

# INTEGRATED APPROACH TO ASSESS ENVIRONMENTAL QUALITY IN HARBOUR AREAS

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## Abstract

Harbors are critical environments with strategic economic importance but also potential environmental impact caused by several anthropogenic activities: the development of quality assessment criteria is thus of key importance in these areas. The aim of the present investigation was a multidisciplinary characterization of the ecological status of Portimão harbor, selected as a model case-study. Levels of priority and specific chemicals in sediments were integrated with the measurement of their bioavailability in the mussels, *Mytilus galloprovincialis*, a battery of ecotoxicological bioassays (sediment toxicity with amphipods, Microtox solid-phase and Stress on Stress (SoS) tests), and a wide array of biomarkers including antioxidant defenses and oxidative stress effects (superoxide dismutase, catalase, glutathione peroxidases and lipid peroxidation), specific biomarkers of exposure to metals (metallothioneins and  $\delta$ -aminolevulinic acid dehydratase), pesticides (acetylcholinesterase), and endocrine disruptors (alkali-labile phosphates). Biomarkers were elaborated in an integrated biomarker index (IBR) and the overall results in a weight of evidence (WOE) model.

The chemical and toxicological evaluations indicated that metals PAHs, PCBs and HCB were not particularly elevated when compared with sediment quality guidelines and standards established for dredging activities under European and national legislation. On the other hand bioavailability was evident for Cd, Cu and Zn. Moreover, biomarkers proved to be more sensitive (higher IBR at sites 2 and 4) and included changes of antioxidant responses, metallothioneins and alkali-labile phosphates .

The assessment of the ecological status of Portimão harbor by WOE approach highlighted the importance of integrating sediment chemistry, bioaccumulation, biomarker responses and bioassays and revealed that despite the existence of some disturbance in the harbor area (sites 2 and 4), the harbor was also affected by the impact of urban effluents from upstream (site 6 at higher risk).

**Keywords:** harbor, sediment, biomarker, IBR, weight of evidence

## **Introduction**

Coastal areas are affected by a variety of anthropogenic pressures, among which harbours represent critical environments with strategic economic importance, often limited hydrodynamism, poor water quality and low biodiversity. Stressors in harbours arise from anthropogenic sources and from economic and commercial activities, such as transports, ship repair or painting, loading and bunkering operations, shipyards, accidental spills, wastewater emissions (Bocchetti et al., 2008). These activities contribute to the generation of chemical inputs such as metals, oxidized and alkylated PAHs, petrol fuel additives, antifoulants, etc. which can pose a risk to aquatic organisms residing in harbour areas. In addition, ports are not independent entities since they are integrated in population centers and can have direct influence on surrounding environments and related interests (i.e. fishing, recreation, etc.) (Grifoll, 2011). In this respect, there is concern on the environmental impact caused by port activities and how these should be properly managed (Dabra et al 2009).

Within the European Water Framework Directive (WFD, 2000/60/EC and 2008/105/EC), the recognized economic and social value of harbours can justify hydro-morphological changes of the water bodies, classified as heavily modified (HMWB) because they fail to meet the good ecological status. The explicit recognition of the importance and development of specific economic activities strongly support an integrative approach to assess the environmental quality in harbour areas within the WFD and the development of new environmental management tools aimed to identify which end points are better suited as proxies for quality evaluation.

Multidisciplinary approaches are required in chronically polluted harbour areas to assess the chemical, biological and toxicological impact of complex mixtures of stressors in different environmental matrices, i.e. water, sediments and biota (Viarengo et al., 2007). Sediments act as sink of contaminants and provide precise records about the type and magnitude of the disturbance (Ondiviela *et al*, 2012): however, changes of physicochemical characteristics (redox potential, pH, dissolved oxygen) or desorption during dredging activities can remobilize contaminants, affecting their mobility, bioavailability and risk for marine organisms (Bocchetti et al., 2008; Ondiviela et al., 2012).. Therefore, a particular attention should be paid to the presence of priority and specific substances in sediments, despite bulk chemical analyses alone do not necessarily reflect the bioavailability and the toxic action of measured compounds (Annicchiarico *et al.*, 2007; Prato *et al.*, 2010). In this respect, ecotoxicological bioassays are important complementary tools to evaluate synergistic effects of contaminant mixtures in sediments. In addition, organisms such as the blue mussels *Mytilus*

*galloprovincialis* are good bioindicators to assess environmental bioavailability, bioaccumulation and biological responses of anthropogenic pressures: at cellular level, biomarkers are excellent early warning signals that can indicate the exposure to specific groups of contaminants, or different levels of cellular unbalance and toxicity due to complex mixtures of chemicals not necessarily identified as being of concern (Cajaraville et al., 2000). An integration of well-established biomarkers and bioassays in current EU decision making criteria is thus expected to be an important component to assess the environmental quality of harbor areas and to establish the link between contaminants and ecological responses.

The main goal of the PORTONOVO project ([www.portonovoproject.org](http://www.portonovoproject.org)) was the selection, development and validation of indicators and methodological procedures for the definition of the good ecological potential and management in ports of the Atlantic Area. Within this project, an environmental quality assessment was carried out in Portimão harbor to identify and quantify spatial variations of WFD priority and specific substances in water, sediments and biota from several sites differently impacted by port activities (Directives 2000/60/EC and 2008/105/EC). Based on the most relevant European and national normative a set of physico-chemical (water and sediments), hydromorphological and biological indicators was selected. Physico-chemical indicators were transparency, oxygenation and nutrients conditions. Bioavailability and biological effects of contaminants were assessed by integrating levels of priority and specific substances (metals and organic compounds) detected in sediments and accumulated in mussels *Mytilus galloprovincialis* with a wide array of bioassays and biomarkers reflecting specific effects of classes of contaminants at different levels of biological organization. The biomarkers selected included biomarkers of oxidative stress (superoxide dismutase SOD, catalase CAT, glutathione peroxidase GPX, lipid peroxidation LPO), biomarkers of exposure (metallothioneins MT,  $\delta$ -aminolevulinic acid dehydratase ALAD, acetylcholinesterase AChE) and of estrogenic effect (alkali-labile phosphates ALP). Besides biomarkers, bioassays (sediment toxicity with amphipods, Microtox solid-phase and Stress on Stress (SoS) tests) were integrated to assess the toxicity of contaminant's mixtures trapped in sediments and accumulated in the biota. Data were integrated in the Integrated Biomarker Response (IBR) index (Serafim et al., 2012) to rank sites according to the disturbance levels and provide environmental managers with a decision-support tool to evaluate the environmental quality of Portimão harbour.

All the chemical and biological data were further elaborated within a quantitative Weight Of Evidence, WOE model (SediquaSoft) which combine various typologies of studies (or lines of evidence, LOEs), including sediment chemistry, ecotoxicological bioassays, bioaccumulation and biomarker results (Piva et al., 2011): logical flowcharts and mathematical algorithms evaluate data from individual LOEs and provide specific hazard indices, before their differential weighting and

integration in a quantitative risk index (Piva et al., 2011). Independent elaborations for different LOEs allow to consider different criteria which better apply to various typologies of data; the hazards for sediment chemistry and bioavailability are based on the number, magnitude and potential toxicity of chemicals which exceed respectively a set of Sediment Quality Guidelines or natural concentrations measured in control organisms (Piva et al., 2011), while biomarkers and bioassays are evaluated considering the biological relevance of measured endpoints (“weight”) and the entity of variations compared to specific “thresholds” defined for several species and tissues (Piva et al., 2011). The use of weighted criteria overcomes the limits of qualitative pass-fail approaches toward normative values, in line with recent European Directives which require to classify the ecological status of water bodies integrating different quality indicators. The Sediqualsoft model was previously applied to different multidisciplinary studies for the characterization of industrial and harbour sediments, the assessment of environmental hazards in coastal and brackish areas, the ecological risk assessment after the Costa Concordia wreck at Giglio Island (Benedetti et al., 2012, 2014; Piva et al., 2011; Regoli et al., 2014).

## **Materials and Methods**

### ***The Portimão Harbour***

The Portimão harbour, located in the Arade river, is the main freshwater input in the South West coast of Portugal and has an area of approximately 987 km<sup>2</sup>. The Arade river crosses several urban areas (Ferragudo/Parchal and Portimão with around 45 000 inhabitants) and the main contamination sources come from municipal and industrial effluents, harbour, marina and all sort of fishing-related activities (shipyards, industries), fish farms, husbandry, agricultural and urban runoff. Located near the river mouth are small harbours for recreational and fishing vessels and the Portimão harbour (DGPA, 2004). Ships facilities exist near the city of Portimão and the port itself is a gateway to the southern region of Portugal and lies on the route to or from the Mediterranean Sea or from the North Atlantic and also on the route of cruise ships that cross the Atlantic Ocean. The port of Portimão offers excellent conditions to dock large vessels and international cruise ships, after appropriate dredging activities carried out since 2008. The port accommodates commerce and tourist quays, several socio-economic activities (maritime traffic, ferry boats, ship building industry, marine culture, beach and tourism) and it actively contributes to the increase of transport and tourism. Nevertheless, the areas inside and outside the harbour are affected by the port water quality.

