Weiss et al., 2007).

In this study, we could not apply the sediment quality triad approach (SQT), which has been widely used in pollution monitoring, for the lack of site replications. Moreover, we did not have the same reference site for all abiotic and biotic parameters. In the present paper we integrated the chemical, toxicological and biological approach attempting to give a general overview of the sediment quality nearby the sewage duct. Differently from SQT, in which macrobenthos is commonly considered as the only biological community, we also introduced the microphytobenthic community as an additional biological indicator of sediment quality. Our results suggest that not only macrobenthos but also marine benthic diatoms can be good indicators of environmental alteration. Although high concentrations of some heavy metals, PCBs and total PAHs were detected at the sewage duct, overall did not appear to influence either the microphytobenthos or the macrobenthos, probably due to moderate organic matter and nutrient inputs which favoured both communities.

In this preliminary survey only one sampling site near the sewage outfall and one reference site were considered. Nevertheless, marked differences between the two stations were highlighted. Consequently, in the next sediment monitoring plan, a more extensive area nearby the sewage duct will be investigated, allowing us to gain information on the variability along a pollution gradient. The present collected data can constitute a baseline for future studies.

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Trace metal concentrations in the aquatic environment of Albay Gulf in the Philippines after a reported mine tailings spill

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The mine tailings pond of a gold mining company in Rapu-rapu Island in the Philippines overflowed and spilled into Albay Gulf during a storm in October 11 and 13, 2005. The fish kill that resulted from the contamination of the coastal water with cyanide and a reported mercury contamination of the fish caught in Albay Gulf, caused a scare among the people of Sorsogon, a province along the coast of Albay Gulf. The people of Sorsogon avoided consuming fish for months after the spill because of the mercury scare; and this caused severe economic losses to fishermen who comprise the majority of the population in the province. A survey of the trace metals in water, sediments and fish collected in Rapu-rapu Island and in the surrounding coastal areas in Sorsogon was conducted in January 2006. The survey was done primarily to determine the mercury contamination in fish caught in the gulf. The mine in Rapu-rapu Island consists of sulphide deposits of gold and copper in the forms of pyrite, chalcopyrite and sphalerites (Watt, 2003). The metals cadmium, lead, arsenic and mercury have good affinity with sulfidic ores (Nash, 2002); it is possible that the mine tailings from the gold and copper mining operations in Rapu-rapu may contain traces of cadmium, lead, arsenic and mercury. Arsenic and cadmium have been proven to be carcinogenic in humans while lead and mercury have multiple adverse effects on the nervous system, renal system and reproductive systems in humans (Goyer and Clarkson, 2001). In addition, these trace metals, if present in the aquatic environment, can bioaccumulate in the fish and can contaminate humans through the food chain (Goyer and Clarkson, 2001). For these reasons, the concentrations of arsenic, cadmium, lead and mercury in water, sediments and some biota in Albay Gulf were also investigated.

Water, sediment, fish and shellfish samples collected in 33 selected coastal sites were analyzed for arsenic, cadmium, copper, lead and mercury. Sampling sites were selected in Rapu-rapu Island near the existing mine site and the abandoned mine site and in creeks and rivers in the island that may have been affected by present and previous mining activities. In Albay Gulf, sampling sites were selected along the coast of Rapu-rapu Island, offshore of Albay Gulf and along the coast of Sorsogon province. Samples were also taken in rivers and creeks in Sorsogon to determine possible sources of trace metals from Sorsogon. The details of the sampling sites and sampling conditions are listed in Table 1.

Water samples were collected by dipping an acid-washed plastic sample bottle about 8 cm below the water surface. Most of the sediment samples were collected using an Eckmann Dredge and in sites where the dredge could not be used, the sediment was collected by divers by hand using a plastic scoop and plastic bag. The fish and shrimp samples and small shells were obtained from fishermen who provided information where the organisms were caught. The cuttlefish and helmet shell from the site near the abandoned Hixbar Mine were collected by hand and net by divers.

Standard methods for analysis of metals in water using atomic absorption spectrometry (AAS) were applied for arsenic, copper and mercury (APHA, 2005). For mercury analysis, the water sample (100 ml) was allowed to react with concentrated H_2SO_4, concentrated HNO_3 and 5% $KMnO_4$ solution at room temperature for 15 min. The sample was digested at 95 °C for 2 h in a water bath after addition of 5% $K_2S_2O_8$ solution. The excess $KMnO_4$ was removed with sodium chloride-hydroxylamine solution and the mercury vapor released from the solution after addition of $SnCl_2$ solution (10% solution in 0.5 N H_2SO_4) was determined in a cold vapor set-up of the AAS. For the analysis of copper, cadmium and lead, the water sample (100 ml) was digested with concentrated HNO_3 on a hotplate to almost dryness; the residue was dissolved in concentrated HNO_3 and HCl (1 + 1), filtered and diluted to volume (100 ml) with ultrapure water. The copper concentration in the solution was determined by Flame AAS; the cadmium and lead concentrations were determined in 20 ml aliquot of the solution by anodic stripping voltammetry with a rotating glass electrode (APHA, 2005). For arsenic analysis, either 10 ml or 20 ml water sample was digested on a hotplate with 1.25 M H_2SO_4 and 5% $K_2S_2O_8$ until the volume was reduced to 10 ml. After addition of 1 M KI, the solution was transferred to the hydride generator reaction flask containing 4% $NaBH_4$ and the arsenic concentration was determined in the AAS by hydride generation. All water samples were analyzed in duplicate trials.

The US EPA methods for sampling and acid extraction for metals in sediments (US EPA, 1994; US EPA, 1996) were applied to the sediment samples. The samples were air dried, homogenized and sieved (US standard sieve no. 10) before digestion with acid. For the analysis of cadmium, copper and lead in sediments, 1 g sediment sample was digested with concentrated HNO_3 and 30% H_2O_2 on a hotplate. The sample was further digested with concentrated HCl. The digested sample was filtered and diluted to a final volume (100 ml) with ultrapure water before determination of the metals using Flame AAS. For arsenic analysis, the sediment was digested with concentrated HNO_3 and concentrated HCl on a hotplate. The digested sample was filtered and collected in a 100 ml volumetric flask. After washing, the filter and residue were further

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