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

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23 **Abstract**

24 The aim of the present work is to demonstrate the practical importance of a multidisciplinary

25 approach and weighted criteria to synthesize and integrate different typologies of data (or lines of

26 evidence, LOEs), including chemical levels in marine sediments, their bioavailability to specific

27 indicator species, ecotoxicological effects measured through subcellular biomarkers and batteries

28 of bioassays, and potential impacts of pollution on local benthic communities. The area of

29 Bagnoli (Gulf of Naples, Southern Italy) was selected as a model case-study, as it is a coastal area

30 chronically impacted by massive industrial contamination (trace metals and hydrocarbons), and

31 dismissed decades ago without any subsequent remediation or habitat restoration. The results of

32 each LOE were elaborated to provide specific hazard indices before their overall integration in a

33 weight of evidence (WOE) evaluation. Levels of some trace metals and PAHs revealed a severe

34 contamination in the entire study area. Bioavailability of hydrocarbons was evident particularly

35 for high molecular weight PAHs, which also caused significant variations of cellular biomarkers,

36 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

endpoints, as well as the number and magnitude of observed variations normalized toward specific thresholds. Synthetic and quantitative hazard indices are calculated for each LOE, before their overall integration in the WOE assessment: the calculated level of risk is assigned to 1 of 5 classes, ranging from absent to severe (Piva et al. 2011, Regoli et al. 2019), which is the basis for different management options associated to each class of environmental risk. Weighted criteria for elaboration of chemical data and ecotoxicological bioassays have been incorporated in the last Italian law for determining quality class and management options for dredged marine sediments, based on the weighted elaboration and integration of their chemical and ecotoxicological characteristics (DM 173/2016).

As part of an extensive research project aimed to characterize the environmental quality and a possible remediation strategy of Bagnoli industrial site (ABBaCo 2018), the objective of the present work was to demonstrate the practical efficacy of the WOE approach to elaborate and integrate huge datasets of heterogeneous results. The combination of rigorous mathematical algorithms with the user-friendly outputs of the Sediqualsoft model was expected to represent an important tool to facilitate site-oriented and scientifically supported management options for sediments of such a polluted area.

2. Materials and methods

2.1 Experimental design and sampling activities

The study area is located in the eastern part of the Bay of Pozzuoli, within the Gulf of Naples. According to knowledge from previous studies (ISPRA-ICRAM 2005; Arienzo et al. 2017, 2019; Romano et al. 2018; Trifuoggi et al. 2017, 2018), it was divided in 11 sub-areas, each containing from 5 to 23 sampling points, depending on the distance from the industrial plant and the expected level of contamination (Figure 1). According to requirements of Italian Ministry of Environment and within "ABBaCo project", 118 sediments samples were collected in the study

area between May 2017 and December 2017 for chemical analyses, ecotoxicological bioassays

and analyses of benthic communities.

Bioaccumulation and biomarker responses were carried out on mussels, *Mytilus galloprovincialis*,

and on the fish species, *Mullus barbatus, Pagellus erythrinus* and *Diplodus vulgaris*, sampled in

December 2017. These fish species are commonly used in the biomonitoring of marine

environment (Regoli et al. 2002; Bonsignore et al. 2013), and they were selected to highlight the

potential influence of their different mobility, contact with sediments and feeding behavior on

bioaccumulation and responsiveness to chemical pollutants. Organisms were sampled from

different sites in the same period to avoid that comparisons of bioaccumulation and biomarkers

responses could be differently influenced by seasonal variations (Bocchetti et al. 2006; Fattorini

et al. 2014). Mussels (shell length 5.5 ± 0.5 cm) were collected in 3 points along the 2 industrial

piers (P2, PGT, PGP) and in additional 4 sites at different distances from the plant (Figure 1).