### ***Sampling Sites***

Seven sites were selected along the Portimão harbour numbered downstream to upstream (Figure 1). The coordinates of these sites are listed in Table 1 along with abiotic parameters (temperature, salinity, pH and dissolved oxygen) measured *in situ* with an YSI probe and turbidity a turbidimeter. Water, sediments and mussel *M. galloprovincialis* were also collected at these sites between November 2010 and February 2011.

Nutrient (silicates, nitrates, nitrites, phosphates and ammonia) concentrations were determined in 0.45 µm filtered seawater by spectrophotometric methods described by Grasshoff *et al.* (1983) and data accuracy assessed using reference standard solutions (Marine Nutrient Standard Kit – OSI). Chlorophyll *a* and phaeopigment were analysed in seawater filtered with a glass fiber filter (0.7 µm) by spectrophotometry, according to the method described by Lorenzen (1967).

### ***Sediments analyses***

Surface sediment samples were collected in triplicate with the aid of a "Van Veen" grab, transported to the laboratory at 4°C and stored at -20°C for subsequent use for bioassays and for the determination of metal and organic contaminant concentrations. Sediment organic content was determined in three replicates from each site as the percentage of weight loss by combustion at 450 °C and drying at 100 °C for 24 h. Total metal content was determined in the < 63 µm fraction after wet digestion with HNO<sub>3</sub> and hydrogen peroxide (30% H<sub>2</sub>O<sub>2</sub>). Cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) concentrations were analysed by atomic absorption spectrophotometry (AAS Analyst 800 - Perkin Elmer) and are expressed by µg.g<sup>-1</sup>dw. Data was validated with certified reference materials.

Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated biphenyls (PCBs) in sediments were determined by isotopic dilution mass spectrometry combined with high resolution gas chromatography using internal standards. Limit of detection was for individual PAHs 0.010 µg.g<sup>-1</sup> d.w., for total PAH 0.160 µg.g<sup>-1</sup>d.w., for individual PCBs 0.0007 µg.g<sup>-1</sup>d.w., for total PCBs 0.0049 µg.g<sup>-1</sup> d.w. and for HCB 0.0005 µg.g<sup>-1</sup> d. w.. Results are expressed as µg.g<sup>-1</sup> d.w..

### ***Sediments Bioassays***

Two sediments bioassays were carried out: the 10 days whole sediment toxicity test with amphipods *Corophium insidiosum* (US EPA, 1994; ASTM, 1999; Prato *et al.*, 2010) and the Microtox solid phase test (MSTP) since it allows an evaluation of re-suspended sediments (Azur Environmental, 1998; Ghirardini *et al.*, 2009).

For the whole sediment toxicity test, a static test was applied where amphipods were exposed to sediments for ten days. Briefly, 20 amphipods (per replicate) were exposed to 200 ml of 1 mm sieved sediments (3 replicates per site) and 800 ml of overlying natural filtered seawater at  $17.7 \pm 0.1^\circ\text{C}$  and salinity ( $35.7 \pm 0.2$ ) under continuous aeration, normal season photoperiod illumination (US EPA, 1994; ASTM, 1999; Ré *et al.*, 2007, 2009; Prato *et al.*, 2010). A negative control using native sediment was also run at the same time. Water temperature, salinity, pH and dissolved oxygen were measured at the beginning and end of the test. After ten days, the number of live animals was registered and the mortality rate calculated (US EPA, 1994).

The MSPT test is based on the inhibition of bioluminescence of the marine bacteria *Vibrio fischeri* exposed to sediments using a serial of dilutions (Azur Environmental, 1998; Ghirardini *et al.*, 2009). The standardize protocol proposed by Azur Environmental (1998) with slight modifications was followed and the endpoint was the effective concentration of sediment that causes a 50% reduction of the bacteria bioluminescence ( $\text{EC}_{50}$ ). (Casado-Martínez *et al.*, 2006; Ghirardini *et al.*, 2009). Briefly, 7.0 g of wet sediment was re-suspended in 35 ml of diluent solution for solid-phase with magnetic stirring for 10 minutes. Subsamples of this suspension were serial-diluted and after 10 minutes equilibration, bacteria was then mixed, incubated for 20 minutes and further separated from the sediments by filtration. A subsample of the liquid phase was equilibrated for 5 minutes and light emission was recorded after 5 and 15 minutes and output data analysed with MicrotoxOmni software (Azur Environmental). Results are expressed as  $\text{EC}_{50}$  ( $\text{g}\cdot\text{L}^{-1}$ ) and in Toxicity Unit ( $\text{TU}_{50}$ ) calculated as the inverse of  $\text{EC}_{50}$  in percentage.

### ***Mussels Mytilus galloprovincialis***

Around 90 mussels *M. galloprovincialis* (medium length  $56.0 \pm 5.6\text{mm}$ ) were only collected at each of the sites 2, 4, 6 and 7 because no mussels were available at sites 1, 3 and 5. After collection, mussels were transported alive to the laboratory and kept in seawater to depurate for the determination of metal concentrations. The condition index (CI) was estimated as a percentage of the ratio between drained weight of soft tissues and e total weight of 15 mussels (Amiard *et al.*, 2004). Another group of fifteen mussels were immediately used to perform the “stress-on-stress” bioassay. The remaining organisms were stored at  $-20^\circ\text{C}$  for metal analysis. Cd, Cr, Cu, Pb and Zn concentrations were determined on mussels whole soft tissues ( $n=20$ ) after wet digestion and analysed by atomic absorption spectrophotometry (AAS AAnalyst 800 – Perkin Elmer). Quality assurance was performed with standard reference material (Lobster Hepatopancreas) provided by the National Research Council of

Canada (TORT II). The values for the reference material and the certified values are in Table 2. All metal concentrations are expressed as  $\mu\text{g}\cdot\text{g}^{-1}$  d.w..

Tissues (gills, digestive gland, gonads and remaining edible tissue) from 30 individuals from each site were dissected and separated for biomarkers determination namely antioxidant enzymes (SOD, CAT, GPX), MT, ALAD, LPO, AChE, and ALP. Tissues were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further use.

### ***Biomarkers analysis***

Three pools of five *M. galloprovincialis* tissues (gills, digestive glands or whole soft tissues) each were used after homogenisation in 20 mM Tris buffer, pH 7.6, containing 1 mM of EDTA, 0.5 M of saccharose, 0.15 M of KCl and 1 mM of DTT. The homogenates were centrifuged at 500 g for 15 min at  $4^{\circ}\text{C}$  to precipitate large particles and re-centrifuged at 12 000 g for 45 min at  $4^{\circ}\text{C}$  to precipitate the mitochondrial fraction. Gel filtration was used to eliminate low molecular weight impurities. Hence, all cytosolic fractions were chromatographed on a Sephadex G-25 column (PD10, Pharmacia) to remove small weight proteins.

Antioxidant defences (SOD, CAT, GPx) were analysed in the gills and digestive gland of mussels from the different sites ( $n=5$ ). SOD activity was determined by measuring the reduction of cytochrome C by the xanthine oxidase/hypoxanthine system at 550 nm (McCord and Fridovich, 1969). SOD activity is expressed in U.SOD  $\text{mg}^{-1}$  total protein concentration. CAT activity was determined according to Greenwald (1985) by the decrease in absorbance at 240 nm. CAT activity is expressed as  $\mu\text{moles}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$  of total protein concentration. GPx activity was measured following NADPH oxidation at 340 nm in the presence of excess glutathione reductase, reduced glutathione and corresponding peroxide (Lawrence and Burk, 1976). Total GPx activity was measured by using  $\text{H}_2\text{O}_2$  as substrate and is expressed as  $\mu\text{moles}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$  of total protein concentration.

MTs were determined in *M. galloprovincialis* gills and digestive gland of three replicates of five tissues each. Tissues were weighed and homogenized in 3 volumes of 20 mM Tris-HCl (pH 8.6) in an ice bath ( $4^{\circ}\text{C}$ ). An aliquot of the homogenate (3 mL) was centrifuged (30 000g for 1 h at  $4^{\circ}\text{C}$ ). Two aliquots of the supernatant were further collected to be used in LPO and protein determinations. The supernatant was then heat treated at  $80^{\circ}\text{C}$  and centrifuged (30 000g for 1 h at  $4^{\circ}\text{C}$ ) to precipitate the denatured proteins. MTs were determined in the heat-treated cytosolic fraction of mussel gills and digestive gland according to the method described by Bebianno and Langston (1989), using differential pulse polarography ( $\mu\text{Autolab II}$  potentiostat/galvanostat). Calibration of MT concentrations was

performed by the standard addition method with rabbit liver MT (Sigma) standard and results are expressed as  $\text{mg.g}^{-1}$  of total protein concentration.

LPO was determined in the same tissues (gills and digestive gland) and in the same homogenate as MT following the method described by Erdelmeier *et al.* (1998) that measures malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) concentrations produced upon the decomposition by polyunsaturated fatty acid peroxides. Results are expressed as micromoles of MDA and 4-HNE per gram of total protein concentration ( $\mu\text{mol.g}^{-1}$  total protein concentrations).

