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# Long-Term Monitoring Programs to Assess Environmental Pressures on Coastal Area: Weighted Indexes and Statistical Elaboration as Handy Tools for Decision-Makers

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## Abstract

Data generated by long-term monitoring programs for coastal areas are intricate and require advanced tools able to identify the factors, natural or anthropogenic, responsible for the observed quality status. In the present study, data stemming from a 5-year monitoring programme of the Apulian coast were utilized for validating a speedy and comprehensive approach to assess the environmental quality of the marine-coastal area. Selecting 12 indicator contaminants controlling the pollution degree of the bottom sediments and attributing to each of them a weighted relevance according to their hazard potential, two multimetric indexes were calculated, helping to establish how and to what extent the selected contaminants could affect the achievement of good chemical and ecological status of coastal area. The relationships between calculated hazard degree values and the main natural factors loading on the study area were addressed through multivariate analyses. The variability of hazard degree values over time was explained by means of combined use of multivariate analyses and multimetric indexes, affording a handy method that allows to differentiate the role of natural factors, such as hydrodynamic and morphological features of the coastal track versus that of anthropogenic pressures. The combined approach adopted supports a reliable hazard assessment at long-term period and at a large spatial scale.

**Keywords**: coastal ecosystem, SQGs, multimetric indexes, long-term monitoring, multivariate analyses, hazard assessment.

## 1. Introduction

Marine ecosystems are extremely exposed to numerous disturbances that associated with the rapid development of industrial and urban activities, cause severe anthropogenic impacts produced by chronic or acute uncontrolled sources of pollution (Rombouts et al. 2013). In addition, natural factors, such as land inputs by hydrographic networks and morphological features of coastal area can often dramatically emphasize or mitigate the adverse effects that the pollution can cause to marine life due to their synergistic combinations with anthropogenic pressures. The main threats resulting by such pressures is the increase of the concentration of trace metals and persistent organic pollutants, as well as excess of nutrients that can cause deleterious effects on marine equilibria. This problem occurs especially in densely populated coastal areas, where land use is intensified and storm-water run-off, effluent discharges from industry and sewage treatment plants are constantly pressing.

In this context, the long-term monitoring program for marine-coastal environment can play a fundamental and irreplaceable role in studying current and future impacts. Analyses of the complex data set generated by such programs with appropriate tool can support distinguishing between impacts from natural factors and those caused by anthropogenic changes and fluctuations occurring over time.(Navarrete et al. 2010; Lohner & Dixon, 2013). A long-term monitoring program can support understanding of the vulnerability of marine habitats and, at the same time, can constitute useful tool for decision makers in designing efficient marine protection strategies at regional level. Introduction of the Water Framework Directive (WFD, 2000/60/EU), containing the broadest ranging and the most specific measure programme for water bodies, constitutes a legislative support for achieving this goal. Article 8 of the Directive emphasized the need of implementing comprehensive programs for the continuous monitoring of water bodies status, suggesting three levels of monitoring systems: *i)* surveillance monitoring; *ii)* operational monitoring and *iii)* investigative monitoring. All EU Member States, in compliance with WFD, have indeed enacted specific transposition laws to activate monitoring programs, delegating the Regional competent authorities for local application.

However, the long-term monitoring of marine-coastal water bodies is a very complex task due to unstable nature of coastal ecosystem affected by unpredictable point and/or diffuse pressures that, combined with hydro-morphological changes in water, can affect the ecological and chemical status of coastal ecosystem. This calls for reliable searching and identification of the main factors influencing the water quality.

It is well known that bottom sediments can be used as valuable indicators of the impact of human activity on the water bodies' quality, being they capable of accumulating both organic and inorganic pollutants over time. Several studies have considered the role of sediments in determining the fate of metals and organic compounds in seawater (Chapman D. 1996; Tomadin, 2000; Spagnoli et al., 2010; Mugnai et al. 2010; Lofu et al. 2016; Chapman et al. 2013; Mali et al. 2016). Moreover, the impact of hydrodynamic processes on the spatial distribution of pollutants in coastal areas has been addressed (Malcangio et al. 2017; Valentini et al. 2017; Mali et al. 2017a, 2017b; 2018). Evaluation of contaminant concentration in marine sediments is a major part of the assessment program for coastal area because it helps to define the hazard degree, to predict the potential threat of pollutants, and to identify the allocation of pollution sources (Morillo et al., 2004; 2008; Borja et al. 2008; Piva et al. 2011; Mali et al. 2017c). For this reason, great efforts have been made to establish Sediment Quality Guidelines (SQGs), using different calculation approaches (Chapman 1989; Del Valls et al. 1998; Wenning et al. 2005; Long et al. 2006; Chapman 2001; Chapman 2007; Ritter et al. 2008; Piva et al. 2011; Regoli et al., 2013, Gredilla et al. 2014; Zahra et al. 2014; Souza et al. 2016).

Among the principal approaches, we cite the index method and the model index method. The index method refers to substituting the actual pollutant concentrations into the mathematical formula to get the pollution indices: comparison of such indices with the corresponding assessment criteria thus gives the pollution degree. On the other hand, the model index method assesses metal pollutions by constructing very complex mathematical models. These models have some advantages than index method when processing the fuzzy boundary effect, but they require a lot of mathematical functions and cumbersome operations, which limits their applications. Thus, the index method is the most preferred one, especially for decision makers that need easy-to use tools for evaluating sediment quality.

In this paper, a handy method for evaluating the pollution degree in marine-coastal sediments is proposed as an integrative approach that can be considered a middle ground between index and

model index methods. The pollution degree assessment by the proposed index was indeed associated with multivariate analyses as wide-ranging tools for interpreting complex data generated by long term monitoring programs.

As case study, marine sediments of Apulia Region coast were investigated, using data obtained by a 5-year monitoring programme activated by the Italian Ministerial Decree (MD) n. 260/2010 Ministerial Decree, the national law transposing the Water Framework Directive (2000/60/EU) at Italian level. The proposed index is a modified version of the environmental Pollution Index (PLI, Tomlison et al. 1984) that in the new proposed version (cPLI) includes two new elements: a Chemical Hazard Index ($HI_{ch}$), that takes into account a revised Contamination Factor ($CF_{ir}$) and an Eco Toxicological Hazard Contribution ($HI_{tox}$), derived by bioassay responses.

The MD 260/2010 has defined Environmental Quality Standards for water bodies, used also for quality sediments in water-coastal bodies. Therefore, using the monitoring data and standards established in compliance with this MD, we calculated the hazard index considering only some the priority substances given by Directive 2013/39/EU (2013/39/EU) as principal contaminants in the field of water policy. We selected 12 contaminants as toxic indicators (6 metals and metalloids and 6 persistent organic pollutants), controlling the pollution status of the coastal waters and we tried to establish how these contaminants could affect the achievement of good ecological status of coastal marine water, attributing to each of them a weighted relevance on the sediment quality, according to their hazard potential. Furthermore, we calculated the relationships between the hazard index values and the main natural factors, such as hydrodynamic and morphological features of the studied coastal track, by using supervised multivariate statistical elaboration to get insight the contamination variability over time and to understand the role of natural and anthropogenic factors.

The novelty of this approach consists in reading the complexity of long-term monitoring program data by using Multivariate Statistical Elaboration and by performing handy multimetric and comprehensive indexes that consider weighted contribution of indicator contaminants measuring their chemical and eco-toxicological impact in the hazard degree. The idea is to propose a speedy, cheap and comprehensive approach for hazard assessment at long-term period and at a large spatial scale capable to improve the objectivity in defining the responsible contaminants within hot spots areas.