Fish were sampled by local fishermen both in area of Bagnoli (INSIN) and in a reference site

outside the bay (OUTSIN). Mean lengths were recorded both in fish from INSIN (*M. barbatus:*

17.5±1.4 cm; *P. erythrinus:* 15.7±1.5 cm; *D. vulgaris:* 18.7±2.1 cm) and from OUTSIN (*M.*

barbatus: 11.5±2.1 cm; *P. erythrinus:* 22.6±4.6 cm; *D. vulgaris:* 16.7±0.9 cm). Analyses on fish

samples (n=5) were performed using tissues of one individual for each replicate, while mussels

samples (n=5) were constituted each by pooling tissues of 3 individuals.

2.2 Chemical characterization of sediments

After collection, sediment samples for chemical analyses were stored at -20 °C, until analysed for

grain-size distribution (gravel, sand, silt, and clay), organic matter (OM), trace metals and

metalloids (Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V, and Zn), hydrocarbons with C>12, polycyclic

aromatic polyaromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), organotin

compounds, organochlorine pesticides, dioxin (PCDDs) and furan (PCDFs). Measurements were

carried out through validated methods by sieves and laser particle sizer, inductively coupled

plasma-mass spectrometry (ICP-MS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), Atomic Absorption Spectrophotometry, gas-chromatography with mass spectrometry and flame ionization detector (GC-MS, GC-FID), high resolution DFS mass spectrometer Dioxine B Thermo Fischer. For the determination of PCDDs and PCDFs the isotopic dilution procedures reported by USEPA 1613B (1994) were performed. Detailed analytical protocols, including QA/QC procedures, have been given elsewhere (Armiento et al., 2020; Molisso et al. 2020).

2.3 Bioaccumulation analyses

Bioaccumulation of trace elements (Al, As, Cd, Cu, Cr, Hg, Ni, Pb and Zn) and polycyclic aromatic hydrocarbons (PAHs) were carried out on the whole soft tissues of wild specimens of *M. galloprovincialis* and in the liver tissues of *M. barbatus, P. erythrinus* and *D. vulgaris*, following previously validated procedures based on atomic absorption spectrophotometry (AAS) and high performance liquid chromatography (HPLC) with diode array (DAD) and fluorimetric detection (Benedetti et al. 2014, Regoli et al. 2019). All the analytical determinations were performed by analysing five replicates, carefully checking for accuracy, precision and recovery by testing a series of blank solutions (reagents only), reference standards and selected certified standard materials.

2.4 Biomarkers analyses

Lysosomal membrane stability was measured in mussels hemocytes through the Neutral Red Retention Time (NRRT) assay, while metallothioneins (MTs) were determined in digestive glands by spectrophotometric analysis after acidic ethanol/chloroform fractionation using GSH as standard (Viarengo et al., 1997). Acetylcholinesterase enzymatic activity (AChE) was spectrophotometrically assayed in mussels haemolymph and fish brain using the Ellman's reaction (Gorbi et al. 2008). Ethoxyresorufin O-deethylase (EROD) was spectrofluorimetrically determined in individual fish livers measuring resorufin formation from 7-ethoxyresorufin

167 (Regoli et al. 2003). Aromatic metabolites in fish bile were measured by fixed fluorescence (FF)

168 spectrofluorimetry and semi-quantitatively assessed as naphthalene-like, pyrene-like and

169 benzo[a]pyrene-like metabolites (Gorbi and Regoli 2004). Micronuclei (MN) frequency was

170 microscopically measured in mussels hemocytes and fish gills, observing 2000 cells with

171 preserved cytoplasm: such genotoxic alterations were defined as round structures, smaller than

172 1/3 of the main nucleus diameter, on the same optical plan and clearly (Gorbi et al. 2008).

173 Detailed analytical procedure have been previously reported (Benedetti et al., 2014).

174 *2.5 Ecotoxicological bioassays*

175 A battery of ecotoxicological bioassays was applied to sediment samples following standardized

176 procedures. The bioluminescence test with *Vibrio fischeri* (Doe et al. 2005) was selected for the

177 solid phase, while the algal growth inhibition of *Skeletonema costatum* (ISO 10253: 2006) and the

178 sea urchin embryotoxicity assay with *Paracentrotus lividus* (ISPRA, 2017) were used to test

179 elutriates, prepared with standard procedure (USEPA 503/8-91/001: 1991). Detailed procedures

180 for sediments treatment and ecotoxicological bioassays have been reported elsewhere (Morroni et

181 al. 2018; Gallo et al. 2020).