ALAD activity was measured in mussels whole soft tissues ( $n=5$ ) after homogenization, with 0.1 M phosphate buffer (pH 6.6) according to a slightly modified version of the European standardized method for ALAD activity determination in blood (Company *et al.*, 2011). Briefly, the homogenates were centrifuged at 10 000 g for 15 minutes at 4°C and the resulting supernatants separated in 5 aliquots (50 ml each). 200 mL of phosphate buffer was added to 2 of the aliquots and 200 mL of ALA-reagent ( $\delta$ -aminolevulinic acid) to the others. The mixture was incubated for 2 hours at room temperature and afterwards 750 mL of the precipitation reagent (containing trichloroacetic acid) was mixed for 30 minutes and centrifuged at 2500 g for 5 minutes. The resulting supernatant was transferred to a plastic cell, mixed with the Ehrlich chromogenic reagent (dimethylaminobenzaldehyde) and incubated for 15 minutes at 25°C. A colorimetric method was used through the reaction of porphobilinogen (PBG), which is enzymatically formed from aminolevulinic acid, with the Ehrlich chromogenic reagent. The UV absorbance of the amount of PBG produced was determined at 550 nm and the ALAD activity is expressed as  $\text{ng of PBG.min}^{-1}.\text{mg}^{-1}$  of total protein concentrations.

AChE activity was determined in the cytosolic fraction of mussels gills ( $n=5$ ), according to the colorimetric method described by Ellman *et al.* (1961). Gills were homogenized on ice in five volumes of Tris-HCl buffer (100 mM, pH 8.0) containing 10% Triton and centrifuged at 12 000g for 30 min (4°C). This method is based on the coupled enzyme reaction of acetylthiocholine as the specific substrate for AChE and 5,5-dithio-bis-2-nitrobenzoate (DTNB) as an indicator for the enzyme reaction at 405 nm. AChE activity is expressed in  $\text{nmol.min.mg}^{-1}$  of total protein concentrations.

The effects of estrogenic contamination was accessed by the levels of Vg-like proteins in males and immature females indirectly determined in sex-differentiated mussels gonad tissues ( $n=15$ , separated by gender in a similar ratio) by applying the ALP method adapted from Gagné *et al.* (2003) following an homogenization with 25 mM HEPES–NaOH buffer (containing 125 mM NaCl + 1 mM DTT + 1 mM EDTA) at pH 7.4 on ice. The levels of alkali-labile phosphates released after acetone-extracted (35%) lipo phosphoproteins after hydrolysis with alkali compounds were determined by the



phosphomolybdenum method using a standard curve of known concentrations of inorganic phosphates (KH<sub>2</sub>PO<sub>4</sub>). Results are expressed as µg PO<sub>4</sub> .mg<sup>-1</sup> of total protein concentrations.

Total protein concentrations were determined in the cytosolic fraction according to Lowry *et al.* (1951) for antioxidant enzymes, LPO, MT, ALAD and AChE, and according to the Bradford method (1976) (gonads) for the Alkali-labile phosphates assay. Bovine Serum Albumin (BSA) was used as a reference standard material for both methods.

### ***SoS bioassay***

The SoS bioassay was performed to measure tolerance of the organisms to anoxic conditions. The endpoint was the average time that half the sample-population takes to die (LT<sub>50</sub>) (Hellou and Law, 2003). SoS was performed with 15 mussels from each site placed without any water, at constant room temperature (≈18°C). Mussel mortality was checked daily: organisms were considered dead when valves gaped failed to close with physical stimuli. Results are expressed as LT<sub>50</sub> in days with confidence intervals for each site.

### ***Statistical Analysis***

All data was tested for normality and homogeneity of variance. Analysis of variance (ANOVA) or Kruskal Wallis One Way Analysis of Variance on Ranks was applied to detect significant differences between sites. If significant, pair wise multiple comparison procedures were conducted using the Tukey or the Dunn's tests, Pearson's correlation analysis was performed to verify existing relationships between environmental parameters, contaminants and biomarkers. A Principal Component Analysis (PCA) was also applied to the mean values from all sites to evaluate the relationships between variables and the relative influence of different parameters in the overall results.

### ***Biomarker index***

The Integrated Biomarker Response (IBR) index was calculated for each site, combining the results of condition index, SoS bioassays and biomarkers in mussels tissues following the method described by Serafim *et al.*, (2012) as follows: individual areas A<sub>i</sub> connecting the *i*th and the (*i* + 1)th radius coordinates of the star plot were obtained in a simpler way, according to the formula:

$$A_i = \frac{1}{2} \sin \left( \frac{2\pi}{n} \right) S_i S_{i+1}$$

where S<sub>i</sub> and S<sub>i+1</sub> represent the individual biomarker scores (calculated from standardised data) and their successive star plot radius coordinates. *n* represent the number of radii corresponding to the parameters used in the survey. The different parameters used for the IBR calculation were ranged clockwise according to their hierarchy of biological organization,

from the subcellular to the individual level, as follows: CI, SoS, SOD, CAT, GPx, MT, ALAD, AChE, ALP and LPO.

### ***Weight Of Evidence Elaboration***

Data on sediment chemistry, bioaccumulation, biomarkers and bioassays measured at sites 2, 4, 6 and 7 were elaborated within the quantitative WOE model, Sediqualsoft. Conceptual elaborations of the model, whole calculations, detailed flow-charts, rationale for weights, thresholds and expert judgments have been fully given elsewhere (Benedetti et al., 2012, 2014; Piva et al., 2011; Regoli et al., 2014).

Briefly, the evaluation of hazard from sediment chemistry is initially based on the calculation for each pollutant of Ratio to Reference (RTR), i.e. the ratio between measured concentrations and those indicated by various sediment quality guidelines (SQGs); this value is further corrected (RTRw) to account for the typology of contaminant, if a “priority” or “priority and hazardous” pollutant according to EC Directive 2008/105. In this study, the considered SQGs were: Effects Range Low/Effects Range Median (ERL/ERM), that provide concentrations below which adverse effects are rarely observed, and above which such effects frequently occur (Long et al., 1995); Threshold Effect Level/Probable Effect Level (TEL/PEL) indicate for each xenobiotic the highest concentration (TEL) corresponding to a limited probability of adverse effects, and the lowest range of values (PEL) frequently or always associated with adverse effects (Macdonald et al., 1996); the 5 levels considered by Portuguese legislation (Portaria nº1450) and the 2 levels of the normative guidelines on dredged sediments in France (Arrêté du 14/06/00), Spain (CEDEX 1994), UK (OSPAR, 2004) and Italy.

In the calculation of the specific Hazard Quotient for chemistry (HQ<sub>C</sub>), an average RTRw is obtained for all of 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:

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

With such calculation the HQ<sub>C</sub> increases according to both the number and the magnitude of the exceeding parameters (for which the specific RTRw are individually added), while not being lowered by the analysis of many ‘not exceeding’ parameters (which are summarized in the averaged

RTRw). Based on expert judgment, the values of HQ<sub>C</sub> are assigned to one class of chemical hazard (absent or negligible, slight, moderate, major, severe) depending on the number, typology and magnitude of exceeding chemicals (Piva et al., 2011).

The bioaccumulation hazard in mussel tissues is based on the calculation for each parameter of the RTRw, i.e. the increase of concentration compared to control specimens, corrected for the typology of pollutant and the statistical significance of the difference (Piva et al., 2011). The cumulative Hazard Quotient for bioavailability (HQ<sub>BA</sub>) does not consider parameters with RTRw <1.3 (concentrations ≤ control value for a priority and hazardous pollutants), calculates the average for those with RTRw ranging between 1.3 and 2.6 (i.e. up to 2 fold increase compared to controls for a priority and hazardous pollutant), and adds the summation (Σ) of all those with RTRw ≥2.6):

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

The level of cumulative HQ<sub>BA</sub> is summarized in one class of hazard for bioavailability, from Absent to Severe, depending on the distribution of analyzed chemicals within the different classes of effect (Benedetti et al., 2012; Piva et al., 2011).

The module for the elaboration of biomarker results contains a wide battery of responses, each assigned with a weight (based on the relevance of biological endpoint) and a threshold for changes of biological relevance which considers species or tissue differences, and the possibility of both induction and/or inhibition for biomarkers potentially showing biphasic responses (Piva et al., 2011). For every analysed biomarker, the measured variation is compared to the threshold (effect), then corrected for the weight of the response and the statistical significance of the difference compared to controls. The calculation of the Hazard Quotient for biomarkers (HQ<sub>BM</sub>) does not consider the contribution of responses with an effect <1 (lower than threshold), calculates the average for those with an effect up to two-fold compared to the threshold and adds the summation (Σ) for the responses more than 2 fold greater than the respective threshold (Piva et al., 2011):

$$HQ_{BM} = \left( \frac{\sum_{j=1}^N Effect_W(j)_{1 < Effect(j) \leq 2}}{num\ biomark_{1 < Effect(j) \leq 2}} + \sum_{k=1}^M Effect_W(k)_{Effect(j) > 2} \right)$$

According to variations measured for various biomarkers, the model summarizes the level of cumulative  $HQ_{BM}$  in one of five classes of hazard for biomarkers, from Absent to Severe (Piva et al., 2011).

In the module for ecotoxicological bioassays, the cumulative hazard quotient ( $HQ_{Battery}$ ) is obtained by the summation ( $\Sigma$ ) 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 ( $w_2$ ):

$$HQ_{Battery}: \Sigma Effect_w(k) w_2$$

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 (if  $<1$ ) to Severe (if  $>6$ ).