## 2. Materials and Methods

### 2.1. Hydrographical and geomorphological features of the study area

The southern Adriatic coastline investigated is extended on 370 km length, from Peschici (PE) in the Gargano promontory up to the Nature Reserve "Le Cesine" (CE) in Salento Peninsula. The coast is composed mainly of micritic and calcarenitic limestone and sands (Spagnoli et al. 2010; Caldara et al. 2013). The sediments contain mainly marine-derived carbonate as well as terrigenous fractions indicating highly heterogeneous composition dictated by different provenance and complex transport processes.

From a morphological point of view, according to the criteria defined in Apulia Coastal Regional Plan (Piscitelli at al. 2011), the analyzed coastal track includes four natural Physiographic Units (UPs), that constitute areas delimiting barriers against the longitudinal transport of solids (Figure 1a).

The northern UP (Physiographic Unit - UP1) extends from the Peschici-Vieste up to Head of Gargano Promontory. It is characterized by generally high cliff rocky coast, with predominantly calcareous sediments and siliceous calcareous rocks. In this track, there are widespread forms of marine and karstic erosion accompanied by cavities and caves.

The UP2 extends from the Head of Gargano Promontory (near Vieste), including the whole Gargano Gulf, heading until near Bari city coast. This coastal track is very heterogeneous with different morphological profiles due to the marine abrasion that acts in a selective form in different areas. Terrigenous filling material, transported to river estuaries affect the marine sediment composition. Different rivers characterized by torrential regime such as Candelaro, Cervaro, Carapelle, contribute with sandy silt sediments originated from the disintegration of limestone and siliceous calcareous rocks that make up the nearby hills inland. Furthermore, the UP2 includes the Ofanto River, one of the main watercourses of Apulia Region that, with its 170 km long, is the most significant river flowing into the Southern Adriatic Sea. Its hydraulic regime is not always constant, with discharges concentrated during the autumn-inter period.

The UP3 extends from the northern coast of the metropolitan city of Bari (BA) heading southward to Monopoli Beach (MA). The first track is constituted by a low sandy coast characterized by sandy beach rock and strongly diagenized limestone strips belonging to different sea terraces. The coastal tract near the metropolitan city of Bari is heavily modified by

large-scale artificial burying work and only short coastal tracks are preserved from human intervention. Several blades and small rivers of seasonal flow regime are also present in this area. The last track (among Polignano and Monopoli) is characterized by a high coast rocky that can end with a vertical wall (cliff) or with a sloping profile. In the first case they are associated with the presence of caves and with evidence of erosion phenomena.

The last physiographic unit investigated, the southern UP4, extents from Torre Guaceto (TG), getting through the coast of Brindisi city, until the Natural Protected Reserve Area, denominated "Le Cesine". The UP4 have two rivers that nourish with terrigenous sediment the coast (Great River and Small River). There are also several barrier works on watercourses. It is worth mentioning the Dam on clay on the Cillarese stream for the supply of water resources for industrial uses and the presence of Royal Channel that flows through different inland municipalities, enriching its watercourses with inland contribution. UP4 ends with sandy stretches often accompanied by the presence of marshes and retrodunal lakes (Fontanelle, Alimini) shallow beaches and dune beds with dunes even 10 m high.

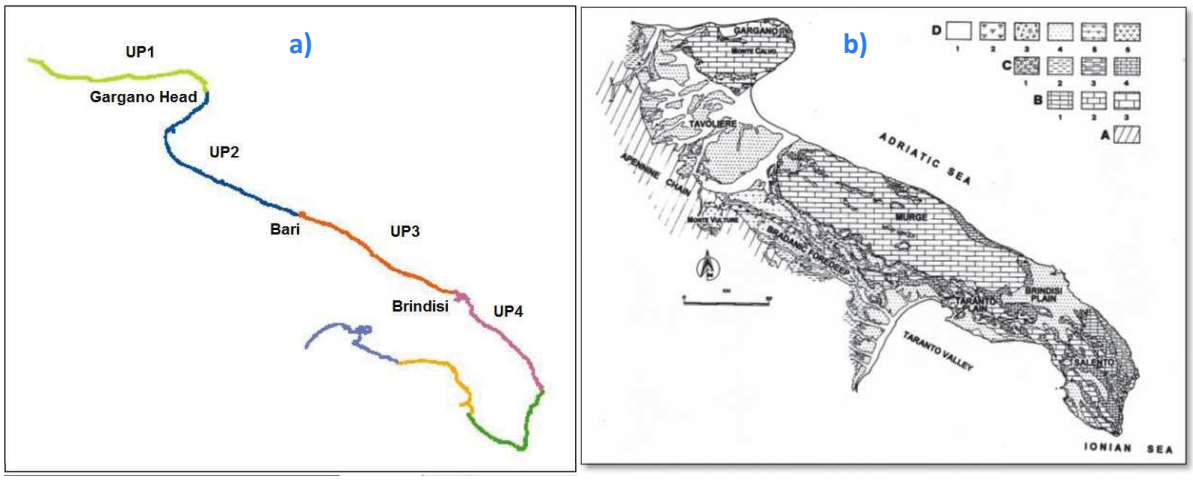



**Figure 1.** a) The Physiographic Unit (UPs) defined by the Regional Coastal Plan of Apulia Region.; b) the Geological features of Apulia platform according to Piscitelli et al, 2011.

### 2.2. Data collection

As required by the Italian Legislation transposing at national level the WFD (D.Lgs. 152/2006; D.M. 260/2010), the long-term monitoring programme for coastal area has been activated by Apulia Region and realized by the Regional Agency for Environment Prevention and Protection

175 (ARPA Puglia) starting from March 2010 and continued, on an annual basis, until April 2015.
176 Within this five-year temporal span, two types of monitoring programs were carried out: *i)*
177 surveillance monitoring (April 2010- March 2011); *ii)* operational monitoring (April 2012-
178 March 2013; April 2013 - March 2014 and March 2014 – April 2015). According to the Italian
179 D.M.260/2010, Regional Monitoring Programme includes Water Courses, Lakes/Reservoir
180 Waters, Transitional Waters and Marine-Coastal Waters. The present work was focused only on
181 Marine-Coastal Water Bodies (MCWB). The quality assessment of this category includes
182 analyses of water column, sediments and biota. To the purpose of the present paper, only
183 sediment analyses were considered.

184 **2.3. Sampling strategy and sediment sample handling**

185 A total of 144 surface sediment samples, collected from 70 coastal sites belonging to 35 marine
186 transects were selected. These transects belong to two different distances from the coast: 200/500
187 m and 1750 m, respectively. The location of the sampling sites with details of their longitudes
188 and latitudes are reported in Supporting Information (Table S1), while the sampling site
189 distribution is reported in Figure 2. Sampling sites are selected as representative of the
190 corresponding Apulian MCWB, as identified according to the Italian Ministerial Decree n.
191 131/2008 taking into account the local morphological and hydrodynamic features as well as the
192 anthropogenic pressures loading.

193 The surficial sediment samples were taken with a "van Veen" bucket having a sampling surface
194 of 0.10 $m^2$. In each station, three sampling buckets, corresponding to three replicates, were
195 collected. Adequate clean plastic jars with Teflon coated lids were used for storage and transport
196 of the samples to the laboratory conserved at 4 °C. Once in laboratory, the collected sediment
197 samples were freeze-dried, gently ground in an agate mortar trying to not alter the grain size
198 features, then passed through a 0.5 mm mesh sieve to remove debris and pebbles, and finally
199 stored at −20 °C prior to analysis.

