Politecnico di Bari

Repository Istituzionale dei Prodotti della Ricerca del Politecnico di Bari

Identification of hot spots within harbour sediments through a new cumulative hazard index. Case study: port of Bari - Italy

This is a post print of the following article

Original Citation:
Identification of hot spots within harbour sediments through a new cumulative hazard index. Case study: port of Bari - Italy / Mali, M., Dell'Anna, M.M., Mastrorilli, P., Damiani, L., Ungaro, N., Gredilla, A., Ortiz de Vallejuelo, S.F.. - In: ECOLOGICAL INDICATORS. - ISSN 1470-160X. - 60:(2016), pp. ECOLIND-4982R2.548-ECOLIND-4982R2.556. [10.1016/j.ecolind.2015.07.024]

Availability:
This version is available at http://hdl.handle.net/11589/1278 since: 2022-06-27

Published version
DOI:10.1016/j.ecolind.2015.07.024

Publisher:

Terms of use:

(Article begins on next page)

17 November 2026

Marked up revised manuscript
Click here to view linked References

Identification of hot spots within harbour sediments through a new cumulative hazard index. Case study: port of Bari, Italy

Matilda Mali[a,], Maria Michela Dell'Anna[a,], Piero Mastrorilli[a], Leonardo Damiani[a], Nicola Ungaro[b], Ainara Gredilla[c], Silvia Fdez-Ortiz de Vallejuelo[c]*

[a]DICATECh, Politecnico di Bari, via Orabona, 4 I-70125 Bari, Italy.

[b]ARPA PUGLIA, Corso Trieste, 27 I-70126 Bari, Italy.

[c]Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country UPV/EHU, P.O. Box 644; 48080 Bilbao, Basque Country.

[*]Corresponding author e-mail address: matilda.mali@poliba.it; Tel:+39 080 5963666; Fax: +39 080 5963414

Abstract

While numerous quality guidelines unquestionably drive the management of harbour sediments, port managers increasingly require easy-to-use tools supporting them in assessing the sediment quality. In this work, a new hazard index, named cumulative Normalized and Weighted Average Concentration (c_NWAC), is proposed, considering the concentrations of main hazardous, toxic and bio-accumulative sediment contaminants. This index is an upgraded version of the previously introduced NWAC index, which considered only the metal concentrations. The c_NWAC values range from 0 to 10 scores, and their visualization on a colour base code map leads to an easy identification of hotspots. The applicability of the new index was verified using a dataset derived from the analyses of 42 samples collected at different depths of the seabed of the Port of Bari (Italy). The concentrations of 58 parameters were considered, namely 11 metal and metalloids, 17 congeners of polychlorinated biphenyls (PCBs), 16 congeners of polycyclic aromatic hydrocarbons (PAHs), further 11 organic micropollutants, total organic carbon (TOC) and nutrients (N_{tot}, P_{tot}). For the study area, the obtained c_NWAC values resulted well correlated to the corresponding values of the commonly used mean Effects Range Median quotient (mERMq), and similar hazard categories were developed for the new index, even if the latter do not necessarily explain the real sediment toxicity, because they were derived from a different sediment dataset and different test species.

Moreover, the hazard predictive ability of the new index was verified (with satisfactory results) testing the considered samples by eco-toxicological assays, using three different biological species, and comparing the obtained results to the corresponding hazard categories developed for c_NWAC.

Keywords: Pollution hazard, Harbour, Sediment quality, Metals, Polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Ecotoxicological tests.

1. Introduction

Sediments are an essential and dynamic part of the port; their quality and quantity are integral to ecosystem health and a fundamental component of the regional economy. Many hazardous, toxic and bioaccumulative chemicals, such as metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorophenols, organotin compounds, organochlorine pesticides and aliphatic petroleum hydrocarbons are continuously leaked into the port due to shipping traffic, loading, repairs, dredging and other activities, as well as rainwater runoff, effluent discharges, dust etc. (Guerra-Garcia and Gomez, 2005; Casado-Martínez et al., 2009; Szlinder-Richert et al., 2012).

The contaminants, which are found only in trace amounts in water, tend to be adsorbed into both inorganic and organic material eventually settled in sediments. If the concentration of these contaminants in the water column is high enough, sediments may accumulate excessive quantities of contaminants that directly and indirectly disrupt the ecosystem. Indeed, many studies have documented the importance of sediment contamination for ecosystem quality (Burton, 2002, 2013; Chapman et al. 2002, 2013; Chapman 2007; Schintu et al 2015). Moreover, numerous efforts, resources and money are continuously spent in many countries in an attempt to clean up or remove contaminated sediments, in order to restore ecosystem quality and develop beneficial uses for dredged materials (SedNet, 2007).

National and international guidelines have unquestionably supported the evaluation and management of sediments (US/EPA Environmental Protection Agency 1998; EPA-905-B02-001-A, 2002), but researchers in this field are still attempting to identify easy-to-use tools that support and facilitate sediment quality assessment. The methodologies proposed in the literature to estimate the quality of sediments can be classified into two categories. The simplest one uses the ratio between the measured concentration of a given contaminant in sediment samples and the

background value estimated for the area (Müller, 1969; Salomons and Forstner, 1984; Hakanson, 1990; Wolska et al, 2007). The other category of methodologies combines chemical parameters with related biological effects, valuating the toxicity degree of sediments (Long et al, 1995; Macdonald et al, 1996; Macdonald et al. 2000). Nevertheless, all such methodologies are contaminant-specific and depend upon the legislation currently in force in each specific area that usually applies specific numerical criteria (threshold levels for each contaminant) when assessing the quality of sediments. Registering an excess of concentration of a given contaminant with respect to the numerical threshold constitutes the necessary condition for assessing the pollution status of the sediment and, consequently, the development of regulatory and remedial actions.

Since none of the existing methodologies take into account the possible synergic or antagonistic effects that the simultaneous occurrence of different hazardous contaminants within sediments may present (Eggen et al., 2004), cumulative indexes that consider the concomitance of different stressors are highly recommended. However, the multidisciplinary approaches that combine multiple typology of investigation (or lines of evidence, LOEs) (Chapman, 1996; Chapman et al., 2002) are often hampered by the lack of standardized procedures for the interpretation and the integration of complicated datasets of heterogeneous results, which typically require various expert judgements (Piva et al., 2011; Benedetti et al., 2012; Regoli et al., 2013). Aiming at overcoming this inconvenience, recently, a software-assisted model has been developed to integrate and differently weight data from various lines of evidence (Piva et al., 2011).

One of the most employed indexes is the mean Effects Range Median quotient (mERMq) (Long et al. 2006), which estimates the toxic effect of selected pollutants on a wide variety of benthic organisms. Furthermore, Gredilla et al. (2014) recently calculated the Normalized and Weighted Average Concentration (NWAC) that, however, takes into account only metal content (14 metals and metalloids) in estuarine systems.

In this paper, an upgraded version of NWAC is proposed, named cumulative NWAC (c_NWAC), able to examine the multiple effects of three categories of contamination indicators: metals and metalloids, persistent organic pollutants and TOC, and nutrients, such as N_{tot} and P_{tot}. We applied the upgraded index to the Port of Bari (southeast Italy) using sediment cores collected at three depths (50 cm, 100 cm and 150 cm) from 23 sampling stations. The predictive ability of the new proposed index was ascertained by comparing the c_NWAC ranges of hazard with the results obtained from three amphipod survival tests.

2. **Materials and methods**

2.1 Features of the study area

The Port of Bari is located in the southeast of the Italian Adriatic coast, at the centre of the Apulia region (Figure 1). The coastal area under study forms part of the westward extension of the carbonatic plateau of the Murge, the lithological platform of southern Italy. Its low-profile morphological features are characterized mainly by sandy sediments with beaches only partially preserved from human intervention. The coastal tract of the metropolitan city of Bari is currently heavily modified by large-scale artificial burying. Numerous ephemeral watercourses, parallel to each other and perpendicular to the coast, form the characteristic hydrographic network that nourishes the coast with terrigenous sediments.

Figure 1 near here

The Port of Bari is one of the most important ports settled along the Adriatic coast and is located within the urban area of the metropolitan city of Bari. It is a multipurpose stopover equipped with docks for handling a range of goods and freight. Water depth in the port varies from 6.9 m to 14.9 m, allowing the movement of large ships and vessels. Several quays for various types of commercial traffic (solid and liquid bulk, containers, packaged goods, steel products, forestry products etc.), as well as Ro-Ro docks for ferries and cruise ships platforms are used daily. High-level port activity seriously impacts the quality of the port and, therefore, the quality of harbour sediments, that act as a final sink for all contaminants entering the port within the water column. Regarding the contamination history of the study area, two important events should be mentioned. The first one is the activity of the National Hydrogenation Fuels Company at the north of Bari which had caused significant impact to the local area, until it ceased activity in 1974, and consequently affected the quality of the port area nearby, in which the waste water was dumped. The concerned area has only recently been subjected to reclamation work in order to eliminate residual hydrocarbons. The second event was the bombing campaign during the Second World War that involved directly the port basin.

2.2 Sampling area and sampling design

Sediment samples were collected during a sampling campaign between 2010-2011 from the most significant basins in the port area (see Figure 1): at the Ro-Ro terminal (sites 42-51), the Container Terminal (sites 53-61), the Passenger Traffic zone (cruise and scheduled shipping- (sites 68-90)

and at the access channel of the port (sites 124-130). A total of 23 core samples were taken from different sampling sites, using a PF2 vibrocorer, equipped with a liner. The vibrocorer operated with the support of a vessel endowed with a differential GPS system for the positioning of core samples. Each core was sectioned into 50 cm length sub-cores and samples were collected at different depth levels up to 150 cm. When the final sub-core was less than 100 cm long, due to the presence of rock on the seabed, only superficial sediment was considered. The letters A, B and C were assigned to each core sample indicating the core depth at 50 cm, 100 cm and 150 cm respectively (Table S1). Aliquots of 250 g of wet sediments were taken from each sub-core and transported in cleaned plastic containers, available for the analyses. Part of the analyses were performed on wet-sediments (microbiological analyses, bioassay tests), while aliquots useful for the other analytical analyses were stored at –20 °C.

2.3 Analytical methods

Before analysis sediments that were stored at –20 ° C after sampling, were dried at 40 °C for 48 h. Each sediment was classified according to Shepard (1954) into four classes: gravel: > 2 mm; sand: 2-0.063 mm; silt: 0.063-0.002 mm; clay < 0.002 mm. A set of ASTM sieves and a granulometer laser were used for the granulometrical separation (Romano et al., 2001).

The trace metal concentrations were measured by inductively coupled plasma mass spectrometry (ICP/MS X Series Thermo Fisher Scientific) after sample mineralization by total acid digestion (HCl, HNO_3 and HF) (Pellegrini et al., 2001). The fraction < 63 μm dried at 105 °C was used for the metal and metalloids determination. The results are expressed on the dry weight basis. The detection limits (LODs) were calculated from 3 replicates of procedural blanks. The LODs estimated were in mg/kg units and were equal to 1 ppb for all metals and metalloids. Marine Sediment References Material NIST 2702 (Inorganics in Marine Sediment) was used to control the analysis quality: the agreement between the analytical results for certified and measured values was satisfactory with recoveries between 80 and 100% for all metals and metalloids.

Total petroleum hydrocarbons amounts were determined by IR spectroscopy analysis after extraction according to US/EPA, Method 418.1 Issued 1978 (US/EPA, 1978).