Results from individual LOEs are finally elaborated within a classical weight of evidence approach which, after normalization of indices to a common scale, integrates and gives a different weight to various lines of evidence. 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).

### ***Results and Discussion***

Harbors are of strategic economic importance but some of their normal activities (shipping, loading and bunkering operations, shipyards, accidental spills, waste water emissions) are cause of concern for their potential impact on the quality of marine ecosystems. The assessment of the ecological status was carried out in Portimão harbor selected as model case-study under the WFD to identify and quantify spatial variations of priority and specific substances in water, sediments and biota (European Community Water Framework Directive 2000/60/EC), different typologies of biological effects, and to integrate such relevant information on the ecological status of the harbor area.

The abiotic parameters (temperature, salinity, pH and dissolved oxygen) measured in water at different sites are shown in Table 1. Temperature ranged from 13.7 °C (minimum at site 7) to 17.5 °C (maximum on the same site); salinity was lowest at site 7 (12.7‰) and maximum at site 2 (36.5‰), while the greatest salinity range (from 12.7 to 35.3‰) was also detected at site 7. The pH values were almost similar ranging from 7.8 (sites 5-7) to 8.1 (site 2); levels of dissolved oxygen were minimum at site 5 (52.8%) and maximum at site 1 (108.9%).

Nutrients (nitrites, nitrates, ammonium, phosphates and silicates) revealed a spatial trend with higher levels in water samples upstream and lower levels downstream (Table 1). The lowest nitrogen concentrations (nitrites, nitrates and ammonium) were at site 2, followed by site 4. Conversely, the highest levels of nitrites, nitrates phosphates and silicates were at sites 6 and 7, where ammonium was maxima. This may be related to a nutrient enrichment due to the proximity of waste water treatment plant (WWTP) discharges near sites 6 and 7, or even to agricultural runoff that would have a strong influence upstream. These results are in agreement with the water quality model data for the same area that indicated that nutrients are consumed in the upper part of the river (Martins et al., 2009).

### *Sediments*

Because sediments are reservoirs of complex mixtures of contaminants, surface signatures were used as a finger print for the type and magnitude of hazards present in the harbor of Portimão. Analyses included priority and specific compounds (European Community Water Framework Directive 2000/60/EC) such as metals (Cd, Cr, Cu, Ni, Pb and Zn) and organic chemicals (PAHs, HCB and PCBs). Levels of these compounds were integrated with ecotoxicological bioassays to better assess sediment quality at different sites of Portimão harbor.

Site 5 revealed the highest organic content in sediments and the minimum dissolved oxygen in water, as opposed to site 2 where organic content was the lowest (Table 1). This suggests low hydrodynamism at site 5 and a spatial trend with lower organic content in sediments near the mouth of the river (sites 1 and 2), as compared to the sites close to the harbour facilities (sites 3- 5) and to site 7.

Concentrations of metals in sediments are shown in Figure 2. A similar trend was observed for Cr, Ni and Pb along the seven sites, with higher levels at site 1 decreasing to site 5, elevated at site 6 and decreasing again at site 7. Also Cd and Zn exhibited similar variations with levels increasing inside the harbour area (from sites 1 to 4) while, outside the harbour, their levels were higher at site 6. A totally different pattern was observed for Cu with the highest levels at site 4 and a decreasing trend towards the ocean, possibly associated with the use of antifouling paints in harbour facilities. Nevertheless, Cd, Cr, Ni, Pb and Zn levels presented a similar trend upstream (from sites 5-7) with higher levels at site 6 while Cu concentrations were similar between these sites. With the exception of Cd and Zn, the other metal levels were higher than those detected in the harbour of Aveiro (Portugal) (Gonçalves et al., 2013)

PAHs concentrations in sediments are given in Table 3. Levels were below the detection limit in sites 2 and 6, and followed a decreasing trend from sites 4 >> 3 > 5 > 7. These results are similar to those already described for Cd, Cu and Zn within the harbour area. The highest PAHs from site 4 (1.69 µg/g d.w.) was especially due to chrysene (0.137 µg/g d.w.), fluoranthene (0.376 µg/g d.w.), phenanthrene (0.379 µg/g d.w.) and pyrene (0.264 µg/g d.w.) among others. With the exception of phenanthrene, 2+3 ring PAHs were only present at site 4, where the sum of carcinogenic PAHs (0.519 µg/g d.w.) was lower than that of non-carcinogenic (1.17 µg/g d.w.); although lower PAHs concentrations were measured at sites 3, 5 and 7, carcinogenic PAHs were comparatively higher to non-carcinogenic. In this respect, high liver EROD activity and elevated levels of 1-pyrenol equivalents in bile were previously detected in sea bass *Dicentrarchus labrax* from site 4, suggesting exposure to AhR agonists like pyrene, chrysene, benzo[a]pyrene, phenanthrene and fluoranthene (Fernandes et al., 2008). The diagnostic ratio measured in this study for PAH in sediments indicated a pyrolytic origin at sites 3-5 and 7 (Flu/Pyr >1), while a mixed source of oil and biomass combustion at site 4. Although PAHs data are not available for site 1 due to sample lost, previous results showed slightly lower values than those measured at site 4 indicating the occurrence of contaminants deposition from the harbour activities (IPIMAR, 2010). Concentrations of HCB and PCBs were below the detection limit at all sites revealing no contamination from these compounds in the area.

Since sediments may be a source of pollution to living organisms potentially causing adverse biological effects (Long et al., 2006), metal levels and organic compounds were compared with sediment quality guidelines (SQGs) from OSPAR Environmental Assessment Criteria (EACs) (OSPAR, 2004), the NOAA Effects Low and Median Range (ERL and ERM) (Long et al., 1995), the Canadian sediment quality guidelines (CSQG) threshold effect (TEL) and Probable Effect levels (PEL) (McDonald et al 2004) ( Table 4), and with those defined by Portuguese legislation for dredging marine and estuarine sediments (Portaria 1450, 12<sup>th</sup> November 2007). Results revealed that except for Cd and Zn whose levels were below ERL and TEL, concentrations in sediments were generally within the range where adverse effects can occasionally occur (ERM and PEL). Ni, Cr and Pb concentrations, however, exceeded the ERM/PEL at site 1 (52, 160 and 218 µg/g for Ni, Cr and Pb respectively), Ni and Pb exceeded ERM at site 6, and Ni at site 2. In addition, total PAH levels (Table 3) were lower than ERL or TEL values (4 µg/g), although the ERL levels of acenaphthene and phenanthrene were exceeded at site 4 (Table 4). According to values of Portuguese legislation (Table 4), sediment from site 5 and 7 were classified as Level II for measured concentrations of Pb and/or Cu (“dredging is allowed taking into account the characteristics of dumping site”), level III at sites 3-4 for Pb (“when dumped, a study at dumping site is needed and the implementation of a monitoring program is essential”), Level IV at sites 1, 2, 6 for Pb (“disposal should be made on land with imperviousness

measures for soil”). The knowledge of the concentrations of these priority and specific compounds in sediments is important to evaluate the contamination of a marine ecosystem but, as alone, they do not provide information on potential toxicity for those organisms exposed to such chemical mixtures.

Although there is no reference to sediment bioassays in the Portuguese legislation, these tests are mandatory in other European Countries like France, Italy Spain and United Kingdom (CEDEX, 1994, Arrêtê du 14/06/00, OSPAR, 2004). In the present study, the results of the 10-days toxicity test with amphipods *C. insidiosum* revealed mortality rates of 58.3% for site 2, 45% for site 4, 50% for site 6 and 48.3% for site 7. Moreover, data from Microtox SP indicated EC<sub>50</sub> values corresponding to low risk (4-6 g.L<sup>-1</sup>) in sediments from sites 3 and 4 (located in front and in the commercial harbour) and upstream at site 7 (possibly related to either harbour activities or to the impact of a recreational marina and residential developments) (Table 4). Sediments from sites 2 and 6 exhibited no risk (EC<sub>50</sub> levels between 32 and 50 g.L<sup>-1</sup>) while those for site 1, located in the river mouth, were in the transition between low to no risk (EC<sub>50</sub> levels between 9 – 10 g.L<sup>-1</sup>). The obtained Microtox data are not fully consistent with concentrations of metals and PAHs measured in sediments. In a previous study, a combination of *in vitro* bioassays focussing on cytotoxicity, CYP1A induction, ROS generation and inhibition of steroidogenic enzymes in fish cell cultures or subcellular fractions, allowed to discriminate differently impacted sediments from middle part and upstream of the Arade estuary, revealing both the presence of endocrine chemicals and antagonistic effects between metals and PAHs which are difficult to characterize from chemical data (Fernandes et al., 2014). The results of the present study highlighted that, although there is a general disturbance due to harbour activities and interactions with the Atlantic Ocean, contamination from upstream in the Arade river is also evident in terms of high turbidity, organic contamination and metals (Pb and Zn); similar inputs came mainly from point or diffuse sources (WWTP, urban and agricultural runoffs) that have a direct impact on sites 6 and 7.