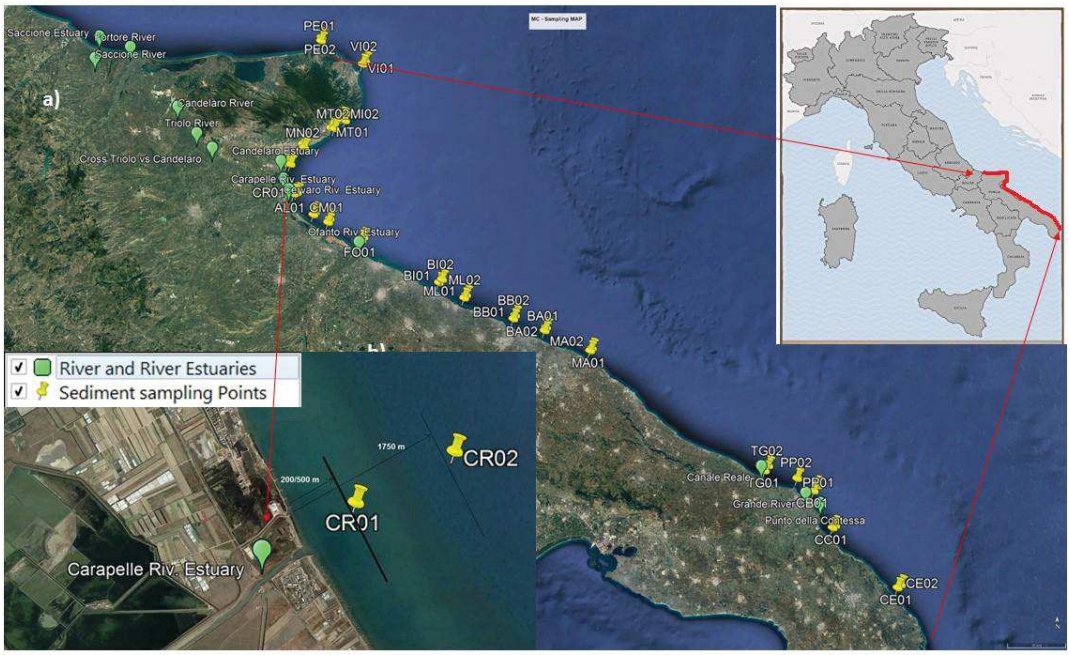



**Figure 2.** The sampling map; a) sediment sampling distribution and river estuaries location alongside coast, b) an inset showing a close-up of the two transects considered (01 and 02 for 200/500 and 1750 m from the coastline respectively)

### 2.4. Analytical methods

Each sample was classified according to Shepard (1954) into four sections: gravel: >2 mm; sand: 2–0.063 mm; silt: 0.063–0.002 mm; clay <0.002 mm. A set of ASTM sieves was used for the granulometric separation (Romano and Gabellini, 2001).

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, HNO3 and HF) (Pellegrini and Lucarotti, 2001). The < 63 μm fraction, dried at 105 °C, was used for the determination of metals in order to reduce the grain size effect. The detection limits (LODs) were calculated from 3 replicates of procedural blanks. The estimated LODs were equal to 1 ppb for all metals. Marine Sediment Reference Materials 2702 (Inorganics in Marine Sediment) were used to control the analysis quality: the agreement between the analytical results for the certified and measured values was satisfactory, with recoveries ranging from 80% to 100% for all metals.

The total nitrogen amount ($N_{tot}$) was determined by an elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer. 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 an elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer, after removal of carbonates by reaction with hydrochloric acid (Giani, 2001).

As for the five high molecular weight Polycyclic Aromatic Hydrocarbons (PAHs) congeners considered in this study, benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), Benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP); indeno[1,2,3-cd] (Ind) the analytical methods performed is according Ausili (Ausili 2000) that foreseen extraction with cyclohexane/methanol mixture and determination in High Pressure Liquid Chromatograph. The sum of Polychlorinated biphenyls (PCBs) congeners (28, 47, 99, 100, 153, 154) considered in this study was determined by the Cicero et al. method (Cicero et al. 2000) through extraction in acetone/petroleum solvent followed by analysis by Gas Chromatograph equipped with an Electron Capture Detector. Ecotoxicological tests (Microtox® SPT with Vibrio Fisheri, fertilization success bioassay with sea urchin Paracentrotus lividus, inhibition of a marine algal growth with *Dunaliella tertiolecta* were performed following procedures defined by Azur Environmental 1994, Onorati and Mecozzi, 2004; Lera et al. 2006; ISO 10253, 2006. All analyses were carried out in the ARPA Puglia (Apulian Regional Agency for Environmental Protection) laboratories.

### 2.5. Statistical analyses

Analysis of variance (ANOVA) was applied to perform the spatial and temporal distribution of contaminants and hazard degree in sediment samples. One-way ANOVA was used to identify the differences in the hazard levels in sediment due to their physicochemical properties and total contents of pollutants based on the relevant physiographic unit (UP). PLS-DA was used to optimize separation between samples belonging to different groups and to identify the main contaminants controlling each UP and each Grain Size classes. The PLS-DA is aimed at maximizing the covariance between the independent variables (in our case the UP and Grain Size features), and the corresponding dependent variable Y (concentration of contaminants). On the other side, Spearman correlation analysis was performed to identify the relationships between

249 the detected sediment properties, contaminant concentrations and hazard index values.
250 Differences were considered statistically significant only for that cases in which the $p < 0.05$.

251 Two different software were utilized for Statistical Analyses: Soft Independent Modeling of
252 Class Analogy (SIMCA) 10.2 for PLS_DA analyses and STATISTICA 10.0 for One way
253 ANOVA and Factorial ANOVA, Correlation Analyses and for normality-test of the raw and log-
254 transformed data. The SIMCA software was selected for its intuitive graphical interphase
255 allowing to extract the main information describing dataset variability, while the non-parametric
256 approaches of STATISTICA were selected as the most powerful tests in case of environmental
257 data for which distribution assumptions are violated due to the presence of outliers or to non-
258 normal distributions.

259 **2.6. Assessment of sediment contamination**

260 All Sediment Quality Guidelines (SQGs) developed in literature and dealing with environmental
261 concerns, provide a simple comparative mean for assessing the risk of contamination in an
262 aquatic ecosystem (Macdonald et al., 2000), mainly by comparing the chemical concentration of
263 individual pollutants with their corresponding limit concentrations. The most exploited and
264 internationally accepted indexes are those used for trace metal pollution assessment (Table 1),
265 widely used by researchers in environmental science (Salomon and Foster 1984; Zhang and Liu,
266 2002; Dassenakis et al. 2003; Spagnoli et al. 2008; Rath et al. 2009; Varol et al. 2011; Desaules
267 et al. 2012; Banu et al. 2013; Mali et al. 2015; Maanan et al. 2015, Zhang et al. 2017). The main
268 limitation of these indexes consists in not considering the potentiality of the combination effects
269 due to the coexistence of different contaminants (organic and inorganic) accumulated in the
270 sediments (Birch et al. 2018). These limitations become more dramatic when dealing with
271 complex matrix, such as marine sediments, affected by "matrix effect" that calls for
272 comprehensive pollution assessment able to consider simultaneously *i)* the level of
273 contamination; *ii)* the combination effects of different groups of contaminants (heavy metals,
274 organic pollutants and nutrients) and *iii)* the occurrence of natural factors that can emphasize
275 such effects.

