Integrated characterization and risk management of marine sediments: The case study of the industrialized Bagnoli area (Naples, Italy)

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Abstract

The aim of the present work is to demonstrate the practical importance of a multidisciplinary approach and weighted criteria to synthesize and integrate different typologies of data (or lines of evidence, LOEs), including chemical levels in marine sediments, their bioavailability to specific indicator species, ecotoxicological effects measured through subcellular biomarkers and batteries of bioassays, and potential impacts of pollution on local benthic communities. The area of Bagnoli (Gulf of Naples, Southern Italy) was selected as a model case-study, as it is a coastal area chronically impacted by massive industrial contamination (trace metals and hydrocarbons), and dismissed decades ago without any subsequent remediation or habitat restoration. The results of each LOE were elaborated to provide specific hazard indices before their overall integration in a weight of evidence (WOE) evaluation. Levels of some trace metals and PAHs revealed a severe contamination in the entire study area. Bioavailability of hydrocarbons was evident particularly for high molecular weight PAHs, which also caused significant variations of cellular biomarkers, such as cytochrome P450 metabolization in fish, lysosomal membrane destabilization in mussels,

genotoxic effects both in fish and molluscs. The results of a battery of bioassays indicated less marked responses compared to those obtained from chemical and biomarkers analyses, with acute toxicity still present in sediments close to the source of contamination. The analysis of benthic assemblages showed limited evidence of impact in the whole area, indicating a good functioning of local ecosystems at chronic contamination. Overall, the results of this study confirm the need of combining chemical and biological data, the quantitative characterization of various typologies of hazard and the importance of assessing an integrated environmental WOE risk, to orientate specific and scientifically-supported management options in industrialized areas.

1. Introduction

Since the first chemical factory built in 1854, the Bagnoli-Coroglio industrial area (Gulf of Naples, Italy) rapidly become a key site for Italian economic growth, with several industrial plants producing steel, cement and asbestos, using fossil coal, iron ores and limestone as raw materials, transported by vessels and processed on site. In the mid-80s, the environmental risk of such activities was recognized, leading to a progressive dismantling of the industrial area, which ended in the mid-90s. However, the drastic impact of industrial activities was never remediated after the plant dismission, with extremely high concentrations of polycyclic aromatic hydrocarbons (PAHs) and trace metals in sediments, especially close to the piers of the plant (Romano et al. 2004).

Nowadays it is widely recognized that the impact of chemical pollution should be evaluated by giving increasing importance to the assessment of biological effects of contaminants, and using an integrated approach with chemical data. The first example of integrated assessment was the Sediment Quality Triad (SQT), which considered chemical analyses, ecotoxicological testing and benthic communities as different Lines of Evidence (LOEs) to describe environmental quality of marine sediments (Chapman, 2007). Integrated strategies have also been proposed by various

63 international agencies, e.g. OSPAR, HELCOM, MEDPOL, ICES. An important advantage of
64 these approaches is the added interpretative value derived from the integration of multiple
65 typologies of studies, thus improving our ability to describe and interpret variations of
66 environmental conditions (Regoli et al. 2019). The chemical approach by itself does not provide
67 information on real bioavailability and biological risk of measured pollutants, often resulting in
68 overestimated and costly management decisions (Bradham et al. 2006). Ecotoxicological batteries
69 of bioassays have progressively been applied to quantify the potential biological hazard caused by
70 bioavailable multi-factorial contamination, thus providing a more relevant response not restricted
71 by a predetermined list of contaminants (Volpi Ghirardini et al. 2005). The benthic studies add
72 information on the functioning of local communities and Ecological Quality Status Descriptors
73 have been developed from these results (Dauvin 2015, Borja et al. 2016). In recent years,
74 additional LOEs have been integrated in a weight of evidence (WOE) framework, such as
75 bioaccumulation and biomarkers investigations. The bioaccumulation LOE quantifies the
76 bioavailable fraction of contaminants, which can be transferred to aquatic organisms, being
77 responsible for potential onset of adverse effects. Biomarkers reflect sub-lethal alterations at
78 molecular and cellular level, representing a sensitive and early warning method to better
79 understand the toxic effects and mechanism of action of environmental contaminants (Regoli &
80 Giuliani 2014, Benedetti et al. 2015, Regoli et al. 2019). The WOE integration of chemical
81 analysis, bioaccumulation, biomarkers, bioassays and analysis of benthic communities provides a
82 more robust basis for environmental control and management in respect to the first SQT. In recent
83 years this approach was synthesized in a quantitative model (Sediqualsoft), validated in several
84 case studies for environmental risk assessment associated with polluted sediments, harbor areas,
85 or complex natural and anthropic impacts on the marine environment (Piva et al. 2011, Benedetti
86 et al. 2012, 2014, Regoli et al. 2014, 2019, Bebianno et al. 2015, Mestre et al. 2017, Pittura et al.
87 2018). In Sediqualsoft different LOEs are independently elaborated, using specific criteria for
88 each data, which weight typology of chemical pollutants and toxicological relevance of measured

89 endpoints, as well as the number and magnitude of observed variations normalized toward
90 specific thresholds. Synthetic and quantitative hazard indices are calculated for each LOE, before
91 their overall integration in the WOE assessment: the calculated level of risk is assigned to 1 of 5
92 classes, ranging from absent to severe (Piva et al. 2011, Regoli et al. 2019), which is the basis for
93 different management options associated to each class of environmental risk. Weighted criteria
94 for elaboration of chemical data and ecotoxicological bioassays have been incorporated in the last
95 Italian law for determining quality class and management options for dredged marine sediments,
96 based on the weighted elaboration and integration of their chemical and ecotoxicological
97 characteristics (DM 173/2016).
98 As part of an extensive research project aimed to characterize the environmental quality and a
99 possible remediation strategy of Bagnoli industrial site (ABBaCo 2018), the objective of the
100 present work was to demonstrate the practical efficacy of the WOE approach to elaborate and
101 integrate huge datasets of heterogeneous results. The combination of rigorous mathematical
102 algorithms with the user-friendly outputs of the Sediqualsoft model was expected to represent an
103 important tool to facilitate site-oriented and scientifically supported management options for
104 sediments of such a polluted area.
105
106 **2. Materials and methods**
107
108 *2.1 Experimental design and sampling activities*
109 The study area is located in the eastern part of the Bay of Pozzuoli, within the Gulf of Naples.
110 According to knowledge from previous studies (ISPRA-ICRAM 2005; Arienzo et al. 2017, 2019;
111 Romano et al. 2018; Trifuoggi et al. 2017, 2018), it was divided in 11 sub-areas, each containing
112 from 5 to 23 sampling points, depending on the distance from the industrial plant and the
113 expected level of contamination (Figure 1). According to requirements of Italian Ministry of
114 Environment and within "ABBaCo project", 118 sediments samples were collected in the study

115 area between May 2017 and December 2017 for chemical analyses, ecotoxicological bioassays
116 and analyses of benthic communities.
117 Bioaccumulation and biomarker responses were carried out on mussels, *Mytilus galloprovincialis*,
118 and on the fish species, *Mullus barbatus, Pagellus erythrinus* and *Diplodus vulgaris*, sampled in
119 December 2017. These fish species are commonly used in the biomonitoring of marine
120 environment (Regoli et al. 2002; Bonsignore et al. 2013), and they were selected to highlight the
121 potential influence of their different mobility, contact with sediments and feeding behavior on
122 bioaccumulation and responsiveness to chemical pollutants. Organisms were sampled from
123 different sites in the same period to avoid that comparisons of bioaccumulation and biomarkers
124 responses could be differently influenced by seasonal variations (Bocchetti et al. 2006; Fattorini
125 et al. 2014). Mussels (shell length 5.5 ± 0.5 cm) were collected in 3 points along the 2 industrial
126 piers (P2, PGT, PGP) and in additional 4 sites at different distances from the plant (Figure 1).
127 Fish were sampled by local fishermen both in area of Bagnoli (INSIN) and in a reference site
128 outside the bay (OUTSIN). Mean lengths were recorded both in fish from INSIN (*M. barbatus:*
129 17.5±1.4 cm; *P. erythrinus:* 15.7±1.5 cm; *D. vulgaris:* 18.7±2.1 cm) and from OUTSIN (*M.*
130 *barbatus:* 11.5±2.1 cm; *P. erythrinus:* 22.6±4.6 cm; *D. vulgaris:* 16.7±0.9 cm). Analyses on fish
131 samples (n=5) were performed using tissues of one individual for each replicate, while mussels
132 samples (n=5) were constituted each by pooling tissues of 3 individuals.
133
134 *2.2 Chemical characterization of sediments*
135 After collection, sediment samples for chemical analyses were stored at -20 °C, until analysed for
136 grain-size distribution (gravel, sand, silt, and clay), organic matter (OM), trace metals and
137 metalloids (Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V, and Zn), hydrocarbons with C>12, polycyclic
138 aromatic polyaromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), organotin
139 compounds, organochlorine pesticides, dioxin (PCDDs) and furan (PCDFs). Measurements were
140 carried out through validated methods by sieves and laser particle sizer, inductively coupled

141 plasma-mass spectrometry (ICP-MS), inductively coupled plasma-optical emission spectroscopy
142 (ICP-OES), Atomic Absorption Spectrophotometry, gas-chromatography with mass spectrometry
143 and flame ionization detector (GC-MS, GC-FID), high resolution DFS mass spectrometer
144 Dioxine B Thermo Fischer. For the determination of PCDDs and PCDFs the isotopic dilution
145 procedures reported by USEPA 1613B (1994) were performed. Detailed analytical protocols,
146 including QA/QC procedures, have been given elsewhere (Armiento et al., 2020; Molisso et al.
147 2020).
148
149 *2.3 Bioaccumulation analyses*
150 Bioaccumulation of trace elements (Al, As, Cd, Cu, Cr, Hg, Ni, Pb and Zn) and polycyclic
151 aromatic hydrocarbons (PAHs) were carried out on the whole soft tissues of wild specimens of *M.*
152 *galloprovincialis* and in the liver tissues of *M. barbatus, P. erythrinus* and *D. vulgaris*, following
153 previously validated procedures based on atomic absorption spectrophotometry (AAS) and high
154 performance liquid chromatography (HPLC) with diode array (DAD) and fluorimetric detection
155 (Benedetti et al. 2014, Regoli et al. 2019). All the analytical determinations were performed by
156 analysing five replicates, carefully checking for accuracy, precision and recovery by testing a
157 series of blank solutions (reagents only), reference standards and selected certified standard
158 materials.
159 *2.4 Biomarkers analyses*
160 Lysosomal membrane stability was measured in mussels hemocytes through the Neutral Red
161 Retention Time (NRRT) assay, while metallothioneins (MTs) were determined in digestive
162 glands by spectrophotometric analysis after acidic ethanol/chloroform fractionation using GSH as
163 standard (Viarengo et al., 1997). Acetylcholinesterase enzymatic activity (AChE) was
164 spectrophotometrically assayed in mussels haemolymph and fish brain using the Ellman's
165 reaction (Gorbi et al. 2008). Ethoxyresorufin O-deethylase (EROD) was spectrofluorimetrically
166 determined in individual fish livers measuring resorufin formation from 7-ethoxyresorufin

(Regoli et al. 2003). Aromatic metabolites in fish bile were measured by fixed fluorescence (FF) spectrofluorimetry and semi-quantitatively assessed as naphthalene-like, pyrene-like and benzo[a]pyrene-like metabolites (Gorbi and Regoli 2004). Micronuclei (MN) frequency was microscopically measured in mussels hemocytes and fish gills, observing 2000 cells with preserved cytoplasm: such genotoxic alterations were defined as round structures, smaller than 1/3 of the main nucleus diameter, on the same optical plan and clearly (Gorbi et al. 2008). Detailed analytical procedure have been previously reported (Benedetti et al., 2014).

2.5 Ecotoxicological bioassays

A battery of ecotoxicological bioassays was applied to sediment samples following standardized procedures. The bioluminescence test with *Vibrio fischeri* (Doe et al. 2005) was selected for the solid phase, while the algal growth inhibition of *Skeletonema costatum* (ISO 10253: 2006) and the sea urchin embryotoxicity assay with *Paracentrotus lividus* (ISPRA, 2017) were used to test elutriates, prepared with standard procedure (USEPA 503/8-91/001: 1991). Detailed procedures for sediments treatment and ecotoxicological bioassays have been reported elsewhere (Morroni et al. 2018; Gallo et al. 2020).

2.6 Benthic communities

Sediment samples were sieved through a mesh net of 1 mm and sorted under the stereomicroscope. All taxa were identified and the main taxa classified to the species level or to the lower possible taxonomic level. For each species, whenever possible, the corresponding biocoenosis was identified. From the list of species, the WOE Sediqualsoft elaborated the available community descriptors, diversity indices and ecological indicators (including abundance, richness, Margalef, Shannon, Pielou, AMBI, BENTHIX, BOPA, BITS, mAMBI) which are reported in Table S4b. The results and the methodological details of these analyses have been reported in Hay Mele et al. (2020).

