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COMBINED ASSESSMENT OF CHEMICAL AND ECOTOXICOLOGICAL DATA FOR THE MANAGEMENT OF CONTAMINATED MARINE SEDIMENTS

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Abstract

Sediments in coastal areas can accumulate a variety of contaminants, acting as both carriers and long-term secondary sources of contamination for aquatic ecosystems. Nowadays, there is a growing interest on developing new assessment criteria of sediment ecological quality for setting priorities and management strategies of contaminated materials. According to the literature, the weight of evidence (WOE) approach has been developed to provide a multidisciplinary characterization which combines different studies such as chemical analyses, laboratory and field-based studies to assess the bioavailability of pollutants and ecotoxicological assays. However, applications on complex case studies are limited. In order to strengthen the current literature, this study presents the first results of the application of a WOE model (Sediqualsoft) to a marine chronically polluted area. To this aim, a laboratory experimental investigation was carried out on the polluted sediments of the Mar Piccolo in Taranto (Southern Italy). The combination of chemical and ecotoxicological data confirmed the results obtained with the conventional approaches, highlighting sediment contamination. Even more, the obtained biological responses highlighted an unexpected toxic effect not revealed by conventional approaches: the level of contamination did not seem to be proportional to the ecotoxicological assessment. All these observations have raised numerous questions about the potential mobility of pollutants and additional risks to the environment. The Sediqualsoft model has proven to be a useful tool for processing complex scientific data, playing an important role in contaminated sediment risk assessment supporting stakeholders and decision makers.

Keywords: chemical/ecotoxicological data, contaminated sediments, Mar Piccolo, Sediqualsoft

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1. Introduction

Marine sediments can accumulate a variety of heavy metals (i.e. As, Cr, Hg, Pb, Zn) as well as organic pollutants, including tributyltin (TBT), polychlorinated biphenyls (PCB), pesticides and polycyclic aromatic hydrocarbons (PAHs), acting as both carriers and long-term secondary sources of contamination to aquatic ecosystems (De Gisi et al., 2017a; Lofrano et al., 2016). Contaminated sediments pose major concerns for human health and the environment, because the pollutants can re-enter the overlying water and become available to benthic organisms and subsequently pass into aquatic food chains (Todaro et al., 2018).

The assessment of contamination level of marine sediments is usually done by comparing the chemical concentrations of samples to reference values (e.g. law limits). However, this approach does not account that contaminant behavior (i.e. remobilize or desorption) in sediments is a dynamic process and bioavailability of contaminants is regulated by physical, chemical and biological properties of marine environment (e.g. Eh, pH).

Chemical analyses alone do not necessarily reflect the bioavailability and the toxic action of

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measured contaminants; multidisciplinary approaches are required to assess the chemical, biological and toxicological impact of complex mixtures of contaminants. In this respect, ecotoxicological bioassays are important complementary tools to evaluate synergistic effects of contaminant mixtures in sediments. Therefore, chemical analyses and ecotoxicological bioassays are important components to assess the environmental quality of contaminated marine areas and to establish the link between contaminants and ecological responses. In this respect, there is a growing interest on developing new assessment criteria of sediment ecological quality for setting priorities and management strategies of contaminated materials. Different sediment quality guidelines (SQGs) have since been developed for use in assessing sediment quality, meaning contaminant concentrations that cause adverse effects (Christopher et al., 2002). As shown in Tables 1, there are several sediment quality guidelines (SQGs) in use today. Additional information on each guideline are available in the papers reported in the references.

In particular, the Weight Of Evidence (WOE) approach has been developed to provide a multidisciplinary characterization which combine different studies, or Lines Of Evidence (LOEs) (i.e. chemical analyses, laboratory and field-based studies to assess the bioavailability of pollutants and ecotoxicological assays) (Benedetti et al., 2012; Chapman, 2007; Dagnino et al., 2008). Chemical and biological data can be elaborated within a quantitative model (Sediqualsoft) which combines various typologies of studies, including sediment chemistry, ecotoxicological bioassays, bioaccumulation and biomarker results. WOE methods are often key components for evaluate and classify the ecological status of water bodies (WFD, 2000) and for Ecological Risk Assessment (ERA) (Bebianno et al., 2015).

The aim of the study was the application of Sediqualsoft model, based on the combination of chemical and ecotoxicological data, to assess the environmental quality in a marine chronically polluted area. For the purpose, thirty-eight sediment samples (taken from 19 boreholes) were tested in the laboratory, and the chemical characterization was integrated with acutely toxic effects by Sediqualsoft. As a consequence, the following subgoals have been investigated: (i) to examine the spatial variation of heavy metals and organic compounds contaminants, (ii) to assess the contamination and the sediment quality with multidisciplinary approach and (iii) to investigate the link between contaminants and ecological responses.