### ***Bioaccumulation in mussels***

Bioaccumulation data and biomarkers in sentinel species represent additional lines of evidence to assess environmental quality, previously applied in several European harbours such as Goteborg, Rotterdam, Lenghorn, Genoa, Piombino and Klaipėda: either autochthonous species or active monitoring strategies were used to assess the impact and toxicological effects of chemicals due to specific anthropogenic harbour activities or sediment remobilization during dredging and disposal operations (Stephensen et al., 2000; Regoli et al., 2002, 2004; Stronkhorst et al., 2003; Frenzilli et al., 2004; Almroth et al., 2005; Sturve et al., 2005; Barsiene et al., 2006, Bocchetti et al., 2008). In the

present study, mussels collected from sites 2, 4, 6 and 7 showed similar CI (17.3 – 18.4%), but revealed a different pattern of metal accumulation compared to sediments (Figure 3.) Tissue levels of Pb were similar among all sites while organisms from site 2 presented lower concentrations of Cd, Cr, Cu, Pb and Zn; this is not in agreement with metal levels in sediments, since only Cu and Zn in sediments were lower at this site. Conversely, Cr and Zn levels were higher in mussels from sites 6 and 7, along with Cu (site 6) and Cd (site 7) indicating a higher metal bioavailability at these sites. These results might be related to sewage inputs (supported by high nutrient levels), the impact of the marina at site 7 along with decreasing values of salinity in upstream sites. Although PAHs and PCBs levels were not determined in mussel tissues in the current study, these compounds were previously detected in mussels at site 4 (Bebiano et al., 2007; Cravo et al., 2009). Discrepancies between sediment chemistry and bioavailability corroborate the importance of analysing different compartments (sediments and biota), and the need of an integrated approach to understand how chemical pollutants affect environmental quality.

## Biomarkers

Biomarkers are sensitive “early warning signals” for specific stressors and they were integrated in this assessment as another line of evidence reflecting potential harmful effects. Antioxidant enzymes (SOD, CAT and GPx) activities, MT concentrations and LPO levels in mussel gills and digestive gland (Figure 4) indicated that biomarkers response was tissue and site dependent. SOD, MT and LPO were higher in the gills than in the digestive gland (Figure 4A, D- E) as opposed to higher activities in the digestive gland for CAT and GPX (Figure 4B-C). In gills SOD activity was higher in mussels from site 6, while CAT and GPX were more elevated at sites 4 and 7 and lower at site 6. The same enzymatic activities measured in digestive gland were comparable in mussels from sites 2, 4 and 6 and generally lower in those from site 7.

Also MT and LPO levels were higher in gills than in the digestive gland (Figure 4D-E), the latter showing similar values in mussels from all sites. On the other hand, branchial MT were lowest in mussels from site 6 while LPO was higher in organisms from site 2 followed by those from sites 4 and 6, and lowest in mussels from site 7: in this respect, organisms from site 7 appeared under less oxidative stress in terms of membrane damage.

ALAD activity was lowest in the whole tissues of mussels from site 6, as opposed to organisms from site 4 showing the highest levels (Figure 5A). The inhibition of ALAD activity is a typical effect of lead exposure: in this study however, lead content in mussel tissues was similar between sites while



in sediments, concentrations were highest at site 6 and lowest at site 4, thus better reflecting variations of ALAD activity. In addition, compared to our results, lower ALAD levels were previously detected at site 4 (Cravo et al., 2009) suggesting a possible improvement of Pb contamination at this site.

AChE levels were similar in gills of mussel from all the sites (2.5-3.5 nmol.min.mg<sup>-1</sup> protein) but significantly lower than those previously measured in organisms from site 4 (Cravo et al., 2009). Different classes of pesticides (fungicides, algaecides and insecticides) were detected at the same time of the present study in water at sites 4 and 7 by Polar Organic Chemical Integrative Samplers (POCIS); the main detected pesticides were triazines and their metabolites and carbendazim (Gonzalez-Rey et al, submitted), and mixtures of such compounds might have affected AChE in mussels from the whole investigated area.

ALP concentrations represent an indirect measurement of vitellogenin-like proteins in the gonads of mussels. No significant differences were observed between males and females specimens from the four sites (Figure 5B). This result might indicate the presence of EDCs compounds since sexually mature females (as those analyzed in the present study) typically contain higher ALP levels than males (Pereira et al., 2013). In this respect, significant levels of nonylphenol were also detected in bile of sea bass *D. labrax* (Fernandes et al 2009), and an *in vitro* assay revealed the presence of endocrine disruptors in sediments from sites 3, 4, 5, 6 and 7 which significantly inhibited CYP19 activity in subcellular fractions of ovaries, thus interfering with synthesis of estrogens (Fernandes et al 2009).

The results of SoS bioassay (Table 6) revealed a lower physiological condition in mussels from site 4 which exhibited a 50% mortality after 4 days in air, while organisms from site 2 resisted almost twice the time (LT<sub>50</sub> of 8 days). A reduced air survival time has been shown as an useful index potentially reflecting the effect of pollution exposure in mussels (Pampanin *et al.*, 2005).

#### Integrated Biomarker Response Index (IBR)

Variations of biomarkers, CI levels and SoS data were evaluated in an integrated biomarker response index (IBR) (Beliaeff and Burgeot, 2002, Serafim et al., 2012). This index was developed to rank sites according to contamination gradients and provide environmental managers with decision-support tools to assess ecosystems “health”. Results indicated that downstream sites, closer to the influence of harbour activities (sites 2 and 4), were more stressed than those upstream and further away from the harbour (sites 6 and 7) (Figure 6). When considering site 2 individually, LPO and CAT in

both tissues had a strong contribution to IBR, coupled with MT (in gills), AChE, ALP in males. At site 4, CI and SoS had the strongest contribution followed by the antioxidant enzymes CAT (gills), SOD (digestive gland), GPX (both tissues), MT (in gills), LPO, AChE and also ALP in both females and males. Mussels from site 4 are affected by the commercial harbour and could be expected under greater stress, also considering the higher contaminant levels in sediments and the bioassays results.

On the opposite, IBR from site 6 was mostly affected by antioxidant enzymes (SOD gills and digestive and CAT and GPx from digestive gland) and LPO. Metal load in organisms from this site was higher and could account for the oxidative damage response, although the concentrations in sediments were lower (except for lead). In mussels from site 7, MT (in both tissues), SoS, condition index and ALP in females showed a higher contribution (Figure 6). At this site, concentrations of Cr, Cu and Zn were higher in mussels but lower in sediments; PAHs were also detected at site 7, in agreement with induced EROD activity and high levels 1-pyrenol equivalents in sea bass collected at this site (Fernandes et al., 2007).

### ***Principal Component Analysis***

PCA was applied to the data from the three compartments (water, sediments and biota) to better visualize the global results and depict the spatial association between sites (Figure 7). PC1 explained 49.5% of the variance and clearly distinguished site 4 on the left quadrant closely associated with organic contaminants, ALAD, ALP in females, CAT and GPx (gills). Sites 2 and 6 were on the right quadrant of PC1 and related to the majority of biomarker responses, metals and abiotic parameters. Finally, site 7 was in the center of PC1, associated with all factors but not particularly distinguished.

PC2 explained 27.6% of the variance and separates site 2 (upper quadrant) from site 6 (lower quadrant), the first under strong influence of biomarkers and the latter linked to metals and environmental parameters. Sites 4 and 7 assumed a centered position in PC2, in closer proximity to organic contaminants and some biomarkers such as MT and SOD in the digestive gland, CAT (gills), ALAD, ALP in females and a few metals in sediments.

Taking into account the SoS data and the IBR index, PCA results also revealed that site 4 was one of the most stressed (Figures 6-7), although only Cd and Pb levels were higher in mussels from this area. Similar results reflect the presence of hazards due to the harbour activities at site 4 where high Cu and Zn concentrations were previously detected in sea bass *D. labrax*, associated with the presence of antifouling paints (Fernandes et al., 2007). Other biocides were also measured at this site,

including diuron and its metabolites, irgarol (or cybutryne), terbutryn, the main metabolites of dichlofluanid (DMSA) and of tolyfluanid (DMST), other pesticides and a dozen of pharmaceutical compounds, namely NSAID (i.e. diclofenac) and antidepressants (Gonzalez-Rey et al submitted).

### ***Weight of Evidence Elaboration***

Data obtained from sediment chemistry, bioassays, bioaccumulation and biomarkers were finally integrated within a WOE model which elaborate specific hazard indices for each typology of data, before their differential weighting in an overall quantitative risk assessment (Piva et al., 2011; Benedetti et al, 2012, 2014; Regoli et al., 2014). An example of the model output for elaboration of different LOEs is given in Figure 8. The chemical characterization of sediments (LOE1) is typically summarized toward various SQGs, providing the quantitative value of chemical hazard quotient (HQ), the parameter which gives the highest contribution (in %) to the HQ, the number of exceeding parameters, the number of parameters (among those analysed) which are considered in that SQG, the total number of analysed parameters and the level (or class) of hazard assigned to HQ (from Absent to Severe). For bioavailability of chemicals (LOE2) and biomarker responses (LOE3) in mussel tissues, the output gives the number of parameters assigned to each class of effect (depending on the entity and statistical significance of variations), the quantitative value of HQ and the assigned level of hazard. The module on ecotoxicological bioassays (LOE4) summarizes results for both individual bioassays and for the integrated battery, including number of tests, threshold of the battery, value of the HQ<sub>Battery</sub> and class of hazard for bioassays. All the elaborations and model outputs obtained for elaborated samples are given in Supplementary Material, while Table 7 provides the classifications obtained for various LOEs and the final WOE integration elaborated through different SQGs, like ERL/ERM, TEL/PEL, the 5 levels of Portuguese legislation for dredging materials and the 2 equivalent levels of normative guidelines from France, Spain, UK and Italy.