2.7 Statistical analyses and WOE elaboration

192 Analysis of variance (ANOVA) was applied to chemical data and biomarker responses of *M.*
193 *galloprovincialis,* to test the significance of the differences between areas (level of significance at
194 the 95% of confidence interval, $\alpha = 0.05$); homogeneity of variance was tested by Cochran C, and
195 post-hoc comparisons (Student-Newman-Keuls) were used to compare means of values. Student's
196 t-test was used to test for statistical significance at the 95% of confidence interval ($\alpha=0.05$)
197 between INSIN and OUTSIN areas for chemical data and biomarker responses on each of the
198 three fish species.
199 All results, for various typologies of data, have been elaborated within the quantitative WOE,
200 Sediqualsoft model, which consists in various modules to summarize specific hazard indices for
201 individual LOEs, before their overall integration in the final WOE assessment (Piva et al. 2011,
202 Benedetti et al. 2012, Regoli et al. 2014, 2019). Logical flow charts, based on expert judgment
203 and legislative constraints, were converted into algorithms for weighted elaboration of data from
204 sediment chemistry, bioavailability of chemicals in bioindicator species, ecotoxicological effects
205 measured at subcellular level (biomarkers), toxicity at organism level (laboratory bioassays) and
206 at the community level (benthic communities): the individual LOEs have been finally integrated
207 for the WOE evaluation (see below).
208
209 *LOE 1: Chemical characterization of sediments*
210 The evaluation of chemical hazard (LOE-1) is initially based on the calculation for each pollutant
211 of Ratio to Reference (*RTR*), i.e. the ratio between concentration measured in sediments and those
212 indicated by a sediment quality guideline (SQG); in the present investigation, reference limits
213 were those indicated by the SQG-L2 of the Italian decree for determining quality class and
214 management options for dredged marine sediments (DM 173/2016). The *RTR* is corrected by a
215 factor (w) which depend on the typology of chemicals (i.e. non priority w=1, priority w=1.1,
216 priority and hazardous pollutants w=1.3). In the calculation of the specific Hazard Quotient

(HQ_C), an average *RTRw* is obtained for all of the parameters with *RTR* ≤1 (i.e. values below the SQG), while for those with *RTR* >1, the *RTRw* are individually added into the summation *Σ*:

$$HQ_C = \frac{\sum_{j=1}^{N} RTR_W(j)_{RTR(j)\leq 1}}{N} + \sum_{k=1}^{M} RTR_W(k)_{RTR(k)>1}$$

Based on expert judgment, the values of HQ_C are assigned to one of six classes of chemical hazard, absent, negligible, slight, moderate, major and severe depending on the number, typology and magnitude of exceeding chemicals (Regoli et al., 2019).

LOE 2: Bioavailability of chemicals

The results on bioaccumulation of chemicals in tissues of mussels and fish (LOE2) are elaborated calculating, for each parameter, the increase of concentration compared to control organisms, corrected for the typology of pollutant and the statistical significance of the difference. The cumulative HQ_{BA} does not consider parameters with RTR_w<1.3, calculates the average for those with RTR_w ranging between 1.3 and 2.6, and adds the summation of all those with RTR_w≥ 2.6):

$$HQ_{BA} = \frac{\sum_{n=1}^{j} RTR_W(n)_{1.3\leq RTR_W(j)<2.6}}{j} + \sum_{n=1}^{K} RTR_W(n)_{RTR(k)\geq 2.6}$$

The HQ_{BA} is assigned to one of five classes of hazard for bioavailability, from Absent to Severe (Regoli et al., 2019).

*LOE 3: Sublethal effects: Biomarkers*The module for the elaboration of biomarkers (LOE3) contains a wide battery of responses, each assigned with a weight (based on the relevance of biological endpoint) and a threshold indicative of changes of biological relevance. For each biomarker, the measured variation is compared to the threshold (Table S5), corrected for

237 statistical significance and importance of biomarker (weight), and assigned to 1 of 5 classes of
238 effect which are then differently weighted in the calculation of cumulative HQ_{BM}.

$$HQ_{BM} = \left(\frac{\sum_{j=1}^{N} Effect_w(j)_{1.5<Effect(j)\leq 2.5}}{num\ biomarker_{1.5<Effect(j)\leq 2.5}} + \sum_{k=1}^{M} Effect_w(k)_{Effect(j)>2.5} \right)$$

239

240 According to the % distribution of biomarkers in the 5 classes, the level of cumulative HQ_{BM} is
241 assigned to 1 of 5 classes of hazard: all the more relevant information are given in the model
242 output (Regoli et al., 2019).

243 *LOE 4: Ecotoxicological Bioassays*

244 Weighted criteria to elaborate results from standardized ecotoxicological bioassays (LOE-4) are
245 based on specific thresholds and weights assigned to each bioassay depending on the biological
246 endpoint, tested matrix, time of exposure, and the possibility of hormetic responses.

247 In the module for ecotoxicological bioassays, the cumulative hazard quotient ($HQ_{Battery}$) is
248 obtained by the summation (Σ) of the weighted effects (Ew), i.e., the variations measured for each
249 test compared to specific thresholds, corrected for the statistical significance of the difference (w),
250 biological importance of the endpoint and exposure conditions (w_2):

$$HQ_{BATTERY} = \sum_{k=1}^{N} Effect_w(k) \cdot w_2$$

251 The $HQ_{Battery}$ is normalized to a scale ranging from 0 to 10, where 1 is the battery threshold (when
252 all the measured bioassays exhibit an effect equal to the threshold, 10 when all the assays exhibit
253 100% of effect); the $HQ_{Battery}$ is then assigned to one of five classes of hazard, from Absent to
254 Severe (Regoli et al., 2019).

255

256 *LOE 5: Benthic Communities*

Data on benthic communities are elaborated within a specific module (LOE-5), which converts the list of identified species in several available univariate and multivariate indices for the classification of ecological quality (Vincent 2002, Dauvin and Ruellet 2007, Muxika et al. 2007, Anderson et al. 2008, Mistri and Munari 2008, Sigovini et al. 2013). Such elaborated indices include total abundance (N), species richness (S), Shannon-Weaver Diversity Index (H'), Margalef index (D), Pielou's evenness index (J), AZTI' Marine Biotic Index (AMBI), multimetric-AZTI Marine Biotic Index (m-AMBI), Bentic Index (BENTIX), Benthic Index based on Taxonomic Sufficiency (BITS) and Benthic Opportunistic Polychaetes Amphipods (BOPA index) (Regoli et al., 2019). In this work, the AMBI index was chosen for the integration with other LOEs in the final WOE elaboration of ecological risk.

WOE integration

The huge datasets of results elaborated from the 5 LOEs have been finally integrated through a WOE approach based on the quantitative model Sediqualsoft. The quantitative hazard quotients (HQs) obtained for each LOEs are normalized to a common scale and given a different weight according to previously validated procedures (Piva et al., 2011; Lethonen et al., 2019; Regoli et al., 2019). LOE-2, summarizing bioavailability of chemicals in mussels and fish had a greater weight (w: 1.2) compared to LOE-1 assessing the presence of such compounds in the sediments (w: 1.0); at the biological level, a greater ecological relevance was assigned to LOE-5 on benthic communities (w: 1.3) compared to LOE-4 reflecting acute ecotoxicological effect at an organismal level (w: 1.2), or LOE-3 on sublethal effects at the cellular level (w: 1). An overall WOE level of risk is thus calculated and assigned to 1 of 5 classes of risk from Absent to Severe (Piva et al., 2011). Scientific criteria, validation of weights and thresholds, expert judgment evaluations and specific flow-charts of each LOE have been validated elsewhere (Piva et al., 2011; Benedetti et al., 2012, 2014; Lethonen et al., 2019; Regoli et al., 2019).

3. Results

3.1 Chemical characterization of sediments

Chemical analyses were performed on sediments from the 11 sub-areas (Figure 1) and measured values are reported by Armiento et al (2020). Several critical values were obtained, especially for PAHs and trace metals (As, Zn, Pb, V and to a lesser extent Cd Cu and Hg). The highest concentrations of ∑ PAHs (approximately 2800 mg/kg) were measured in some samples collected within the sub-area 7.

When data were elaborated according to weighted criteria, 100 samples on 118 exhibited a "Severe" chemical Hazard Quotient (HQ) indicating a widespread contamination in the whole investigated area (Figure 2a).

3.2 Bioaccumulation in mussels and fish

Concentrations of trace metals and PAHs measured in whole tissues of mussels *M. galloprovincialis* sampled from 7 sampling stations are shown in Supplementary Materials (Table S1a). Among inorganic elements, concentrations of As, Cd, Fe, Mn, Pb and V were typically higher in mussels sampled from the industrial piers (P2, PGT and PGP), exhibiting a gradual decrease in organisms collected at increasing distances from the industrial plant. .

Mussels from the piers (P2, PGT and PGP) were also characterized by elevated concentrations of high molecular weight (HMW) PAHs, which confirm the industrial origin of such bioavailable organic chemicals. In contrast, low molecular weight (LMW) PAHs did not exhibit significant differences between organisms from the piers or the other sampling sites (BRM, BBM and BNF). Results on bioaccumulation of chemicals in fish are given in the Supplementary Materials (Table S1b). *M. barbatus* and *D. vulgaris* exhibited comparable concentrations of trace metals and PAHs in specimens collected in the area of Bagnoli and in those from the reference site, while concentrations of HMW-PAHs (*M. barbatus*), LMW-PAHs and total PAHs (*D. vulgaris*) were significantly higher in organisms from the industrial area ($p<0.05$). Bioaccumulation of chemicals

in *P. erythrinus* did not reveal significant variations for all the analysed parameters comparing the specimens collected in the industrial and reference areas (Table S1b).

The weighted elaboration of these results summarized as "Major" the HQ for bioavailability in *M. galloprovincialis* from sub-area 1, essentially due to concentrations of HMW-PAHs (supplementary Table S1), while "Moderate" in mussels collected from the sub-areas 7 and 9 (Figure 2b); a lower level of HQ was assigned to bioavailability for mussels in sub-areas 2, 6, 10, 11 (Figure 2b).

Considering results obtained in fish species, the higher bioavailability HQ was elaborated for *M. barbatus*, appearing as "Moderate" in fish collected within the industrial sub-areas 3, 4, 5 and 8.

3.3 Biomarkers responses in mussels and fish

Biomarkers analyzed in native mussels revealed a higher sensitivity of mussels collected in sites PGP and PGT (included in sub-areas 1, 7 and 9) which exhibited a decreased lysosomal membrane stability and increased micronuclei frequency compared to organisms from other areas (Supplementary Table S2a). No variations were observed for the enzymatic activity of acetylcholinesterase in hemolymph, nor for metallothioneins in digestive gland of mussels from various sites (Supplementary Table S2a).

Results on acetylcholinesterase in brain, EROD enzymatic activity in liver, aromatic bile metabolites and frequency of branchial micronuclei in fish species (*M. barbatus*, *P. erythrinus* and in *D. vulgaris*) are reported in supplementary Table S2b. Acetylcholinesterase enzymatic activity was not affected in *M. barbatus* sampled in the industrial area while a decrease of this biomarker was observed in *P. erythrinus* and *D. vulgaris*. The EROD enzymatic activity was significantly induced in *M. barbatus* ($p<0.05$) and *P. erythrinus* ($p<0.05$) sampled in the industrial area compared to specimens from the reference site, while the cytochrome P450 biotransformation pathway was unaffected in *D. vulgaris*. At the same time, all the fish species exhibited higher levels of aromatic metabolites, particularly B[a]P-like and pyrene-like, in

organisms sampled in the industrial compared to reference area. The frequency of micronuclei significantly ($p<0.05$) increased in gills of *M. barbatus* from the industrial area.

Combining the weighted elaboration of biomarker results obtained in mussels and fish, it was possible to assign a hazard index in all the investigated sub-areas: such HQ resulted as "Major" in sub-areas 1, 3, 4, 5 and 8, "Moderate" in 9, "Slight" or "Absent" in the remaining sub-areas (Figure 2c).

3.4 Ecotoxicological bioassays

Ecotoxicological characteristics of the sediments, evaluated through a battery of three bioassays (*V. fischeri*, *S. costatum* and *P. lividus*), are detailed in Supplementary Table S3. The weighted elaboration revealed a "Slight" or "Absent" toxicity for most samples; a "Moderate" HQ was summarized in sub-areas 1, 2 and 7, where some individual samples exhibited a "Major" level of hazard (Figure 2d). The embryotoxicity of *P. lividus* was the most sensitive bioassay, often in combination with the inhibition of the algal growth in *S. costatum* (Supplementary Table S3).

3.5 Benthic communities

The analyses of benthic communities carried out in 15 stations allowed to identify 1796 organisms belonging to 164 taxonomic groups (Supplementary Table S4). The AMBI index was selected as the most appropriate for the study area and the results indicated a "Slight" or "Absent" level of HQ at this level of biological organization (Table 1, Figure 2e).

3.6 Weight of evidence integration

The elaborated WOE risk indices were "Moderate" for the majority of sub-areas (1-5, 7-9 with calculated values between 40.48 and 54.61), and "Slight" in sub-areas 6, 10, 11 (WOE values between 29.61 and 37.03; Table 1 and Figure 3).

360 **4. Discussion**

361 The weight of evidence (WOE) approach, integrating individual lines of evidence through
362 qualitative or quantitative methods, has been widely used in ecological and risk assessments to
363 draw conclusions and justify selection of regulatory benchmarks (Linkov et al., 2009, 2015).
364 Procedures for integration of different typologies of data must be quantitative and transparent for
365 their acceptance in regulatory normative. As part of a decision-making process, various WOE
366 methodologies have been recently formalized in different fields, e.g. by US-EPA (Linkov et al.,
367 2009, 2011, 2015), European Food Safety Authority (Suter et al., 2017), or by the last Italian law
368 on management of dredged sediments (DM 173/2016). The latter is based on the same weighting
369 criteria for chemical analyses and ecotoxicological bioassays presented in this work. The
370 combination of chemical and biological analyses, is recognized as an added value to the use of
371 individual lines of evidence (LOEs), and in line with European Directives which recommend the
372 use of multiple quality indicators for aquatic ecosystems (Lyons et al. 2010; Lethtoten et al.
373 2014). The application of quantitative weighted criteria to process and integrate huge amounts of
374 heterogeneous data from different LOEs allowed to summarize complex scientific information for
375 an easier interpretation by policymakers or environmental managers (Piva et al. 2011; Borja et al.
376 2017; Regoli et al. 2019).