In order to apply Sediqualsoft, the case study of the Mar Piccolo in Taranto (Southern Italy) was addressed (Fig. 1). The Mar Piccolo is an inner sea located on the North of the city of Taranto (south of Italy), with a surface area of 20.72 km^2. Several researchers (Cardellicchio et al., 2007; De Gisi et al., 2017; Di Leo et al., 2014; Mali et al., 2017; Petronio et al., 2012; Vitone et al., 2016) have shown that also the submarine sediments in the Mar Piccolo contain high concentrations of heavy metals (Hg, Pb, Cd, Cu and Zn) and organic pollutants (PCBs and PAHs). The Mar Piccolo basin is part of a large area that has been declared "at high risk of environmental crisis" and included into the list of the Sites of National Interest (SIN Site) (Italian Law, 1998).

2. Material and methods

2. 1. Sediment chemical analyses

Sediment samples were collected to depths of about 3.0 m from the seafloor, transported to the laboratory at 4 °C and stored for subsequent use for bioassays and for the determination of contaminant concentrations. In particular, chemical analyses to determine the concentrations of metals, PAHs and PCBs in the sediments were carried out.

Table 1. Types of SQGs in use

SQG Category	Approach	References
Theoretical	Equilibrium Partitioning	Di Toro and McGrath (2000)
	Spiked Sediment Toxicity Test	Simpson et al., (2004)
	Porewater Effect Concentration	Hübner et al., (2009)
	Equilibrium Partitioning	Yun-Zeng et al., (2007)
	Acid Volatile Sulfides	De Jonge et al., (2009)
	Tissue Residue Approach	Meador (2006)
Empirical	Screening-Level Concentration	Von Stackelberg and Menzie (2002)
	Effects Range-Low and Effects Range-Median	US EPA (1997)
	Threshold-Effects Level and Probable-Effects Level	US EPA (1997)
	Apparent-Effects Threshold	Cubbage et al., (1997)
	Consensus-Based Evaluation	MacDonald, Ingersoll et al., (2000)
	Logistic Regression Modeling	Field et al., (2002)

(a)

(b)

Fig. 1. (a) Gulf of Taranto; (b) location of the samples taken within the First Bay of Mar Piccolo

The pH, Eh and conductivity were measured with electrodes in a Multi-Liner instrument (CyberScan PC5000, Eutech Instruments). The grain size distribution of the sediments has been estimated through sieving for particles larger than 75 μm (i.e. retained on the N. 200 sieve) and by sedimentation process (i.e. hydrometer analysis) for the finer ones. The grain size distribution was then obtained either by including or excluding the gravel fraction, mainly represented by mussels, shells and fossils. In the latter case, the weight of the material passing the 1 mm (No. 18) sieve was set to be equal to the total soil weight. Organic content was determined from each sediment samples as the percentage of weight loss by drying at 100 °C for 24 h and combustion at 450 °C for 5 h. Total metal content was determined in the < 63 μm fraction after wet digestion with HNO_3 and ultrapure water. The concentrations of metals were obtained by ICP-OES, Inductively Coupled Plasma - Optical Emission Spectrometry (iCAP 7000 Series - Thermo Scientific). Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in sediments were determined by a Gas Chromatograph - Mass Spectrometer (GC-MS, Thermo Scientific TRACE 1300).

2. 2. Sediment toxicity bioassays

Toxicity bioassays were performed according to standardized protocols, in terms of exposure conditions, matrix and biological endpoint, with bioindicator species that are widely applied and recognized to assess potential ecotoxicological effects to marine biota. Selected species were the bacterium *Vibrio fischeri*, the algae *Phaedactylum tricornutum*, and the shellfish *Mitilus galloprovincialis*. These were selected considering phylogenetic diversity and within the most widespread organisms already used in the scientific literature for marine sediments monitoring (Simpson et al., 2017) as well as required by Italian legislations (MD, 2016).

The sediment samples were mixed in a 1:4 (v/v) ratio of dry material to seawater and placed on a jar test for 24 h, at a speed of 200 rpm. After mixing the samples were centrifuged at 3800 rpm for 15 min,

to separate the water from the sediment. The aqueous fraction (elutriate sample) was poured off and stored at 20 °C for use. Prior to use, seawater was filtered (0.45 μm membrane filters), analysed and stored in the refrigerator at +4 °C.

The acute toxicity tests on the sediments were performed according to the ISO 11348-3 (2007). This bioassay exposes luminescent bacteria (*V. fischeri*) to aqueous samples and measures the increase or decrease in light output by the test organisms. Bioluminescence at 15 °C was measured with a Microtox luminescence meter after 30 min of incubation.

Toxicity tests with *P. tricornutum* were carried out according to the ISO 10253 (2016) determining the growth inhibition (or biostimulation) of microalgae exposed to sediment elutriate samples. The algal culture was exposed for 72 ± 2 h at 20 ± 1 °C and 6000–10,000 lux and cellular density was evaluated by spectrophotometer. Toxicity and stimulation effect data were determined as percentages of growth inhibition or growth stimulation (biostimulation) in the observed population.