According to data from sediment chemistry (LOE1), site 7 appeared as the less impacted with a chemical hazard summarized as Moderate towards the more restrictive normative levels below which negative biological effects should not occur (ERL, TEL, Portuguese Lev.1, UK Lev.1, Italy Lev.1); it was classified as Absent when compared to all the other levels or SQGs, i.e. those related to remediation activities or reflecting the probable onset of adverse biological consequences (Table 7). Conversely, sites 6 and 2 typically exhibited the higher chemical hazard, classified as Moderate toward PEL, Portuguese Lev.2, France Lev.1, Italy Lev.2, and Slight toward ERM, Portuguese Lev.3, Spain Lev.1, France Lev.2 (Table 7); the chemical hazard was Absent in all the sites when higher normative limits were considered.

Despite some statistically significant differences obtained for bioaccumulation data, their overall elaboration within LOE2 such variations were quite limited, i.e. compared to natural fluctuations of chemicals tissue levels; the model considered as Slight the hazard in mussels from site 6 (mostly due to Cu accumulation), Absent in those from other locations (Table 7).

Data on biomarker responses (LOE3) were generally more sensitive than those on bioaccumulation with a specific hazard quotient summarized as Major in mussels from site 6, Moderate in sites 2 and 4, Absent in site 7 (Table 7). Such elaborations were based on the magnitude and biological relevance of responding biomarkers which primarily included ALAD, GPx (digestive gland), SOD (gills) in site 6; with a lower and variable contribution, variations of SOD and GPx (in gills), catalase in digestive gland, LPO in gills and SOS reflected the Moderate classification of hazard for sites 4 and/or 2.

Results from Microtox STP were used within the model providing for LOE4 an hazard Absent or Slight for sites 2-6 and 4-7 respectively (Table 7).

The overall WOE integration of data from sediment chemistry, bioaccumulation, biomarkers and bioassays discriminated the higher risk at site 6, followed by sites 2 and 4 while site 7 typically appeared as not impacted. Considering the quality objectives of ERM, PEL or Level 3 of Portuguese SQGs the overall risk from the 4 considered LOEs was summarized as Moderate in site 6, Slight in sites 2 and 4 (Table 7).

This work confirmed that multidisciplinary studies combining chemical and biological measurements represent an added value to monitoring and management protocols in highly complex and heterogeneous environments like harbor areas. The WOE approaches have been often included in procedures of Ecological Risk Assessment (ERA), being also appropriate for requirements of European Directives and classification of the ecological status through different quality elements (Chapman, 2007; Chapman et al., 2013). The WOE model used in this study confirmed that different evaluations can be obtained in field conditions when comparing results from sediment chemistry, bioaccumulation, biomarkers responses and bioassays. The greater sensitivity of biomarkers observed in mussels from the different sites of Portimão could be explained by synergistic effects of chemicals in complex mixtures, different time-courses of biological effects, occurrence of persistent secondary effects, influence of non-chemical stressors; these are a few examples which can account for potential discrepancies, strongly supporting the necessity of an integrative, rather than alternative, application of multiple forms of investigations (Benedetti et al., 2014). Weighted criteria have also the great advantage to overcome the limits of pass-fail approach and classifications based on the worst results; further, the possibility to summarize large datasets of chemical and biological data in a synthetic evaluation of risk, represent an important tool providing scientifically sound information in a simple

format, thus supporting non-expert stakeholders in a more comprehensive process of “site-oriented” management decisions.

## Conclusions

The assessment of the ecological status of Portimão harbor supported by the weight of evidence approached highlighted the importance of combining sediment chemistry, bioaccumulation, biomarker responses and bioassays and revealed that despite the existence of some disturbance in the harbor area (sites 2 and 4), the harbor is also affected by the impact of urban effluents from upstream (site 6 at higher risk).

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## References

- Almroth, B.C., Sturve, J., Berglund, A., Forlin, L., 2005. Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. *Aquat. Toxicol.* 73, 171–180.
- Amiard, J.-C., Bacheley, H., Barillé, A.-L., Barillé, L., Geffard, A., Himery, N. 2004. Temporal changes in nickel and vanadium concentrations and in condition index and metallothionein levels in three species of molluscs following the “Erika” oil spill. *Aquat. Living Res.* 17, 281 – 288.
- Annicchiarico, C., Biandolino, F., Cardellicchio, N., Di Leo, A., Giandomenico, S., Prato, E. 2007. Predicting toxicity in marine sediment in Taranto Gulf (Ionian Sea, Southern Italy) using Sediment Quality Guidelines and a battery bioassay. *Ecotox.* 16, 239 – 246.
- Arrêté du 14/06/00. Relative to Baseline Levels to Consider in an Analysis of Marine or Estuarine Sediment Present in the Wild or Port (Arrêté du 14/06/00 relatif aux niveaux de référence à prendre en compte lors d’une analyse de sédiments marins ou estuariens présents en milieu naturel ou portuaire (JO n\_ 184 du 10 août 200)).
- ASTM, 1999. Standard Guide for Conducting 10-day Static Sediment Toxicity Test with Marine and Estuarine Amphipods. American Society of Testing and Materials, USA, E 1367-99.
- Azur Environmental, 1998. Microtox®Solid-Phase Test (SPT) Manual. AZUR Environmental, Carlsbad, CA, 16pp.

- Barsiene, J., Lehtonen, K.K., Koehler, A., Broeg, K., Vuorinen, P.J., Lang, T., Pempkowiak, J., Šyvokièene, J., Dedonytea, V., Rybakovasa, A., Repečka, R., Vuontisjärvi, H., Kopecka, J., 2006. Biomarker responses in flounder (*Platichthys flesus*) and mussel (*Mytilus edulis*) in the KlaipedaButinge area (Baltic Sea). *Mar. Pollut. Bull.* 53, 422–436.
- Bebianno, M.J., Langston, W.J. 1989. Quantification of metallothioneins in marine invertebrates using differential pulse polarography. *Portug. Electrochim. Acta* 7, 59 – 64.
- Bebianno, M. J., Lopes, B., Guerra, L., Hoarau, P., Ferreira, A. M., 2007. Glutathione-S-transferases and cytochrome P450 activities in *Mytilus galloprovincialis* from the South coast of Portugal: effect of abiotic factors. *Environ. Int.* 33, 550-558.
- Benedetti, M., Ciaprini, F., Piva, F., Onorati, F., Fattorini, D., Notti, A., Ausili, A., Regoli, F., 2012. A multidisciplinary weight of evidence approach toward polluted sediments: integrating sediment chemistry, bioavailability, biomarkers responses and bioassays. *Environ. Int.* 38, 17-28.
- Benedetti, M., Gorbi, S., Fattorini, D., Pacitti, D., D’Errico, G., Piva, F., Regoli, F. 2014. Environmental hazards from natural seepage: integrated classification of risk from sediment chemistry, bioavailability and biomarkers responses in sentinel species. *Environ. Pollut.* 185, 116-126.
- Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D., Regoli, F., 2008. Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas. *Aquat. Toxicol.* 89, 257 – 266.
- Bradford, M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* 72, 248 – 254.
- Cajaraville, M.P., Bebianno, M.J., Blasco, J., Porte, C., Sarasquete, C., Viarengo, A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *Sci. Total Environ.* 247, 295 – 311.
- Casado-Martínez, M. C., Campisi, T., Díaz, A., Lo Re, R., Obispo, R., Postma, J. F., Riba, I., Sneekes, A. C., Buceta, J. L., DelValls, T. A. 2006b. Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. II. Bioluminescence inhibition test for rapid sediment toxicity assessment. *Ciencias Marinas* 32 (1B), 129 – 138.
- CEDEX, 1994. Recommendations for the Management of Dredged Material in Spanish Ports. Ministry of Public Works, Transport and Environment (Recomendaciones para la gestión del material