377 In the present study, a WOE assessment has been carried out in Bagnoli industrial site, chosen as
378 model area to demonstrate the practical applicability in a complex environmental scenario of a
379 model integrating chemical characterization of sediments, bioavailability of pollutants to key
380 bioindicator species and the onset of effects at different levels of biological organization, from
381 cellular responses to status of benthic communities.

382 Concentrations of trace metals and especially PAHs in sediments revealed a severe chemical
383 contamination still present in the entire study area (Armiento et al 2020), with concentrations
384 extremely higher than baseline levels and maximum limits indicated by Italian legislation for
385 harbor-dredged sediments (L2 values, DM 173/2016).

386 The integrative approach of this study showed that toxicity of sediments, measured through
387 standardized batteries of ecotoxicological bioassays, was often not in accordance with chemical
388 characterization. In fact, despite an evident contamination, sediments from the area of Bagnoli
389 inlet as well as those from the southern and the northern stations, showed low levels of acute
390 toxicity; only some samples, particularly those collected close to the industrial plant, revealed
391 evidence of a major acute toxicity, but the overall HQ for ecotoxicological bioassays resulted as
392 "Moderate" in these sub-areas.
393 Despite the lack of an elevated acute toxicity, the bioavailability of contaminants was evident in
394 terms of bioaccumulation of PAHs, and of HMW hydrocarbons, both in mussels and fish from the
395 industrialized area. For this reason, the level of hazard for bioavailability was calculated as
396 "Moderate" in the greatest part of the study area, with major effects in proximity of industrial
397 piers. The significant accumulation of HMW-PAHs confirmed a pyrolytic origin of such
398 pollution, related to combustion processes of the plant. A significant accumulation of these PAHs
399 was observed also in liver of the benthic *M. barbatus*, indicating sediments as a still active source
400 of these chemical contaminants to local biota.
401 Bioavailability of PAHs was further confirmed by biotransformation-related biomarkers, such as
402 the induction of EROD enzymatic activity and the accumulation of aromatic metabolites in the
403 bile of *M. barbatus* and *P. erythrinus*. The latter species confirmed the higher sensitivity of these
404 biomarkers compared to tissue concentrations, in revealing bioavailability and metabolism of
405 PAHs in fish. The comparison of the three fish species highlighted *M. barbatus* as the most
406 affected by PAHs, both in terms of accumulation and of cellular responses related to their
407 biotransformation; in *P. erythrinus* the lack of PAHs accumulation did not reflect a lack of
408 exposure but the active metabolization and excretion of these chemicals, as indicated by the
409 significant EROD induction and accumulation of aromatic bile metabolites. Finally, the slight
410 PAHs effects in the more pelagic *D. vulgaris* further confirm the significant role of sediments in
411 direct transfer of chemicals to benthic biota and, indirectly, to trophic webs.

412 Additional adverse effects of PAHs were revealed by lysosomal membrane destabilization in
413 mussels, and the onset of genotoxic effects in both fish and mussels. The enhancement of
414 micronuclei frequency is a well-known effect of PAHs, partly related to increased formation of
415 oxyradicals during their metabolization pathway (Benedetti et al., 2015).
416 The overall biological significance of observed biomarkers responses in biota corresponded to a
417 "Major" level of subcellular hazard in organisms collected from the industrial sub-areas.
418 Concerning the ecological effects on benthic communities, the application of AMBI index (Borja
419 et al. 2000) allowed to classify as "Absent" the hazard in the samples collected in the majority of
420 study area, except in sub-areas 4, 5, 10 and 7, located near the plant, where the impact was
421 summarized as "Slight". Such evaluation is in accordance with Fasciglione et al. (2016), who
422 reported an unexpected evidence of biodiversity in this area, indicating a good functioning of
423 local ecosystems at high levels of chemical contamination.
424 The overall integration of hazard quotients elaborated from sediment chemistry, bioassays,
425 bioaccumulation, biomarkers and benthic communities provided a more holistic assessment of the
426 environmental quality in the investigated area. The results indicate a clear pollution, especially
427 near the piers. Beside a "Slight" level of hazard summarized in sub-areas 6, 10 and 11, the WOE
428 elaboration increased to a "Moderate" level in all the other zones: interestingly the highest hazard
429 coefficients were obtained near the plant: values close to the limit between "Moderate" and
430 "Major" level of risk were obtained in sub-areas 1 and 7 (Table 1), further confirming the impact
431 of discharged material from the industrial activities.
432 The possibility of converting complex scientific information into simple hazard indices easily
433 understandable from policy makers and environmental managers could facilitate and orientate
434 site-specific decisions on environmental sediment management. This study confirm the need of
435 performing multidisciplinary approaches to assess the health status of marine ecosystems. In this
436 perspective the use of the WOE integration, which combine and weight different kinds of data
437 and analyses allows to better discriminate the presence of contaminants and their short or long-

term consequences. The importance of WOE models is particularly evident in complex environmental scenarios where apparently contrasting results are provided by various LOEs. The present study also highlights that different conclusions (with consequent different management scenarios) can be made on the basis of the analyses chosen: from a severe hazard derived from the use of the sediment contamination alone to the lack of hazard if we consider only the data from the analyses of the benthic assemblages. The multidisciplinary approach used here confirms that different typologies of pollutants are bioavailable and selectively transferred from sediments to local biota; such chemicals do not always exert acute toxicological effects, but can induce cellular responses that, being highly sensitive, might be prognostic of future adverse effects. Although the analysis of local sentinel organisms confirms that the contaminants of the sediments are still transferred to biota, the results of the WOE approach suggests that the management of the sediments from the industrial area of Bagnoli should not be based only on its chemical characterization.

Acknowledgments

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655

Tables

Table 1 - Classification of sub-areas within Bagnoli-Coroglio industrial site, according to the weight of evidence elaboration. Levels of hazard are reported for each LOE and for their overall WOE integration.

Area	LOE1 (Level of chemical hazard)	LOE2 (Level of hazard for bioavailability)	LOE3 (Level of hazard for biomarkers)	LOE4 (Level of hazard for bioassays)	LOE5 (Level of hazard for Benthic Communities)	WOE (Weight of Evidence integration)
Area 1	SEVERE	MAJOR	MAJOR	MODERATE	ABSENT	MODERATE (54.61)
Area 2	SEVERE	SLIGHT	SLIGHT	MODERATE	ABSENT	MODERATE (40.48)
Area 3	SEVERE	MODERATE	MAJOR	ABSENT	ABSENT	MODERATE (43.40)
Area 4	SEVERE	MODERATE	MAJOR	ABSENT	SLIGHT	MODERATE (45.36)
Area 5	SEVERE	MODERATE	MAJOR	ABSENT	SLIGHT	MODERATE (46.54)
Area 6	SEVERE	ABSENT	ABSENT	ABSENT	ABSENT	SLIGHT (29.61)
Area 7	SEVERE	MODERATE	MAJOR	MODERATE	SLIGHT	MODERATE (53.56)
Area 8	SEVERE	MODERATE	MAJOR	ABSENT	ABSENT	MODERATE (41.30)
Area 9	SEVERE	MODERATE	MODERATE	ABSENT	ABSENT	MODERATE (41.73)
Area 10	SEVERE	SLIGHT	SLIGHT	ABSENT	ABSENT	SLIGHT (34.83)
Area 11	SEVERE	ABSENT	ABSENT	SLIGHT	ABSENT	SLIGHT (37.03)

670 **Legends of Figures**

671 **Figure 1 -** Localization of sampling sites in the Bagnoli-Coroglio industrial area. In each sub-area
672 were performed: chemical characterization of sediments; bioavailability of trace metals and
673 organic pollutants in mussels and fish, biomarkers in mussels and fish, benthic communities
674 analysis; ecotoxicological bioassays.

675

676 **Figure 2 -** Classification of sub-areas within Bagnoli-Coroglio industrial area for each LOE
677 according to the weight of evidence elaboration. Levels of hazard are reported in different colors
678 on the areas, as detailed in the legend, considering as LOE: chemical characterization of
679 sediments (A); bioavailability of trace metals and organic pollutants in mussels and fish (B),
680 biomarkers in mussels and fish (C); ecotoxicological bioassays (D); benthic communities analysis
681 (E).

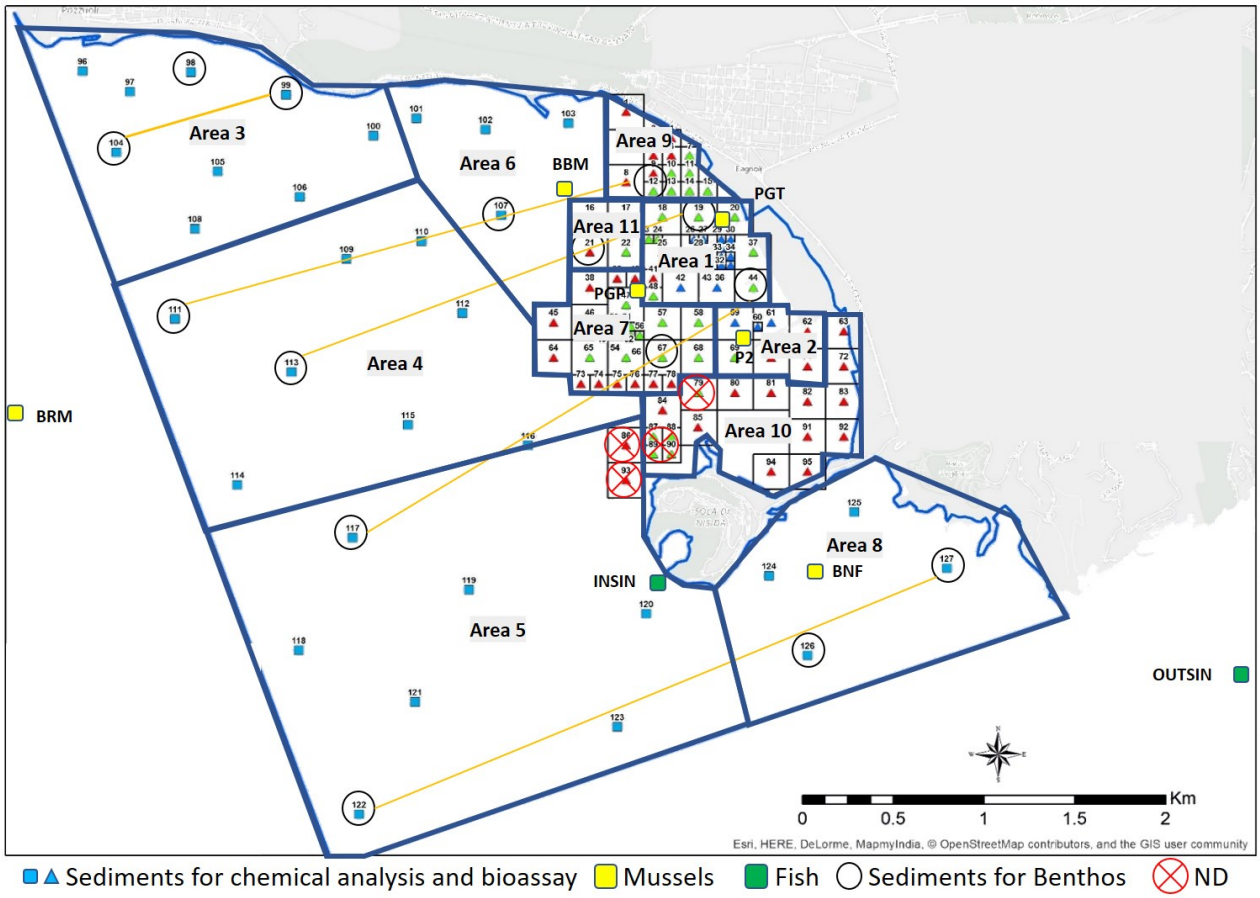
682

683 **Figure 3 –** Classification of areas within Bagnoli-Coroglio industrial area considering the overall
684 weight of evidence integration. Levels of hazard are reported in different colors on the areas as
685 detailed in the legend.