Polychlorinated biphenyls (PCBs) concentrations were determined after acetone/petroleum ether extraction and analysed by Gas-Chromatograph equipped with an Electron Capture Detector, following the procedure proposed by Cicero et al. (2000). The congeners considered are 17:

PCB28, PCB52, PCB77, PCB101, PCB105, PCB114, PCB118, PCB123, PCBB126, PCB128, PCB138, PCB153, PCB156, PCB157, PCB167, PCB180 and PCB189. The same methodology was used for the determination of chlorinated organic compounds.

The polycyclic aromatic hydrocarbons (PAHs) considered for this study were: naphthalene, acenaphthene, acenaphthylene, phenanthrene, anthracene, fluoranthene, benz[a] anthracene, chrysene, benz[b]fluoranthene, benz[k]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, dibenz[a,h]anthracene, fluorine, indeno[1,2,3-cd] and pyrene. They were determined after extraction with cyclohexane/methanol mixture and analyzed by High Pressure Liquid Chromatograph, according to Ausili (2001) method.

N_{tot} was determined by elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer and the total phosphorus concentration (P_{tot}) was determined by colorimetric titration using the molybdenum-blue method (Aspila et al., 1976).

Total organic carbon (TOC) was determined by elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer, after removing of carbonates by reaction with hydrochloric acid (Nieuwenhuize et al., 1994). The amount of organotin compounds was determined by the procedure proposed by Morabito (2001).

The ecotoxicological tests with bioluminescent bacteria *Vibrio Fisheri* were carried out using Onorati et al. (1999b) and Azur Environmental (1994) protocols, while fertilization success bioassay test with sea urchin *Paracentrotus lividus* and the marine algal growth inhibition test with *Phaeodactylum tricornutum* were performed according to standardized procedure applied by Onorati and Mecozzi (2004), Lera et al. (2006) and ISO 1253 (2006).

2.4 Statistical elaborations

The correlation matrixes and Pearson coefficients were calculated using Statistica Software v. 10. The spatial modelling of the elements considered was done by means of kriging method (3D-Field Program, v. 3.8.8.0.) as predictor tool. The kriging method is a spatial modelling that extrapolates information from discrete stations to cover the whole area of study. Likewise some other non-interpolators, kriging generally produces a smoother surface over the area of interest and it assumes stationary and isotropy within the sampling sites, which implies that spatial correlation within the sampling points is independent from direction (Leecaster, 2003; Liu et al., 2012; Fdez-Ortiz de Vallejuelo et al., 2014).

3. Results and discussion

3.1. Preliminary study of the area

3.1.1 Horizontal distribution of contaminants

According to the analysis of contaminant distribution at different sites of the port area, the Ro-Ro, Container and Passenger Terminal sites were revealed as those of most concern.

The highest concentrations of As were identified at sites 61_B (with a concentration value of 34.64 mg/kg) and 88_A (30.10 mg/kg), followed by 44_A (29.56 mg/kg), 51_C (28.29 mg/kg) and 72_A (26.59 mg/kg). The lowest As concentrations were registered at the access channel of the port, due to the renewal of water flow. The most polluted sites in the case of Cd were 61_C (3.32 mg/kg), 88_A (2.09 mg/kg) and 79_A (2.05 mg/kg). The sites of most concern for Hg concentrations were 76_B (5.99 mg/kg) and 88_A (5.09 mg/kg). Other high concentrations of Hg were also registered at sites 82_B (4.69 mg/kg) and 82_C (3.50 mg/kg). Two sites registered elevated concentrations of Pb: 79_A (454.03 mg/kg) and 51_B (386.94 mg/kg), while in the case of Cu, sites 44_A (227.52 mg/kg) and 51_B (169.80 mg/kg) resulted as the most polluted. However, high concentrations of Cu were also found at 82_B/C, 88_A (in the range of 330-332 mg/kg). In terms of Zn distribution, the highest concentration was detected at 61_B (2390.37 mg/kg). High concentrations were again registered at 88_A (561 mg/kg) and 42_A (464 mg/kg). Generally, the lowest Zn concentrations were revealed at the access channel of the port (sites 128, 130).

The distribution of Ni, V and Cr was relatively homogeneous within the area of study with average concentration values comparable to the concentration of crustal shale as determined by Wedepohl and Turekian (1961).

With respect to organic compounds, the highest concentrations of PCBs were registered at 88_B (1.99 μg/g) and 79_A (1.59 μg/g). High concentrations were also registered at 44_B (1.18 μg/g). The highest PAH concentrations were detected at 61_B (61.18 μg/g), 79_A (18.04 μg/g) and 88_A (14.53 μg/g).

The sites with the highest presence of high molecular weight hydrocarbons (HW THC) were generally the same in which the highest presence of PCB and PAH was registered, showing probably the same source of pollution such as fuel losses: 79_A (9.001 mg/g), 51_B (6.172 mg/g), 61_B 5.235 mg/g) and 88_A (4.188 mg/g).

This tendency was also observed for organotin compounds (expressed as sum of mono-, di-, and tributyltin), yet in this case high concentrations were also found at 53_A (0.884 μg/g). The highest concentration of organochlorine compounds was registered at 79_A (1.18 μg/g).

As with metals, the sites with the lowest concentrations of organic pollutants were those located at the access channel of the port (sites 124-130) and those located at the entrance of the Ro-Ro terminal (42), with the exception of ∑PAH and ∑ HW THC, for which elevated concentrations were identified also at the access channel to the port, probably due to fuel losses during the entrance/manoeuvring of vessels.

3.1.2 Vertical distribution of contaminants

A heterogeneous distribution of contaminants was also revealed within the vertical profiles of core samples. At sites 44, 47, 108, 128 and 130 higher concentrations of metals, metalloids, ∑PCBs and ∑Organochlorine compounds were detected at the surface levels of the cores with respect to the deeper levels (with the exception of Pb at site 108 and Cu at site 130).

This trend was not confirmed in the case of ∑PAHs and ∑Organotin compounds for which higher concentrations were revealed at deeper levels in sites located at the entrance to the port (at the sites 126 and 130). Unusual degrees of pollution were registered at site 42 (entrance to the Ro-Ro terminal): higher concentrations for all considered metals and metalloids were found at the depth of 100 cm compared to the superficial layer (50 cm) and to the deeper one (150 cm). This latter registered, in any case, slightly higher amounts of metals with respect to those revealed at superficial depth (50 cm). This anomalous contamination trend could be explained by previous accidental spill of contaminants at this site (contamination caused by vessels battery, discharge of wastewater, runoff waters etc.).

The internal sites of the port area, 44, 51, 61, 76, 82 and 85, presented increasing metal concentrations the deeper the layer from the seabed, with the exception of Ni, Cr and V, for which the distribution of concentrations in vertical profiles follows the Al distribution trend (revealed by the strong correlation between Al and Cr concentrations). It may therefore be assumed that concentrations of Ni, Cr and V are naturally present in the area (Loring and Rantala. 1997).

In terms of organic pollutants, ∑PCBs and ∑Organochlorine compounds generally resulted higher in the superficial layer (with the exception of sites 51, 61 and 82). At nearly all sites the concentration of ∑PAHs increased in correspondence with the distance from the surface. In the

case of ∑Organotin compounds, distribution within the vertical profile is highly heterogeneous and it is therefore difficult to determine a specific trend.

3.2 Calculation of the c_NWAC. Case study: Port of Bari

The cumulative index proposed in the present work is an upgraded version of NWAC (Gredilla et al., 2014). In fact, while the original NWAC is based on the concentration of only 14 metals and metalloids, our cumulative NWAC (c_NWAC) index is based on the multiple effects of three categories of contamination indicators, present in the US/EPA list of toxic and priority pollutants in sediment (US/EPA, 2011): *i*) 11 metals and metalloids; *ii*) persistent organic pollutants, such as 19 congeners of PCBs, 16 congeners of PAHs, 11 chlorinated organic compounds, organostannanes, petroleum hydrocarbons, other organic micropollutants and *iii*) N_{tot}, total organic carbon (TOC) and P_{tot}, that clearly affect the toxicity of sediments (Coulon et al., 2005). Furthermore, the visualization of the cumulative Normalized Average Weighted Concentrations (c_NWAC) in a colour-based map (Kriging methodology) is proposed, which may facilitate the identification of hotspots with the highest levels of contamination present within the harbour area.

The entire dataset was considered in calculating the cumulative NWAC index, compiling the results of the analysis carried out on the 42 sediment samples collected from the Port of Bari. The overall database was therefore composed of 42 samples (23 superficial, 14 collected at 100 cm depth and 5 collected at 150 cm depth) and 58 variables (Table S2). In the calculation of the c_NWAC, data were initially divided into two sub-datasets according to the variables considered. A first sub-set was defined including the concentrations of 11 metals and metalloids (As, Al, Fe, Cd, Cr, Cu, Hg, Ni, Pb, V and Zn). The second one comprised the quantities of organic pollutants (17 congeners of PCBs, 16 congeners of PAHs, 11 organic micropollutants), total organic carbon and sediment nutrients (N_{tot}, P_{tot}). The nutrients and TOC were included within the second sub-set, due to their well-known effects in the microbiological activity that affect the organic pollutants degradation (Coulon et al., 2005).

The procedure proposed by Gredilla et al. (2014) was followed for each sub-dataset and three normalization steps were carried out in order to obtain equal statistical weights for the considered variables. First, for each contaminant, the concentrations (C) found within sampling sites were normalised (C_{norm}) by linear scaling transformation ($C_{norm}= [(C - C_{min})/(C_{max} - C_{min})]$. Then, the normalized concentration C_{norm} were weighted by calculating for each contaminant the dispersion

of the concentrations of that contaminant within the sampling sites. In fact, the relative standard deviations (RSD) were calculated for each contaminant within sampling sites and normalised (RSD_{norm}) by linear scaling transformation according to: [RSD_{norm}= (RSD − RSD_{min})/(RSD_{max}− RSD_{min})]. The resulting RSD_{norm} varied from 0 to 1 and were used to weight the normalised concentrations (C^{w}_{norm}) according to: (C^{w}_{norm}=C_{norm}·RSD_{norm}) of the contaminants. The average of the obtained values ($\overline{C^{w}}_{norm}$) leads to the Normalised-and-Weighted Average Concentration (partial index) for each of the two groups of variables (metals and metalloids on one hand and organic pollutants+total organic carbon+nutrients on the other). The average value of the partial indexes multiplied times ten provided the c_NWAC value for each sample (on a scale of 0 to 10). The results are shown in Table 1.

Table 1 near here

Since sediment sampling was performed at different depths, the c_NWAC value obtained for each depth was also calculated (Table 1, column A, B and C, on the left).

Analyzing the surficial layer c_NWAC values (Table 1, column A on the left), the highest hazard degrees were obtained at the Passenger Terminal basin for sediments collected from sampling points 79 and 88, showing index values of 8.52 and 6.78, respectively. High values were also obtained for other sampling sites: 85 (Passenger Terminal basin), 42, 44 (the two latters at the entrance of Ro-Ro terminal) and 69 (Cruise Terminal area), having c_NWAC values ranging from 3.03 to 4.68, showing a high pollution risk concentrated in these basins. The lowest index values were registered in sediments collected from the port access channel (sampling points 124, 126, 128 and 130).

Moving from surficial to deeper layer cores (Table 1, column B and C, on the left), several differences in the distribution of the c_NWAC values were observed. For example, at sampling site 51 (Ro-Ro terminal area) the c_NWAC value increased from 0.64 for the surface sediment to 5.23 for the 100 cm depth sample core, and subsequently, it decreased down to 1.17 for the 150 cm depth core. Also for site 61 (Container Terminal area), the c_NWAC value registered at 100 cm depth (8.88) was higher than the corresponding value obtained at surficial layer (1.26). Site 42 (at the entrance of Ro-Ro Terminal basin) c_NWAC vertical distibution values did not follow the above mentioned trend, decreasing on passing from surficial (4.68) to the 100 cm depth layer (0.68), and increasing again up to 1.96 at 150 cm depth level. In sites belonging to the Cruise

Terminal area (sampling points 68 and 69), to the entrance of the old dock/private port (site 108) and to the port access channel (sites 128 and 130), the relevant c_NWAC values were roughly the same at all depth levels.