The embryotoxicity test with *Mitilus galloprovincialis* was performed according to ASTM (2004). Thermal shock technique (18 ± 1°C and 28 ± 1°C) was then employed to induce the mussels to spawn. At the end of the spawning, gametes derived from a batch of three males and three females have been removed by filtration at 32 μm (sperm cells) and 100 μm (eggs) to remove impurities. Eggs suspended in 500 mL seawater were fertilized and fertilization success was qualitatively checked by microscopy. At the end of the test, fertilized eggs were fixed in a 4% NBF (Neutral Buffered Formalin) and 100 larvae were observed with a microscope to verify the presence of abnormalities (e.g. malformed or damaged shell). Toxicity effect data were calculated as the ration of the number of retarded and malformed larvae to the 100 counts (percentages of abnormal larvae).

Toxicity tests were performed in triplicate and negative (test without contaminants) and positive (test with reference toxicant) controls were included in each experiment (ASTM, 2004).

2.3. Weight of evidence elaboration

Data on sediment chemistry and toxicity bioassays measured 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).

The evaluation of hazard from sediment chemistry is based on the calculation for each pollutant of Ratio to Reference (RTR), i.e., the ratio between measured concentrations and those indicated by various SQGs; depending to the typology of chemicals (i.e. "non-priority", "priority" or "priority and hazardous" pollutant according to EC Directive 2008/105) this value is further corrected (RTR_W). In this study, the considered SQGs derived from a database of marine sediment chemistry, provide values indicative of concentrations below which adverse effects are rarely observed.

The HQ_C (Hazard Quotient for chemistry) is calculated with Eq. 1 (Piva et al., (2011). RTR_W is is the sum of the parameters with RTR < 1 (i.e. values below the sediment normative limit), while for those with RTR > 1, the RTR_W are individually added into the summation (Eq. 1):

$$HQc = (\sum RTR_{W \cdot (RTR<1)} / N + \sum RTR_{W \cdot (RTR>1)}) \qquad (1)$$

where N is the number of parameters with RTR < 1.

HQ_C increases according to both the number and the magnitude of the exceeding parameters. Based on expert judgment, the values of HQ_C were assigned to one of six classes of chemical hazard identified according to different colours: absent (white) < 0.7; negligible (green) 0.7 ÷ 1.3; slight (azure) 1.3 ÷ 2.6; moderate (yellow) 2.6 ÷ 6.5; major (red) 6.5 ÷ 13; severe (black) > 13.

For ecotoxicological bioassays, the cumulative hazard quotient ($HQ_{Battery}$) is calculated by the summation of the weighted effects obtained by the summation of the weighted effects ($Effect_W$). The biological importance of the endpoint of each test and the exposure conditions (w2) (Eq. 2):

$$HQ_{Battery} = \sum Effect_w(k) \cdot w_2$$

The HQBattery is normalized to a scale ranging from Absent (i.e. HQBattery < 1) to severe (i.e. HQBattery > 10) when all the assays exhibit 100% of effect; the HQBattery is then assigned to one of five classes: i) absent; ii) slight; iii) moderate; iv) major; v) severe.

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, named Integrated Hazard Index (IHI), is thus calculated and associated to 1 of 5 classes (absent, slight, moderate, major, severe) (Piva et al., 2011).

3. Results and discussion

3.1. Sediment chemical characterization

Sediment samples are essentially fine-grained soils, for which the clay fraction, CF, varies between 37.21% (S17 sample) and 56.98% (S9 sample), sand fraction, SF, between 3.35% (S8 sample) and 14.84% (S14 sample) and the silt fraction, MF, ranges from 39.04% of the S9 sample to 55.33% of the S11 sample (Todaro et al., 2018).

The sediments used for the tests were characterised by the physical-chemical properties reported in Table 2. pH ranged from 8.6 (minimum at site 4) to 9.9 (maximum on the site 18); while Eh ranged from -86.8 (site 4) to -174.6 (site 1). The parameters have a similar trend; they increase with depth. Moisture was ranging from 37.8% (sites 9) to 53.9 (site 17) while the percentage of organic matter (OM) was minimum at site 1 (10.9%) and maximum at site 4 (21.1%). Even if few data were available, it should be noticed a tendency of OM to decrease with depth; if the data detected are compared, it could be noticed that Mar Piccolo basin has similar or slight higher organic matter values then other sites (Sollecito et al., 2019). pH values and organic matter concentration in sediments influence the mobility of metals in the sediments, and consequently their distribution (Calace et al., 2008).

Analyses included priority and specific compounds (WDF, 2000) such as metals (As, Cd, Cu, Cr, Hg, Ni, Pb, V and Zn) and organic chemicals (PAHs and PCBs). Levels of these compounds were integrated with ecotoxicological bioassays to better assess sediment quality at different sites of Mar Piccolo. The contents of contaminants were better discriminated in comparison to the limits of site-specific law (ICRAM, 2004), highlighting differences between various sediment samples.