| Multimetrix Index | Formula | Reference |
|---|---|---|
| **1. $I_{geo}$ (Geoaccumulation Index)** | $Igeo = Ig0 + \frac{Cn}{1.5 + Bn}$ where $C_n$ and $B_n$ are metal concentration in sediment sample and in the reference material, respectively. | Muller G., (1969) |
| **2. E.F. (Enrichment Factor)** | $E.F. = \frac{\left(\frac{Cx}{Bx}\right)sample}{\left(\frac{Cb}{Bb}\right)background}$ where $C_n$ and $B_n$ are metal concentration in sediment sample and in the reference material, respectively | Muller et al. (1974); |
| **3. RAC (Risk Assessment Code)** | $RAC = \frac{F1}{Ctot}$ where F1 is the percentage of metal fraction extracted by $CH_3COOH$ 0.11 M solution with respect to the metal total concentrations. | Perin et al. (1985); |
| **4. CF (Contamination factor)** | $CF = \frac{Cmetal}{Cb}$ where $C_{metal}$ is the metal concentration determined in the sample and $C_b$ is the reference values (according to the specific SQG considered) | Muller, (1979); |
| **5. PLI (Pollution load index)** | $PLI = (CF1xCF2xCF3x \ldots CFn)^{1/n}$ where CF Contamination Factor for each metal (see 4.) | Tomlinson et al. (1980); |
| **6. RI (Risk Index)** | $RI = \sum_{i=1}^{k}(Ei)$ where Ei is the ecological risk index for given metal (according $Ei = Ti\,x\,CF$); Ti is the toxicity response factor and CF the Contamination Factor (see 4) | Hakanson. (1980) |

276

277 **Table 1.** Sediment quality guidelines used globally.

## 3. Results

In this study, a modified Pollution Index (cPLI) is proposed, based on two levels of evidence: a Chemical Hazard Index ($HI_{ch}$), calculated through a revised Contamination Factor ($CF_{ir}$), that takes into account the chemical hazard and an Eco toxicological Hazard Contribution ($HI_{tox}$) derived by bioassay responses. The responses of cPLI index were associated with the results obtained by a new Ecological Risks (RI) to provide a comprehensive evaluation of chemical and ecological status of marine coastal area investigated.

### 3.1.1. Chemical Hazard Index for pollution degree assessment ($HI_{ch}$)

It is known that conventional contamination factor ($CF_i$), called also single-factor pollution index, can be obtained by dividing each contaminant concentration by a baseline value defined for each of them. As mentioned above, the Contamination Factor cannot reflect the whole pollution degree deriving comprehensively from various pollutants and is only applicable to a single factor pollution assessment. However, it can be the basis of environmental quality standards, as in our case. Our Chemical Hazard Index ($HI_{ch}$) takes as baseline values the environmental quality standards established by MD 260/2010 for coastal water quality. The novelty of this index ($HI_{ch)}$ stands in two aspects:

- it is a speedy index for a comprehensive chemical pollution evaluation since it considers only the main Priority Substances considered by Directive 2013/39/EU for water quality purposes (six metals and six persistent organic pollutants) selected as indicator contaminants controlling the chemical status of sediments;

- the modified Contamination Factor used to calculate the $HI_{ch}$, includes a "weighted" value, introduced by Directive 2013/39/EU for water quality assessment. This value ranges from 1 to 1.3, depending on whether the contaminant is not covered by the Directive (weight = 1), or, on the contrary it is included in the list of "priority" substances (weight = 1,1) or in the list of "dangerous and priority" substances (weight = 1,3), aiming therefore to attribute to each pollutant a proportional relevance on the chemical status of sediment according to their hazard potential.

Thus, the modified Contamination Factor ($CF_i$) for a single pollutant is calculated according to the formula (Eq.1):

$$CF_{ir} = \frac{C_{mi}}{C_{si}} \; x \; W_0 \qquad \text{(Eq. 1)}$$

where $C_{mi}$ is the concentration of the contaminant "*i*" determined within sediment samples and expressed in ppm ds, $C_{si}$ is the Environmental Quality Standard value defined for the given pollutant by the MD 260/2010 within WFD, and $W_o$ is the weighting factor attributed to each pollutant. The revised $CF_{ir}$ calculated in this way considers the contamination factor of the single element: when $CF_{ir}>1$, the substance is considered as contaminating or enriched; when $CF_{ir}\leq 1_i$, the substance cannot be considered as contaminating or (anthropogenically or naturally) enriched.

Subsequently, the specific chemical contamination degree ($HI_{ch}$) was obtained following Bebianno method for quality assessment of harbor sediments (Bebianno et al. 2015). The $HI_{ch}$ calculated according to Eq 2, is based on the average of all the $CF_{ir} \leq 1$, added of the sum of the $CF_{ir}$ of all contaminants displaying a $CF_{ir} > 1$ (aiming at stressing the contribution of the elements considered contaminants or anthropogenically enriched). In formula:

$$HI_{ch} = \sum_{j=1}^{k} \frac{(CF_{ir} \leq 1)}{k} + \sum_{i=1}^{n} (CF_{ir} > 1) \qquad \text{(Eq. 2)}$$

*k* is the number of $CF_{ir} < 1$ and *n* the number of $CF_{ir} >1$.

### 3.1.2. Toxicological contribution to the comprehensive hazard evaluation

The eco-toxicological results foreseen by MD 260/2010 were exploited for defining the toxicological contribution to the comprehensive Pollution Index. To this purpose, 144 sediment samples, were subjected to a bioassay battery of three biological indicators: bioluminescence of *Vibrio Fishery*, (in both elutriate and solid phase), inhibition of a marine algal growth (in our case *Dunaliella tertiolecta*, determined on the elutriate phase) and embryo-toxicity test with echinoderm *Paracentotus lividus*. The eco-toxicological results and the eco-toxicological classification according to the national legislation (expressed in EC20 and/or EC50 results) are given in Table S2 and Table S3, respectively. There are four toxic classes degree: Classes A that indicates "no toxic" response; Classes B for "moderately" toxic; Classes C for "high toxic" and classes D for "severe toxic" response.

It is known that each bio-indicator has a different pollutant sensitivity. The eco-toxicological results do not correspond to the chemical hazard levels, since the total concentration of contaminants is not the unique factor contributing to the toxicity response (Burton 2002; Wenning 2005). Many other factors need to be considered, most of them unpredictable. Therefore, in order to attribute to each sample results an eco-toxicological contribution to the comprehensive hazard evaluation according to the bioassay responses, we calculated a Toxic Hazard Coefficient ($HI_{tox}$) utilizing an approach similar to that adopted for chemical hazard degree, consisting in the following steps:

1. for each toxicological class defined by the Italian legislation normative (ICRAM 2009), an arbitrary coefficient "k" was given, ranging from 1 to 2.5: k = 1.0 for Class A; k=1.5 for Class B; k = 2 for Class C; k=2.5 for Class D.

2. An "eco-toxicological weighted factor", $W_{oe,}$ was given also to each result, as function of:

   a) the severity of the effect "En", intended as the severity of the biological damage measured by the specific end-point considered: En = 2.4 was given for bioluminescence; En = 2.1 was given for the inhibition of growth rate test; En = 1.9 was given for development rate test);

   b) the type of exposure T (acute or short-term T= 1 while T= 0.8 for chronic or long-term exposure);

   c) the type of test matrix M: M=0.8 when the elutriate phase was considered; M=1 when the sediment or solid phase was considered as matrix for eco-toxicological tests.

   d) a representative weight given for the potential hormesis for each experimental type. For the bioluminescence on solid phase and elutriate on Vibro-fisheri a representativeness weight W=0.25 is defined; for the inhibition of algal growth rate on elutriate a W=0.10 is considered, and for embryo-toxicity with *Paracentrotus Lividus* is given W=0.15.

   The above reported values given to elements constituting the weighting factor $W_{oe}$ comply with what proposed by Piva et al., (Piva et al. 2011). Actually, these are taken as guidelines in the most recent Italian National Laws on Sediment Handling (D.Lgs. 172/2016 and 173/2016) for the integrated quality assessment of dredged sediments.