182 *2.6 Benthic communities*

183 Sediment samples were sieved through a mesh net of 1 mm and sorted under the

184 stereomicroscope. All taxa were identified and the main taxa classified to the species level or to

185 the lower possible taxonomic level. For each species, whenever possible, the corresponding

186 biocoenosis was identified. From the list of species, the WOE Sediqualsoft elaborated the

187 available community descriptors, diversity indices and ecological indicators (including

188 abundance, richness, Margalef, Shannon, Pielou, AMBI, BENTHIX, BOPA, BITS, mAMBI)

189 which are reported in Table S4b. The results and the methodological details of these analyses

190 have been reported in Hay Mele et al. (2020).

191 *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, α= 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 (α=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

217 (*HQ_C*), an average *RTRw* is obtained for all of the parameters with *RTR* ≤1 (i.e. values below the

218 SQG), while for those with *RTR* >1, the *RTRw* are individually added into the summation *Σ*:

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

220 Based on expert judgment, the values of *HQ_C* are assigned to one of six classes of chemical

221 hazard, absent, negligible, slight, moderate, major and severe depending on the number, typology

222 and magnitude of exceeding chemicals (Regoli et al., 2019).

223 *LOE 2: Bioavailability of chemicals*

224 The results on bioaccumulation of chemicals in tissues of mussels and fish (LOE2) are elaborated

225 calculating, for each parameter, the increase of concentration compared to control organisms,

226 corrected for the typology of pollutant and the statistical significance of the difference. The

227 cumulative *HQ_{BA}* does not consider parameters with *RTR_w*<1.3, calculates the average for those

228 with *RTR_w* ranging between 1.3 and 2.6, and adds the summation of all those with *RTR_w*≥ 2.6):

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

230 The *HQ_{BA}* is assigned to one of five classes of hazard for bioavailability, from Absent to Severe

231 (Regoli et al., 2019).

232

233 *LOE 3: Sublethal effects: Biomarkers*The module for the elaboration of biomarkers (LOE3)

234 contains a wide battery of responses, each assigned with a weight (based on the relevance of

235 biological endpoint) and a threshold indicative of changes of biological relevance. For each

236 biomarker, the measured variation is compared to the threshold (Table S5), corrected for

statistical significance and importance of biomarker (weight), and assigned to 1 of 5 classes of

effect which are then differently weighted in the calculation of cumulative HQ_{BM}.

$$HQ_{BM} = \left(\frac{\sum_{j=1}^{N} Effect_w(j)_{1.5<Effect(j)\leq2.5}}{num\ biomarker_{1.5<Effect(j)\leq2.5}} + \sum_{k=1}^{M} Effect_w(k)_{Effect(j)>2.5} \right)$$

According to the % distribution of biomarkers in the 5 classes, the level of cumulative HQ_{BM} is

assigned to 1 of 5 classes of hazard: all the more relevant information are given in the model

output (Regoli et al., 2019).

LOE 4: Ecotoxicological Bioassays

Weighted criteria to elaborate results from standardized ecotoxicological bioassays (LOE-4) are

based on specific thresholds and weights assigned to each bioassay depending on the biological

endpoint, tested matrix, time of exposure, and the possibility of hormetic responses.

In the module for ecotoxicological bioassays, the cumulative hazard quotient ($HQ_{Battery}$) is

obtained by the summation (Σ) of the weighted effects (Ew), i.e., the variations measured for each

test compared to specific thresholds, corrected for the statistical significance of the difference (w),

biological importance of the endpoint and exposure conditions (w_2):

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

The $HQ_{Battery}$ is normalized to a scale ranging from 0 to 10, where 1 is the battery threshold (when

all the measured bioassays exhibit an effect equal to the threshold, 10 when all the assays exhibit

100% of effect); the $HQ_{Battery}$ is then assigned to one of five classes of hazard, from Absent to

Severe (Regoli et al., 2019).