Concentrations of metals in sediments are shown in Fig. 2. In general, metal concentrations decrease with sampling depth. A different pattern was observed for site 1 (i.e., As, Cd, Cu, Hg, Ni, V and Zn concentration increases with depth) and site 9 (i.e. As, Cd, Ni and Zn concentration increases with depth). Nevertheless, As, Cd, Cu, Hg, Pb and Zn presented higher concentrations in the south of the Mar Piccolo (from sites 4, 6 and 7). With the exception of Ni, Cu and V, all the other metal levels (in at least one site) were higher than limits of site-specific law.
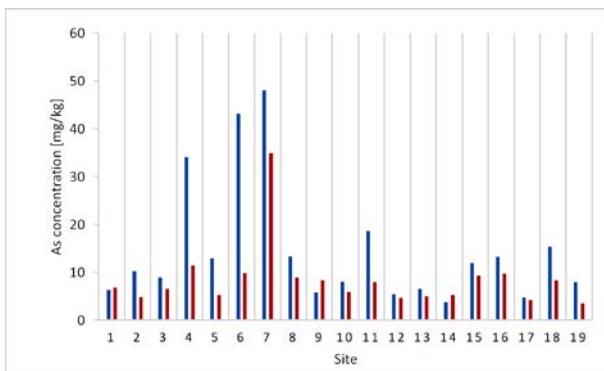
PAHs' and PCBs' concentrations in sediments are in Fig. 3. Levels were higher the detection limit in sites 1, 7, 8, 10, 13, 14, 15, 16, 17, 18 and 19 for PAHs and in sites 2 and 6 for PCBs; organic contaminants followed a decreasing trend with sampling depth, excluding the site 7. The highest PAHs from site 1 (9391 µg/g d.w.) was especially due to fluoranthene (624 µg/kg d.w.), antracene (268 µg/kg d.w.), pyrene (562 µg/kg d.w.), chrysene (311 µg/kg d.w.), benzo[k]fluoranthene (408 µg/kg d.w.),

indeno[a,h]pyrene (280 µg/kg d.w.) among others. The sum of carcinogenic PAHs was lower than the non-carcinogenic; although lower PAHs' concentrations were measured at sites 15 and 16, carcinogenic PAHs were comparatively higher to non-carcinogenic. However, PAHs' concentrations are always lower to limits of site-specific law (4000 µg/kg). The knowledge of the concentrations of these compounds in sediments is important to evaluate the contamination of a marine ecosystem but alone, they do not provide information on potential toxicity for those organisms exposed to such chemical mixtures.

Table 2. Chemical parameters in sediments measured in different sampling site

Site	Depth from seafloor (m)	pH	Eh	Moisture (%)	Organic Matter (%)
1	0.0-1.5	8.8	-161.1	40.3	10.9
	1.5-3.0	9.1	-174.6	44.6	17.1
2	0.0-1.5	9.2	-117.3	48.3	15.5
	1.5-3.0	9.4	-125.2	45.9	16.9
3	0.0-1.5	9.3	-122.0	46.0	16.1
	1.5-3.0	9.2	-114.1	41.8	15.1
4	0.0-1.5	8.6	-86.8	47.7	21.1
	1.5-3.0	9.3	-120-2	39.7	19.6
5	0.0-1.5	9.2	-115.6	49.6	20.0
	1.5-3.0	9.1	-109.8	48.1	16.8
6	0.0-1.5	9.1	-110.8	46.1	15.3
	1.5-3.0	9.3	-126.6	40.3	10.7
7	0.0-1.5	9.4	-127.1	45.7	15.5
	1.5-3.0	9.4	-122.4	48.2	19.7
8	0.0-1.5	9.2	-116.2	50.2	15.3
	1.5-3.0	9.2	-108.7	52.0	15.5
9	0.0-1.5	9.4	-124.9	39.8	18.6
	1.5-3.0	9.3	-124.8	37.8	16.2
10	0.0-1.5	9.3	-121.6	49.4	17.2
	1.5-3.0	9.2	-119.4	46.7	16.9
11	0.0-1.5	9.2	-119.3	51.2	20.2
	1.5-3.0	9.3	-119.6	49.1	19.5
12	0.0-1.5	9.4	-131.1	48.3	16.3
	1.5-3.0	9.6	-137.1	36.4	11.1
13	0.0-1.5	9.3	-123.9	50.3	17.1
	1.5-3.0	9.5	-132.4	47.5	16.8
14	0.0-1.5	9.3	-120.9	50.7	17.0
	1.5-3.0	9.5	-132.2	43.1	15.1
15	0.0-1.5	9.4	-126.5	50.9	16.1
	1.5-3.0	9.5	-132.5	49.5	16.6
16	0.0-1.5	9.3	-122.3	48.1	19.8
	1.5-3.0	9.4	-126.0	43.6	17.5
17	0.0-1.5	9.2	-120.0	53.9	16.3
	1.5-3.0	9.3	-123.7	48.3	17.1
18	0.0-1.5	8.9	-101.3	52.6	18.3
	1.5-3.0	9.9	-104.5	48.7	15.2
19	0.0-1.5	9.4	-126.9	49.5	14.5
	1.5-3.0	9.1	-113.1	47.9	17.7