3. With the above information, the $HI_{tox}$ contribution was calculated according to the following linear formula:

$$Woe_i = En_i \, x \, T_i \, x \, M_i x \, W_i$$

$$HI_{tox} = \sum_{i=1}^{j} (k_i \, x \, Woe_i) \quad \text{(Eq. 3)}$$

where, $k_i$ is the coefficient given to each of the four-classes. *En*, *T* and *M* and *W* are the weighting factor values given to the severity effects (En), type of exposure (T) , type of text matrix (W) and the environmental representative of the tested Matrix (M).

### 3.1.3. Comprehensive pollution Index (cPLI)

With the abovementioned results, the comprehensive modified Pollution Index (cPLI) can now be calculated by considering chemical characterization ($HI_{ch}$) and toxicological contribution ($HI_{tox}$), as Levels of Evidence according to the formula:

$$cPLI = HI_{ch} * HI_{tox} \quad \text{(Eq. 4)}$$

where $HI_{ch}$ is the chemical hazard Index of Eq 2 and $HI_{tox}$ the Toxicological Coefficient defined by Eq. 3.

The comprehensive cPLI is conceived to measure marine environmental impact integrating chemical and eco-toxicological results, being therefore a practical, reliable and predictive tool for assessing sediment quality.

## 3.2. Potential ecological Risk Index for comprehensive pollution degree

Aiming at assessing the ecological risk degree and comparing its estimation ability with those of the cPLI, a revised ecological Risk Index (cRI) was implemented using formulae reported in Eq.5 and Eq. 6, modifying what originally proposed by Hakanson (Hakanson, L. 1980).

$$cE_i = T_i \, x \, CF_{ir} \quad \text{(Eq. 5)}$$

$$cRI = \sum_{i=1}^{n} (T_i \, x \, CF_{ir}) \quad \text{(Eq. 6)}$$

where $cE_i$ is the individual risk for each contaminant, $CF_{ir}$ is the revised contamination factor that considers the weighted factor for each contaminant, $T_i$ is the toxic factor requirement on the individual contaminant defined by Hakanson. (Hakanson, 1980) and $n$ is the number of the pollutants considered in the calculation of the potential ecological Risk Index. The cRI calculated according to this approach allows to assess the pollution status in sediment, combining the environment effects with ecological toxicity, providing thus a better evaluation of the potential risk of contamination with the index level.

Some consideration should be made before discussing the reliability of the hazard assessment according to the cPLI and cRI indexes:

1. the formulae for calculation of each pollutant toxic factor requirement, $T_{i,}$ were those defined by Hakanson, therefore the Ti values are: $T_{Hg} = 80$; $T_{Cd} = 30$; $T_{As} = 10$, $T_{Pb} = 5$; $T_{Ni} = T_{Cr} = 2$; $T_{PCB} = 40$.

2. the value of $T_i$ of PCB was selected as representative for the other lipophilic toxic substances considered in this study. Indeed, being the organic pollutants included in the same list of Priority substances, the value $T_i = 40$ given for PCB is considered as appropriate also for BaP (Benzo(a)Pyrene), BbF (Benzo(b)Floranthene), BghiP (Benzo[ghi]perylene) , BkF (Benzo(k)Floranthene), InD (Indeno, 1.2.3 c.d.pyrene).

3. the evaluation of Risk Index (cRI) and Pollution index (cPLI) includes only the priority Substances given by European Decree (39/2008/CE) for water bodies quality namely 6 metals/metalloids and 6 organic pollutants (Sum of for congeners of PCB and 5 high molecular PAHs). Therefore, the discussion about the hazard risk evaluation is referred to the presence of these contaminants (As, Hg, Cd, Pb, Cr, Ni, Sum of PCB, BaP, BbF, BghiP, BkF, InD). Nevertheless, being these contaminants considered by the Water Framework Directive as the main “weighted” pollutants to be monitored in the long term programs for water quality, the assessment results can also be extended to other contaminants having the same behavior in coastal ecosystem;

4. since some physico-chemical properties of the surficial sediments are very important, especially when the sensitivity of different marine basins was considered, we used the organic content (TOC) and grain size distribution as necessary co-parameters in the calculation of Risk Indexes (see the discussion below).

Table 2 includes the grades of potential pollution degree and potential ecological index for environment.

**Table 2.** Potential pollution degree of cPLI and cRI according Tomlinson et al. 1980 and Hakanson, L. (1980) respectively, proposed in Maanan et al. 2015.

| ***cPLI*** | ***Comprehensive Pollution Hazard degree as*** $(HI_{ch} \cdot HI_{toc})$ | ***cRI*** | ***Potential ecological risk degree as*** $\Sigma Ei \cdot Ti$ |
|---|---|---|---|
| *cPLI<1.9* | Low hazard degree | cRI<95 | Low risk |
| *1<cPLI<2.8* | Moderate hazard degree | 95<cRI<190 | Moderately risk |
| *2.8<cPLI<6.5* | Considerable Hazard degree | 190<cRI<380 | Considerable risk |
| *cPLI>6.5* | High hazard degree | cRI>380 | Very high risk |

### 3.3. Comparative assessment of different hazard indexes

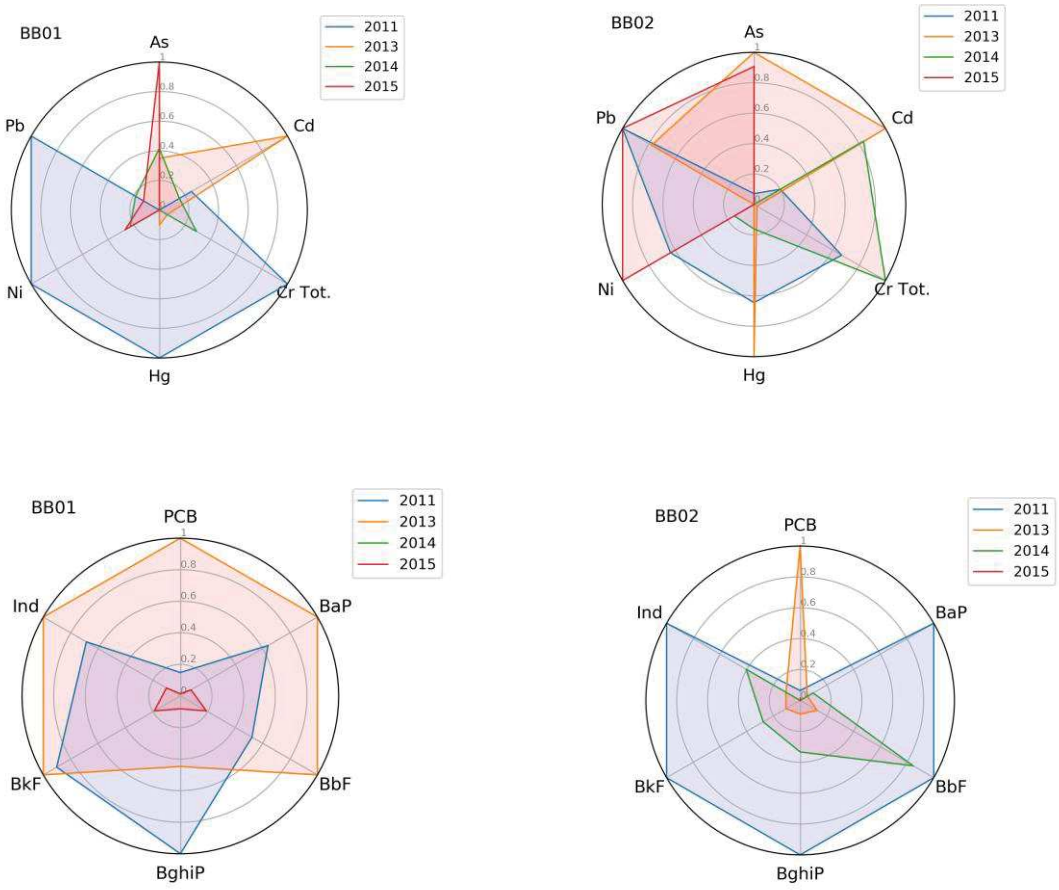
We compared the results obtained with cPLI and cRI with those achieved with the cumulative index c-NWAC (SWRCB, 2006; Mali et al., 2016) and mean ERM quotient (mERMq), respectively. In particular, c-NWAC considered different classes of contaminants (heavy metals and organic pollutants) while mERMq has been applied to determine the possible biological effect of contaminant toxic groups (Long et al. 2006). The hazard degree classifications of the two indexes are reported in Table S4.