686

687 **Figure 1**

688



689

690

Figure 2

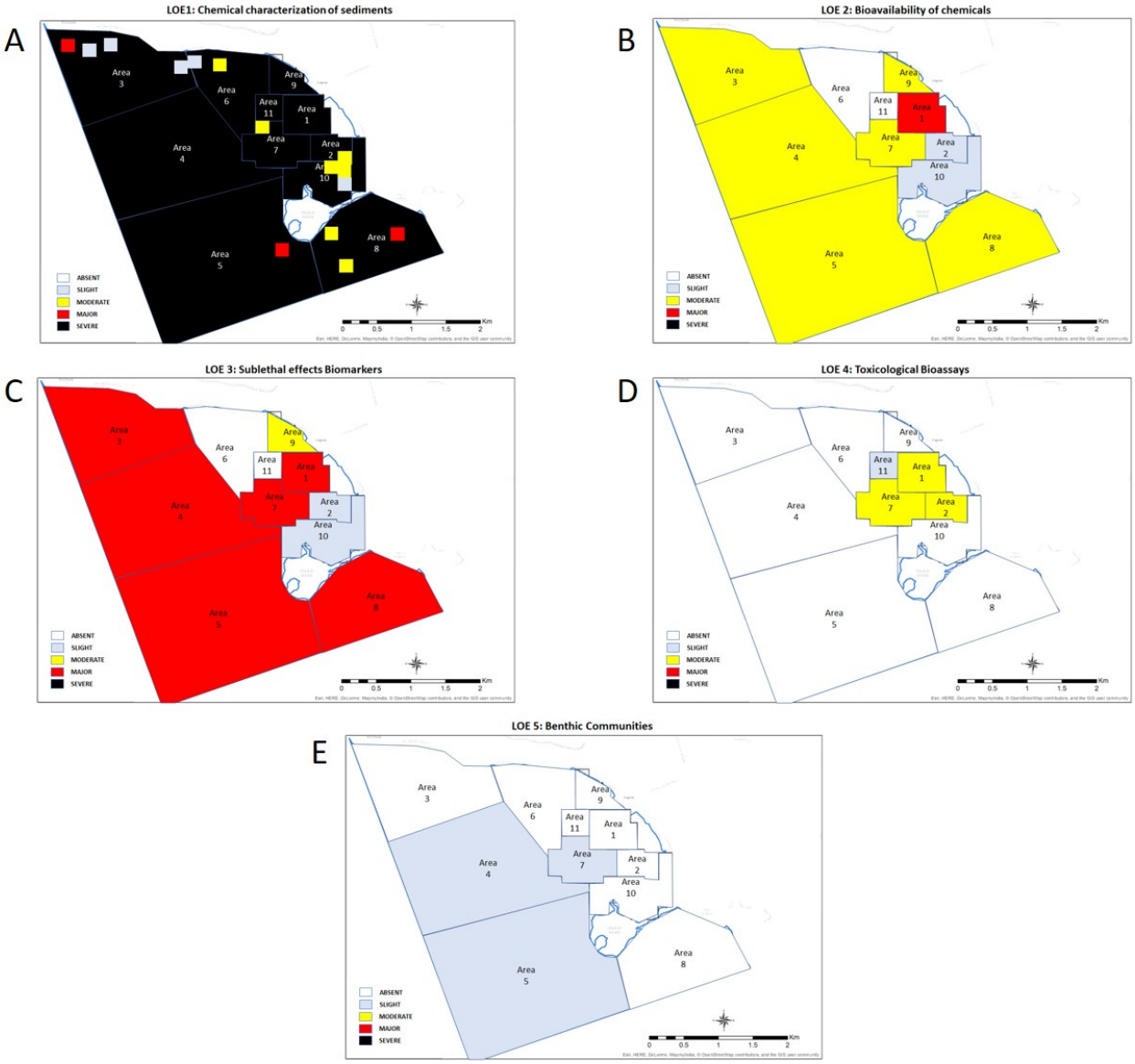
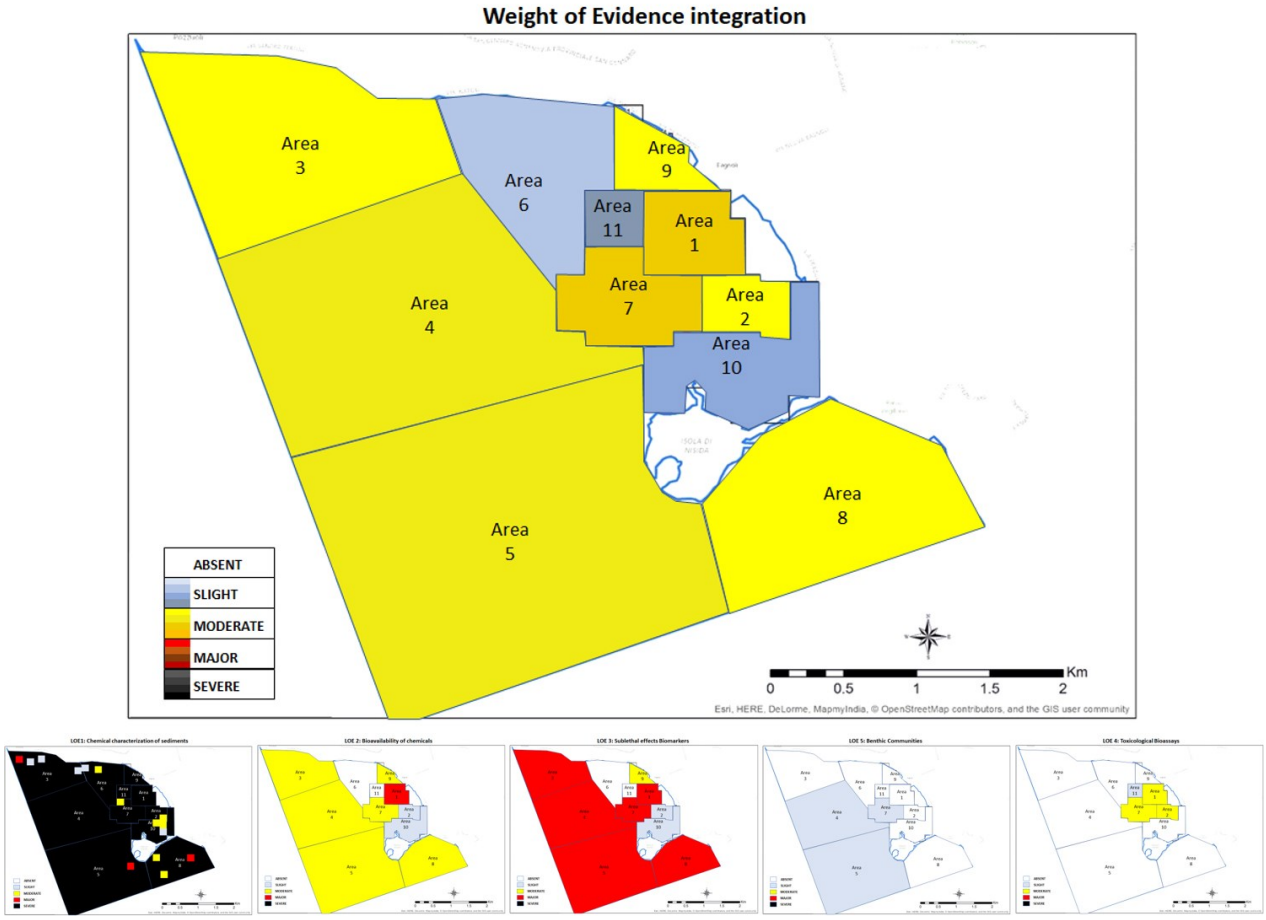

A
LOE1: Chemical characterization of sediments
B
LOE 2: Bioavailability of chemicals
C
LOE 3: Sublethal effects Biomarkers
D
LOE 4: Toxicological Bioassays
E
LOE 5: Benthic Communities
Area 1
Area 2
Area 3
Area 4
Area 5
Area 6
Area 7
Area 8
Area 9
Area 10
Area 11
ABSENT
SLIGHT
MODERATE
MAJOR
SEVERE
0 0.5 1 1.5 2 Km

Figure 3


Weight of Evidence integration
Area 3
Area 6
Area 9
Area 11
Area 1
Area 4
Area 7
Area 2
Area 10
Area 8
Area 5
ABSENT
SLIGHT
MODERATE
MAJOR
SEVERE
0
0.5
1
1.5
2
Km
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

SUPPLEMENTARY MATERIAL

Table S1a. Bioaccumulation of trace metals and polycyclic aromatic hydrocarbons (PAHs) concentrations in tissues of mussels sampled. Data expressed as mean ± standard deviation. Different letters (a, b, c) indicate significant differences (*p<0.05*) between sites, as determined by one way analyses of variance (ANOVA) and Student Newman Keuls post-hoc test.

		PGT (Area 1;9)	PGP (Area 1;7)	P2 (Area 2;10)	BBM (Area 6;9;11)	BNF (Area 8)	BRM (Reference area)	Sig.
Al	µg/g (dw)	85 ± 15.4	61.6 ± 5.48	52.6 ± 15.4	58.2 ± 17	49.2 ± 47.5	46.5 ± 14.7	*n.s.*
As		22.4 ± 5.87 *bc*	25.6 ± 2.29 *c*	22.2 ± 0.783 *bc*	17.1 ± 1.81 *ab*	11.9 ± 1.86 *a*	19.8 ± 0.827 *bc*	*p < 0.0001*
Cd		0.873 ± 0.031 *c*	0.83 ± 0.0943 *c*	0.671 ± 0.0924 *b*	0.579 ± 0.107 *b*	0.394 ± 0.0692 *a*	0.519 ± 0.014 *ab*	*p < 0.0001*
Cr		2.08 ± 0.315 *c*	1.65 ± 0.263 *b*	1.17 ± 0.0242 *a*	1.17 ± 0.127 *a*	1.1 ± 0.221 *a*	0.944 ± 0.0518 *a*	*p < 0.0001*
Cu		6.88 ± 0.872 *bc*	4.07 ± 1.95 *ab*	7.88 ± 2.89 *bc*	6.93 ± 1 *c*	1.19 ± 0.712 *a*	8.68 ± 1.16 *c*	*p < 0.005*
Fe		626 ± 174 *b*	604 ± 96.5 *b*	362 ± 95.5 *a*	340 ± 103 *a*	230 ± 118 *a*	218 ± 43.4 *a*	*p < 0.005*
Hg		0.171 ± 0.011 *b*	0.17 ± 0.00796 *b*	0.157 ± 0.0326 *b*	0.146 ± 0.0123 *b*	0.11 ± 0.0115 *a*	0.139 ± 0.0112 *b*	*p < 0.005*
Mn		68.1 ± 11.7 *c*	62 ± 9 *c*	42.2 ± 12.2 *b*	33.7 ± 1.83 *ab*	14.8 ± 4.53 *a*	31.7 ± 4.04 *ab*	*p < 0.0001*
Ni		1.1 ± 0.0932 *b*	0.997 ± 0.0895 *ab*	0.718 ± 0.0991 *a*	1.32 ± 0.259 *b*	0.725 ± 0.164 *a*	1.22 ± 0.0497 *b*	*p < 0.0001*
Pb		10.4 ± 0.585 *c*	10.5 ± 0.484 *c*	6.16 ± 0.655 *b*	4.1 ± 0.705 *a*	3.57 ± 2.32 *a*	2.9 ± 0.569 *a*	*p < 0.0001*
V		2.65 ± 0.531 *c*	2.42 ± 0.218 *bc*	1.68 ± 0.326 *ab*	1.52 ± 0.472 *a*	1.33 ± 0.387 *a*	1.43 ± 0.259 *a*	*p < 0.005*
Zn		53.2 ± 11	90.7 ± 74.5	41.1 ± 6.91	123 ± 116	38.7 ± 5.54	42.2 ± 20.4	*n.s.*

n.s. = not significant

Table S1a. Continues.

		PGT (Area 1;9)	PGP (Area 1;7)	P2 (Area 2;10)	BBM (Area 6;9;11)	BNF (Area 8)	BRM (Reference area)	Sig.
Naphthalene	ng/g (dw)	18.7 ± 3.19	35.5 ± 24.1	18.2 ± 5.99	14.1 ± 0.932	16.1 ± 0.508	16.6 ± 0.965	*n.s.*
Acenapthylene		< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	33.8 ± 58.5	*n.s.*
1-methylnaphthalene		< 0.05	< 0.05	1.96 ± 3.3 *a*	16 ± 1.88 *b*	17.3 ± 1.94 *b*	18.4 ± 2.57 *b*	*p < 0.0001*
2-methylnaphthalene		< 0.05	< 0.05	2.58 ± 4.38 *a*	10.8 ± 1.67 *b*	8.14 ± 0.336 *b*	9.42 ± 0.391 *b*	*p < 0.0001*
Acenaphthene		< 0.01	< 0.01	< 0.01	< 0.01	0.981 ± 0.0632 *b*	< 0.01	*p < 0.005*
Fluorene		0.778 ± 0.138 *ab*	0.832 ± 0.186 *ab*	1.13 ± 0.229 *b*	0.596 ± 0.136 *a*	0.68 ± 0.204 *ab*	0.684 ± 0.206 *ab*	*p < 0.05*
Phenanthrene		< 0.01	< 0.01	4.31 ± 4.51	4.04 ± 0.723	4.46 ± 0.689	4.18 ± 0.381	*n.s.*
Anthracene		2.74 ± 2.67	0.775 ± 1.32	0.768 ± 0.694	0.057 ± 0.0567	< 0.01	< 0.01	*n.s.*
Fluoranthene		10 ± 17.3	4.63 ± 8.01	0.225 ± 0.372	< 0.01	< 0.01	< 0.01	*n.s.*
Pyrene		34.5 ± 28.6	10 ± 16.2	7.11 ± 6.13	1.39 ± 0.433	0.379 ± 0.082	0.342 ± 0.291	*n.s.*
Benzo(a)anthracene		13.2 ± 9.95 *b*	4.02 ± 6.95 *a*	1.64 ± 2.71 *a*	< 0.01	0.016 ± 0.0103 *a*	0.0766 ± 0.115 *a*	*p < 0.05*
Chrysene		9.93 ± 7.03 *b*	2.95 ± 5.1 *a*	< 0.01	< 0.01	< 0.01	0.354 ± 0.119 *a*	*p < 0.05*
7,12-dimethylbenzo(a)anthracene		10.9 ± 15.7	4.88 ± 8.38	2.29 ± 1.95	3.68 ± 4.02	1.59 ± 0.779	1.21 ± 1.04	*n.s.*
Benzo(b)fluoranthene		13.6 ± 9.71	10.1 ± 9.37	0.0446 ± 0.0756	0.306 ± 0.0459	0.179 ± 0.01	0.321 ± 0.106	*n.s.*
Benzo(k)fluoranthene		8.78 ± 9.5	7.05 ± 6.11	0.0273 ± 0.0275	0.401 ± 0.316	0.0706 ± 0.0366	0.307 ± 0.0646	*n.s.*
Benzo(a)pyrene		5.99 ± 3.88 *b*	2.66 ± 2.36 *a*	1.13 ± 0.893 *a*	0.321 ± 0.0481 *a*	0.0946 ± 0.0465 *a*	0.142 ± 0.0989 *a*	*p < 0.05*
Dibenzo(ah)anthracene		0.677 ± 0.391	14 ± 18.8	0.454 ± 0.327	0.094 ± 0.161	0.189 ± 0.0267	0.236 ± 0.0669	*n.s.*
Benzo(ghi)perylene		0.227 ± 0.0937	0.158 ± 0.271	0.105 ± 0.0136	0.032 ± 0.0536	0.0346 ± 0.0583	< 0.001	*n.s.*
Indeno(123cd)pyrene		1.09 ± 1.8	0.043 ± 0.00608	< 0.05	0.0426 ± 0.0234	0.0393 ± 0.0184	< 0.05	*n.s.*
Low MW PAHs		43.5 ± 9.17 *a*	72.1 ± 47.4 *a*	57.8 ± 24.1 *a*	99.3 ± 5.56 *a*	99.4 ± 5.76 *a*	170 ± 113 *b*	*p < 0.05*
High MW PAHs		172 ± 123 *b*	121 ± 30.5 *b*	32.7 ± 10.5 *a*	13.3 ± 8.24 *a*	5.31 ± 1.67 *a*	6.07 ± 2.23 *a*	*p < 0.05*
Total PAHs		216 ± 129	194 ± 67.6	90.5 ± 27	112 ± 11.4	104 ± 6.33	176 ± 115	*n.s.*