(a)

(b)

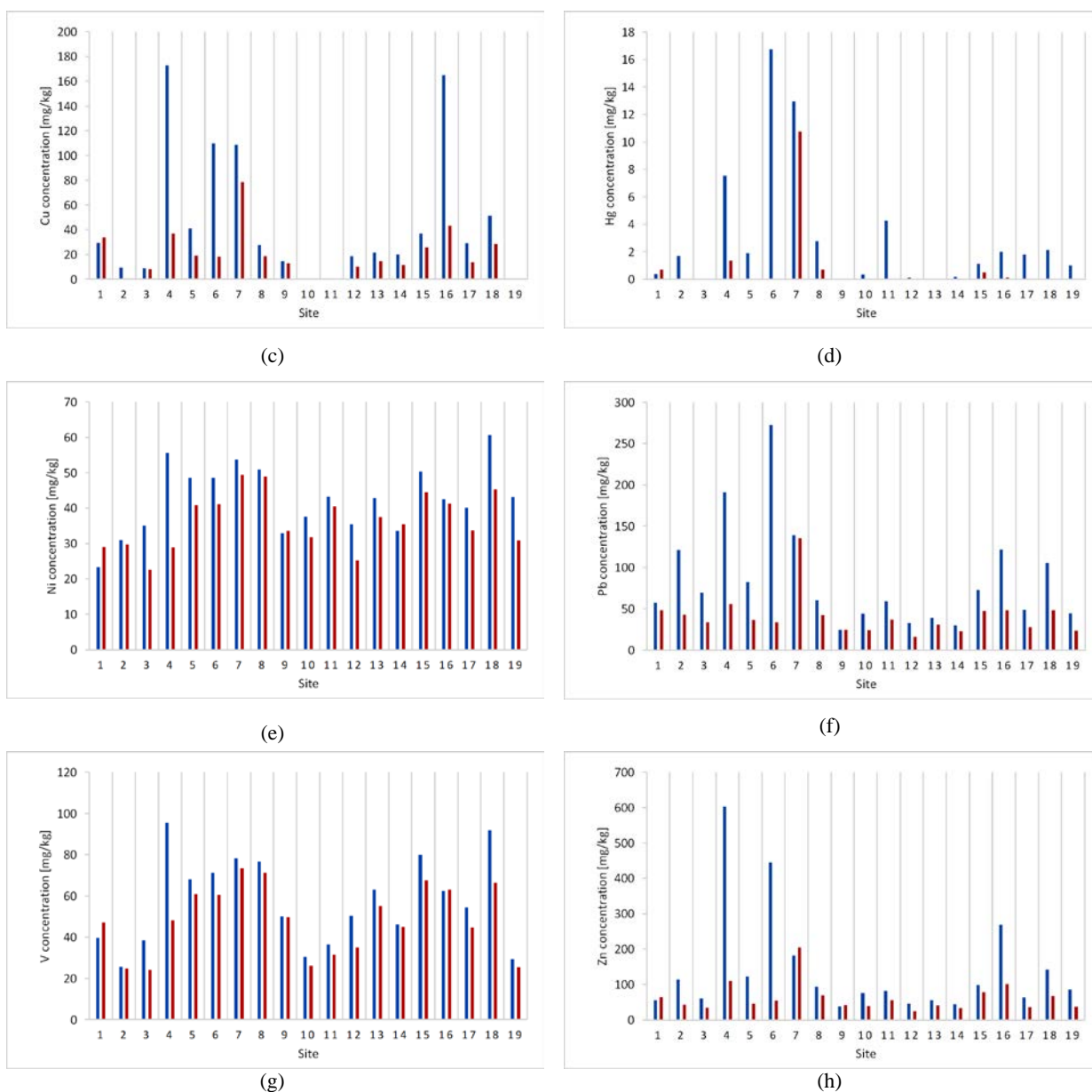(c)

(d)

(e)

(f)

(g)