For easy identification of hotspots within harbour area, all the obtained results were projected as three layer maps (for the depths of 50 cm, 100 cm and 150 cm) presented in Figure 2, a), b) and c), respectively.

Figure 2 near here

3.3 Correlation between the c_NWAC values and mERMq values

In order to validate the obtained results, the mean ERM quotients were calculated based on the same dataset, but considering only those contaminants for which the relevant ERM values were assessed by Long et al, 1995. The mERMq values for each depth are also reported in Table 1, columns A, B and C, on the right.

The toxic categories of mERMq according to the ranges proposed by Long et al. (2006), namely, mERMq<0.1 (non-toxic, nt), 0.1<mERMq<0.5 (slightly toxic, st), 0.5<mERMq<1.5 (moderately toxic, mt) and mERMq>1.5 (highly toxic, ht) are reported in Table 2, on the right.

The c_NWAC values and the corresponding mERMq values were plotted and the square correlation coefficient between c_NWAC and the global mERMq values resulted highly significant, with $R^2 = 0.953$, $p < 0.0005$ (Figure 3). This reveals a similar diagnosis of the study area by both indexes. The first index identifies the zones of major concern due to the simultaneous presence of metals and metalloids, organic compounds and other parameters, while the second one reveals sediment sites with the greatest probability of toxicity.

Figure 3 near here

By analyzing all obtained results, a number of considerations can be made. It is apparent from the visualization of the 2D maps of the c_NWAC values for the different considered depth levels (Figure 2) that hot spots can be easily identified in maps even by non-specialists, highlighting that the basin of the Container (sampling sites 44, 51, 61) and Passenger Terminal (sampling points 76, 79, 82, 88) resulted as those of most concern. In addition, for some hotspots, deep sediments appear as more contaminated than superficial ones such as 51, 61 and 82. These different degrees

of concern regarding the synergic presence of the considered contaminants could possibly be due to adsorption phenomena that allow contaminants of the surface sediments to form chemical equilibria with organic and inorganic species dissolved or suspended in the column water, thus decreasing their toxicity level enhanced by the leaching of contaminants in the water column. Furthermore, surficial layers are probably disturbed by mechanical agitations and turbidity currents caused by the movement of great size ships (Cruise and Container Ships) and other hydrodynamic conditions that may promote contaminant leaching processes. These phenomena do not affect the deeper sediments.

However, a high concentration of a certain contaminant does not always correspond to high c_NWAC value at the corresponding site. For example, considering the high As concentration at the sampling sites 72_B and 51_A (26.56 mg/kg and 27.48 mg/kg, respectively), the corresponding c_NWAC values were not of high concern, being 0.33 and 0.68, respectively. Moreover, the Pb and Hg concentrations registered at site 51_B (386.56 mg/kg for Pb and 2.34 mg/kg for Hg) are higher with respect of those registered at site 61_B (95 mg/kg for Pb and 2.15 mg/kg for Hg), yet the c_NWAC value registered at 51_B (5.23) is lower with respect to the value calculated for 61_B (8.88).

In addition, despite the similarity between the average concentrations of ∑PAHs registered at sampling sites 126_A (2.05-5.62 mg/kg) and 88_A (3-6.7 mg/kg), the relevant c_NWAC values established for the two sites are very different (1.59 for site 126_A and 6.78 for site 88_A). This would indicate the ability of the cumulative index to take into account a combination of different factors (contaminants and other chemical parameters considered in its calculation).

3.4. *Establishing hazard categories of c_NWACs*

The values obtained by the c_NWAC index were compared with the relevant mERMq values in order to assess a magnitude degree of the issue terms of toxicity also for the new proposed index. Thus, based on the equation of the linear correlation between c_NWAC and mERMq (and the classes of toxicity suggested by Long et al., 2006 for mERMq values), different hazard categories were defined also for the c_NWAC index.

Indeed, by using the equation $y = 5.192\ x + 0.2231$ (Figure 3) established for the whole dataset, and defining x as the threshold value of hazard categories established for mERMq (x=0.1; x=0.5; x=1.5), the obtained c_NWAC hazard categories were as follows: “No hazard” is considered for

samples with a c_NWAC range of 0< c_NWAC <0.74; "Low hazard" for 0.74<c_NWAC<2.8; "Moderate hazard" for 2.8<c_NWAC<8.01; "High/severe hazard" for c_NWAC>8.2.

3.5. *Real predictive ability of c_NWACs*

The predictability of the new index was tested using a battery of bio-assays which includes three tests: microtox Solid Phase Test (SPT) with *Vibrio fisheri*, fertilization success bioassay with *Paracentrotus lividus*; algal growth inhibition test with diatom *Phaeodactylum tricornutum*. The microtox SPT is an acute toxicity test for solid matrices based on inhibition of natural bioluminescence of the marine bacterium *Vibrio fischeri* in the presence of pollutants. The test was applied to two phases of sediment: solid and liquid (elutriate). The former gives information about those contaminants that are bound to the sediment particles, for their chemical nature, hydrophobia, adsorption and complexing degree with organic substance (Onorati and Volpi Ghirardini, 2001), while elutriate gives information about the hydro-soluble contaminants that may recirculate by mechanical agitation. The latter component is most representative of the toxic potentialities of marine sediments when they are to be dredged (US/EPA, 1991). The results of test on solid phase were expressed as S.T.I. (Sediment Toxicity Index) that represents real acute toxicity of analyzed sample with respect to the natural toxicity of a sample with the same granulometric characteristics (Onorati et al., 1999a, b).

The test using the sea urchin *Paracentrotus lividus* was conducted on the extracted liquid phase (elutriate) of the sediments and the same method was performed for algal growth inhibition test (using *P. Tricormutum*). The results of the two latter tests are expressed in EC_{50}/EC_{20}. The EC_{50} is the median effective concentration (EC_{50}), a statistically derived concentration of a substance in an environmental medium (in this case the elutriate of sediments) expected to produce a certain effect (algal growth inhibition or fertilization success) in 50% of test organisms in a given population under a defined set of conditions. The EC_{20}, is referred to the concentration for which 20% of the population is affected. They are determined by establishing a dose-response curve which plots the concentration of a given compound in water (x axis) against the effects of this compound on a given species population (y axis). The analytical methods and toxicity classification criteria according Italian guidelines are reported in Table 2, while the obtained results, conditions and methodologies applied are reported in Table S3.

Prior to assessing the agreement between the predicted toxicity established by the c_NWAC index and the real measurement, the samples were grouped in two sub-groups categories: the

"unaffected samples" including those classified as "Non-toxic" and "Low toxicity" according to both the c_NWAC and toxicity tests and the second group of the "affected" samples, including those samples with moderate to high toxicity, thus simplifying the data set in two score orders belonging to two new categories: "affected" or "non-affected". By calculating the number of samples belonging to one of the two groups according to c_NWAC index, the number of samples that agree with the prediction according real biological tests were plotted (Figure 4).

Figure 4 near here

The agreement percentage (A) was calculated by the formula:

$$A = (P_i/P_{tot})\cdot 100$$

where the P_{tot} is the number of samples classified as "affected" or "not affected" according to the c_NWAC index and P_i is the number of samples that really resulted as "affected" or "not affected", according to the biological tests.

As expected (Long et al., 2006; Ritter et al., 2008, 2012), significant variability was revealed in the agreement percentages among different species, showing differences in relative sensitivity to toxicity. Nevertheless, satisfactory percentage agreements were obtained (up to 71%). *Vibrio Fisheri* tests correspond more accurately than the other tests in terms of the foreseen predictability. Indeed, Vibrio Fischeri as luminescence bacteria is particularly appropriate for the toxicity assessment of marine sediment (elutriate and solid phase). Its sensitivity is lower than the one of P. Lividus but its reproducible capability is higher (Guzzella, et al., 1998; Van Beelen, 2003).

Nevertheless, some limitations should be highlighted in the use of cumulative indexes. Wenning et al. (2005) extensively describe that despite the scientific underpinnings of the determination of different sediment quality guidelines (SQG) approaches and evaluation indexes, none of them appear to be intrinsically flawless and able to clearly delineate between sediments that cause biological effects and those that do not. At present, algorithms are unable to predict toxic effects with absolute certainty. The indexes established by the above

approaches can define the ranges of chemical concentrations where biological effects are rare, possible or highly probable, but they are unable to attain a complete distinction.

Factors that cause an overlap between effect and non-effect data are numerous and assorted. They include the effects of other chemicals not considered, the combination of effects, unaccounted for differences in chemical availability, differences in response among organisms and errors in measurements of either chemicals or responses.

Furthermore, the new index proposed herein was based on units of dry weight sediments; therefore it does not take into account the potential effects of hydrodynamic factors acting within harbour sediments, that may influence contaminant bioavailability. The c_NWAC index is thus not intended for use in predicting effects in wildlife or humans through bioaccumulation pathways and it is not intended as a toxicological threshold test. There is no assurance that it will always correctly predict either toxicity or non-toxicity. In addition, it should be underlined that in the present study the c_NWAC is compared to non-site specific SQGs. Therefore, the corresponding hazard categories may not necessarily explain the sediment toxicity of the study area, because they were derived from a different sediment dataset and different test species. For this reason, the mERMq values were calculated only to check the applicability of the new index.

The c_NWAC index constitutes a useful tool for an easy interpretation of data, especially when decision-makers must define regulatory or remedial action in the sites with high concern. However, other techniques that take other factors into account should be developed when an assessment of toxicity levels for humans and the benthic community is required.

4. Conclusions

A new cumulative index (c_NWAC) that takes into account the synergism and antagonism of different contaminants (inorganic and organic) affecting the quality of harbour sediments was proposed. The novelty of this index consists in the possibility of establishing for each harbour site a hazard index value, based on the concentration of several contaminants, including those for which the ERM values are lacking. Noteworthy, for the study area (Port of Bari, Italy), the calculated c_NWAC values resulted well correlated to the corresponding values of the commonly used mERMq, and similar hazard categories were developed. Even though the c_NWAC categories (differently from mERMq) do not necessarily explain the real sediment toxicity, they allow us to get an idea of the hazard of sediments, for the future environmental management of the area under study.

In addition, the visualization on 2D maps of c_NWAC values at different depths facilitated the immediate identification of hotspots or sites of most concern and allowed for a more in-depth investigation of the harbour bed. Differences among depth-layers were obtained, in some cases confirming the presence of earlier contamination phenomena. In other cases, dynamic physical-chemical processes occurring in sediment-water interfaces and combinations of different stressors impacting the toxicity of the hotspot points were identified.

The reliability and the predictive ability of the proposed cumulative index were verified through a comparative analysis between the calculated index values and the results of three biological tests, based on three different bio-indicators. The obtained results were satisfactory and it can be concluded that the cumulative index is a useful tool for the easy interpretation of data, especially when decision-makers are required to define regulatory or remedial action.

Nevertheless, the proposed cumulative index must be implemented taking into account other stressors affecting the toxicity of sediments, such as hydrodynamic factors that identify stagnation points and poor circulation of pollutants, for which the same visualization procedure on 2D maps can be carried out.

Acknowledgments.

This study was supported by ARPA Puglia, the Port Authority of Bari and fund of TEN ECOPORT project within SEE programme (SEE/A/0189/2.2/X). The authors thank the staff of ARPA Puglia for the implementation of eco-toxicological analysis.