n.s. = not significant

Table S1b. Bioaccumulation of trace metals and polycyclic aromatic hydrocarbons (PAHs) concentrations in tissues of fish sampled. Data expressed as mean ± standard deviation. Different letters (a, b, c) indicate significant differences (*p<0.05*) between sites, as determined by one way analyses of variance (ANOVA) and Student Newman Keuls post-hoc test. Significant differences between areas and reference area (*p < 0.05*), as determined by t-student test.

		Mullus barbatus			*Diplodus vulgaris*			*Pagellus sp.*		
		OUTSIN	INSIN	*Sig.*	OUTSIN	INSIN	*Sig.*	OUTSIN	INSIN	*Sig.*
Al	µg/g (dw)	n/a	22.3 ± 14.4	*n.t.*	12.6 ± 7.34	18 ± 7.43	*n.s.*	24.7 ± 26.9	36.6 ± 32.5	*n.s.*
As		61.1 ± 8.3	58.6 ± 15.9	*n.s.*	17.2 ± 0.936	20.6 ± 6.46	*n.s.*	13.7 ± 3.74	9.05 ± 1.48	*p < 0.05*
Cd		0.272 ± 0.156	0.4 ± 0.161	*n.s.*	0.313 ± 0.0418	1.23 ± 0.617	*p < 0.05*	0.657 ± 0.302	0.671 ± 0.571	*n.s.*
Cr		0.0573 ± 0.0279	0.647 ± 0.323	*p < 0.05*	1.39 ± 0.808	0.674 ± 0.45	*p < 0.05*	0.585 ± 0.274	0.633 ± 0.324	*n.s.*
Cu		16.9 ± 3.11	9.89 ± 2.61	*p < 0.05*	25.7 ± 6.17	32.1 ± 7.42	*n.s.*	25.6 ± 11.9	26.1 ± 13	*n.s.*
Fe		366 ± 150	646 ± 223	*p < 0.05*	1148 ± 357	1069 ± 328	*n.s.*	536 ± 111	538 ± 255	*n.s.*
Hg		n/a	0.372 ± 0.216	*n.t.*	0.611 ± 0.215	0.9 ± 0.552	*n.s.*	0.725 ± 0.208	0.62 ± 0.335	*n.s.*
Mn		n/a	13.3 ± 2.25	*n.t.*	10.1 ± 2.2	18.1 ± 7.1	*p < 0.05*	15.3 ± 3.89	13.1 ± 5.2	*n.s.*
Ni		n/a	0.533 ± 0.39	*n.t.*	0.392 ± 0.235	0.744 ± 0.379	*p < 0.05*	0.417 ± 0.147	0.386 ± 0.0409	*n.s.*
Pb		0.786 ± 0.376	2.28 ± 2.27	*p < 0.05*	1.56 ± 0.47	4.19 ± 0.852	*p < 0.05*	4.68 ± 1.22	7.14 ± 3.21	*n.s.*
V		n/a	0.315 ± 0.194	*n.t.*	0.166 ± 0.0422	6.92 ± 3.63	*p < 0.05*	0.292 ± 0.184	0.497 ± 0.244	*p < 0.05*
Zn		61 ± 7.58	98.3 ± 13.6	*p < 0.05*	142 ± 0.473	171 ± 48	*p < 0.05*	129 ± 12	126 ± 13.9	*n.s.*

n.s. = not significant
n.t. = not tested

Table S1b. Continues.

		Mullus barbatus			*Diplodus vulgaris*			*Pagellus sp.*		
		OUTSIN	INSIN	*Sig.*	OUTSIN	INSIN	*Sig.*	OUTSIN	INSIN	*Sig.*
Naphthalene	ng/g (dw)	88.7 ± 36.1	89.1 ± 32.8	*n.s.*	196 ± 42.9	65.8 ± 13.7	*p < 0.05*	92.5 ± 44.3	129 ± 42.7	*n.s.*
Acenapthylene		< 0.05	< 0.05	*n.t.*	< 0.05	< 0.05	*n.t.*	< 0.05	< 0.05	*n.t.*
1-methylnaphthalene		80.7 ± 20.7	50.7 ± 10.6	*n.s.*	115 ± 18.7	38.4 ± 7.8	*p < 0.05*	66.6 ± 35.1	79.9 ± 33	*n.s.*
2-methylnaphthalene		62.8 ± 16.1	39.3 ± 11.9	*n.s.*	105 ± 6.69	40.2 ± 9.11	*p < 0.05*	42.4 ± 22.3	59.5 ± 21.9	*n.s.*
Acenaphthene		3.79 ± 1.15	9.77 ± 1.36	*p < 0.05*	< 0.01	12.7 ± 1.58	*n.t.*	< 0.01	8.35 ± 3.23	*n.t.*
Fluorene		13 ± 2.8	6.11 ± 0.385	*p < 0.05*	7.68 ± 0.0539	6.47 ± 0.81	*n.s.*	5.83 ± 1.87	6.46 ± 1.62	*n.s.*
Phenanthrene		36.6 ± 9.98	23.5 ± 18.4	*n.s.*	31.2 ± 26.7	3.16 ± 1.18	*p < 0.05*	27 ± 21.8	38.4 ± 18.8	*n.s.*
Anthracene		0.38 ± 0.489	0.728 ± 0.121	*p < 0.05*	< 0.01	1.08 ± 0.0569	*n.t.*	0.473 ± 0.329	0.52 ± 0.079	*n.s.*
Fluoranthene		4.05 ± 1.76	< 0.01	*n.t.*	< 0.01	< 0.01	*n.t.*	< 0.01	7.96 ± 2.82	*n.t.*
Pyrene		1.81 ± 0.858	2.32 ± 1.54	*n.s.*	6.77 ± 1.12	3.26 ± 1.67	*n.s.*	9.23 ± 12.4	2.69 ± 1.58	*p < 0.05*
Benzo(a)anthracene		< 0.01	< 0.01	*n.t.*	< 0.01	< 0.01	*n.t.*	< 0.01	< 0.01	*n.t.*
Chrysene		< 0.05	0.795 ± 0.722	*n.t.*	< 0.01	0.456 ± 0.4	*n.t.*	4.18 ± 6.44	0.473 ± 0.287	*p < 0.05*
7,12-dimethylbenzo(a)anthracene		< 1	20.8 ± 11.2	*n.t.*	< 0.05	16.2 ± 12.3	*n.t.*	< 0.05	7.91 ± 2.05	*n.t.*
Benzo(b)fluoranthene		0.944 ± 0.765	2.07 ± 2.27	*n.s.*	2.76 ± 0.342	0.85 ± 0.299	*p < 0.05*	5.13 ± 7.96	1.12 ± 0.84	*n.s.*
Benzo(k)fluoranthene		0.611 ± 0.684	1.71 ± 1.99	*n.s.*	1.48 ± 1.75	1.66 ± 0.849	*n.s.*	2.69 ± 3.95	0.789 ± 0.657	*n.s.*
Benzo(a)pyrene		0.872 ± 0.627	1.06 ± 1.01	*n.s.*	1.27 ± 0.00229	1.02 ± 0.459	*n.s.*	2.3 ± 3.67	0.73 ± 0.461	*n.s.*
Dibenzo(ah)anthracene		< 0.001	2,391	*n.t.*	2.92 ± 0.00591	< 0.001	*n.t.*	1.12 ± 0.749	1.79 ± 1.33	*n.s.*
Benzo(ghi)perylene		0.631 ± 0.297	2,648	*n.t.*	0.513 ± 0.725	0.376 ± 0.0947	*n.s.*	0.858 ± 0.464	0.251 ± 0.434	*p < 0.05*
Indeno(123cd)pyrene		< 0.001	0,084	*n.t.*	< 0.05	< 0.05	*n.t.*	< 0.05	0.192 ± 0.333	*n.t.*
Low MW PAHs		286 ± 79.1	219 ± 71.2	*n.s.*	456 ± 41.7	167 ± 28.9	*p < 0.05*	234 ± 120	319 ± 119	*n.s.*
High MW PAHs		8.39 ± 0.742	30.1 ± 16.1	*p < 0.05*	15.7 ± 2.5	23.3 ± 15.9	*n.s.*	25 ± 34.3	23.3 ± 8.57	*n.s.*
Total PAHs		294 ± 79.4	249 ± 86.6	*n.s.*	472 ± 44.2	190 ± 44.8	*p < 0.05*	259 ± 125	343 ± 127	*n.s.*

n.s. = not significant

n.t. = not tested

Table S2a. Results of biomarkers analyzed in mussels sampled. Data expressed as mean ± standard deviation (n=5). Asterisks (*) indicate significant differences between areas and reference area (*p < 0.05*), as determined by t-student test.

		PGT (Area 1;9)	PGP (Area 1;7)	P2 (Area 2;10)	BB (Area 6;9;11)	BNF (Area 8)	BRM (Reference area)	*Sig.*
Lysosomal membranes stability	(min)	53.1 ± 8.0		55.2 ± 19.8	76.8 ± 5.0	61.8 ± 15.5	73.5 ± 28.9	*n.s.*
Acetylcholinesterase enzyme activity	(nmol/min/mg prt)	88.1 ± 10.1	94.5 ± 21.7	99.9 ± 23.8	82.7 ± 19.4	99.1 ± 30.5	105.1 ± 25.1	*n.s.*
Metallothioneins	(nmol eq.(G)SH/mg prt)	3.2 ± 1.7	1.7 ± 0.5	2.4 ± 0.6	2.00 ± 1.2	2.8 ± 0.5	2.3 ± 0.7	*n.s.*
Micronuclei frequency	(‰)	1.3 ± 0.3	0.8 ± 0.1	0.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.3	0.3 ± 0.1	*n.s.*

n.s. = not significant

Table S2b. Results of biomarkers analyzed in fish species. Data expressed as mean ± standard deviation (n=5). Asterisks (*) indicate significant differences between areas and reference area (*p < 0.05*), as determined by t-student test.

		Mullus barbatus			*Diplodus vulgaris*			*Pagellus sp.*		
		OUTSIN (Reference area)	INSIN (Area 3;4;5;8)	*Sig.*	OUTSIN (Reference area)	INSIN (Area 3;4;5;8)	*Sig.*	OUTSIN (Reference area)	INSIN (Area 3;4;5;8)	*Sig.*
Acetylcholinesterase enzyme activity	(nmol/min/mg prt)	63.3 ± 14.3	64.9 ± 13.1	*n.s.*	85.5 ± 10.6	61.2 ± 12.9	*n.s.*	91.4 ± 16.3	66.5 ± 11.8	*n.s.*
EROD enzyme activity	(pmol/min/mg prt)	85.3 ± 23.4	162.6 ± 47	*p < 0.05*	7.3 ± 4.1	4.3 ± 2.6	*n.s.*	27.2 ± 20.1	67.7 ± 15.3	*p < 0.05*
Pyrene-like metabolites	(µg/µmol biliverdina)	0.4 ± 0.3	6.7 ± 2.3	*p < 0.01*	1.3 ± 1.0	5.4 ± 5.9	*n.s.*	0.1 ± 0.1	3.8 ± 2.7	*p < 0.05*
B[a]P-like metabolites	(µg/µmol biliverdina)	3.6 ± 1.7	20.2 ± 4.7	*p < 0.01*	7.1 ± 3.9	10.5 ± 7.8	*n.s.*	0.6 ± 0.5	11.8 ± 3.6	*p < 0.01*
Naphtalene-like metabolites	(mg/µmol biliverdina)	9.0 ± 5.9	8.6 ± 1.8	*n.s.*	3.9 ± 2.0	8.2 ± 3.4	*n.s.*	1.1 ± 0.6	6.5 ± 4.0	*p < 0.05*
Micronuclei frequency	(‰)	6.3 ± 0.5	10.6 ± 1.8	*p < 0.05*	4.8 ± 0.6	5.3 ± 1.1	*n.s.*	5.5 ± 0.8	6.3 ± 0.8	*n.s.*

n.s. = not significant

Table S3a. Result of the bioassay with *Vibrio fischeri.* Data are expressed in toxic units (TU), reporting mean ± standard deviation.