(h)
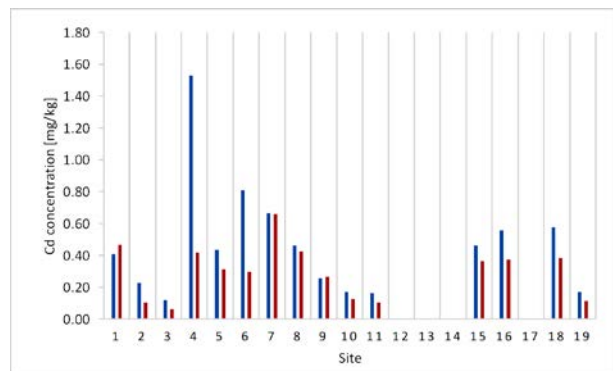
Fig. 2. Metal concentrations in sediments: (a) As; (b) Cd; (c) Cu; (d) Hg; (e) Ni; (f) Pb;(g) V; (h) Zn. (Blue bars are used for samples taken at 0.0-1.5 m below seafloor and red bars for samples taken at 1.5 - 3.0 m below seafloor. The dashed lines indicate the site-specific law (ICRAM, 2004))
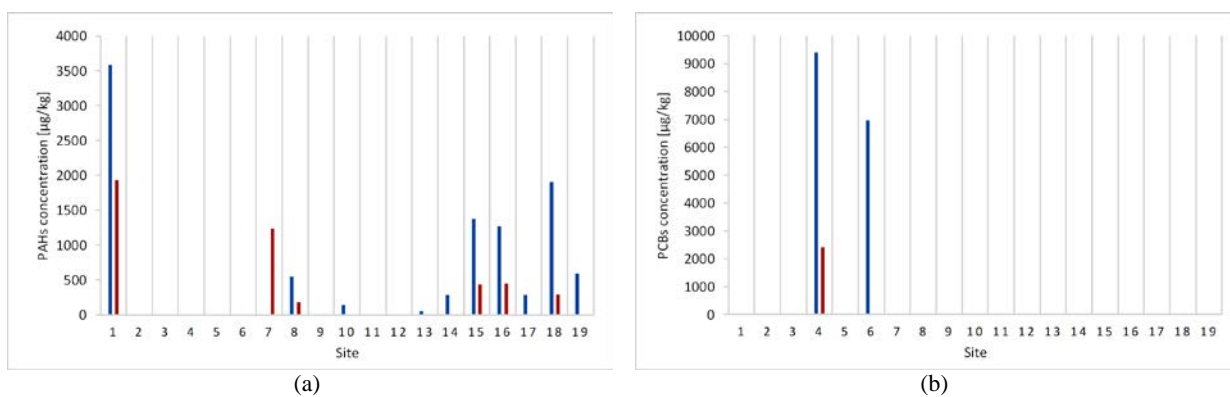

(a)

(b)

Fig. 3. Organic contaminants concentrations in sediments: (a) PAHs; (b) PCBs. (Blue bars are used for samples taken at 0.0-1.5 m below seafloor and red bars for samples taken at 1.5 - 3.0 m below seafloor. The dashed lines indicate the site-specific law (ICRAM, 2004))

3.2. Sediment ecotoxicological characterization

Toxicity results are shown in Table 3 for both bioassays, as well as integrated toxicity judgements according to Ministerial Decree (2016). The toxicity score was based on five toxicity classes: absent, low, medium, high and very high. Only the bioassay measuring the inhibition of larval development in *M. Galloprovincialis* was markedly affected by all the sediment samples, while more heterogeneous responses were obtained for other bioassays. Several elutriates were stimulatory relative to the negative control (i.e. test that is essentially free of contaminated sediment), presumably because of the release of nutrients such as ammonia.

The possible contribution of ammonia, as confounding factor, was investigated according to Losso et al., (2007). Total ammonia values in elutriates were under the no observed effect concentration (NOEC = 5 mg/l), which is considered as the sensitivity threshold limit value of the test towards ammonia. They were therefore not considered as confounding factors.

The elaboration by Italian Law summarized the hazard indicated by the whole battery of tests as "Slight" for sediments S1 (0.0-1.5 m), S9 (0.0-1.5 m), S5 (0.0-1.5 m) and Moderate for the other samples (see Table 3). The ranking indicated that all leachate samples could be deemed as moderate toxic.

The results have put in evidence that not always the presence of contaminants in sediments is a toxicity index. In the case of S1 also having found high concentration of PAHs, they are not found to be the main responsible of the toxicity of sediments.

3. 3. WOE combined assessment

Data obtained from sediment chemistry and bioassays were finally integrated within a WOE model which elaborates specific hazard indices for each typology of data, before their differential weighting in an overall quantitative risk assessment. An example of the model output for elaboration of different LOEs is given in Fig. 4. The chemical characterization of sediments (LOE1) is typically summarized toward various SQG, providing the quantitative value of chemical hazard quotient (HQ_C), the parameter which gives the highest contribution (in %) to the HQ_C, 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_C (from Absent to Severe).

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 $HQ_{Battery}$ and class of hazard for bioassays.