## 4. Discussion of results

### 4.1 Chemical contamination factors

The basic statistical parameters and the measured concentrations of pollutants are shown in **Table 3**. The distribution of the metal and organic pollutant concentrations during different years are illustrated through radial graphs as reported Figure 3. Comparing the concentration of contaminants with the Environmental Standard Values (simple contamination factor, $CF_i$) revealed that As, Cd and Ni are the pollutants of most concern, followed by Hg, BaP, BkF since for all of them CFi>1. The respective hazard degree for these pollutants indicates hazard levels ranging from “moderate” to “high”. In detail, 28% and 6% of samples display respectively

436 “moderate” and “high” hazard degree for Arsenic. The contamination factor of Cd shows “low”
437 to “moderate” hazard degree for 18% of sediment samples, while the $CF_i$ of Ni reaches “low” to
438 “moderate” hazard degree in 10% of samples. As to Hg, even if for only two specific sites, the
439 hazard degree resulted that of the most concern, with value of $CF_{Hg}$=8 in ML site in 2013 and
440 $CF_{Hg}$= 2 in VL site in 2014, indicating a “severe” hazard degree in these points. As to the organic
441 pollutants, only for BaP and BkF and in some specific cases, “low” or “moderate” hazard degree
442 was registered (respectively for 2% and 3% of the samples). For the sum of PCB and other
443 organic contaminants, no risk level was observed.




444
445
446

447 **Figure 3.** The distribution of the normalized concentration of six metals and six organic
448 pollutant considered, determined within sediments of one of the UP3 sites, named Bari Balice
449 (BB01; BB02), during the monitoring period.

450

451 **Table 3.** Basic Statistic Data of the concentration of contaminants during investigated years. The concentration of $N_{tot}$, $P_{tot}$ are expressed in mg/kg
452 d.s.; the concentration of As, Cd, $Cr_{tot}$, Hg, Ni, Pb are expressed in ppm; the concentration of organic contaminants are expressed in ppb.

| Year | 2011 | | | | 2013 | | | | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *MEAN* | *MIN* | *MAX* | *S.D.* | *MEAN* | *MIN* | *MAX* | *S.D.* | *MEAN* | *MIN* | *MAX* | *S.D.* | *MEAN* | *MIN* | *MAX* | *S.D.* |
| TOC | 542 | 100 | 1290 | 339.36 | 491 | 70 | 1470 | 387 | 475 | 100 | 1700 | 398.84 | 394 | 100 | 1300 | 353.71 |
| Coarse (%) | 3 | 0 | 25.2 | 6.06 | 5 | 0 | 42.1 | 10 | 6 | 0 | 32 | 10.13 | 6 | 0 | 33 | 10 |
| Sand (%) | 67 | 4.17 | 100 | 30.20 | 74 | 17 | 100 | 25 | 78 | 3.5 | 100 | 23.29 | 83 | 21.9 | 100 | 21 |
| Fine Fraction (*Pelite*) (%) | 30 | 0 | 95.83 | 31.71 | 20 | 0 | 83 | 25 | 16 | 0.0001 | 94.5 | 24.39 | 12 | 0.0001 | 78.2 | 21 |
| TOC (%) | 0.542 | 0.1 | 1.29 | 0.34 | 0 | 0.07 | 1.47 | 0 | 0.475 | 0.1 | 1.7 | 0.40 | 0.394 | 0.1 | 1.3 | 0.35 |
| $N_{tot}$ | 187 | 5 | 638 | 214 | 1067 | 15 | 9362 | 1980 | 1085 | 150 | 5250 | 1442.49 | 140 | 39 | 535 | 128 |
| $P_{tot}$ | 606 | 88 | 2700 | 521 | 281.16 | 22 | 1718 | 305 | 460 | 17.2 | 1381 | 361.86 | 225 | 37 | 650 | 151 |
| As | 10.37 | 2.05 | 19.86 | 4.88 | 15.36 | 1.63 | 69.9 | 16 | 7.30 | 0.1 | 23 | 7.27 | 12.46 | 0.30 | 40.00 | 11.28 |
| Cd | 0.17 | 0.05 | 0.43 | 0.13 | 0.25 | 0.02 | 0.67 | 0.17 | 0.14 | 0.025 | 0.3 | 0.08 | 0.08 | 0.03 | 0.20 | 0.04 |
| Cr Tot. | 23.86 | 3.06 | 70.46 | 18.62 | 12.68 | 1.69 | 46.4 | 10.73 | 14.21 | 0.90 | 57.00 | 12.86 | 9.96 | 2.04 | 34.29 | 8.30 |
| Hg | 0.04 | 0.00 | 0.26 | 0.06 | 0.02 | 0.005 | 0.1 | 0.02 | 0.11 | 0.01 | 2.40 | 0.40 | 0.07 | 0.00 | 0.60 | 0.12 |
| Ni | 19.05 | 2.15 | 42.65 | 12.85 | 10.54 | 0.255 | 46.5 | 10.26 | 7.65 | 0.20 | 30.00 | 7.71 | 9.28 | 1.10 | 30.66 | 7.43 |
| Pb | 10.65 | 0.55 | 31.44 | 7.53 | 6.07 | 1.15 | 18.99 | 4.30 | 6.28 | 2.00 | 15.00 | 3.55 | 8.41 | 1.90 | 23.20 | 5.15 |
| Sum PCB (28-47-99-100-153-154) | 0.06 | 0.01 | 0.19 | 0.04 | 0.08 | 0.01 | 0.67 | 0.15 | 0.05 | 0.02 | 0.08 | 0.03 | 0.02 | 0.01 | 0.10 | 0.02 |
| benzo(a)pyrene | 9.45 | 0.26 | 62.00 | 14.46 | 5.82 | 2.50 | 26.30 | 4.07 | 3.63 | 0.50 | 17.00 | 2.99 | 4.61 | 2.50 | 15.50 | 3.46 |
| benzo(b)fluoranthene | 5.95 | 0.17 | 39.00 | 7.64 | 5.41 | 2.50 | 11.30 | 2.26 | 4.22 | 0.50 | 19.00 | 4.06 | 4.59 | 2.50 | 21.16 | 4.06 |
| benzo(ghi)perylene | 8.08 | 0.17 | 41.50 | 9.74 | 6.25 | 2.50 | 13.50 | 3.14 | 3.64 | 0.50 | 12.00 | 2.59 | 4.43 | 2.50 | 13.22 | 2.75 |
| benzo(k)fluoranthene | 6.22 | 0.24 | 34.40 | 7.53 | 4.93 | 2.50 | 11.40 | 1.81 | 3.15 | 0.00 | 9.00 | 2.34 | 5.27 | 2.50 | 28.82 | 5.90 |
| indeno(1,2,3-cd)pyrene | 6.68 | 0.28 | 33.40 | 7.59 | 6.06 | 2.50 | 20.19 | 3.53 | 3.46 | 0.50 | 12.00 | 2.52 | 4.10 | 2.50 | 10.90 | 2.20 |

453 Considering the "weighted" contamination factors ($CF_{ir}$), the situation become more critical. For
454 As, being the $W_0$ as high as 1.1, the percentage of samples with "moderate" risk passed from 28
455 to 34%, increasing the number of samples with "high" hazard degree that reach 7% of total
456 samples. The same consideration for Cd, having a $W_0$=1.3, for which the percentage of samples
457 presenting "low "and "moderate" increase substantially, passing from 10% to 23%. For Ni
458 ($W_0$=1.1) the samples reaching the "low to moderate" risk pass from 10% to 22%. Also the
459 number of samples having "high" hazard degree for Hg increased as did the percentage of
460 samples with "moderate" degree due to BaP and BkF ($W_0$=1.3).