References

Aspila, K.I., Agemian, H., Chau, A.S.Y., 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst. 101, 197–197

Ausili, A., 2001. Analisi degli idrocarburi policiclici aromatici – Sedimenti. scheda 9. In: Cicero AM, Di Girolamo I (eds).Metodologie Analitiche di Riferimento del Programma di Monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001–2003). Ministero dell'Ambiente e della Tutela del Territorio. ICRAM© Roma 2001. file:///E:/dati/Downloads/10087_metodologie%20(1).pdf . Accessed 20 March 2015.

Azur Environmental. 1994 Microtox® Acute Toxicity Basic Test procedures, 63.

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

Burton, G.A. Jr., 2002. Sediment quality criteria in use around the world. Limnology Rev. 3, 65-75

Burton, G.A. Jr., 2013. Assessing sediment toxicity: past, present, and future. Environ. Tox. Chem., 32 (7) 1438–1440

Casado-Martìnez, M.C., Forja, J.M., DelValls, T.A. 2009. A multivariate assessment of sediment contamination in dredged materials from Spanish ports. J. Hazard. Mater. 163, 1353–1359

Chapman, P M. 1996. Presentation and interpretation of sediment quality triad data. Ecotoxicology, 5 (5), 327-339.

Chapman, P.M., McDonald, B.G., Lawrence, G.S.. 2002. Weight-of-evidence issues and frameworks for sediment quality (and other) assessments Hum. Ecol. Risk Assess., 8, 1489–1515

Chapman, P.M., 2007. Determining when contamination is pollution e weight of evidence determinations for sediments and effluents. Environ. Int. 33, 492-501.

Chapman, P.M., Wang, F., Caeiro, S.S., 2013. Assessing and managing sediment contamination in transitional waters. Environ. Int. 55, 71-91

Cicero, A.M., Pietrantonio, E., Romanelli, G., Di Muccio, A., 2000. Comparison of soxhlet, shaking, and microwave assisted extraction techniques for determination of PCB congeners in a marine sediment. Bull Environ. Contam. Toxicol. 65, 07–313

Coulon, F., Pelletier, E., Gourhant, L., Delille, D., 2005. Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. Chemosphere, 58, 1439–1448

Eggen, R.J.J., Behra, R., Burkhardt-Holm, P., Escher, B. I., Schweigert, N., 2004. Challenges in ecotoxicology. Env. Science. & Tecnology. 38, 58A-64A.

Fdez-Ortiz de Vallejuelo, S., Gredilla, A., de Diego, A., Arana, G., Madariaga, J.M., 2014. Methodology to assess the mobility of trace elements between water and contaminated estuarine sediments as a function of the site physico-chemical characteristics. Sci. Total Environ. 473–474, 359–371.

Gredilla, A., Fdez-Ortiz de Vallejuelo, S., De Diego, A., Arana, G., Madariaga, J.M. 2014. A new index to sort estuarine sediments according to their contaminant content; Ecol. Indic. 45, 364–370

Guerra-Garcia, J.M., Garcia Gomez, J.C., 2005. Assessing pollution levels in sediments of harbours with two opposit entrances. Enviromental Implication. J. Environment. Manage 77, 1-11.

Guzzella, L., 1998. Comparison of test procedures for sediment toxicity evaluation with Vibrio fischeri bacteria. Chemosphere, 37, 2895-2909.

Hakanson, L. 1990. A new functional view of Baltik Sea. Ambio. Special Report. 7, 19-24.

ISO 1253 2006. Water quality—marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum. ISO 10253:2006.

Leecaster, M., 2003. Spatial analysis of grain size in Santa Monica Bay. Mar. Environ. Res. 56, 67–78.

Lera, S., Macchia, S., Pellegrini, D., 2006. Standardizing the methodology of sperm cell test with Paracentrotus lividus. Environ Monit Assess. 122, 101–109.

Liu, F., Peng, J., Zhang, C.Y., 2012. A non-parametric indicator Kriging method for generating coastal sediment type map. Marin Sci. Bull. 14 (1), 57-67.

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., Ingersoll, C.G., MacDonald, D.D., 2006. Calculation and uses of mean sediment quality guideline quotients: A critical review. Env. Science. & Tecnology 40, 1726-1736

MacDonald D. D., Ingersoll C. G., Berger T. A. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Arch. Envirom. Contam. Toxicol. 39, 20-31.

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. Ecotoxicology 5, 253-278

Morabito A., 2001. Metodo per la determinazione di composti organostannici in sedimenti e matrici biologiche tramite GC-MS e GC-FPD—Appendice 1. In: Cicero AM, Di Girolamo I (eds)

Metodologie Analitiche di Riferimento del Programma di Monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001–2003). Ministero dell'Ambiente e della Tutela del Territorio. ICRAM© Roma file:///E:/dati/Downloads/10087_metodologie%20(1).pdf . Accessed 20 March 2015.

Müller, G. 1969. Index of geoaccumulation in sediments of the Rhine River. Geol J. 2, 109-118

Nieuwenhuize, J., Maas, Y.E.M., Middleburg, J.J. 1994. Rapid analysis of organic carbon and nitrogen in particulate materials. Mar. Chem., 45, 217-224.

Onorati, F, Volpi Ghirardini, A., 2001. Informazioni fornite dalle diverse matrici da testare con i saggi biologici: applicabilità di Vibrio fischeri. Biol Mar Medit 8, 31–40.

Onorati, F., Bigongiari, N., Pellegrini, D., Giuliani, S., 1999a The suitability of Corophium orientale (Crustacea, Amphipoda) in harbour sediment toxicity bioassessment. Aquat Ecosyst Health Manag 2, 465 –473.

Onorati, F., Pellegrini, D., Ausili, A. 1999b. Valutazione della tossicità naturale nel saggio Microtox® in fase solida: la normalizzazione pelitica, Acqua & Aria 6, 83-91.

Piva, F., Ciaprini, 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.

Regoli, F., Pellegrini, D., Cicero, A.M., Nigro, M., Benedetti, M., Gorbi, S., Fattorini, D., D'Errico, G., Di Carlo, M., Nardi, A., Gaion, A., Scuderi, A., Giuliani, S., Romanelli, G., Berto, D., Trabuco, B., Giudi, P., Bernardeschi, M., Scarcelli, V., Frenzili, G., 2013 A multidisciplinary weight of evidence approach for environmental risk assessment at the Costa Concordia wreck: Integrative indices from Mussel Watch. Marine Environmental Research, 1-13.

Ritter, K.J., Bay, S.M., Smith Y.R.W., Vidal-Dorsch Yy D.W., Jay, L. 2012. Field Development and Evaluation of Sediment Quality Guidelines Based on Benthic Macrofauna Responses; Integr. Enviro. Assess. Manage. 8, 125-148,

Ritter, K.J., Bay, S.M., Smith, R.W., Vidal-Dorsch, D.E., Field, L.J., 2008. Development and evaluation of sediment quality guidelines based on benthic macrofauna responses. In: Weisberg

SB, Miller K, (Editors). Southern California Coastal Water Research Project annual report 2008. Costa Mesa (CA): Southern California Coastal Water Research Project

Romano, E., Gabellini, M., 2001. Analisi delle caratteristiche granulometriche—Sedimenti, scheda 3 In: Cicero A. M. , Di Girolamo I. (eds) Metodologie Analitiche di Riferimento del Programma di Monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001–2003). Ministero dell'Ambiente e della Tutela del Territorio. ICRAM, Rome file:///E:/dati/Downloads/10087_metodologie%20(1).pdf. Accessed 20 March 2015

Salomons, W., Forstner, U., 1984. Metals in the hydrocycle. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, ISBN 3540127550.

Schintu, M., Buosi, C., Galgani, F., Marrucci, A., Marras, A., Ibba, A., Cherchi, A. 2015. Interpretation of coastal sediment quality based on trace metal and PAH analysis, benthic foraminifera, and toxicity tests (Sardinia, Western Mediterranean). Mar. Poll. Bull. 94, 72–83.

Sustainable Management of Sediment Resources. 2007. Sediment and Dredged Material Treatment, Vol.2 Bortne G. Palumbo L. (Editors) Elsevier B.V.

Szlinder-Richert, J., Usydusa, Z., Drgasb, A., 2012. Persistent organic pollutants in sediment from the southern Baltic: Risk Assessment. J. Environ. Monit., 14, 2100-2107.

Turekian, K.K., Wedepohl, K.H., 1961. Distribution of the elements in some major units of the earth's crust. Bull. Geol. Soc. Amer. 72, 175–192.

US/EPA United States Environmental Protection Agency. 1991. Evaluation of dredged material proposed for ocean disposal (Testing manual). Report n. EPA-503/8-91/001, Washington, D.C

US/EPA United States Environmental Protection Agency. 1998. EPA's contaminated sediment management strategy. EPA-823-R-98-001. Washington, DC

US/EPA United States Environmental Protection Agency. 1978. Petroleum hydrocarbons Method 418_1.

US/EPA United States Environmental Protection Agency. 2000a. Draft implementation framework for the use of equilibrium partitioning sediment quality guidelines. Office of Water of Science and Technology. Washington, District of Columbia.

US/EPA. United States Environmental Protection Agency. 2000b. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition. EPA/600/R-99/064. Washington, District of Columbia

US/EPA United States Environmental Protection Agency. 2011. List of toxic and priority pollutants http://water.epa.gov/scitech/methods/cwa/pollutants-background.cfm (accessed 18 may 2015)

Van Beelen, P. 2003. A review of the application of microbial toxicity test for deriving sediment quality guidelines' Chemosphere. 53, 795-808.

Wenning, R.J., Batley, G.E. Ingersoll, C.G. and Moore D.W., 2005 Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments Executive Summary Booklet of a SETAC Pellston Workshop. Edited by Richard J. Wenning,Christopher G. Ingersoll . Society of Environmental Toxicology and Chemistry. Pensacola, FL

Wolska L., Sagajdakow A., Kaczynski A., Namiesnik J (2007). Application of ecotoxicological studies in integrated environmental monitoring; possibilities and problems. Trend. Anal. Chem. 26, 332-344.

Revised Manuscript
Click here to view linked References

Identification of hot spots within harbour sediments through a new cumulative hazard index. Case study: port of Bari, Italy

Matilda Mali[a,*], *Maria Michela Dell'Anna*[a], *Piero Mastrorilli*[a], *Leonardo Damiani*[a], *Nicola Ungaro*[b], *Ainara Gredilla*[c], *Silvia Fdez-Ortiz de Vallejuelo*[c]

[a]DICATECh, Politecnico di Bari, via Orabona, 4 I-70125 Bari, Italy.

[b]ARPA PUGLIA, Corso Trieste, 27 I-70126 Bari, Italy.

[c]Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country UPV/EHU, P.O. Box 644; 48080 Bilbao, Basque Country.

[*]Corresponding author e-mail address: matilda.mali@poliba.it; Tel:+39 080 5963666; Fax: +39 080 5963414

Abstract

While numerous quality guidelines unquestionably drive the management of harbour sediments, port managers increasingly require easy-to-use tools supporting them in assessing the sediment quality. In this work, a new hazard index, named cumulative Normalized and Weighted Average Concentration (c_NWAC), is proposed, considering the concentrations of main hazardous, toxic and bio-accumulative sediment contaminants. This index is an upgraded version of the previously introduced NWAC index, which considered only the metal concentrations. The c_NWAC values range from 0 to 10 scores, and their visualization on a colour base code map leads to an easy identification of hotspots. The applicability of the new index was verified using a dataset derived from the analyses of 42 samples collected at different depths of the seabed of the Port of Bari (Italy). The concentrations of 58 parameters were considered, namely 11 metal and metalloids, 17 congeners of polychlorinated biphenyls (PCBs), 16 congeners of polycyclic aromatic hydrocarbons (PAHs), further 11 organic micropollutants, total organic carbon (TOC) and nutrients (N_{tot}, P_{tot}). For the study area, the obtained c_NWAC values resulted well correlated to the corresponding values of the commonly used mean Effects Range Median quotient (mERMq), and similar hazard categories were developed for the new index, even if the latter do not necessarily explain the real sediment toxicity, because they were derived from a different sediment dataset and different test species.