The results of analysis were compared by Sediqualsoft to baseline chemical levels (LCL) from the Italian law for the management of dredged sediments, that are respectively values typical for national coastal sediments potentially causing negative effects on aquatic communities (MD, 2016). In the top layer (0.0-1.5 m), the concentration of several heavy metals (i.e. Cd, Cr, Cu, Hg, Ni, Pb and Zi) was exceeding than LCL. PCBs showed a very high concentration in the south area (with maximum concentrations of 9300 µg/kg d.w. in station S04 and 6900 µg/kg d.w. in station S06). The PHAs exceed limits in several areas (i.e. S01, S02, S04, S05, S06, S07, S015, S016 and S18), reaching maximum value of 18000 µg/kg d.w in area S06.

Sediments from the superficial layers result more contaminated than those from deeper layer. Biological responses highlight an unexpected low toxic effect for model organisms exposed; Hazard Index for biomarkers range from "absent" (north area) to "moderate" (south area). Fig. 5 show the distributions of the Integrated Hazard Index (IHI) at various depths below the seafloor (i.e. 0.0-1.5 m in the Fig. 5a and 1.5-3.0 m in the Fig. 5b). In the top layer, the integration of data identified the higher risk at areas influenced by industrial activity (i.e. sites 4, 6, 7 near naval arsenal); while only site 9 appeared as not impacted. To some extent, the polluted areas (i.e., sites 10, 12, 14 and 19) are close to the areas of mussel-culture activity and fishery areas, raising concerns with respect to environmental and health issues. In general, the class of hazard tend to decrease with depth; only in the stations S03 and S09 the index is lower in the top layer (Fig. 5).
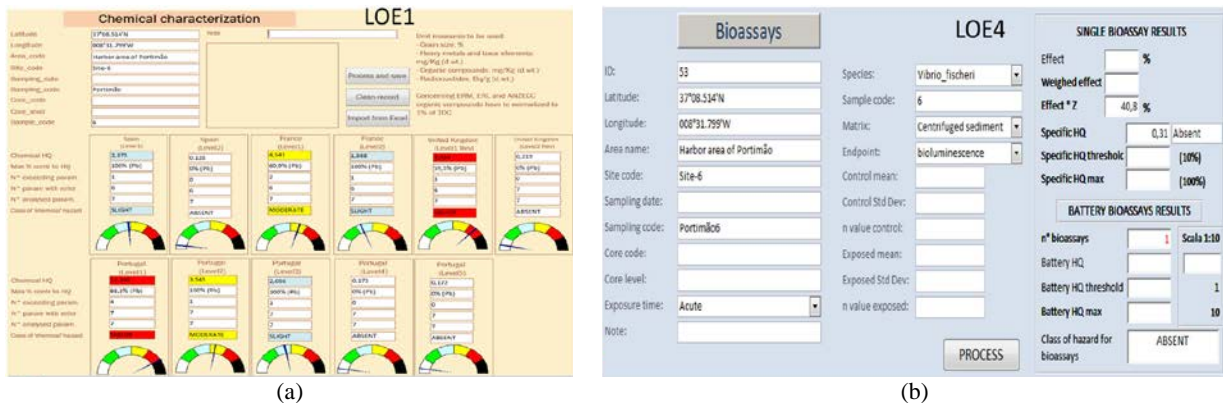

| (a) | (b) |

Fig. 4. Representative model output of hazard elaborations from various Lines of Evidence: (a): LOE1, sediment chemistry; (b): LOE4, bioassays

Table 3. Toxicity effect (in percentage, %) resulting from leachate samples prepared in seawater (positive values: toxic effects; negative values: stimulation effects)