LOE 5: Benthic Communities

257 Data on benthic communities are elaborated within a specific module (LOE-5), which converts

258 the list of identified species in several available univariate and multivariate indices for the

259 classification of ecological quality (Vincent 2002, Dauvin and Ruellet 2007, Muxika et al. 2007,

260 Anderson et al. 2008, Mistri and Munari 2008, Sigovini et al. 2013). Such elaborated indices

261 include total abundance (N), species richness (S), Shannon-Weaver Diversity Index (H'),

262 Margalef index (D), Pielou's evenness index (J), AZTI' Marine Biotic Index (AMBI),

263 multimetric-AZTI Marine Biotic Index (m-AMBI), Bentic Index (BENTIX), Benthic Index based

264 on Taxonomic Sufficiency (BITS) and Benthic Opportunistic Polychaetes Amphipods (BOPA

265 index) (Regoli et al., 2019). In this work, the AMBI index was chosen for the integration with

266 other LOEs in the final WOE elaboration of ecological risk.

267 *WOE integration*

268 The huge datasets of results elaborated from the 5 LOEs have been finally integrated through a

269 WOE approach based on the quantitative model Sediqualsoft. The quantitative hazard quotients

270 (HQs) obtained for each LOEs are normalized to a common scale and given a different weight

271 according to previously validated procedures (Piva et al., 2011; Lethonen et al., 2019; Regoli et

272 al., 2019). LOE-2, summarizing bioavailability of chemicals in mussels and fish had a greater

273 weight (w: 1.2) compared to LOE-1 assessing the presence of such compounds in the sediments

274 (w: 1.0); at the biological level, a greater ecological relevance was assigned to LOE-5 on benthic

275 communities (w: 1.3) compared to LOE-4 reflecting acute ecotoxicological effect at an

276 organismal level (w: 1.2), or LOE-3 on sublethal effects at the cellular level (w: 1). An overall

277 WOE level of risk is thus calculated and assigned to 1 of 5 classes of risk from Absent to Severe

278 (Piva et al., 2011). Scientific criteria, validation of weights and thresholds, expert judgment

279 evaluations and specific flow-charts of each LOE have been validated elsewhere (Piva et al.,

280 2011; Benedetti et al., 2012, 2014; Lethonen et al., 2019; Regoli et al., 2019).

281

282 **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 $\sum$ 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

308   in *P. erythrinus* did not reveal significant variations for all the analysed parameters comparing the

309   specimens collected in the industrial and reference areas (Table S1b).

310   The weighted elaboration of these results summarized as "Major" the HQ for bioavailability in *M.*

311   *galloprovincialis* from sub-area 1, essentially due to concentrations of HMW-PAHs

312   (supplementary Table S1), while "Moderate" in mussels collected from the sub-areas 7 and 9

313   (Figure 2b); a lower level of HQ was assigned to bioavailability for mussels in sub-areas 2, 6, 10,

314   11 (Figure 2b).

315   Considering results obtained in fish species, the higher bioavailability HQ was elaborated for *M.*

316   *barbatus*, appearing as "Moderate" in fish collected within the industrial sub-areas 3, 4, 5 and 8.

317

318   **3.3 Biomarkers responses in mussels and fish**

319   Biomarkers analyzed in native mussels revealed a higher sensitivity of mussels collected in sites

320   PGP and PGT (included in sub-areas 1, 7 and 9) which exhibited a decreased lysosomal

321   membrane stability and increased micronuclei frequency compared to organisms from other areas

322   (Supplementary Table S2a). No variations were observed for the enzymatic activity of

323   acetylcholinesterase in hemolymph, nor for metallothioneins in digestive gland of mussels from

324   various sites (Supplementary Table S2a).