Area	Sample	CONTROL T.U.	TREATED T.U.
Area 1	18	100	100
	19	100	100
	20	100	100
	23	100	100
	24	100	100
	25	100	100
	26	81 .2 ± 1 .1	240 .8 ± 18 .6
	27	63 ± 0 .9	36 .7 ± 4 .7
	28	108 .8 ± 2 .5	118 .6 ± 41 .4
	29	100	100
	30	100	100
	31	100	100
	32	100	100
	33	92 .2 ± 2	4799 .1 ± 824 .1
	34	100	100
	35	67 .8 ± 3 .9	108 .2 ± 12
	36	100	100
	37	100	100
	41	100	100
	42	117 .5 ± 2 .6	3006 .3 ± 4338 .4
	43	130 ± 7 .5	366 .3 ± 77
	44	100	100
	48	71 .5 ± 8	172 .9 ± 14 .5
Area 2	59	100	100
	60	63 .8 ± 4 .8	93 .4 ± 22 .8
	61	100	100
	62	100	100
	69	100	100
	70	100	100
	71	100	100
Area 3	96	96 ± 1 .9	69 .8 ± 3 .5
	97	96 ± 1 .9	80 .3 ± 2
	98	96 ± 1 .9	74 ± 11 .3
	99	96 ± 1 .9	64 .2 ± 1 .5
	100	96 ± 1 .9	86 .8 ± 1 .4
	104	96 ± 1 .9	37 .6 ± 0 .2
	105	96 ± 1 .9	52 .8 ± 1 .7
	106	96 ± 1 .9	37 ± 0 .4
	108	96 ± 1 .9	17 .9 ± 3
Area 4	109	93 .9 ± 1 .4	67 .7 ± 0 .9
	110	93 .9 ± 1 .4	78 .8 ± 1 .5
	111	96 ± 1 .9	10 .6 ± 0 .2
	112	91 .5 ± 2 .1	7 .5 ± 0 .7
	113	91 .5 ± 2 .1	2 .5 ± 0 .7
	114	106 ± 8 .4	58 ± 4 .2
	115	91 .5 ± 2 .1	7 ± 2 .8
	116	91 .5 ± 0 .7	12 .5 ± 3 .5

Table S3a. Continues.

Area	Sample	CONTROL T.U.	TREATED T.U.
Area 5	117	99 ± 1 .4	34 .5 ± 6 .3
	118	99 ± 1 .4	21 .5 ± 0 .7
	119	91 .5 ± 0 .7	5 .5 ± 0 .7
	120	94 .5 ± 0 .7	56 ± 1 .4
	121	95	15 .5 ± 0 .7
	122	94 .5 ± 0 .7	14 ± 1 .4
	123	94 .5 ± 0 .7	25 ± 1 .4
Area 6	101	96 ± 1 .9	65 .5 ± 2 .9
	102	96 ± 1 .9	83 ± 1 .9
	103	96 ± 1 .9	83 ± 2 .1
	107	96 ± 1 .9	61 .8 ± 2 .7
Area 7	38	100	100
	39	100	100
	40	100	100
	45	216 .2 ± 20 .5	293 .3 ± 45 .2
	46	100	100
	47	75 .9 ± 8 .5	78 .8 ± 8 .7
	49	138 .6 ± 1	1269 .7 ± 358 .7
	50	158 .4 ± 1 .2	6641 .8 ± 1243 .6
	51	151 .3 ± 0	9510 .6 ± 1182 .3
	52	199 .9 ± 22 .8	3434 .2 ± 663 .3
	53	209 .7 ± 7 .7	1263 ± 426 .4
	54	239 .3 ± 19 .4	228 .7 ± 20 .3
	55	214 .4 ± 6 .5	18384 .7 ± 1613 .5
	56	165 .5 ± 2 .4	335 .3 ± 35
	57	100	100
	58	197 .7 ± 10 .4	1058 .7 ± 323 .1
	64	216 .6 ± 15 .7	77 .2 ± 4 .7
	65	220 .7 ± 13 .2	2401 .4 ± 563 .3
	66	243 .7 ± 14 .6	168 .4 ± 11 .2
	67	100	100
	68	100	100
	73	217 .1 ± 12 .7	1866 .9 ± 688 .8
	74	171 .9 ± 7 .5	881 .5 ± 166 .7
	75	156 .2 ± 12 .7	298 .9 ± 62 .1
	77	100	100
Area 8	124	94 .5 ± 0 .7	50 ± 1 .4
	125	94 .5 ± 0 .7	65 ± 2 .8
	126	94 .5 ± 0 .7	47 ± 2 .8
	127	94 .5 ± 0 .7	41 ± 1 .4

Table S3a. Continues.

Area	Sample	CONTROL	TREATED
		T.U.	T.U.
Area 9	1	100	100
	2	100	100
	3	100	100
	4	100	100
	5	100	100
	6	100	100
	7	100	100
	8	100	100
	9	100	100
	10	100	100
	11	100	100
	12	100	100
	13	100	100
	14	100	100
	15	100	100
Area 10	63	100	100
	72	100	100
	80	92 .4 ± 0 .6	133 .9 ± 13 .7
	81	100	100
	82	100	100
	83	100	100
	84	100	100
	85	100	100
	91	100	100
	92	100	100
	94	71 .3 ± 2 .6	109 .1 ± 17 .1
	95	101 .2 ± 3 .7	108 .3 ± 6 .1
Area 11	16	100	100
	17	100	100
	21	100	100
	22	100	100

Table S3b. Result of the bioassay with *Skeletonema costatum.* Algal growth values are expressed as mean cell density (cells/mL) ± standard deviation.

Area	Sample	CONTROL cell/ml	TREATED cell/ml
Area 1	18	19563 .4 ± 247849 .7	27307 .3 ± 68014 .5
	19	14653 .7 ± 210384 .1	15619 .4 ± 98947 .7
	20	19971 ± 226955 .4	1222 .7 ± 166722 .2
	23	19971 ± 226955 .4	5909 .8 ± 165425 .3
	24	33828 .5 ± 222488 .4	4483 .2 ± 45343
	25	14653 .7 ± 210384 .1	8312 .8 ± 87900 .2
	26	14653 .7 ± 210384 .1	815 .1 ± 213266
	27	8359 .3 ± 189441 .7	8287 .3 ± 312406
	28	19563 .4 ± 247849 .7	10153 .9 ± 74162 .8
	29	23948 .7 ± 169844 .3	4991 .7 ± 65709
	30	14653 .7 ± 210384 .1	29751 .8 ± 193572 .6
	31	19971 ± 226955 .4	3750 .2 ± 110975 .2
	32	19971 ± 226955 .4	27307 .3 ± 245928 .4
	33	23948 .7 ± 169844 .3	11699 .5 ± 132955
	34	14653 .7 ± 210384 .1	53828 .4 ± 153705 .2
	35	2512 .4 ± 182601 .8	8438 .4 ± 176146 .2
	36	19563 .4 ± 247849 .7	14471 .2 ± 350832 .3
	37	19563 .4 ± 247849 .7	4075 .7 ± 141793 .1
	41	2512 .4 ± 182601 .8	3668 .1 ± 244391 .4
	42	19563 .4 ± 247849 .7	12763 .3 ± 121811 .4
	43	13042 .3 ± 212497 .5	13449 .8 ± 136413 .4
	44	13042 .3 ± 212497 .5	25946 .3 ± 458041 .7
	48	2512 .4 ± 182601 .8	10049 .7 ± 135568
Area 2	59	33828 .5 ± 222488 .4	6647 .3 ± 55333 .9
	60	19563 .4 ± 247849 .7	14547 .5 ± 401170 .8
	61	6349 ± 174455 .5	9246 .3 ± 136413 .4
	62	2512 .4 ± 182601 .8	3260 .5 ± 229635 .7
	69	33828 .5 ± 222488 .4	1199 .8 ± 39963 .3
	70	2512 .4 ± 182601 .8	8823 .4 ± 212113 .3
	71	6349 ± 174455 .5	18650 .6 ± 216724 .4
Area 3	96	4890 .8 ± 204966	10316 .2 ± 199048 .4
	97	4890 .8 ± 204966	11412 ± 232863 .5
	98	4890 .8 ± 204966	5598 .2 ± 172534 .2
	99	4890 .8 ± 204966	8671 .5 ± 201353 .9
	100	4890 .8 ± 204966	4048 .4 ± 181948 .6
	104	4890 .8 ± 204966	20760 .8 ± 221527 .8
	105	4890 .8 ± 204966	6113 .5 ± 204043 .8
	106	4890 .8 ± 204966	18748 .3 ± 205580 .8
	108	4890 .8 ± 204966	14986 .2 ± 205773
Area 4	109	4890 .8 ± 204966	17862 ± 188289
	110	4890 .8 ± 204966	17118 ± 242278
	111	4890 .8 ± 204966	10596 .8 ± 186137 .1
	112	4890 .8 ± 204966	7743 .8 ± 189518 .6
	113	4890 .8 ± 204966	2512 .4 ± 130649 .5
	114	4890 .8 ± 204966	15935 .3 ± 202276 .2
	115	4890 .8 ± 204966	9046 .5 ± 235553 .4
	116	4890 .8 ± 204966	15690 .2 ± 190786 .7

Table S3b. Continues.

Area	Sample	CONTROL	TREATED
		cell/ml	cell/ml
Area 5	117	4890 .8 ± 204966	13449 .9 ± 221912
	118	4890 .8 ± 204966	9374 .1 ± 231518 .6
	119	4890 .8 ± 204966	10507 .6 ± 176569
	120	4890 .8 ± 204966	5763 .9 ± 192515 .9
	121	4890 .8 ± 204966	16152 .7 ± 205580 .8
	122	4890 .8 ± 204966	4075 .7 ± 180699 .8
	123	4890 .8 ± 204966	16387 .6 ± 218645 .8
Area 6	101	4890 .8 ± 204966	13857 .4 ± 186944 .1
	102	4890 .8 ± 204966	17933 .2 ± 181948 .6
	103	4890 .8 ± 204966	14494 .1 ± 172918 .5
	107	4890 .8 ± 204966	4924 .7 ± 180988
Area 7	38	8359 .3 ± 189441 .7	18528 .5 ± 301646 .6
	39	2512 .4 ± 182601 .8	7321 .1 ± 178912 .9
	40	2512 .4 ± 182601 .8	2853 ± 127267 .9
	45	19563 .4 ± 247849 .7	5706 ± 300109 .5
	46	8359 .3 ± 189441 .7	9719 .2 ± 307410 .5
	47	19563 .4 ± 247849 .7	58690 .4 ± 236321 .8
	49	23948 .7 ± 169844 .3	14721 .5 ± 258609 .1
	50	19971 ± 226955 .4	40443 .7 ± 77813 .3
	51	6349 ± 174455 .5	38311 .8 ± 264373 .1
	52	14653 .7 ± 210384 .1	24454 .3 ± 271385 .9
	53	14653 .7 ± 210384 .1	25946 .3 ± 402707 .8
	54	13042 .3 ± 212497 .5	7043 .6 ± 208270 .6
	55	8359 .3 ± 189441 .7	8559 ± 169844 .3
	56	14653 .7 ± 210384 .1	3174 .5 ± 127767 .5
	57	14653 .7 ± 210384 .1	47278 .4 ± 340553 .2
	58	14653 .7 ± 210384 .1	4483 .2 ± 181564 .3
	64	6349 ± 174455 .5	5198 .2 ± 209807 .7
	65	33828 .5 ± 222488 .4	1222 .7 ± 6916 .7
	66	6349 ± 174455 .5	7411 .4 ± 294345 .6
	67	2512 .4 ± 182601 .8	4483 .2 ± 262836
	68	13042 .3 ± 212497 .5	6099 .9 ± 504921 .8
	73	13042 .3 ± 212497 .5	25946 .3 ± 402707 .8
	74	2512 .4 ± 182601 .8	18340 .7 ± 209346 .6
	75	6349 ± 174455 .5	8372 .6 ± 396559 .6
	77	2512 .4 ± 182601 .8	9374 .1 ± 177760 .1
Area 8	124	4890 .8 ± 204966	15487 .7 ± 174455 .5
	125	4890 .8 ± 204966	15487 .7 ± 177913 .9
	126	4890 .8 ± 204966	14744 .1 ± 182332 .9
	127	4890 .8 ± 204966	20378 .6 ± 173610 .1

Table S3b. Continues.