Site	Depth from seafloor (m)	*Vibrio fischeri*	*Phaeodactylum tricornutum*	*Mitilus Galloprovincialis*	Toxicity data integration
1	0.0 – 1.5	-21.7	-27.6	2	Slight Toxicity
	1.5 – 3.0	-23.3	-31.5	60	Moderate Toxicity
2	0.0 – 1.5	14.5	18.6	100	Moderate Toxicity
	1.5 – 3.0	5.0	16.2	100	Moderate Toxicity
3	0.0 – 1.5	17.0	18.3	100	Moderate Toxicity
	1.5 – 3.0	37.5	17.3	100	Moderate Toxicity
4	0.0 – 1.5	21.7	-55.7	100	Moderate Toxicity
	1.5 – 3.0	11.3	-38.2	100	Moderate Toxicity
5	0.0 – 1.5	18.0	-32.3	100	Moderate Toxicity
	1.5 – 3.0	26.0	-39.8	100	Moderate Toxicity
6	0.0 – 1.5	7.0	-42.4	100	Moderate Toxicity
	1.5 – 3.0	25.0	-32.0	100	Moderate Toxicity
7	0.0 – 1.5	-12.7	-45.0	100	Moderate Toxicity
	1.5 – 3.0	-22.0	-38.5	100	Moderate Toxicity
8	0.0 – 1.5	-38.7	-46.7	100	Moderate Toxicity
	1.5 – 3.0	-15.0	-44.4	100	Moderate Toxicity
9	0.0 – 1.5	4.5	6.0	28	Sligh Toxicity
	1.5 – 3.0	-4.0	17.2	73	Moderate Toxicity
10	0.0 – 1.5	11.0	16.7	87	Moderate Toxicity
	1.5 – 3.0	-7.0	16.3	100	Moderate Toxicity
11	0.0 – 1.5	46.0	16.8	100	Moderate Toxicity
	1.5 – 3.0	21.5	15.8	100	Moderate Toxicity
12	0.0 – 1.5	1.5	16.4	100	Moderate Toxicity
	1.5 – 3.0	9.5	15.6	100	Moderate Toxicity
13	0.0 – 1.5	7.0	17.1	100	Moderate Toxicity
	1.5 – 3.0	5.0	17.6	63	Moderate Toxicity
14	0.0 – 1.5	27.0	18.5	100	Moderate Toxicity
	1.5 – 3.0	40.5	15.9	100	Moderate Toxicity
15	0.0 – 1.5	8.0	17.2	33	Slight Toxicity
	1.5 – 3.0	-15.5	12.3	59	Moderate Toxicity
16	0.0 – 1.5	21.3	-27.9	87	Moderate Toxicity
	1.5 – 3.0	19.0	-20.7	84	Moderate Toxicity
17	0.0 – 1.5	3.50	16.4	100	Moderate Toxicity
	1.5 – 3.0	17.0	14.8	100	Moderate Toxicity
18	0.0 – 1.5	18.0	-44.5	100	Moderate Toxicity
	1.5 – 3.0	23.7	-41.9	100	Moderate Toxicity
19	0.0 – 1.5	37.5	18.9	100	Moderate Toxicity
	1.5 – 3.0	38.0	15.8	100	Moderate Toxicity

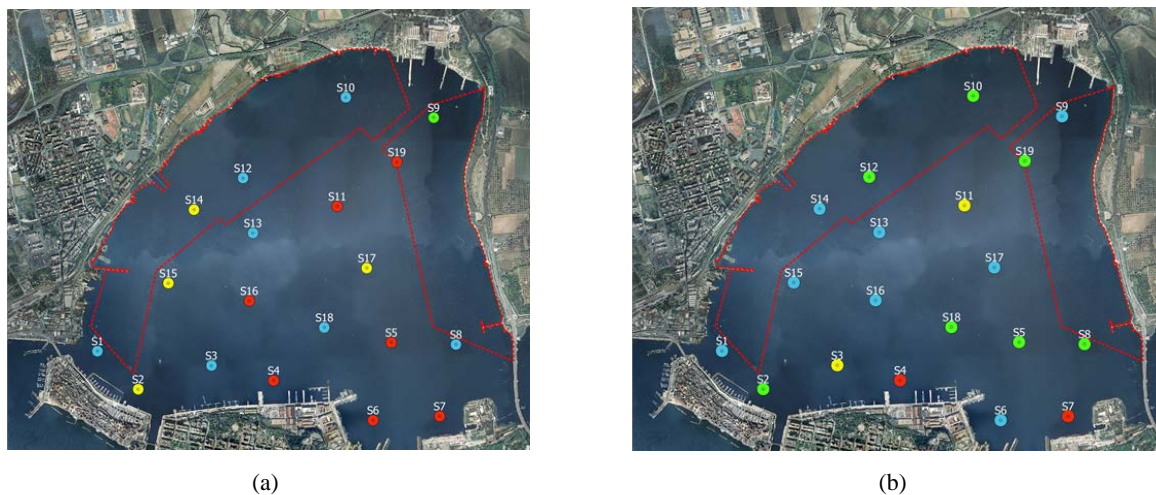(a)   (b)

Fig. 5. Spatial distributions of the Integrated Hazard Index: (a) 0.0-1.5 m below seafloor; (b) 1.5-3.0 m below seafloor. (Class of hazard: ● Absent; ● Slight; ● Moderate; ● Major and ● Severe. In red the mussel-culture areas)

One reason can be the dynamic conditions (site 3 is in the direction of Navigable Channel and site 9 is near submarine freshwater springs, called "Citro Citrello") that might promote a mixing of sediments. The IHI confirmed the critical situations in the Mar Piccolo; the level typically ranged from Slight to Major.

4. Conclusions

The assessment of the health status of sediment of Mar Piccolo highlighted the importance of combining sediment chemistry and ecotoxicological assays and revealed the existence in the marine basin of areas strongly compromised (sites at higher risk) due to industrial and harbor activities.

Results confirmed that sediments are strongly contaminated; nevertheless, the obtained biological responses highlighted an unexpected toxic effect: level of contamination seems to not affect in a proportional manner the biological compartment. All these observations raise numerous questions about the potential mobility of pollutants and further risks for the surrounding environment, making this site a model environment to study.

Sediqualsoft model was confirmed a useful tool to elaborate complex scientific data in integrative indices for stakeholders and decision makers, supporting a more comprehensive process of site-oriented management decisions.

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