325   Results on acetylcholinesterase in brain, EROD enzymatic activity in liver, aromatic bile

326   metabolites and frequency of branchial micronuclei in fish species (*M. barbatus*, *P. erythrinus*

327   and in *D. vulgaris*) are reported in supplementary Table S2b. Acetylcholinesterase enzymatic

328   activity was not affected in *M. barbatus* sampled in the industrial area while a decrease of this

329   biomarker was observed in *P. erythrinus* and *D. vulgaris*. The EROD enzymatic activity was

330   significantly induced in *M. barbatus* ($p<0.05$) and *P. erythrinus* ($p<0.05$) sampled in the industrial

331   area compared to specimens from the reference site, while the cytochrome P450

332   biotransformation pathway was unaffected in *D. vulgaris*. At the same time, all the fish species

333   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).

4. Discussion

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

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

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

The integrative approach of this study showed that toxicity of sediments, measured through standardized batteries of ecotoxicological bioassays, was often not in accordance with chemical characterization. In fact, despite an evident contamination, sediments from the area of Bagnoli inlet as well as those from the southern and the northern stations, showed low levels of acute toxicity; only some samples, particularly those collected close to the industrial plant, revealed evidence of a major acute toxicity, but the overall HQ for ecotoxicological bioassays resulted as "Moderate" in these sub-areas.

Despite the lack of an elevated acute toxicity, the bioavailability of contaminants was evident in terms of bioaccumulation of PAHs, and of HMW hydrocarbons, both in mussels and fish from the industrialized area. For this reason, the level of hazard for bioavailability was calculated as "Moderate" in the greatest part of the study area, with major effects in proximity of industrial piers. The significant accumulation of HMW-PAHs confirmed a pyrolytic origin of such pollution, related to combustion processes of the plant. A significant accumulation of these PAHs was observed also in liver of the benthic *M. barbatus*, indicating sediments as a still active source of these chemical contaminants to local biota.

Bioavailability of PAHs was further confirmed by biotransformation-related biomarkers, such as the induction of EROD enzymatic activity and the accumulation of aromatic metabolites in the bile of *M. barbatus* and *P. erythrinus*. The latter species confirmed the higher sensitivity of these biomarkers compared to tissue concentrations, in revealing bioavailability and metabolism of PAHs in fish. The comparison of the three fish species highlighted *M. barbatus* as the most affected by PAHs, both in terms of accumulation and of cellular responses related to their biotransformation; in *P. erythrinus* the lack of PAHs accumulation did not reflect a lack of exposure but the active metabolization and excretion of these chemicals, as indicated by the significant EROD induction and accumulation of aromatic bile metabolites. Finally, the slight PAHs effects in the more pelagic *D. vulgaris* further confirm the significant role of sediments in direct transfer of chemicals to benthic biota and, indirectly, to trophic webs.

Additional adverse effects of PAHs were revealed by lysosomal membrane destabilization in mussels, and the onset of genotoxic effects in both fish and mussels. The enhancement of micronuclei frequency is a well-known effect of PAHs, partly related to increased formation of oxyradicals during their metabolization pathway (Benedetti et al., 2015).

The overall biological significance of observed biomarkers responses in biota corresponded to a "Major" level of subcellular hazard in organisms collected from the industrial sub-areas.

Concerning the ecological effects on benthic communities, the application of AMBI index (Borja et al. 2000) allowed to classify as "Absent" the hazard in the samples collected in the majority of study area, except in sub-areas 4, 5, 10 and 7, located near the plant, where the impact was summarized as "Slight". Such evaluation is in accordance with Fasciglione et al. (2016), who reported an unexpected evidence of biodiversity in this area, indicating a good functioning of local ecosystems at high levels of chemical contamination.

The overall integration of hazard quotients elaborated from sediment chemistry, bioassays, bioaccumulation, biomarkers and benthic communities provided a more holistic assessment of the environmental quality in the investigated area. The results indicate a clear pollution, especially near the piers. Beside a "Slight" level of hazard summarized in sub-areas 6, 10 and 11, the WOE elaboration increased to a "Moderate" level in all the other zones: interestingly the highest hazard coefficients were obtained near the plant: values close to the limit between "Moderate" and "Major" level of risk were obtained in sub-areas 1 and 7 (Table 1), further confirming the impact of discharged material from the industrial activities.