- dragado en los puertos Españoles. Ministério de Obras Públicas, Transportes y Medio Ambiente), Madrid, p. 45.
- Chapman, P.M., 2007. Determining when contamination is pollution – weight of evidence determinations for sediments and effluents. *Environ. Internat.* 33, 492-501.
- Chapman, P. M., Wang, F., Caeiro S. S., 2013. Assessing and managing sediment contamination in transitional waters. *Environ. Internat.* 55, 71-91.
- Company, R., Serafim, A., Lopes, B., Cravo, A., Kalman, J., Riba, I., DelValls, T. A., Blasco, J., Delgado, J., Sarmiento, A. M., Nieto, J. M., Shepherd, T. J., Nowell, G., Bebianno, M. J., 2011. Source and impact of lead contamination on  $\delta$ -aminolevulinic acid dehydratase activity in several marine bivalve species along the Gulf of Cadiz. *Aquat. Toxicol.* 101, 146 – 154.
- Cravo, A., Lopes, B., Serafim, A., Company, R., Barreira, L., Gomes, T., Bebianno, M.J., 2009. A multibiomarker approach in *Mytilus galloprovincialis* to assess environmental quality. *J. Environ. Monit.* 11, 1673 – 1686.
- Dagnino, A., Sforzini, S., Dondero, F., Fenoglio, S., Bona, E., Jensen, J., Viarengo, A., 2008. A "Weight-of-Evidence" approach for the integration of environmental "Triad" data to assess ecological risk and biological vulnerability. *Integrat. Environ. Assess. Manag* 4, 314-326.
- Darbra, R.M., Pittam, N., Royston, K.A., Darbra, J.P., Journee H. Survey on environmental monitoring requirements of European ports. *J. Environ. Manage.* 2009;9:1396–403
- Diário da República, 2007. Portaria nº1450/2007 de 12 de Novembro de 2007. Diário da República n\_217/07 – I Série. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Lisboa, Portugal.
- Direção Geral das Pescas e Aquicultura (DGPA), 2004. Recursos da pesca. Série estatística. Vol 17 A-B, 169 p.
- Ellman, G.L., Courtney, K.O., Anders, V., Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol*, 7, 88 – 95.
- Erdelmeier, I., Gerard-Monnier, D., Yadan J. C., Acudiere, J., 1998. Reactions of N-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. *Chem. Res. Toxicol.* 11, 1184 – 1194.
- European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Off. J. Eur. Union* L 327,1-72.

- European Commission, 2008. Directive 2008/105/EC of the European Parliament and the Council of 16 December 2008 on environmental quality standards in the field of water policy. *Off. J. Eur. Com. L*, 348, 84.
- European Commission, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Com. L*. 226/1.
- Fernandes D., Porte C., Bebianno M. J., 2007. Chemical residues and biochemical responses in wild and cultured European sea bass (*Dicentrarchus labrax* L.). *Environ. Res.* 103, 247–256.
- Fernandes, D., Zanuy, S., Bebianno, M. J., Porte, C., 2008. Chemical and biochemical tools to assess pollution exposure in cultured fish. *Environ. Pollut.* 152, 138-146.
- Fernandes, D., Bebianno, M. J., Porte, C., 2009 Assessing pollutant exposure in cultured and wild sea bass (*Dicentrarchus labrax*) from the Iberian Peninsula. *Ecotoxicol.* 18, 1043-1050.
- Fernandes, D., Pujol, S., Pérez-Albaladejo, E., Tauler, R., Bebianno, M. J., Porte, C., 2014 Characterization of the environmental quality of sediments from two estuarine systems based on different in-vitro bioassays. *Mar. Environ. Res.* 96, 127-135.
- Frenzilli, G., Scarcelli, V., Barga, I.D., Nigro, M., Forlin, L., Bolognesi, C., Sturve, J., 2004. DNA damage in eelpout (*Zoarces viviparus*) from Goteborg harbour. *Mutat. Res.* 552, 187–195.
- Gagné, F., Blaise, C., Pellerin, J., Pelletier, E., Douville, M., Gauthier-Clerc, S., Viglino, L. 2003. Sex alteration in soft-shell clams (*Mya arenaria*) in an intertidal zone of the Saint Lawrence River (Quebec, Canada). *Comp. Biochem. Physiol. C* 134, 189 – 198.
- Ghirardini, A. V., Girardini, M., Marchetto, D., Pantani, C. 2009. Microtox® solid phase test: Effect of diluent used in toxicity test. *Ecotoxicol. Environ. Saf.* 72, 851 – 861.
- Gonçalves, S.F., Calado, R., Gomes, N.C.M., Soares, A. M. V. M. , Loureiro S., 2013. An ecotoxicological analysis of sediments quality in a European Atlantic harbour emphasis current limitations of the Water Framework Directive. *Mar. Pollut. Bull.* 72, 197–204
- Gonzalez-Rey M., Tapie, N., Le Menarch, T., Devier, M-H., Budzinski, H., Bebianno M.J. Occurrence of APIs and pesticides in aquatic systems. *Chmosphre* (submitted)
- Grasshoff, K., Erkhart, M., Kremling, K., 1983. *Methods of Seawater Analysis*. Verlag Chemie, New York, 419 pp.
- Grifoll, M., Jordà, G., Espino, M., Romo, J., García-Sotillo, M., 2011. A management system for accidental water pollution risk in a harbour: The Barcelona case study. *J. Mar. Syst.* 88, 60–73.



- Hellou, J., Lawm R. J., 2003. Stress on stress response of wild mussels, *Mytilus edulis* and *Mytilus trossulus*, as an indicator of ecosystem health. *Environ. Pollut.* 126, 407 – 416.
- Hubner, R., Astin, K. B., Herbert, R. J. H. (2009). Comparison of sediment quality guidelines (SQGs) for the assessment of metal contamination in marine and estuarine environments. *J. Environ. Monit.* 11,(4), 713–722.
- IPIMAR, 2010. Caracterização do estado químico e ecológico das massas de água de transição da região hidrográfica do Algarve. Relatório final.
- Lawrence, R. A., Burk, R. F., 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.* 71 (4), 952 – 958.
- Linkov, I., Loney, D., Cormier, S., Satterstrom, F.K., Bridges, T., 2009. Weight-of-evidence evaluation in environmental assessment: Review of qualitative and quantitative approaches. *Sci. Total Environ.* 407, 5199-5205.
- Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manag.* 19, 81-97.
- Long, E. R., FIELD, L. J., MACDONALD, D. D., 2009. Predicting toxicity in marine sediments with numerical sediment quality guidelines. Lorenzen C. J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations, *Limnol. Oceanog.* 12.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J., 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265 – 275.
- MacDonald, D.D., Carr, R.S., Eckenrod, D., Greening, H., Grabe, S., Ingersoll, C.G., Janicki, S., Janicki, T., Lindskoog, R.A., Long, E.R., Pribble, R., Sloane, G., Smorong, D.E., 2004. Development, evaluation, and application of sediment quality targets for assessing and managing contaminated sediments in Tampa Bay, Florida. *Archives of Environmental Contamination and Toxicology* 46, 147-161.
- Martins, F. A., Janeiro, J., Gabriel, S., Venâncio A. Neves R., 2009. Integrated monitoring of South Portugal water bodies: a methodology towards WFD. *Wat. Sci. Technol.* 60, 1979-1988.
- Macdonald, D. D., Carr, R. S., Calder, F. D., Long, E. R., Ingersoll, C.G., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicol.* 5, 253-278.
- McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymatic function for erythrocyte hemocuprein (hemocuprein). *J. Biol. Chem.* 244, 6049 – 55.

- Mudge, S. M., Hooper, L., Icely, J. D., 1998. Biomarkers associated with sewage in the Arade River, Portugal. *Env. Tech.* 19, 1055-1060.
- Ondiviela B., Juanes, J. A., Gómez, A. G., Sámano, M. L., Revilla, J. A., 2012. Methodological procedure for water quality management in port areas at the EU level. –*Ecol. Indicat.* 13, 117-128.
- OSPAR, 2004. Overview of Contracting Parties' National Action Levels for Dredged Material. Biodiversity Series, 22.
- Pampanin, D. M., Volpato, E., Marangon, I., Nasci, C., 2005. Physiological measurements from native and transplanted mussel (*Mytilus galloprovincialis*) in the canals of Venice. Survival in air and condition index. *Comp. Biochem. Physiol. A* 140, 41 – 52.
- Pereira, C., Gomes, T., Cardoso, C., Almeida, A. C., Araújo, O., Bebianno, M. J., Cravo, A., 2013. Interspecific variability of endocrine disruption and oxidative stress in two bivalve species from the Ria Formosa Lagoon (south coast of Portugal). *Scientia Marina*, 77, 79-89.
- Piva, F., Ciapriani, F., Onorati, F., Benedetti, M., Fattorini, D., Ausili, A., Regoli, F., 2011. Assessing sediment hazard through a Weight Of Evidence approach with bioindicator organisms: a practical model to elaborate data from sediment chemistry, bioavailability, biomarkers and ecotoxicological bioassays. *Chemosphere* 83, 475-485.
- Prato, E., Bigongiari, N., Barghihiani, C., Biandolino, F., 2010. Comparison of amphipods *Corophium insidiosum* and *C. orientale* (Crustacea: Amphipoda) in sediment toxicity testing. *J. Environ. Sci. Health A* 45, 1461 – 1467.
- Ré, A., Freitas, R., Sampaio, L., Rodrigues, A. M., Quintino, V., 2009. Estuarine sediment acute toxicity testing with the European amphipod *Corophium multisetosum* Stock, 1952. *Chemosphere* 76, 1323 – 1233.
- Ré, A., Rodrigues, A.M., Quintino, V. 2007. Acute toxicity testing with the European estuarine amphipod *Corophium multisetosum*. *Hydrobiol.* 587, 89 – 99.
- Regoli, F., Pellegrini, D., Winston, G.W., Gorbi, S., Giuliani, S., VirnoLamberti, C., Bompadre, S., 2002. Application of biomarkers for assessing the biological impact of dredged materials in the Mediterranean: the relationship between antioxidant responses and susceptibility to oxidative stress in the red mullet (*Mullus barbatus*). *Mar. Pollut. Bull.* 44, 912–922.
- Regoli, F., Frenzilli, G., Bocchetti, R., Annarumma, F., Scarcelli, V., Fattorini, D., Nigro, M., 2004. Timecourse variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, *Mytilus galloprovincialis*, during a field translocation experiment. *Aquat. Toxicol.* 68, 167–178.