Moreover, the hazard predictive ability of the new index was verified (with satisfactory results) by testing the considered samples by eco-toxicological assays, using three different biological species, and comparing the obtained results to the corresponding hazard categories developed for c_NWAC.

Keywords: Pollution hazard, Harbour, Sediment quality, Metals, Polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Ecotoxicological tests.

1. Introduction

Sediments are an essential and dynamic part of the port; their quality and quantity are integral to ecosystem health and a fundamental component of the regional economy. Many hazardous, toxic and bioaccumulative chemicals, such as metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorophenols, organotin compounds, organochlorine pesticides and aliphatic petroleum hydrocarbons are continuously leaked into the port due to shipping traffic, loading, repairs, dredging and other activities, as well as rainwater runoff, effluent discharges, dust etc. (Guerra-Garcia and Gomez, 2005; Casado-Martínez et al., 2009; Szlinder-Richert et al., 2012).

The contaminants, which are found only in trace amounts in water, tend to be adsorbed into both inorganic and organic material eventually settled in sediments. If the concentration of these contaminants in the water column is high enough, sediments may accumulate excessive quantities of contaminants that directly and indirectly disrupt the ecosystem. Indeed, many studies have documented the importance of sediment contamination for ecosystem quality (Burton, 2002, 2013; Chapman et al. 2002, 2013; Chapman 2007; Schintu et al 2015). Moreover, numerous efforts, resources and money are continuously spent in many countries in an attempt to clean up or remove contaminated sediments, in order to restore ecosystem quality and develop beneficial uses for dredged materials (SedNet, 2007).

National and international guidelines have unquestionably supported the evaluation and management of sediments (US/EPA Environmental Protection Agency 1998; EPA-905-B02-001-A, 2002), but researchers in this field are still attempting to identify easy-to-use tools that support and facilitate sediment quality assessment. The methodologies proposed in the literature to estimate the quality of sediments can be classified into two categories. The simplest one uses the ratio between the measured concentration of a given contaminant in sediment samples and the

background value estimated for the area (Müller, 1969; Salomons and Forstner, 1984; Hakanson, 1990; Wolska et al, 2007). The other category of methodologies combines chemical parameters with related biological effects, valuating the toxicity degree of sediments (Long et al, 1995; Macdonald et al, 1996; Macdonald et al. 2000). Nevertheless, all such methodologies are contaminant-specific and depend upon the legislation currently in force in each specific area that usually applies specific numerical criteria (threshold levels for each contaminant) when assessing the quality of sediments. Registering an excess of concentration of a given contaminant with respect to the numerical threshold constitutes the necessary condition for assessing the pollution status of the sediment and, consequently, the development of regulatory and remedial actions.

Since none of the existing methodologies take into account the possible synergic or antagonistic effects that the simultaneous occurrence of different hazardous contaminants within sediments may present (Eggen et al., 2004), cumulative indexes that consider the concomitance of different stressors are highly recommended. However, the multidisciplinary approaches that combine multiple typology of investigation (or lines of evidence, LOEs) (Chapman, 1996; Chapman et al., 2002) are often hampered by the lack of standardized procedures for the interpretation and the integration of complicated datasets of heterogeneous results, which typically require various expert judgements (Piva et al., 2011; Benedetti et al., 2012; Regoli et al., 2013). Aiming at overcoming this inconvenience, recently, a software-assisted model has been developed to integrate and differently weight data from various lines of evidence (Piva et al., 2011).

One of the most employed indexes is the mean Effects Range Median quotient (mERMq) (Long et al. 2006), which estimates the toxic effect of selected pollutants on a wide variety of benthic organisms. Furthermore, Gredilla et al. (2014) recently calculated the Normalized and Weighted Average Concentration (NWAC) that, however, takes into account only metal content (14 metals and metalloids) in estuarine systems.

In this paper, an upgraded version of NWAC is proposed, named cumulative NWAC (c_NWAC), able to examine the multiple effects of three categories of contamination indicators: metals and metalloids, persistent organic pollutants and TOC, and nutrients, such as N_{tot} and P_{tot}. We applied the upgraded index to the Port of Bari (southeast Italy) using sediment cores collected at three depths (50 cm, 100 cm and 150 cm) from 23 sampling stations. The predictive ability of the new proposed index was ascertained by comparing the c_NWAC ranges of hazard with the results obtained from three amphipod survival tests.

2. **Materials and methods**

2.1 Features of the study area

The Port of Bari is located in the southeast of the Italian Adriatic coast, at the centre of the Apulia region (Figure 1). The coastal area under study forms part of the westward extension of the carbonatic plateau of the Murge, the lithological platform of southern Italy. Its low-profile morphological features are characterized mainly by sandy sediments with beaches only partially preserved from human intervention. The coastal tract of the metropolitan city of Bari is currently heavily modified by large-scale artificial burying. Numerous ephemeral watercourses, parallel to each other and perpendicular to the coast, form the characteristic hydrographic network that nourishes the coast with terrigenous sediments.

Figure 1 near here

The Port of Bari is one of the most important ports settled along the Adriatic coast and is located within the urban area of the metropolitan city of Bari. It is a multipurpose stopover equipped with docks for handling a range of goods and freight. Water depth in the port varies from 6.9 m to 14.9 m, allowing the movement of large ships and vessels. Several quays for various types of commercial traffic (solid and liquid bulk, containers, packaged goods, steel products, forestry products etc.), as well as Ro-Ro docks for ferries and cruise ships platforms are used daily. High-level port activity seriously impacts the quality of the port and, therefore, the quality of harbour sediments, that act as a final sink for all contaminants entering the port within the water column. Regarding the contamination history of the study area, two important events should be mentioned. The first one is the activity of the National Hydrogenation Fuels Company at the north of Bari which had caused significant impact to the local area, until it ceased activity in 1974, and consequently affected the quality of the port area nearby, in which the waste water was dumped. The concerned area has only recently been subjected to reclamation work in order to eliminate residual hydrocarbons. The second event was the bombing campaign during the Second World War that involved directly the port basin.

2.2 Sampling area and sampling design

Sediment samples were collected during a sampling campaign between 2010-2011 from the most significant basins in the port area (see Figure 1): at the Ro-Ro terminal (sites 42-51), the Container Terminal (sites 53-61), the Passenger Traffic zone (cruise and scheduled shipping- (sites 68-90)

and at the access channel of the port (sites 124-130). A total of 23 core samples were taken from different sampling sites, using a PF2 vibrocorer, equipped with a liner. The vibrocorer operated with the support of a vessel endowed with a differential GPS system for the positioning of core samples. Each core was sectioned into 50 cm length sub-cores and samples were collected at different depth levels up to 150 cm. When the final sub-core was less than 100 cm long, due to the presence of rock on the seabed, only superficial sediment was considered. The letters A, B and C were assigned to each core sample indicating the core depth at 50 cm, 100 cm and 150 cm respectively (Table S1). Aliquots of 250 g of wet sediments were taken from each sub-core and transported in cleaned plastic containers, available for the analyses. Part of the analyses were performed on wet-sediments (microbiological analyses, bioassay tests), while aliquots useful for the other analytical analyses were stored at –20 °C.

2.3 Analytical methods

Before analysis sediments that were stored at –20 ° C after sampling, were dried at 40 °C for 48 h. Each sediment was classified according to Shepard (1954) into four classes: gravel: > 2 mm; sand: 2-0.063 mm; silt: 0.063-0.002 mm; clay < 0.002 mm. A set of ASTM sieves and a granulometer laser were used for the granulometrical separation (Romano et al., 2001).

The trace metal concentrations were measured by inductively coupled plasma mass spectrometry (ICP/MS X Series Thermo Fisher Scientific) after sample mineralization by total acid digestion (HCl, HNO_3 and HF) (Pellegrini et al., 2001). The fraction < 63 μm dried at 105 °C was used for the metal and metalloids determination. The results are expressed on the dry weight basis. The detection limits (LODs) were calculated from 3 replicates of procedural blanks. The LODs estimated were in mg/kg units and were equal to 1 ppb for all metals and metalloids. Marine Sediment References Material NIST 2702 (Inorganics in Marine Sediment) was used to control the analysis quality: the agreement between the analytical results for certified and measured values was satisfactory with recoveries between 80 and 100% for all metals and metalloids.

Total petroleum hydrocarbons amounts were determined by IR spectroscopy analysis after extraction according to US/EPA, Method 418.1 Issued 1978 (US/EPA, 1978).

Polychlorinated biphenyls (PCBs) concentrations were determined after acetone/petroleum ether extraction and analysed by Gas-Chromatograph equipped with an Electron Capture Detector, following the procedure proposed by Cicero et al. (2000). The congeners considered are 17:

PCB28, PCB52, PCB77, PCB101, PCB105, PCB114, PCB118, PCB123, PCBB126, PCB128, PCB138, PCB153, PCB156, PCB157, PCB167, PCB180 and PCB189. The same methodology was used for the determination of chlorinated organic compounds.

The polycyclic aromatic hydrocarbons (PAHs) considered for this study were: naphthalene, acenaphthene, acenaphthylene, phenanthrene, anthracene, fluoranthene, benz[a] anthracene, chrysene, benz[b]fluoranthene, benz[k]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, dibenz[a,h]anthracene, fluorine, indeno[1,2,3-cd] and pyrene. They were determined after extraction with cyclohexane/methanol mixture and analyzed by High Pressure Liquid Chromatograph, according to Ausili (2001) method.

N_{tot} was determined by elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer and the total phosphorus concentration (P_{tot}) was determined by colorimetric titration using the molybdenum-blue method (Aspila et al., 1976).

Total organic carbon (TOC) was determined by elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer, after removing of carbonates by reaction with hydrochloric acid (Nieuwenhuize et al., 1994). The amount of organotin compounds was determined by the procedure proposed by Morabito (2001).

The ecotoxicological tests with bioluminescent bacteria *Vibrio Fisheri* were carried out using Onorati et al. (1999b) and Azur Environmental (1994) protocols, while fertilization success bioassay test with sea urchin *Paracentrotus lividus* and the marine algal growth inhibition test with *Phaeodactylum tricornutum* were performed according to standardized procedure applied by Onorati and Mecozzi (2004), Lera et al. (2006) and ISO 1253 (2006).

2.4 Statistical elaborations

The correlation matrixes and Pearson coefficients were calculated using Statistica Software v. 10. The spatial modelling of the elements considered was done by means of kriging method (3D-Field Program, v. 3.8.8.0.) as predictor tool. The kriging method is a spatial modelling that extrapolates information from discrete stations to cover the whole area of study. Likewise some other non-interpolators, kriging generally produces a smoother surface over the area of interest and it assumes stationary and isotropy within the sampling sites, which implies that spatial correlation within the sampling points is independent from direction (Leecaster, 2003; Liu et al., 2012; Fdez-Ortiz de Vallejuelo et al., 2014).

3. Results and discussion

3.1. Preliminary study of the area

3.1.1 Horizontal distribution of contaminants

According to the analysis of contaminant distribution at different sites of the port area, the Ro-Ro, Container and Passenger Terminal sites were revealed as those of most concern.