The possibility of converting complex scientific information into simple hazard indices easily understandable from policy makers and environmental managers could facilitate and orient site-specific decisions on environmental sediment management. This study confirm the need of performing multidisciplinary approaches to assess the health status of marine ecosystems. In this perspective the use of the WOE integration, which combine and weight different kinds of data and analyses allows to better discriminate the presence of contaminants and their short or long-

17

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.

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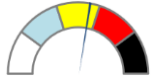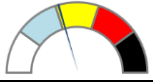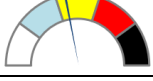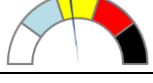
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Tables

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)	

687   **Figure 1**

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689   ■▲ Sediments for chemical analysis and bioassay □ Mussels ■ Fish ○ Sediments for Benthos ⊗ ND

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Figure 2

696   **Figure 3**

697

Weight of Evidence integration
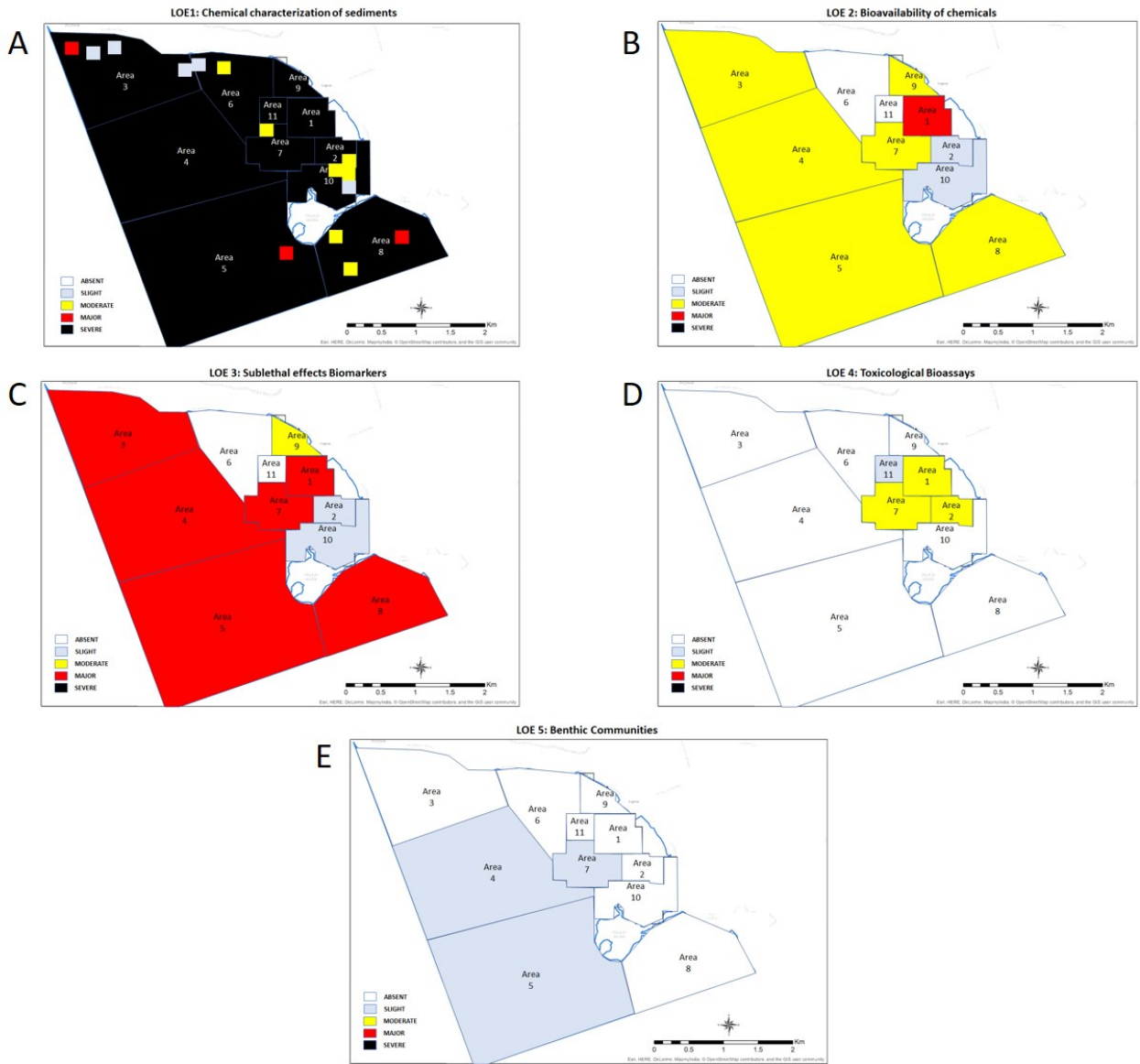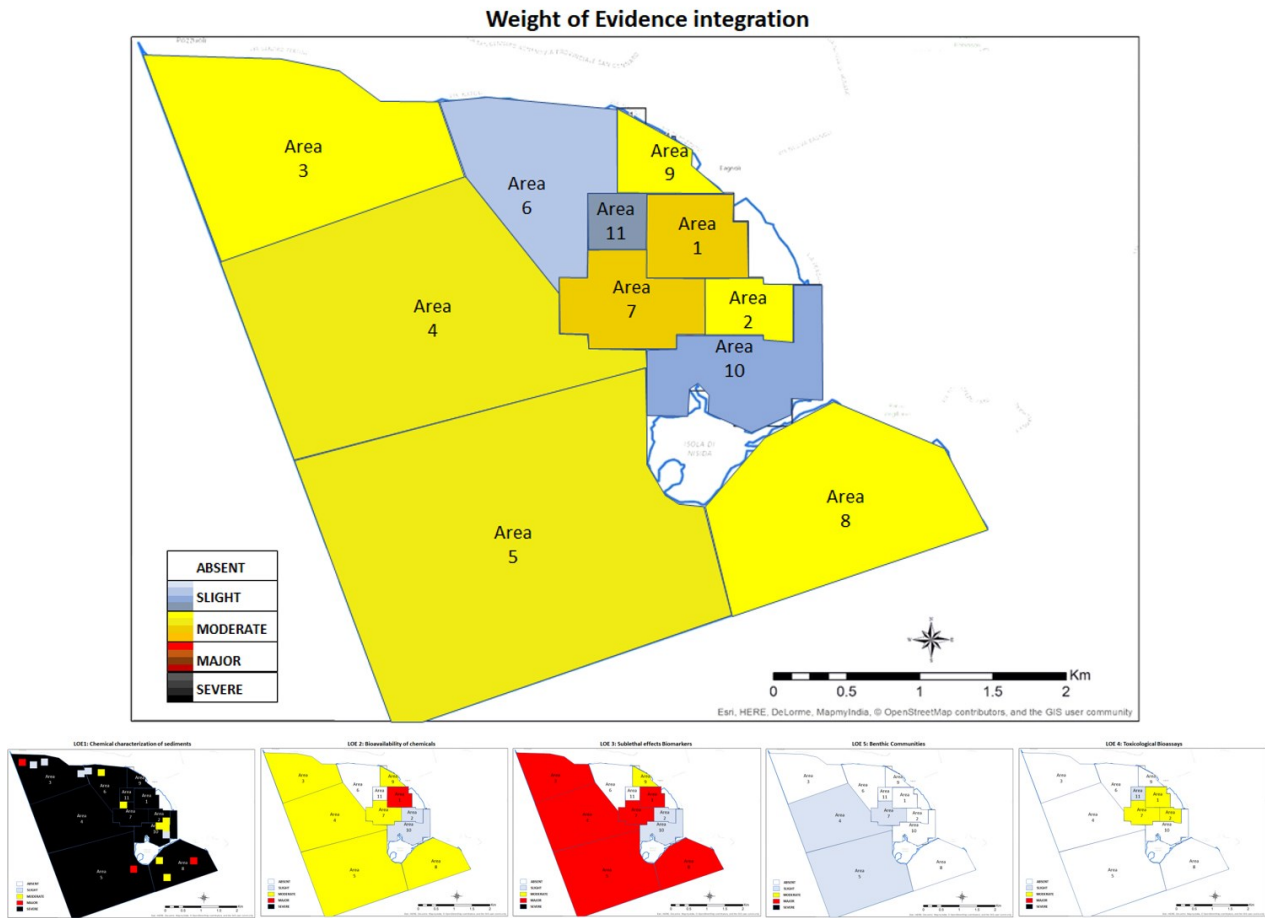
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SUPPLEMENTARY MATERIAL