- Regoli F., Pellegrini D., Cicero A.M., Nigro N., Benedetti M., Gorbi S., Fattorini D., D'Errico G., Di Carlo M., Nardi A., Gaion A., Scuderi A., Giuliani S., Romanelli G., Berto D., Trabucco B., Guidi, Bernardeschi M., *Scarcella V.*, Frenzilli G., 2014. A multidisciplinary weight of evidence approach for environmental risk assessment at the Costa Concordia wreck: integrative indices from Mussel Watch. *Mar. Environ. Res.* 96, 92-104.
- Schiff, K., Diehl, D., Valkirs, A.O., 2004. Copper emissions from antifouling paint on recreational vessels. *Mar. Pollut. Bull.* 48, 371–377.
- Serafim, A., Company, R., Lopes, B., Fonseca, V.F., França, S., Vasconcelos, R.P., Bebianno, M.J., Cabral, H.N., 2012, Application of an integrated biomarker response index (IBR) to assess temporal variation of environmental quality in two Portuguese aquatic systems. *Ecol. Indic.* 19, 215-225.
- Stephensen, E., Svavarsson, J., Sturve, J., Ericson, G., Adolfsson-Erici, M., Forlin, L., 2000. Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in four harbours on the southwest coast of Iceland. *Aquat. Toxicol.* 48, 431–442.
- Stronkhorst, J., Ariese, F., Van Hattum, B., Postma, J.F., De Kluijver, M., Den Besten, P.J., Bergman, M.J.N., Daane, R., Murk, A.J., Vethaak, A.D., 2003. 2003 Environmental impact and recovery at two dumping sites for dredged material in the North Sea. *Environ. Pollut.* 124, 17–31.
- Sturve, J., Berglund, A., Balk, L., Broeg, K., Bohmert, B., Massey, S., Savva, D., Parkkonen, J., Stephensen, E., Koehler, A., Forlin, L., 2005. Effects of dredging in Goteborg harbor, Sweden, assessed by biomarkers in Eelpout (*Zoarces viviparus*). *Environ. Toxicol. Chem.* 24, 1951–1961.
- US EPA, 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. United States Environmental Protection Agency, EPA/600/R-94/025.
- Viarengo A., Lowe D., Bolognesi C., Fabbri E., Koehler A. 2007. The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comp. Biochem.* 146C, 281–300.



**Table 2** – Metal concentrations (mean  $\pm$  standard deviation) of standard reference material (TORT II) and respective certified values ( $\mu\text{g}\cdot\text{g}^{-1}$ ).

	<b>Cadmium</b>	<b>Chromium</b>	<b>Copper</b>	<b>Lead</b>	<b>Zinc</b>
<b>Values obtained</b>	26.2 $\pm$ 9.5	1.1 $\pm$ 0.4	105.2 $\pm$ 4.8	0.2 $\pm$ 0.1	241.9 $\pm$ 12.6
<b>Certified values</b>	26.7 $\pm$ 0.6	0.8 $\pm$ 0,2	106.0 $\pm$ 10.0	0.4 $\pm$ 0.1	180.0 $\pm$ 6.0

Table 3 – PAH concentrations ( $\mu\text{g/g d.w.}$ ) in sediments from all sites. Concentrations above the detection limit are shaded grey

PAHS	Sites					
	2	3	4	5	6	7
Naphthalene	<0.010	<0.010	0.028	<0.010	<0.010	<0.010
Acenaphthylene	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthene	<0.010	<0.010	0.06	<0.010	<0.010	<0.010
Fluorene	<0.010	<0.010	0.019	<0.010	<0.010	<0.010
Phenanthrene	<0.010	0.019	0.379	0.015	<0.010	<0.010
Anthracene	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluoranthene	<0.010	0.067	0.376	0.053	<0.010	0.038
Pyrene	<0.010	0.061	0.264	0.048	<0.010	0.035
Benz(a)anthracene	<0.010	0.032	0.057	0.024	<0.010	0.018
Chrysene	<0.010	0.032	0.137	0.025	<0.010	0.02
Benzo(b)fluoranthene	<0.010	0.034	0.097	0.016	<0.010	0.022
Benzo(k)fluoranthene	<0.010	0.027	0.072	0.017	<0.010	0.016
Benzo(a)pyrene	<0.010	0.044	0.077	0.03	<0.010	0.031
Dibenz(a,h)anthracene	<0.010	<0.010	0.013	<0.010	<0.010	<0.010
Benzo(g,h,i)perylene	<0.010	0.03	0.046	0.017	<0.010	0.016
Indeno(1.2.3.cd)pyrene	<0.010	0.022	0.066	0.023	<0.010	0.022
<b>Total PAHs</b>	<0.160	0.368	1.69	0.268	<0.160	0.218
Sum of carcinogenic PAHs	<0.070	0.191	0.519	0.135	<0.070	0.129
Sum of non-carcinogenic PAHs	<0.090	0.177	1.17	0.133	<0.090	<0.090

Table 4 – Sediment Quality Criteria (SQC) for metals, PAHs, PCBs and HCB (mg/kg d.w.)

SQC (mg/kg d.w.)	OSPAR <sup>a</sup>		NOAA <sup>b</sup>		Canada <sup>c</sup>	
	BAC	EAC	ERL	ERM	TEL	PEL
Cd	0.31	0.06	2.2	9.6	0.7	4.2
Cr	81		81	370	52.3	160
Cu	27		34	270	18.7	108
Ni	36		21	52	15.9	42.8
Pb	38	22	47	218	30	112
Zn	122		150	410	124	271
Naphthalene	0.008	0.043	0.16	2.1	0.035	0.391
Acenaphthylene			0.044	0.64	0.006	0.128
Acenaphthene			0.016	0.5	0.007	0.089
Fluorene			0.019	0.54	0.021	0.144
Phenanthrene	0.032	1.25	0.24	1.5	0.087	0.544
Anthracene	0.005	0.08	0.085	1.1	0.047	0.245
Fluoranthene	0.039	0.25	0.6	5.1	0.113	1.490
Pyrene	0.024	0.35	0.665	2.6	0.153	1.400
Benz(a)anthracene	0.016	0.0015	0.261	1.6	0.075	0.693
Chrysene	0.020		0.384	2.8	0.108	0.846
Benzo(b)fluoranthene						
Benzo(k)fluoranthene			0.24	1.34		
Benzo(a)pyrene	0.030	0.625	0.43	1.6	0.089	0.763
Dibenz(a,h)anthracene			0.063	0.26	0.0062	0.135
Benzo(g,h,i)perylene	0.080	0.0021	0.17	0.32		
Indeno(1.2.3.cd)pyrene	0.103	0.0015	0.2	0.32		
Total PAHs			4	45	17	16.8
Total PCBs			0.023	0.18	0.0216	0.189
HCB			0.02	24		

OSPAR, 2009; b) Long et al., 2005; c) CCME, 2001

BAC (background assessment criteria): statistical tools defined in relation to the background

concentrations which enable testing of whether mean observed concentrations can be considered near background concentrations;

EAC (environmental assessment criteria) - contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive species;

ERL (effect range low) and ISQG – chemical concentrations below which adverse effects would be rarely observed

ERM (effect range medium) and PEL (probable effects level) – chemical concentrations above which adverse effects are expected to occur frequently.



Table 5–Hexachlorobenzene (HCB) and PCBs (µg/g d.w.) in sediments from all sites.

Site	2	3	4	5	6	7
Hexachlorobenzene (HCB)	0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050
PCB 101	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
PCB 118	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
PCB 138	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
PCB 153	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
PCB 180	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
PCB 28	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
PCB 52	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070	<0.00070
Sum of 7 PCBs	<0.00490	<0.00490	<0.00490	<0.00490	<0.00490	<0.00490

**Table I** – EC<sub>50</sub> (g.L<sup>-1</sup> w. w.) and TU (toxicity units) after 5, 15 and 30 minutes, obtained with the Microtox SPT for sediments from all sites in the Portimão harbour.

Site	EC <sub>50</sub> (g.L <sup>-1</sup> )			TU <sub>50</sub>		
	5 min	15 min	30 min	5 min	15 min	30 min
<b>1</b>	90.22	9.53	10.60	1	1	1
<b>2</b>	32.43	49.02	39.56	3	2	2
<b>3</b>	4.02	3.89	3.94	2	2	2
<b>4</b>	.80	3.98	3.62	2	2	2
<b>5</b>	16.05	14.75	-	6	6	-
<b>6</b>	37.79	32.41	40.84	2	3	2
<b>7</b>	6.17	5.47	5.02	1	1	1

**Table II** – SoS bioassay results (LT50, in days, with confidence intervals (upper and lower limits)) performed with mussels *M. galloprovincialis* from 4 sites in Portimão harbour area.

Site	LT50	Upper Limit	Lower Limit
2	8.0	10.6	6.0
4	4.3	4.8	3.8
6	7.1	8.4	5.9
7	6.7	9.8	4.4