461 The comprehensive pollution risk calculated according to cPLI, indicates that almost all sites
462 were interested by different pollution potential, starting from low, moderate and severe degree,
463 as reported in Figure 4. The highest cPLI values were observed near the Molfetta coastal track
464 (at ML01 site with cPLI=22) alongside UP3, while the lowest value of 0.18 registered in CE02
465 (Cerano coastal track) in the southern UP4. The Pollution degree varies also during the different
466 years considered. The temporal hazard distribution along years indicates the persistence of hot-
467 spot area, especially those located near river estuaries (CR and FO, respectively close to
468 Candelaro river estuary and Ofanto river Estuary) and near the highly populated centers, (ML,
469 BB, BA, respectively for Molfetta, Barletta-Bisceglie and Bari coasts). By analyzing the nutrient
470 distribution, it is apparent that the sites close to coastal cities registered also high concentration
471 of $N_{tot}$, $P_{tot}$, TOC, probably due to the municipality wastewater discharges and run-off from
472 agriculture areas, which are widely occurring in the area.

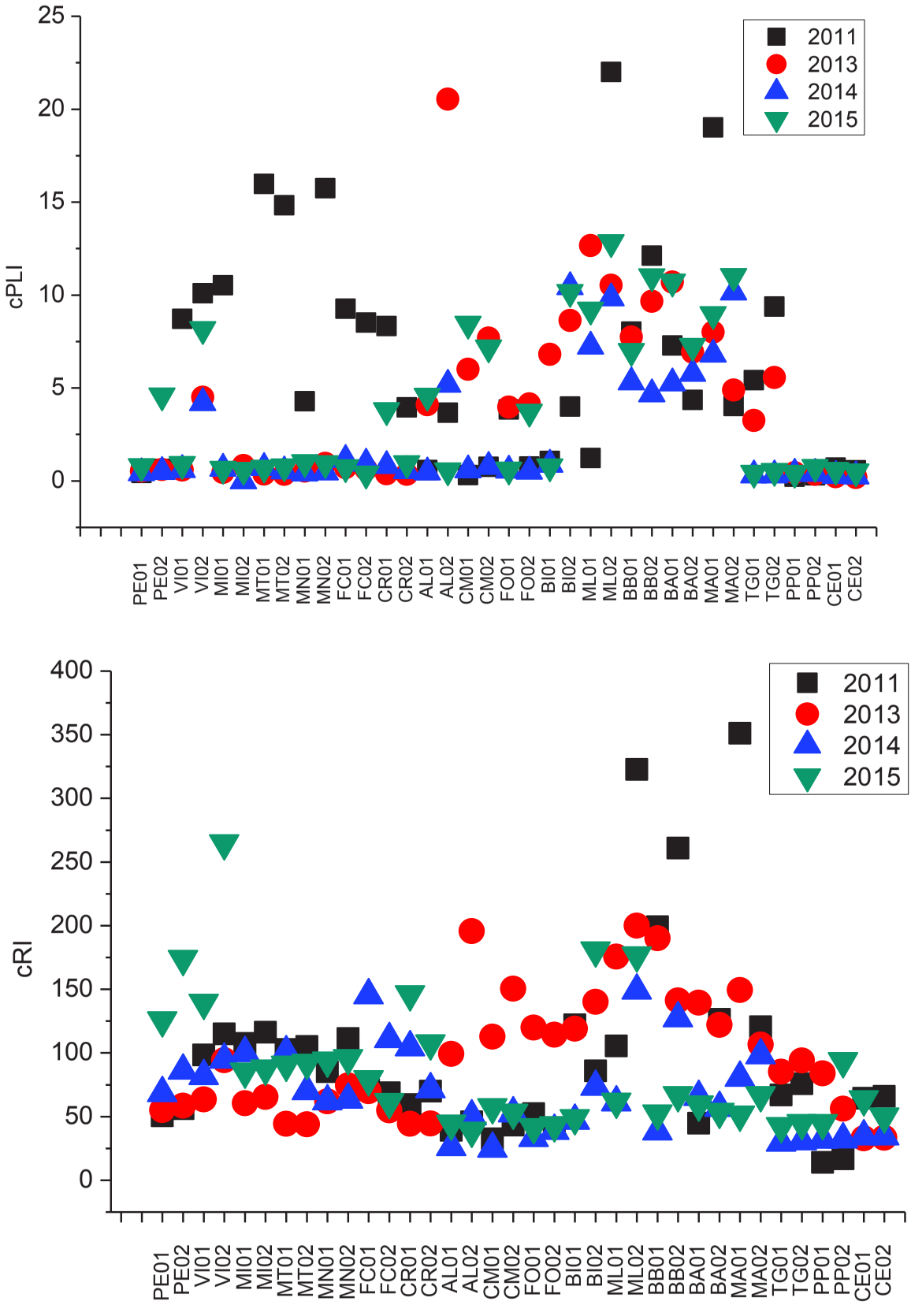



**Figure 4**. cPLI (a) and cRI (b) values in sediment samples during the monitored years.

476 Results deriving by the analyses of Risk Factor (cRI) values, confirm the pollution assessment
477 made according to cPLI. Nevertheless, the comparison pointed out the most severe evaluation
478 made by cPLI that considers both levels of evidence (chemical and toxicological) with respect to
479 cRI that takes into account the toxicological contribution, suggesting a greater objectivity of the
480 comprehensive pollution assessment (cPLI) based on chemical and toxic potential.

481 As to the single contaminants, analyzing the $E_i$ values for the individual ecological risk, it was
482 found that Cd, Hg and BkF result the contaminants of most concern, demonstrating the severe
483 toxicity associated to such contaminants. Nevertheless, it needs to be specified that for Hg only
484 some samples registered a high ecological risk. In general, the order of risks for the contaminant
485 analyzed through individual $E_i$, is the following:

486 Cd>Hg>BkF>As>BaP>BbF>BhgiP>InP>Pb>Ni>Cr>PCB

487 showing the highest individual ecological risk for Cd, Hg, BkF and As and the lowest ones for
488 Pb, Ni, Cr and sum of 5 congeners of PCB.

489

490 **4.2 Spatial and temporal contamination trend by ANOVA and PCA/PLS**

491 The spatial and temporal contamination trend was assessed through the combination of two
492 statistical elaboration, PCA/CA and ANOVA that are considered two complementary techniques
493 for apprehending the impact of multi-sources and multi-factors acting simultaneously in the
494 spatial contamination pattern. The importance of the combination of these two techniques was
495 previously explained (Mali et al. 2017c).