The highest concentrations of As were identified at sites 61_B (with a concentration value of 34.64 mg/kg) and 88_A (30.10 mg/kg), followed by 44_A (29.56 mg/kg), 51_C (28.29 mg/kg) and 72_A (26.59 mg/kg). The lowest As concentrations were registered at the access channel of the port, due to the renewal of water flow. The most polluted sites in the case of Cd were 61_C (3.32 mg/kg), 88_A (2.09 mg/kg) and 79_A (2.05 mg/kg). The sites of most concern for Hg concentrations were 76_B (5.99 mg/kg) and 88_A (5.09 mg/kg). Other high concentrations of Hg were also registered at sites 82_B (4.69 mg/kg) and 82_C (3.50 mg/kg). Two sites registered elevated concentrations of Pb: 79_A (454.03 mg/kg) and 51_B (386.94 mg/kg), while in the case of Cu, sites 44_A (227.52 mg/kg) and 51_B (169.80 mg/kg) resulted as the most polluted. However, high concentrations of Cu were also found at 82_B/C, 88_A (in the range of 330-332 mg/kg). In terms of Zn distribution, the highest concentration was detected at 61_B (2390.37 mg/kg). High concentrations were again registered at 88_A (561 mg/kg) and 42_A (464 mg/kg). Generally, the lowest Zn concentrations were revealed at the access channel of the port (sites 128, 130).

The distribution of Ni, V and Cr was relatively homogeneous within the area of study with average concentration values comparable to the concentration of crustal shale as determined by Wedepohl and Turekian (1961).

With respect to organic compounds, the highest concentrations of PCBs were registered at 88_B (1.99 μg/g) and 79_A (1.59 μg/g). High concentrations were also registered at 44_B (1.18 μg/g). The highest PAH concentrations were detected at 61_B (61.18 μg/g), 79_A (18.04 μg/g) and 88_A (14.53 μg/g).

The sites with the highest presence of high molecular weight hydrocarbons (HW THC) were generally the same in which the highest presence of PCB and PAH was registered, showing probably the same source of pollution such as fuel losses: 79_A (9.001 mg/g), 51_B (6.172 mg/g), 61_B 5.235 mg/g) and 88_A (4.188 mg/g).

This tendency was also observed for organotin compounds (expressed as sum of mono-, di-, and tributyltin), yet in this case high concentrations were also found at 53_A (0.884 μg/g). The highest concentration of organochlorine compounds was registered at 79_A (1.18 μg/g).

As with metals, the sites with the lowest concentrations of organic pollutants were those located at the access channel of the port (sites 124-130) and those located at the entrance of the Ro-Ro terminal (42), with the exception of ∑PAH and ∑ HW THC, for which elevated concentrations were identified also at the access channel to the port, probably due to fuel losses during the entrance/manoeuvring of vessels.

3.1.2 Vertical distribution of contaminants

A heterogeneous distribution of contaminants was also revealed within the vertical profiles of core samples. At sites 44, 47, 108, 128 and 130 higher concentrations of metals, metalloids, ∑PCBs and ∑Organochlorine compounds were detected at the surface levels of the cores with respect to the deeper levels (with the exception of Pb at site 108 and Cu at site 130).

This trend was not confirmed in the case of ∑PAHs and ∑Organotin compounds for which higher concentrations were revealed at deeper levels in sites located at the entrance to the port (at the sites 126 and 130). Unusual degrees of pollution were registered at site 42 (entrance to the Ro-Ro terminal): higher concentrations for all considered metals and metalloids were found at the depth of 100 cm compared to the superficial layer (50 cm) and to the deeper one (150 cm). This latter registered, in any case, slightly higher amounts of metals with respect to those revealed at superficial depth (50 cm). This anomalous contamination trend could be explained by previous accidental spill of contaminants at this site (contamination caused by vessels battery, discharge of wastewater, runoff waters etc.).

The internal sites of the port area, 44, 51, 61, 76, 82 and 85, presented increasing metal concentrations the deeper the layer from the seabed, with the exception of Ni, Cr and V, for which the distribution of concentrations in vertical profiles follows the Al distribution trend (revealed by the strong correlation between Al and Cr concentrations). It may therefore be assumed that concentrations of Ni, Cr and V are naturally present in the area (Loring and Rantala. 1997).

In terms of organic pollutants, ∑PCBs and ∑Organochlorine compounds generally resulted higher in the superficial layer (with the exception of sites 51, 61 and 82). At nearly all sites the concentration of ∑PAHs increased in correspondence with the distance from the surface. In the

case of ∑Organotin compounds, distribution within the vertical profile is highly heterogeneous and it is therefore difficult to determine a specific trend.

3.2 Calculation of the c_NWAC. Case study: Port of Bari

The cumulative index proposed in the present work is an upgraded version of NWAC (Gredilla et al., 2014). In fact, while the original NWAC is based on the concentration of only 14 metals and metalloids, our cumulative NWAC (c_NWAC) index is based on the multiple effects of three categories of contamination indicators, present in the US/EPA list of toxic and priority pollutants in sediment (US/EPA, 2011): *i*) 11 metals and metalloids; *ii*) persistent organic pollutants, such as 19 congeners of PCBs, 16 congeners of PAHs, 11 chlorinated organic compounds, organostannanes, petroleum hydrocarbons, other organic micropollutants and *iii*) N_{tot}, total organic carbon (TOC) and P_{tot}, that clearly affect the toxicity of sediments (Coulon et al., 2005). Furthermore, the visualization of the cumulative Normalized Average Weighted Concentrations (c_NWAC) in a colour-based map (Kriging methodology) is proposed, which may facilitate the identification of hotspots with the highest levels of contamination present within the harbour area.

The entire dataset was considered in calculating the cumulative NWAC index, compiling the results of the analysis carried out on the 42 sediment samples collected from the Port of Bari. The overall database was therefore composed of 42 samples (23 superficial, 14 collected at 100 cm depth and 5 collected at 150 cm depth) and 58 variables (Table S2). In the calculation of the c_NWAC, data were initially divided into two sub-datasets according to the variables considered. A first sub-set was defined including the concentrations of 11 metals and metalloids (As, Al, Fe, Cd, Cr, Cu, Hg, Ni, Pb, V and Zn). The second one comprised the quantities of organic pollutants (17 congeners of PCBs, 16 congeners of PAHs, 11 organic micropollutants), total organic carbon and sediment nutrients (N_{tot}, P_{tot}). The nutrients and TOC were included within the second sub-set, due to their well-known effects in the microbiological activity that affect the organic pollutants degradation (Coulon et al., 2005).

The procedure proposed by Gredilla et al. (2014) was followed for each sub-dataset and three normalization steps were carried out in order to obtain equal statistical weights for the considered variables. First, for each contaminant, the concentrations (C) found within sampling sites were normalised (C_{norm}) by linear scaling transformation ($C_{norm}= [(C - C_{min})/(C_{max} - C_{min})]$. Then, the normalized concentration C_{norm} were weighted by calculating for each contaminant the dispersion

of the concentrations of that contaminant within the sampling sites. In fact, the relative standard deviations (RSD) were calculated for each contaminant within sampling sites and normalised (RSD_{norm}) by linear scaling transformation according to: [RSD_{norm}= (RSD − RSD_{min})/(RSD_{max}− RSD_{min})]. The resulting RSD_{norm} varied from 0 to 1 and were used to weight the normalised concentrations (C^{w}_{norm}) according to: (C^{w}_{norm}=C_{norm}·RSD_{norm}) of the contaminants. The average of the obtained values ($\overline{C^{w}_{norm}}$) leads to the Normalised-and-Weighted Average Concentration (partial index) for each of the two groups of variables (metals and metalloids on one hand and organic pollutants+total organic carbon+nutrients on the other). The average value of the partial indexes multiplied times ten provided the c_NWAC value for each sample (on a scale of 0 to 10). The results are shown in Table 1.

Table 1 near here

Since sediment sampling was performed at different depths, the c_NWAC value obtained for each depth was also calculated (Table 1, column A, B and C, on the left).

Analyzing the surficial layer c_NWAC values (Table 1, column A on the left), the highest hazard degrees were obtained at the Passenger Terminal basin for sediments collected from sampling points 79 and 88, showing index values of 8.52 and 6.78, respectively. High values were also obtained for other sampling sites: 85 (Passenger Terminal basin), 42, 44 (the two latters at the entrance of Ro-Ro terminal) and 69 (Cruise Terminal area), having c_NWAC values ranging from 3.03 to 4.68, showing a high pollution risk concentrated in these basins. The lowest index values were registered in sediments collected from the port access channel (sampling points 124, 126, 128 and 130).

Moving from surficial to deeper layer cores (Table 1, column B and C, on the left), several differences in the distribution of the c_NWAC values were observed. For example, at sampling site 51 (Ro-Ro terminal area) the c_NWAC value increased from 0.64 for the surface sediment to 5.23 for the 100 cm depth sample core, and subsequently, it decreased down to 1.17 for the 150 cm depth core. Also for site 61 (Container Terminal area), the c_NWAC value registered at 100 cm depth (8.88) was higher than the corresponding value obtained at surficial layer (1.26). Site 42 (at the entrance of Ro-Ro Terminal basin) c_NWAC vertical distibution values did not follow the above mentioned trend, decreasing on passing from surficial (4.68) to the 100 cm depth layer (0.68), and increasing again up to 1.96 at 150 cm depth level. In sites belonging to the Cruise

Terminal area (sampling points 68 and 69), to the entrance of the old dock/private port (site 108) and to the port access channel (sites 128 and 130), the relevant c_NWAC values were roughly the same at all depth levels.

For easy identification of hotspots within harbour area, all the obtained results were projected as three layer maps (for the depths of 50 cm, 100 cm and 150 cm) presented in Figure 2, a), b) and c), respectively.

Figure 2 near here

3.3 Correlation between the c_NWAC values and mERMq values

In order to validate the obtained results, the mean ERM quotients were calculated based on the same dataset, but considering only those contaminants for which the relevant ERM values were assessed by Long et al, 1995. The mERMq values for each depth are also reported in Table 1, columns A, B and C, on the right.

The toxic categories of mERMq according to the ranges proposed by Long et al. (2006), namely, mERMq<0.1 (non-toxic, nt), 0.1<mERMq<0.5 (slightly toxic, st), 0.5<mERMq<1.5 (moderately toxic, mt) and mERMq>1.5 (highly toxic, ht) are reported in Table 2, on the right.

The c_NWAC values and the corresponding mERMq values were plotted and the square correlation coefficient between c_NWAC and the global mERMq values resulted highly significant, with $R^2 = 0.953$, $p < 0.0005$ (Figure 3). This reveals a similar diagnosis of the study area by both indexes. The first index identifies the zones of major concern due to the simultaneous presence of metals and metalloids, organic compounds and other parameters, while the second one reveals sediment sites with the greatest probability of toxicity.

Figure 3 near here

By analyzing all obtained results, a number of considerations can be made. It is apparent from the visualization of the 2D maps of the c_NWAC values for the different considered depth levels (Figure 2) that hot spots can be easily identified in maps even by non-specialists, highlighting that the basin of the Container (sampling sites 44, 51, 61) and Passenger Terminal (sampling points 76, 79, 82, 88) resulted as those of most concern. In addition, for some hotspots, deep sediments appear as more contaminated than superficial ones such as 51, 61 and 82. These different degrees

of concern regarding the synergic presence of the considered contaminants could possibly be due to adsorption phenomena that allow contaminants of the surface sediments to form chemical equilibria with organic and inorganic species dissolved or suspended in the column water, thus decreasing their toxicity level enhanced by the leaching of contaminants in the water column. Furthermore, surficial layers are probably disturbed by mechanical agitations and turbidity currents caused by the movement of great size ships (Cruise and Container Ships) and other hydrodynamic conditions that may promote contaminant leaching processes. These phenomena do not affect the deeper sediments.