Area	Sample	CONTROL	TREATED
		cell/ml	cell/ml
Area 9	1	13042 .3 ± 212497 .5	8873 .4 ± 397328 .1
	2	23948 .7 ± 169844 .3	33417 .6 ± 269368 .5
	3	19563 .4 ± 247849 .7	2445 .4 ± 152168 .2
	4	6349 ± 174455 .5	3049 .9 ± 175224
	5	14653 .7 ± 210384 .1	9228 .3 ± 191170 .9
	6	19563 .4 ± 247849 .7	18492 .6 ± 157547 .9
	7	6349 ± 174455 .5	37806 .9 ± 199048 .3
	8	8359 .3 ± 189441 .7	15640 .7 ± 143714 .4
	9	6349 ± 174455 .5	7500 .5 ± 174455 .5
	10	23948 .7 ± 169844 .3	40660 .6 ± 278975 .1
	11	19563 .4 ± 247849 .7	45847 .8 ± 141793 .1
	12	14653 .7 ± 210384 .1	6555 ± 249194 .7
	13	8359 .3 ± 189441 .7	6730 ± 309716 .1
	14	19971 ± 226955 .4	20582 .4 ± 205556 .8
	15	13042 .3 ± 212497 .5	14944 .3 ± 428069 .2
Area 10	63	2512 .4 ± 182601 .8	1729 .1 ± 196435 .3
	72	6349 ± 174455 .5	6754 .7 ± 142945 .9
	80	6349 ± 174455 .5	14270 .8 ± 230557 .9
	81	2512 .4 ± 182601 .8	3260 .5 ± 202890 .9
	82	6349 ± 174455 .5	2641 .3 ± 262836
	83	2512 .4 ± 182601 .8	2037 .8 ± 172918 .4
	84	2512 .4 ± 182601 .8	20378 .6 ± 269291 .6
	85	6349 ± 174455 .5	22303 .8 ± 389642 .9
	91	6349 ± 174455 .5	21661 .5 ± 185983 .4
	92	6349 ± 174455 .5	16475 .2 ± 319322 .7
	94	6349 ± 174455 .5	9922 .2 ± 219030
	95	6349 ± 174455 .5	23472 .3 ± 276669 .5
Area 11	16	14653 .7 ± 210384 .1	12789 .3 ± 76852 .6
	17	19971 ± 226955 .4	8966 .5 ± 213611 .9
	21	33828 .5 ± 222488 .4	11819 .6 ± 192285 .3
	22	33828 .5 ± 222488 .4	3923 .4 ± 226715 .3

Table S3c. Result of bioassay with *Paracentrotus lividus.* Values are expressed as mean % of normal embryos ± standard deviation.

Area	Sample	CONTROL	TREATED
		%	%
Area 1	18	84 .6 ± 1 .5	82 .6 ± 2 .5
	19	84 .6 ± 1 .5	3 .3 ± 1 .5
	20	84 .6 ± 1 .5	68 ± 2
	23	84 .6 ± 0 .5	84 .6 ± 0 .5
	24	84 .6 ± 1 .5	82 .3 ± 0 .5
	25	81 .3 ± 1 .5	59 .6 ± 1 .5
	26	81 .3 ± 1 .5	18 .6 ± 2
	27	81 .3 ± 1 .5	70 .3 ± 0 .5
	28	84 .6 ± 0 .5	70 ± 2 .6
	29	84 .6 ± 0 .5	85 .3 ± 2 .5
	30	84 .6 ± 0 .5	9 ± 1
	31	81 .3 ± 1 .5	80 .6 ± 1 .1
	32	81 .3 ± 1 .5	62 .3 ± 2 .5
	33	84 .6 ± 0 .5	61 .6 ± 1 .5
	34	84 .6 ± 0 .5	38 .3 ± 3 .5
	35	79 .6 ± 3	79 .6 ± 3 .5
	36	84 .6 ± 0 .5	83 .3 ± 2 .8
	37	79 .6 ± 3	79 .3 ± 1 .1
	41	84 .6 ± 0 .5	16 .6 ± 2 .8
	42	81 .3 ± 1 .5	0
	43	81 .3 ± 1 .5	0 .6 ± 0 .5
	44	79 .6 ± 3	80 .3 ± 3 .5
	48	84 .6 ± 0 .5	5 .3 ± 0 .5
Area 2	59	84 .6 ± 0 .5	23 ± 1
	60	84 .6 ± 1 .5	32 .6 ± 2
	61	84 .6 ± 0 .5	84 .3 ± 0 .5
	62	84 .6 ± 0 .5	61 .6 ± 2 .8
	69	81 .3 ± 1 .5	80 .6 ± 1 .1
	70	79 .6 ± 3	78 .6 ± 1 .1
	71	79 .6 ± 3	61 ± 3 .6
Area 3	96	89 ± 2	0 .6 ± 1 .1
	97	89 ± 2	82 ± 2 .6
	98	89 ± 2	88 .6 ± 0 .5
	99	89 ± 2	0
	100	89 ± 2	0
	104	89 ± 2	73 ± 2 .6
	105	89 ± 2	23 ± 5 .2
	106	89 ± 2	89 ± 1
	108	89 ± 2	89 .3 ± 0 .5
Area 4	109	89 ± 2	17 .3 ± 6 .8
	110	89 ± 2	88 .3 ± 1 .5
	111	89 ± 2	89 ± 1
	112	89 ± 2	88 .3 ± 2
	113	89 ± 2	46 .3 ± 1 .5
	114	89 ± 2	88 .6 ± 0 .5
	115	89 ± 2	89 .3 ± 0 .5
	116	89 ± 2	87 .6 ± 0 .5

Table S3c. Continues.

Area	Sample	CONTROL	TREATED
		%	%
Area 5	117	89 ± 2	88 .3 ± 2 .5
	118	89 ± 2	0
	119	89 ± 2	88 ± 1
	120	89 ± 2	21 .3 ± 11 .7
	121	89 ± 2	77 .3 ± 2 .5
	122	89 ± 2	89 ± 1
	123	89 ± 2	15 .3 ± 0 .5
Area 6	101	89 ± 2	82 .3 ± 2 .5
	102	89 ± 2	80 .6 ± 1 .1
	103	89 ± 2	87 ± 1 .7
	107	89 ± 2	89 .6 ± 1 .1
Area 7	38	84 .6 ± 0 .5	84 .6 ± 0 .5
	39	84 .6 ± 0 .5	73 .3 ± 2 .8
	40	79 .6 ± 3	79 .6 ± 1 .5
	45	79 .6 ± 3	74 ± 5 .2
	46	79 .6 ± 3	79 .3 ± 2
	47	84 .6 ± 1 .5	65 ± 3
	49	84 .6 ± 1 .5	0
	50	81 .3 ± 1 .5	0
	51	84 .6 ± 1 .5	17 .6 ± 2 .5
	52	84 .6 ± 0 .5	7 .3 ± 1 .5
	53	81 .3 ± 1 .5	0 .6 ± 0 .5
	54	79 .6 ± 3	0
	55	79 .6 ± 3	0
	56	84 .6 ± 0 .5	52 .3 ± 2 .5
	57	81 .3 ± 1 .5	70 .6 ± 1 .1
	58	81 .3 ± 1 .5	72 ± 2 .6
	64	84 .6 ± 0 .5	84 .6 ± 0 .5
	65	84 .6 ± 0 .5	53 .6 ± 1 .5
	66	84 .6 ± 1 .5	0
	67	84 .6 ± 0 .5	36 ± 1 .7
	68	81 .3 ± 1 .5	81 .3 ± 1 .1
	73	84 .6 ± 1 .5	77 .6 ± 2 .5
	74	84 .6 ± 1 .5	86 .6 ± 1 .5
	75	84 .6 ± 1 .5	84 .6 ± 0 .5
	77	79 .6 ± 3	81 .3 ± 3 .2
Area 8	124	89 ± 2	58 .6 ± 4
	125	89 ± 2	0
	126	89 ± 2	89 .3 ± 1 .1
	127	89 ± 2	88 .3 ± 1 .1

Table S3c. Continues.

Area	Sample	Area	CONTROL	TREATED
			%	%
Area 9	1	Area 9	84 .6 ± 0 .5	84 .6 ± 1 .5
	2	Area 9	84 .6 ± 0 .5	84 .6 ± 1 .5
	3	Area 9	84 .6 ± 0 .5	84 .3 ± 0 .5
	4	Area 9	84 .6 ± 0 .5	84 .6 ± 0 .5
	5	Area 9	84 .6 ± 0 .5	84 .6 ± 1 .1
	6	Area 9	84 .6 ± 0 .5	84 .6 ± 0 .5
	7	Area 9	84 .6 ± 0 .5	84 .3 ± 3
	8	Area 9	84 .6 ± 0 .5	85 .3 ± 1 .5
	9	Area 9	84 .6 ± 0 .5	84 .6 ± 0 .5
	10	Area 9	84 .6 ± 1 .5	83 .6 ± 1 .1
	11	Area 9	84 .6 ± 0 .5	84 .6 ± 0 .5
	12	Area 9	84 .6 ± 0 .5	84 .6 ± 4 .1
	13	Area 9	84 .6 ± 0 .5	80 .3 ± 1 .5
	14	Area 9	84 .6 ± 0 .5	84 .6 ± 0 .5
	15	Area 9	84 .6 ± 1 .5	54 .6 ± 2 .5
Area 10	63	Area 10	79 .6 ± 3	68 .6 ± 1 .5
	72	Area 10	79 .6 ± 3	62 .6 ± 4 .6
	80	Area 10	81 .3 ± 1 .5	80 .6 ± 1 .1
	81	Area 10	79 .6 ± 3	80 .6 ± 4
	82	Area 10	79 .6 ± 3	78 .6 ± 4 .1
	83	Area 10	79 .6 ± 3	79 .3 ± 0 .5
	84	Area 10	84 .6 ± 1 .5	87 ± 1 .7
	85	Area 10	79 .6 ± 3	6 .3 ± 1 .5
	91	Area 10	79 .6 ± 3	63 .6 ± 3 .2
	92	Area 10	79 .6 ± 3	80 .6 ± 1 .1
	94	Area 10	79 .6 ± 3	79 .6 ± 2 .5
	95	Area 10	79 .6 ± 3	80 .6 ± 1 .1
Area 11	16	Area 11	84 .6 ± 0 .5	60 .3 ± 0 .5
	17	Area 11	84 .6 ± 0 .5	84 .6 ± 3 .5
	21	Area 11	84 .6 ± 0 .5	84 .6 ± 0 .5
	22	Area 11	84 .6 ± 1 .5	0

Table S4a. List of observed species in benthic communities analyses.

Phylum	Class	Order	Family	Species
Annelida	Polychaeta	Eunicida	Dorvilleidae	*Protodorvillea kefersteini* (McIntosh, 1869)
			Eunicidae	*Eunice vittata* (Delle Chiaje, 1828)
			Eunicidae	*Lysidice unicornis* (Grube, 1840)
			Eunicidae	*Marphysa bellii* (Audouin & Milne-Edwards, 1833)
			Lumbrineridae	*Lumbrineris latreilli* Audouin & Milne-Edwards, 1834
			Lumbrineridae	*Ninoe armoricana* Glémarec, 1968
			Oenonidae	*Drilonereis filum* (Claparède, 1868)
			Onuphidae	*Aponuphis bilineata* (Baird, 1870)
			Onuphidae	*Hyalinoecia tubicola* (O.F. Müller, 1776)
			Onuphidae	*Onuphis eremita* Audouin & Milne Edwards, 1833
		Phyllodocida	Aphroditidae	*Pontogenia chrysocoma* (Baird, 1865)
			Glyceridae	*Glycera tridactyla* Schmarda, 1861
			Glyceridae	*Glycera unicornis* Lamarck, 1818
			Goniadidae	*Goniada maculata* Örsted, 1843
			Hesionidae	*Psamathe fusca* Johnston, 1836
			Nephtyidae	*Nephtys hombergii* Savigny in Lamarck, 1818
			Nereididae	*Nereis rava* Ehlers, 1868
			Paralacydoniidae	*Paralacydonia paradoxa* Fauvel, 1913
			Phyllodocidae	*Mysta picta* (Quatrefages, 1866)
			Phyllodocidae	*Nereiphylla rubiginosa* (Saint-Joseph, 1888)
			Phyllodocidae	*Phyllodoce lineata* (Claparède, 1870)
			Phyllodocidae	Phyllodocidae indet.
			Pilargidae	*Sigambra tentaculata* (Treadwell, 1941)
			Polynoidae	*Harmothoe antilopes* McIntosh, 1876
			Polynoidae	*Harmothoe longisetis* (Grube, 1863)
			Polynoidae	*Harmothoe* sp.
			Polynoidae	Polynoidae indet.
			Sigalionidae	*Sigalion mathildae* Audouin & Milne Edwards in Cuvier, 1830
			Sigalionidae	*Sthenelais boa* (Johnston, 1833)
			Syllidae	*Exogone* sp.
			Syllidae	Syllidae indet.
		Sabellida	Oweniidae	*Owenia fusiformis* Delle Chiaje, 1844
			Sabellidae	*Acromegalomma claparedei* (Gravier, 1906)
			Sabellidae	*Dialychone acustica* Claparède, 1870
			Sabellidae	*Dialychone arenicola* (Langerhans, 1881)
			Serpulidae	*Ditrupa arietina* (O. F. Müller, 1776)
		Spionida	Magelonidae	*Magelona alleni* Wilson, 1958
			Magelonidae	*Magelona johnstoni* Fiege, Licher & Mackie, 2000
			Poecilochaetidae	*Poecilochaetus serpens* Allen, 1904
			Spionidae	*Dipolydora coeca* (Örsted, 1843)
			Spionidae	*Paraprionospio pinnata* (Ehlers, 1901)
			Spionidae	*Prionospio ehlersi* Fauvel, 1928
			Spionidae	*Prionospio fallax* Soderstrom, 1920
			Spionidae	*Pseudopolydora antennata* (Claparède, 1869)
			Spionidae	*Scolelepis (Scolelepis) squamata* (O.F. Muller, 1806)
			Spionidae	*Spio filicornis* (Müller, 1776)

Table S4a. Continues.