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	PGP	P2	BBM	BNF	BRM	Sig.
		(Area 1;9)	(Area 1;7)	(Area 2;10)	(Area 6;9;11)	(Area 8)	(Reference area)	
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.
	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
Area 1	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
	59	100	100
	60	63 .8 ± 4 .8	93 .4 ± 22 .8
	61	100	100
Area 2	62	100	100
	69	100	100
	70	100	100
	71	100	100
	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
Area 3	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
	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
Area 4	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.
	1	100	100
	2	100	100
	3	100	100
	4	100	100
	5	100	100
	6	100	100
	7	100	100
Area 9	8	100	100
	9	100	100
	10	100	100
	11	100	100
	12	100	100
	13	100	100
	14	100	100
	15	100	100
	63	100	100
	72	100	100
	80	92 .4 ± 0 .6	133 .9 ± 13 .7
	81	100	100
	82	100	100
	83	100	100
Area 10	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
	16	100	100
Area 11	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 cell/ml	TREATED cell/ml
	117	4890 .8 ± 204966	13449 .9 ± 221912
	118	4890 .8 ± 204966	9374 .1 ± 231518 .6
	119	4890 .8 ± 204966	10507 .6 ± 176569
Area 5	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
	101	4890 .8 ± 204966	13857 .4 ± 186944 .1
Area 6	102	4890 .8 ± 204966	17933 .2 ± 181948 .6
	103	4890 .8 ± 204966	14494 .1 ± 172918 .5
	107	4890 .8 ± 204966	4924 .7 ± 180988
	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
Area 7	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
	124	4890 .8 ± 204966	15487 .7 ± 174455 .5
Area 8	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 cell/ml	TREATED 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 %
	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
Area 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
	59	84 .6 ± 0 .5	23 ± 1
	60	84 .6 ± 1 .5	32 .6 ± 2
	61	84 .6 ± 0 .5	84 .3 ± 0 .5
Area 2	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
	96	89 ± 2	0 .6 ± 1 .1
	97	89 ± 2	82 ± 2 .6
	98	89 ± 2	88 .6 ± 0 .5
	99	89 ± 2	0
Area 3	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
	109	89 ± 2	17 .3 ± 6 .8
	110	89 ± 2	88 .3 ± 1 .5
	111	89 ± 2	89 ± 1
Area 4	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 %
	117	89 ± 2	88 .3 ± 2 .5
	118	89 ± 2	0
	119	89 ± 2	88 ± 1
Area 5	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
	101	89 ± 2	82 .3 ± 2 .5
Area 6	102	89 ± 2	80 .6 ± 1 .1
	103	89 ± 2	87 ± 1 .7
	107	89 ± 2	89 .6 ± 1 .1
	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
Area 7	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
	124	89 ± 2	58 .6 ± 4
Area 8	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)

1 **Table S4b**. Ecological quality indices values: organisms abundance (N), speces richness (S), Margalef index (d), Shannon index (H'), Pielou index (J'), AMBI
2 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
3 through conventional colours (blu: elevated; green: good; yellow: sufficient; orange: scarce; red: bad)

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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

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6

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

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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

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