However, a high concentration of a certain contaminant does not always correspond to high c_NWAC value at the corresponding site. For example, considering the high As concentration at the sampling sites 72_B and 51_A (26.56 mg/kg and 27.48 mg/kg, respectively), the corresponding c_NWAC values were not of high concern, being 0.33 and 0.68, respectively. Moreover, the Pb and Hg concentrations registered at site 51_B (386.56 mg/kg for Pb and 2.34 mg/kg for Hg) are higher with respect of those registered at site 61_B (95 mg/kg for Pb and 2.15 mg/kg for Hg), yet the c_NWAC value registered at 51_B (5.23) is lower with respect to the value calculated for 61_B (8.88).

In addition, despite the similarity between the average concentrations of ∑PAHs registered at sampling sites 126_A (2.05-5.62 mg/kg) and 88_A (3-6.7 mg/kg), the relevant c_NWAC values established for the two sites are very different (1.59 for site 126_A and 6.78 for site 88_A). This would indicate the ability of the cumulative index to take into account a combination of different factors (contaminants and other chemical parameters considered in its calculation).

3.4. *Establishing hazard categories of c_NWACs*

The values obtained by the c_NWAC index were compared with the relevant mERMq values in order to assess a magnitude degree of the issue terms of toxicity also for the new proposed index. Thus, based on the equation of the linear correlation between c_NWAC and mERMq (and the classes of toxicity suggested by Long et al., 2006 for mERMq values), different hazard categories were defined also for the c_NWAC index.

Indeed, by using the equation $y = 5.192\ x + 0.2231$ (Figure 3) established for the whole dataset, and defining x as the threshold value of hazard categories established for mERMq (x=0.1; x=0.5; x=1.5), the obtained c_NWAC hazard categories were as follows: “No hazard” is considered for

samples with a c_NWAC range of 0< c_NWAC <0.74; “Low hazard” for 0.74<c_NWAC<2.8; “Moderate hazard” for 2.8<c_NWAC<8.01; “High/severe hazard” for c_NWAC>8.2.

3.5. *Real predictive ability of c_NWACs*

The predictability of the new index was tested using a battery of bio-assays which includes three tests: microtox Solid Phase Test (SPT) with *Vibrio fisheri*, fertilization success bioassay with *Paracentrotus lividus*; algal growth inhibition test with diatom *Phaeodactylum tricornutum*. The microtox SPT is an acute toxicity test for solid matrices based on inhibition of natural bioluminescence of the marine bacterium *Vibrio fischeri* in the presence of pollutants. The test was applied to two phases of sediment: solid and liquid (elutriate). The former gives information about those contaminants that are bound to the sediment particles, for their chemical nature, hydrophobia, adsorption and complexing degree with organic substance (Onorati and Volpi Ghirardini, 2001), while elutriate gives information about the hydro-soluble contaminants that may recirculate by mechanical agitation. The latter component is most representative of the toxic potentialities of marine sediments when they are to be dredged (US/EPA, 1991). The results of test on solid phase were expressed as S.T.I. (Sediment Toxicity Index) that represents real acute toxicity of analyzed sample with respect to the natural toxicity of a sample with the same granulometric characteristics (Onorati et al., 1999a, b).

The test using the sea urchin *Paracentrotus lividus* was conducted on the extracted liquid phase (elutriate) of the sediments and the same method was performed for algal growth inhibition test (using *P. Tricormutum*). The results of the two latter tests are expressed in EC_{50}/EC_{20}. The EC_{50} is the median effective concentration (EC_{50}), a statistically derived concentration of a substance in an environmental medium (in this case the elutriate of sediments) expected to produce a certain effect (algal growth inhibition or fertilization success) in 50% of test organisms in a given population under a defined set of conditions. The EC_{20}, is referred to the concentration for which 20% of the population is affected. They are determined by establishing a dose-response curve which plots the concentration of a given compound in water (*x* axis) against the effects of this compound on a given species population (*y* axis). The analytical methods and toxicity classification criteria according Italian guidelines are reported in Table 2, while the obtained results, conditions and methodologies applied are reported in Table S3.

Prior to assessing the agreement between the predicted toxicity established by the c_NWAC index and the real measurement, the samples were grouped in two sub-groups categories: the

"unaffected samples" including those classified as "Non-toxic" and "Low toxicity" according to both the c_NWAC and toxicity tests and the second group of the "affected" samples, including those samples with moderate to high toxicity, thus simplifying the data set in two score orders belonging to two new categories: "affected" or "non-affected". By calculating the number of samples belonging to one of the two groups according to c_NWAC index, the number of samples that agree with the prediction according real biological tests were plotted (Figure 4).

Figure 4 near here

The agreement percentage (A) was calculated by the formula:

$$A = (P_i/P_{tot})\cdot 100$$

where the P_{tot} is the number of samples classified as "affected" or "not affected" according to the c_NWAC index and P_i is the number of samples that really resulted as "affected" or "not affected", according to the biological tests.

As expected (Long et al., 2006; Ritter et al., 2008, 2012), significant variability was revealed in the agreement percentages among different species, showing differences in relative sensitivity to toxicity. Nevertheless, satisfactory percentage agreements were obtained (up to 71%). *Vibrio Fisheri* tests correspond more accurately than the other tests in terms of the foreseen predictability. Indeed, Vibrio Fischeri as luminescence bacteria is particularly appropriate for the toxicity assessment of marine sediment (elutriate and solid phase). Its sensitivity is lower than the one of P. Lividus but its reproducible capability is higher (Guzzella, et al., 1998; Van Beelen, 2003).

Nevertheless, some limitations should be highlighted in the use of cumulative indexes. Wenning et al. (2005) extensively describe that despite the scientific underpinnings of the determination of different sediment quality guidelines (SQG) approaches and evaluation indexes, none of them appear to be intrinsically flawless and able to clearly delineate between sediments that cause biological effects and those that do not. At present, algorithms are unable to predict toxic effects with absolute certainty. The indexes established by the above

approaches can define the ranges of chemical concentrations where biological effects are rare, possible or highly probable, but they are unable to attain a complete distinction.

Factors that cause an overlap between effect and non-effect data are numerous and assorted. They include the effects of other chemicals not considered, the combination of effects, unaccounted for differences in chemical availability, differences in response among organisms and errors in measurements of either chemicals or responses.

Furthermore, the new index proposed herein was based on units of dry weight sediments; therefore it does not take into account the potential effects of hydrodynamic factors acting within harbour sediments, that may influence contaminant bioavailability. The c_NWAC index is thus not intended for use in predicting effects in wildlife or humans through bioaccumulation pathways and it is not intended as a toxicological threshold test. There is no assurance that it will always correctly predict either toxicity or non-toxicity. In addition, it should be underlined that in the present study the c_NWAC is compared to non-site specific SQGs. Therefore, the corresponding hazard categories may not necessarily explain the sediment toxicity of the study area, because they were derived from a different sediment dataset and different test species. For this reason, the mERMq values were calculated only to check the applicability of the new index.

The c_NWAC index constitutes a useful tool for an easy interpretation of data, especially when decision-makers must define regulatory or remedial action in the sites with high concern. However, other techniques that take other factors into account should be developed when an assessment of toxicity levels for humans and the benthic community is required.

4. Conclusions

A new cumulative index (c_NWAC) that takes into account the synergism and antagonism of different contaminants (inorganic and organic) affecting the quality of harbour sediments was proposed. The novelty of this index consists in the possibility of establishing for each harbour site a hazard index value, based on the concentration of several contaminants, including those for which the ERM values are lacking. Noteworthy, for the study area (Port of Bari, Italy), the calculated c_NWAC values resulted well correlated to the corresponding values of the commonly used mERMq, and similar hazard categories were developed. Even though the c_NWAC categories (differently from mERMq) do not necessarily explain the real sediment toxicity, they allow us to get an idea of the hazard of sediments, for the future environmental management of the area under study.

In addition, the visualization on 2D maps of c_NWAC values at different depths facilitated the immediate identification of hotspots or sites of most concern and allowed for a more in-depth investigation of the harbour bed. Differences among depth-layers were obtained, in some cases confirming the presence of earlier contamination phenomena. In other cases, dynamic physical-chemical processes occurring in sediment-water interfaces and combinations of different stressors impacting the toxicity of the hotspot points were identified.

The reliability and the predictive ability of the proposed cumulative index were verified through a comparative analysis between the calculated index values and the results of three biological tests, based on three different bio-indicators. The obtained results were satisfactory and it can be concluded that the cumulative index is a useful tool for the easy interpretation of data, especially when decision-makers are required to define regulatory or remedial action.

Nevertheless, the proposed cumulative index must be implemented taking into account other stressors affecting the toxicity of sediments, such as hydrodynamic factors that identify stagnation points and poor circulation of pollutants, for which the same visualization procedure on 2D maps can be carried out.

Acknowledgments.

This study was supported by ARPA Puglia, the Port Authority of Bari and fund of TEN ECOPORT project within SEE programme (SEE/A/0189/2.2/X). The authors thank the staff of ARPA Puglia for the implementation of eco-toxicological analysis.

References

Aspila, K.I., Agemian, H., Chau, A.S.Y., 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst. 101, 197–197

Ausili, A., 2001. Analisi degli idrocarburi policiclici aromatici – Sedimenti. scheda 9. In: Cicero AM, Di Girolamo I (eds).Metodologie Analitiche di Riferimento del Programma di Monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001–2003). Ministero dell'Ambiente e della Tutela del Territorio. ICRAM© Roma 2001. file:///E:/dati/Downloads/10087_metodologie%20(1).pdf . Accessed 20 March 2015.

Azur Environmental. 1994 Microtox® Acute Toxicity Basic Test procedures, 63.

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

Burton, G.A. Jr., 2002. Sediment quality criteria in use around the world. Limnology Rev. 3, 65-75

Burton, G.A. Jr., 2013. Assessing sediment toxicity: past, present, and future. Environ. Tox. Chem., 32 (7) 1438–1440

Casado-Martìnez, M.C., Forja, J.M., DelValls, T.A. 2009. A multivariate assessment of sediment contamination in dredged materials from Spanish ports. J. Hazard. Mater. 163, 1353–1359

Chapman, P M. 1996. Presentation and interpretation of sediment quality triad data. Ecotoxicology, 5 (5), 327-339.

Chapman, P.M., McDonald, B.G., Lawrence, G.S.. 2002. Weight-of-evidence issues and frameworks for sediment quality (and other) assessments Hum. Ecol. Risk Assess., 8, 1489–1515

Chapman, P.M., 2007. Determining when contamination is pollution e weight of evidence determinations for sediments and effluents. Environ. Int. 33, 492-501.

Chapman, P.M., Wang, F., Caeiro, S.S., 2013. Assessing and managing sediment contamination in transitional waters. Environ. Int. 55, 71-91

Cicero, A.M., Pietrantonio, E., Romanelli, G., Di Muccio, A., 2000. Comparison of soxhlet, shaking, and microwave assisted extraction techniques for determination of PCB congeners in a marine sediment. Bull Environ. Contam. Toxicol. 65, 07–313

Coulon, F., Pelletier, E., Gourhant, L., Delille, D., 2005. Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. Chemosphere, 58, 1439–1448

Eggen, R.J.J., Behra, R., Burkhardt-Holm, P., Escher, B. I., Schweigert, N., 2004. Challenges in ecotoxicology. Env. Science. & Tecnology. 38, 58A-64A.