Phylum	Class	Order	Family	Species
Annelida	Polychaeta	Spionida	Spionidae	*Spio multioculata* (Rioja, 1918)
			Spionidae	*Spiophanes reyssi* Laubier, 1964
		Terebellida	Ampharetidae	*Adercodon pleijeli* Mackie, 1994
			Ampharetidae	*Adercodon pleijeli* Mackie, 1994
			Ampharetidae	*Ampharete acutifrons* (Grube, 1860)
			Ampharetidae	*Melinna palmata* Grube, 1870
			Cirratulidae	*Aphelochaeta marioni* (Saint-Joseph, 1894)
			Cirratulidae	*Chaetozone caputesocis* (Saint-Joseph, 1894)
			Cirratulidae	Cirratulidae indet.
			Cirratulidae	*Kirkegaardia dorsobranchialis* (Kirkegaard, 1959)
			Flabelligeridae	*Diplocirrus glaucus* (Malmgren, 1867)
			Sternaspidae	*Sternaspis scutata* (Ranzani, 1817)
			Terebellidae	*Pista cretacea* (Grube, 1860)
			Trichobranchidae	*Terebellides stroemii* Sars, 1835
			Capitellidae	*Leiocapitella dollfusi* (Fauvel, 1936)
			Capitellidae	*Notomastus latericeus* Sars, 1851
			Capitellidae	*Pseudoleiocapitella fauveli* Harmelin, 1964
			Chaetopteridae	*Phyllochaetopterus socialis* Claparède, 1868
			Cossuridae	*Cossura soyeri* Laubier, 1962
			Maldanidae	*Chirimia biceps* (M. Sars, 1861)
			Maldanidae	*Euclymene lombricoides* (Quatrefages, 1866)
			Maldanidae	*Euclymene oerstedi* (Claparède, 1863)
			Maldanidae	*Leiochone leiopygos* (Grube, 1860)
			Maldanidae	*Metasychis gotoi* (Izuka, 1902)
			Maldanidae	*Praxillella* sp.
			Ophelidae	*Armandia cirrhosa* Filippi, 1861
			Opheliidae	*Ophelia* sp.
			Orbiniidae	*Phylo foetida ligustica* (Orlandi, 1896)
			Paraonidae	*Aricidea (Acmira) catherinae* Laubier, 1967
			Paraonidae	*Levinsenia gracilis* (Tauber, 1879)
Arthropoda	Hexanauplia	Sessilia	Balanidae	*Balanus trigonus* Darwin, 1854
	Malacostraca	Amphipoda	Ampeliscidae	*Ampelisca brevicornis* (Costa, 1853)
			Ampeliscidae	*Ampelisca ledoyeri* Bellan-Santini & Kaim-Malka, 1977
			Ampeliscidae	*Ampelisca ruffoi* Bellan-Santini & Kaim-Malka, 1977
			Ampeliscidae	*Ampelisca* sp.
			Ampeliscidae	*Ampelisca spinifer* Reid, 1951
			Ampeliscidae	*Ampelisca typica* (Spence Bate, 1856)
			Aoridae	*Autonoe spiniventris* Della Valle, 1893
			Aoridae	*Microdeutopus versiculatus* (Spence Bate, 1857)
			Bathyporeiidae	*Bathyporeia lindstromi* Stebbing, 1906
			Cheirocratidae	*Cheirocratus sundevallii* (Rathke, 1843)
			Dexaminidae	*Dexamine spinosa* (Montagu, 1813)
			Dexaminidae	*Guernea (Guernea) coalita* (Norman, 1868)
			Leucothoidae	*Leucothoe pachycera* Della Valle, 1893
			Maeridae	*Othomaera schmidti* (Stephensen, 1915)
			Oedicerotidae	*Deflexilodes gibbosus* (Chevreux, 1888)

Table S4a. Continues.

Phylum	Class	Order	Family	Species
Arthropoda	Malacostraca	Amphipoda	Oedicerotidae	*Kroyera carinata* Spence Bate, 1857
			Oedicerotidae	*Perioculodes longimanus* (Spence Bate & Westwood, 1868)
			Oedicerotidae	*Synchelidium haplocheles* (Grube, 1864)
			Photidae	*Photis longicaudata* (Spence Bate & Westwood, 1862)
			Phoxocephalidae	*Harpinia antennaria* Meinert, 1890
			Phoxocephalidae	*Harpinia truncata* Sars, 1891
			Phoxocephalidae	*Metaphoxus gruneri* Karaman, 1986
			Tryphosidae	*Hippomedon ambiguus* Ruffo, 1946
			Tryphosidae	*Hippomedon massiliensis* Bellan-Santini, 1965
			Urothoidae	*Urothoe elegans* (Spence Bate, 1857)
		Cumacea	Bodotriidae	*Bodotria scorpioides* (Montagu, 1804)
			Bodotriidae	*Iphinoe serrata* Norman, 1867
		Decapoda	Alpheidae	*Alpheus glaber* (Olivi, 1792)
			Callianassidae	*Callianassa* sp.
			Carcinidae	*Xaiva biguttata* (Risso, 1816)
			Diogenidae	*Diogenes pugilator* (Roux, 1829)
			Solenoceridae	*Solenocera membranacea* (Risso, 1816)
				Decapoda indet.
		Isopoda	Sphaeromatidae	*Cymodoce tuberculata* Costa in Hope, 1851
		Mysida		Mysida indet.
		Stomatopoda	Nannosquillidae	*Platysquilla eusebia* (Risso, 1816)
		Tanaidacea	Apseudidae	*Apseudopsis latreillii* (Milne Edwards, 1828)
			Leptocheliidae	*Chondrochelia savignyi* (Kroyer, 1842)
Echinodermata	Echinoidea	Clypeasteroida	Echinocyamidae	*Echinocyamus pusillus* (O.F. Müller, 1776)
	Ophiuroidea	Ophiurida	Amphiuridae	*Amphipholis squamata* (Delle Chiaje, 1828)
			Amphiuridae	*Amphiura chiajei* Forbes, 1843
			Amphiuridae	*Amphiura filiformis* (O.F. Müller, 1776)
			Ophiotrichidae	*Ophiothrix* sp.
			Ophiuridae	*Ophiura ophiura* (Linnaeus, 1758)
	Echinoidea	Spatangoida	Loveniidae	*Echinocardium cordatum* (Pennant, 1777)
Mollusca	Bivalvia	Arcida	Glycymerididae	*Glycymeris bimaculata* (Poli, 1795)
			Noetiidae	*Striarca lactea* (Linnaeus, 1758)
		Cardiida	Cardiidae	*Acanthocardia echinata* (Linnaeus, 1758)
			Cardiidae	*Fulvia australis* (G. B. Sowerby II, 1834)
			Cardiidae	*Laevicardium oblongum* (Gmelin, 1791)
			Cardiidae	*Papillicardium papillosum* (Poli, 1791)
			Cardiidae	*Parvicardium exiguum* (Gmelin, 1791)
			Donacidae	*Donax venustus* Poli, 1795
			Tellinidae	*Fabulina fabula* (Gmelin, 1791)
			Tellinidae	*Moerella pulchella* (Lamarck, 1818)
			Astartidae	*Astarte fusca* (Poli, 1791)
		Littorinimorpha	Naticidae	*Neverita josephinia* Risso, 1826
		Lucinida	Lucinidae	*Lucinella divaricata* (Linnaeus, 1758)
			Thyasiridae	*Thyasira biplicata* (Philippi, 1836)
		Myida	Corbulidae	*Corbula gibba* (Olivi, 1792)
		Nuculanida	Nuculanidae	*Saccella commutata* (Philippi, 1844)

Table S4a. Continues.

Phylum	Class	Order	Family	Species
Mollusca	Bivalvia	Nuculida	Nuculidae	*Nucula nitidosa* Winckworth, 1930
		Venerida	Veneridae	*Callista chione* (Linnaeus, 1758)
			Veneridae	*Chamelea gallina* (Linnaeus, 1758)
			Veneridae	*Chamelea striatula* (da Costa, 1778)
			Veneridae	*Clausinella fasciata* (da Costa, 1778)
			Veneridae	*Dosinia lupinus* (Linnaeus, 1758)
			Veneridae	*Pitar rudis* (Poli, 1795)
			Mactridae	*Spisula subtruncata* (da Costa, 1778)
			Thraciidae	*Thracia phaseolina* (Lamarck, 1818)
			Ungulinidae	*Diplodonta trigona* (Scacchi, 1835)
	Gastropoda	Cephalaspidea	Philinidae	*Philine* sp.
		Littorinimorpha	Naticidae	*Naticarius hebraeus* (Martyn, 1786)
		Neogastropoda	Mangeliidae	*Mangelia costata* (Pennant, 1777)
			Muricidae	*Hexaplex trunculus* (Linnaeus, 1758)
			Nassariidae	*Tritia mutabilis* (Linnaeus, 1758)
			Nassariidae	*Tritia pygmaea* (Lamarck, 1822)
			Ringiculidae	*Ringicula auriculata* (Ménard de la Groye, 1811)
	Polyplacophora			Polyplacophora indet.
	Scaphopoda	Dentaliida	Dentaliidae	*Antalis inaequicostata* (Dautzenberg, 1891)
	Bivalvia	Arcida	Arcidae	*Anadara gibbosa* (Reeve, 1844)
		Cardiida	Tellinidae	*Peronidia albicans* (Gmelin, 1791)
		Lucinida	Lucinidae	*Loripinus fragilis* (Philippi, 1836)
			Mactridae	*Lutraria lutraria* (Linnaeus, 1758)
Sipuncula	Phascolosomatidea	Aspidosiphonida	Aspidosiphonidae	*Aspidosiphon (Aspidosiphon) muelleri muelleri* Diesing, 1851
	Sipunculidea	Golfingiida	Golfingiidae	*Golfingia (Golfingia) elongata* (Keferstein, 1862)
			Phascolionidae	*Phascolion (Phascolion) strombus strombus* (Montagu, 1804)

Table S4b. Ecological quality indices values: organisms abundance (N), speces richness (S), Margalef index (d), Shannon index (H'), Pielou index (J'), AMBI index, BENTHIX index, BOPA index, BITS index, m AMBI index, HQ and level of hazard for Benthic Communities. Quality classes of AMBI index are expressed through conventional colours (blu: elevated; green: good; yellow: sufficient; orange: scarce; red: bad)

Area	Sample	N	H'	S	D	J'	AMBI	BENTIX	BOPA	BITS	m-AMBI	HQ	Level of hazard
Area 1	19	37.7	3.2	13.3	3.4	1.3	0.8	2.6	0.1	1.1	0.7	13.2	Absent
Area 1 - 2 - 10	44	42.3	3.1	14.3	3.6	1.2	0.6	2.9	0.1	1.3	0.7	10.5	Absent
Area 3	98	23	3.1	10.7	3.1	1.3	0.6	3.9	0.2	1.2	0.7	10.8	Absent
Area 3	99	51.3	3.7	21.3	5.2	1.2	0.8	3.8	0.2	1.2	0.9	12.7	Absent
Area 3	104	57	3.9	22	5.2	1.3	0.8	2.5	0.2	1.1	0.9	13.7	Absent
Area 4	111	24	3.7	15.7	4.6	1.4	1.6	2.5	0.3	0.6	0.7	23.6	Slight
Area 4	113	42	3.5	17.7	4.5	1.2	2.6	2.6	0.3	0.6	0.7	32.9	Slight
Area 5	117	17.7	3.4	12	3.8	1.4	1.6	3.7	0.3	0.9	0.7	23.7	Slight
Area 5	122	18.7	2.7	9.3	2.9	1.2	3	2.7	0.2	0.6	0.5	36.8	Slight
Area 6	107	43.7	3.3	17.7	4.5	1.2	0.5	3.6	0.2	0.8	0.8	8.5	Absent
Area 7 - 10	67	51	3.9	21	5.1	1.3	1.2	3.1	0.2	1.1	0.8	20.3	Slight
Area 8	126	74	3.4	25	5.6	1.1	0.5	4	0.2	1	0.9	8.7	Absent
Area 8	127	49.3	3.5	15.7	3.8	1.3	0.4	4.1	0.1	1.2	0.8	6.8	Absent
Area 9	12	34.7	3.5	13.7	3.6	1.3	0.9	2.8	0.2	0.9	0.7	15.5	Absent
Area 11	21	32.3	3.6	17	4.6	1.3	1.1	3.1	0.2	0.8	0.8	18.1	Absent

Table S5. Weight and threshold (%) of biomarkers used for elaboration LOE3: Sublethal effects.

Biomarkers	Species	Weight	Inhibition threshold	Induction threshold
Lysosomal membranes stability	*M. galloprovincialis*	1.2	25	
Acetylcholinesterase enzyme activity		1.5	25	60
Metallothioneins		1		40
Micronuclei frequency		1.9		50
Acetylcholinesterase enzyme activity	*Fish species*	1.5	25	60
EROD enzyme activity		1.5		200
Pyrene-like metabolites		1		150
B[a]P-like metabolites		1		100
Naphtalene-like metabolites		1		200
Micronuclei frequency		1.9		50