Fdez-Ortiz de Vallejuelo, S., Gredilla, A., de Diego, A., Arana, G., Madariaga, J.M., 2014. Methodology to assess the mobility of trace elements between water and contaminated estuarine sediments as a function of the site physico-chemical characteristics. Sci. Total Environ. 473–474, 359–371.

Gredilla, A., Fdez-Ortiz de Vallejuelo, S., De Diego, A., Arana, G., Madariaga, J.M. 2014. A new index to sort estuarine sediments according to their contaminant content; Ecol. Indic. 45, 364–370

Guerra-Garcia, J.M., Garcia Gomez, J.C., 2005. Assessing pollution levels in sediments of harbours with two opposit entrances. Enviromental Implication. J. Environment. Manage 77, 1-11.

Guzzella, L., 1998. Comparison of test procedures for sediment toxicity evaluation with Vibrio fischeri bacteria. Chemosphere, 37, 2895-2909.

Hakanson, L. 1990. A new functional view of Baltik Sea. Ambio. Special Report. 7, 19-24.

ISO 1253 2006. Water quality—marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum. ISO 10253:2006.

Leecaster, M., 2003. Spatial analysis of grain size in Santa Monica Bay. Mar. Environ. Res. 56, 67–78.

Lera, S., Macchia, S., Pellegrini, D., 2006. Standardizing the methodology of sperm cell test with Paracentrotus lividus. Environ Monit Assess. 122, 101–109.

Liu, F., Peng, J., Zhang, C.Y., 2012. A non-parametric indicator Kriging method for generating coastal sediment type map. Marin Sci. Bull. 14 (1), 57-67.

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., Ingersoll, C.G., MacDonald, D.D., 2006. Calculation and uses of mean sediment quality guideline quotients: A critical review. Env. Science. & Tecnology 40, 1726-1736

MacDonald D. D., Ingersoll C. G., Berger T. A. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Arch. Envirom. Contam. Toxicol. 39, 20-31.

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. Ecotoxicology 5, 253-278

Morabito A., 2001. Metodo per la determinazione di composti organostannici in sedimenti e matrici biologiche tramite GC-MS e GC-FPD—Appendice 1. In: Cicero AM, Di Girolamo I (eds)

Metodologie Analitiche di Riferimento del Programma di Monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001–2003). Ministero dell'Ambiente e della Tutela del Territorio. ICRAM© Roma file:///E:/dati/Downloads/10087_metodologie%20(1).pdf . Accessed 20 March 2015.

Müller, G. 1969. Index of geoaccumulation in sediments of the Rhine River. Geol J. 2, 109-118

Nieuwenhuize, J., Maas, Y.E.M., Middleburg, J.J. 1994. Rapid analysis of organic carbon and nitrogen in particulate materials. Mar. Chem., 45, 217-224.

Onorati, F, Volpi Ghirardini, A., 2001. Informazioni fornite dalle diverse matrici da testare con i saggi biologici: applicabilità di Vibrio fischeri. Biol Mar Medit 8, 31–40.

Onorati, F., Bigongiari, N., Pellegrini, D., Giuliani, S., 1999a The suitability of Corophium orientale (Crustacea, Amphipoda) in harbour sediment toxicity bioassessment. Aquat Ecosyst Health Manag 2, 465 –473.

Onorati, F., Pellegrini, D., Ausili, A. 1999b. Valutazione della tossicità naturale nel saggio Microtox® in fase solida: la normalizzazione pelitica, Acqua & Aria 6, 83-91.

Piva, F., Ciaprini, 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.

Regoli, F., Pellegrini, D., Cicero, A.M., Nigro, M., Benedetti, M., Gorbi, S., Fattorini, D., D'Errico, G., Di Carlo, M., Nardi, A., Gaion, A., Scuderi, A., Giuliani, S., Romanelli, G., Berto, D., Trabuco, B., Giudi, P., Bernardeschi, M., Scarcelli, V., Frenzili, G., 2013 A multidisciplinary weight of evidence approach for environmental risk assessment at the Costa Concordia wreck: Integrative indices from Mussel Watch. Marine Environmental Research, 1-13.

Ritter, K.J., Bay, S.M., Smith Y.R.W., Vidal-Dorsch Yy D.W., Jay, L. 2012. Field Development and Evaluation of Sediment Quality Guidelines Based on Benthic Macrofauna Responses; Integr. Enviro. Assess. Manage. 8, 125-148,

Ritter, K.J., Bay, S.M., Smith, R.W., Vidal-Dorsch, D.E., Field, L.J., 2008. Development and evaluation of sediment quality guidelines based on benthic macrofauna responses. In: Weisberg

SB, Miller K, (Editors). Southern California Coastal Water Research Project annual report 2008. Costa Mesa (CA): Southern California Coastal Water Research Project

Romano, E., Gabellini, M., 2001. Analisi delle caratteristiche granulometriche—Sedimenti, scheda 3 In: Cicero A. M. , Di Girolamo I. (eds) Metodologie Analitiche di Riferimento del Programma di Monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001–2003). Ministero dell'Ambiente e della Tutela del Territorio. ICRAM, Rome file:///E:/dati/Downloads/10087_metodologie%20(1).pdf. Accessed 20 March 2015

Salomons, W., Forstner, U., 1984. Metals in the hydrocycle. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, ISBN 3540127550.

Schintu, M., Buosi, C., Galgani, F., Marrucci, A., Marras, A., Ibba, A., Cherchi, A. 2015. Interpretation of coastal sediment quality based on trace metal and PAH analysis, benthic foraminifera, and toxicity tests (Sardinia, Western Mediterranean). Mar. Poll. Bull. 94, 72–83.

Sustainable Management of Sediment Resources. 2007. Sediment and Dredged Material Treatment, Vol.2 Bortne G. Palumbo L. (Editors) Elsevier B.V.

Szlinder-Richert, J., Usydusa, Z., Drgasb, A., 2012. Persistent organic pollutants in sediment from the southern Baltic: Risk Assessment. J. Environ. Monit., 14, 2100-2107.

Turekian, K.K., Wedepohl, K.H., 1961. Distribution of the elements in some major units of the earth's crust. Bull. Geol. Soc. Amer. 72, 175–192.

US/EPA United States Environmental Protection Agency. 1991. Evaluation of dredged material proposed for ocean disposal (Testing manual). Report n. EPA-503/8-91/001, Washington, D.C

US/EPA United States Environmental Protection Agency. 1998. EPA's contaminated sediment management strategy. EPA-823-R-98-001. Washington, DC

US/EPA United States Environmental Protection Agency. 1978. Petroleum hydrocarbons Method 418_1.

US/EPA United States Environmental Protection Agency. 2000a. Draft implementation framework for the use of equilibrium partitioning sediment quality guidelines. Office of Water of Science and Technology. Washington, District of Columbia.

US/EPA. United States Environmental Protection Agency. 2000b. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition. EPA/600/R-99/064. Washington, District of Columbia

US/EPA United States Environmental Protection Agency. 2011. List of toxic and priority pollutants http://water.epa.gov/scitech/methods/cwa/pollutants-background.cfm (accessed 18 may 2015)

Van Beelen, P. 2003. A review of the application of microbial toxicity test for deriving sediment quality guidelines' Chemosphere. 53, 795-808.

Wenning, R.J., Batley, G.E. Ingersoll, C.G. and Moore D.W., 2005 Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments Executive Summary Booklet of a SETAC Pellston Workshop. Edited by Richard J. Wenning,Christopher G. Ingersoll . Society of Environmental Toxicology and Chemistry. Pensacola, FL

Wolska L., Sagajdakow A., Kaczynski A., Namiesnik J (2007). Application of ecotoxicological studies in integrated environmental monitoring; possibilities and problems. Trend. Anal. Chem. 26, 332-344.

Figure 1

Click here to download high resolution image

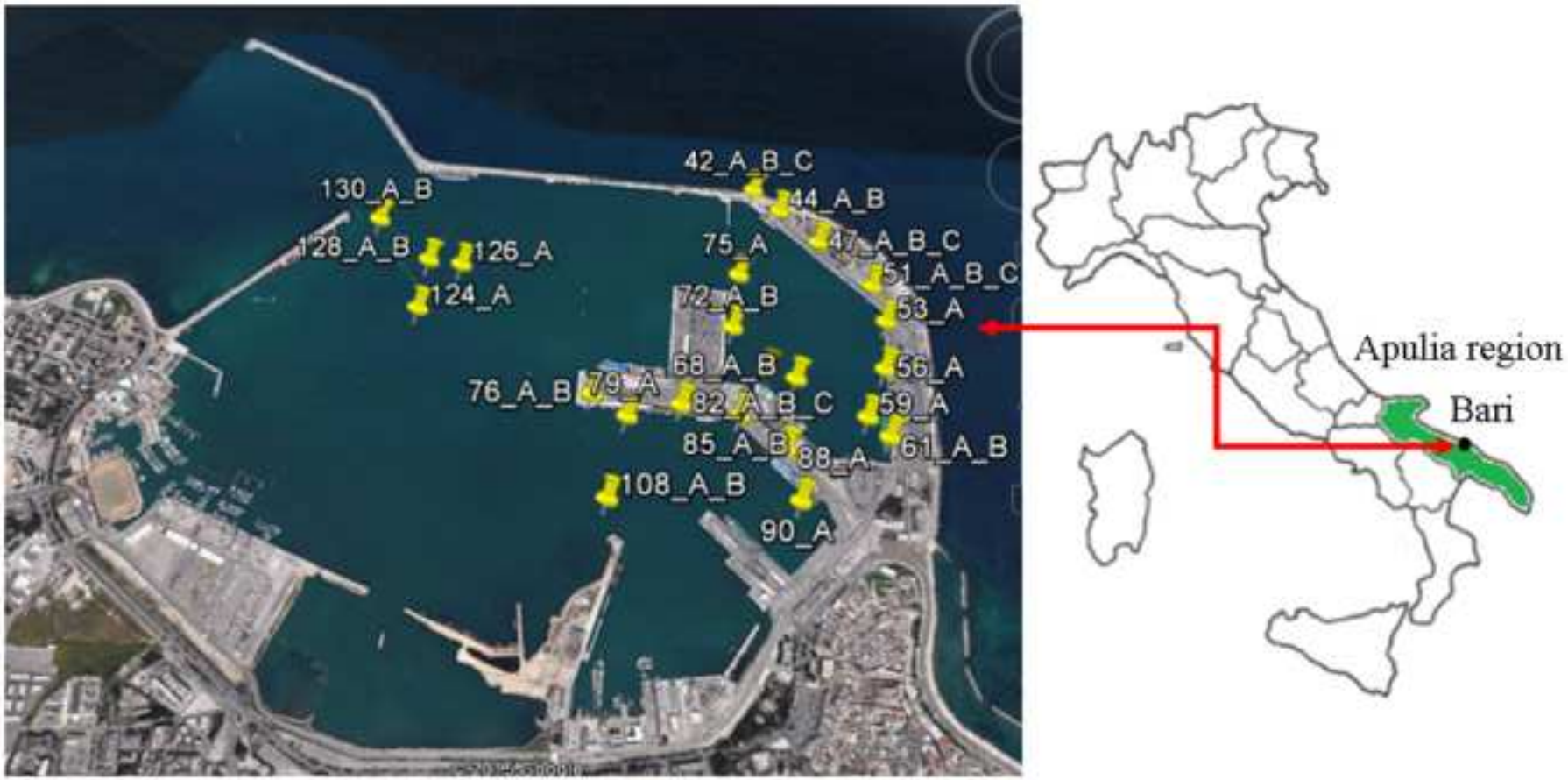


Figure 2

Click here to download high resolution image