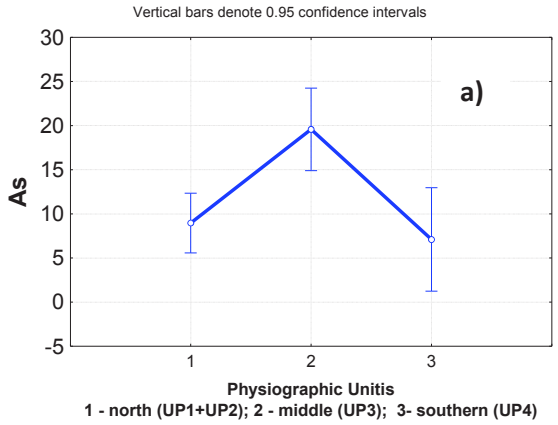
496 **4.2.1 ANOVA responses**

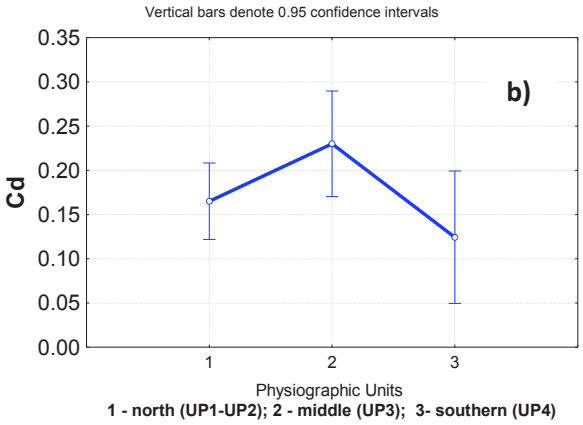
497 Plotting the cPLI and cRI values during different years and within different physiographic units
498 (Figure 3a, b) shows a variable trend of the hazard and ecological risk, that seems to decrease on
499 going from 2011 to 2014, when it starts to increase. The trend confirms also the persistence of
500 some hot-spot areas during years. Aiming at analyzing more deeply these differences, trying to
501 understand the influence of different hydrodynamic and morphological features of the coastal
502 area in the contaminant trend, one-way analysis of variance (ANOVA) was performed.
503 Homogeneity of variance was tested by Levene's Test, and post-hoc comparisons (Tukey HSD
504 test) were applied to discriminate between the means of values. Indeed, the one-way ANOVA

505 evaluates the variance of contaminant concentration in sediments within each class characterized
506 by an independent factor (in our case the belonging to one of the UPs). Outputs of the ANOVA
507 analyses are the degrees of freedom (df), sums of squares (SS), mean squares (MS) and the F
508 value for each independent variable considered. The F-value is the ratio between the mean of a
509 dependent variable (in our case the concentration of contaminants or hazard and risk level)
510 within each class of independent factor (UP) with the mean of the same variable in all dataset.
511 The F value is usually associated with p-value that measures the goodness/power with which the
512 analyses can verify or reject the null hypotheses, namely, that there is no difference between the
513 mean variable within each level of UP: thus the mean variable is μUPa= μUP3= μUP4 (where
514 $UP_a$=UP1+UP2). An estimated probability (p) lower than 0.01 and F>1 means that the
515 independent factor selected makes a significant difference in the variable (*i.e.* contaminant
516 concentration).

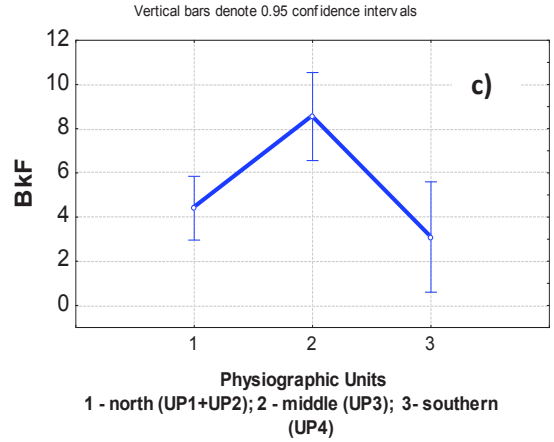
517 The ANOVA results confirmed the significant differences within the physiographic units
518 investigated, both in terms of cluster of prevalent contaminants for each UP and in terms of
519 average of contaminant concentration and hazard levels within each UP. As shown in Figure 5
520 a÷i, As, Cd and almost all the organic pollutants are the most relevant contaminants for UP3, in
521 which they display high levels of average concentration with respect to the northern coastal
522 track, $UP_a$ ($UP_a$=UP1+UP2) and to the southern UP (UP4). In addition, $UP_a$ and UP3, display
523 similar concentration trend of Ni, Cr and Pb, contrary to what happened for UP4.

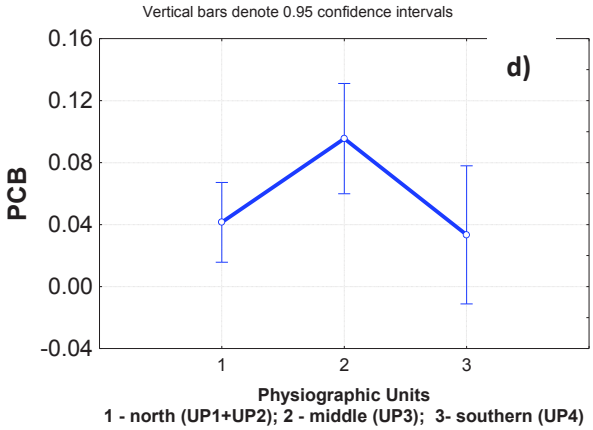
524


Vertical bars denote 0.95 confidence intervals
As
a)
Physiographic Unitis
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

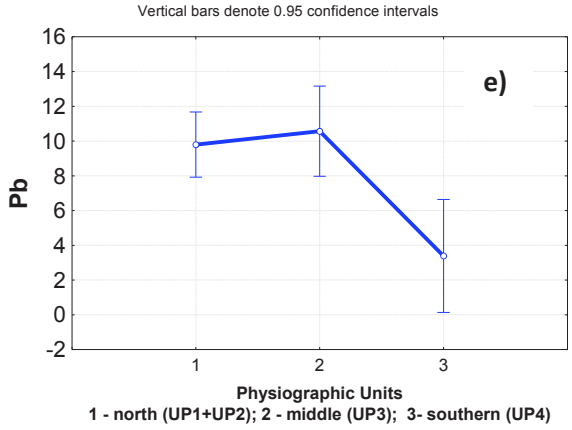

Vertical bars denote 0.95 confidence intervals
Cd
b)
Physiographic Units
1 - north (UP1-UP2); 2 - middle (UP3); 3- southern (UP4)

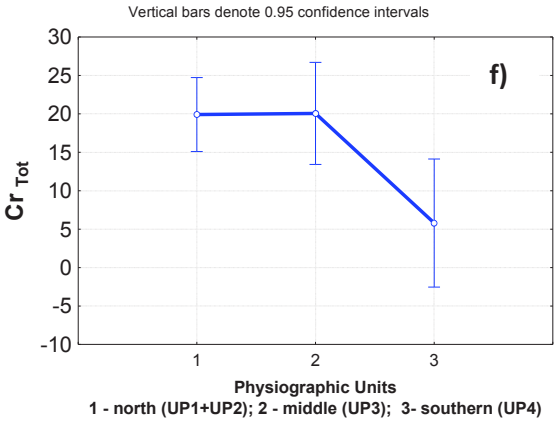
525


Vertical bars denote 0.95 confidence intervals
BkF
c)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)


Vertical bars denote 0.95 confidence intervals
PCB
d)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

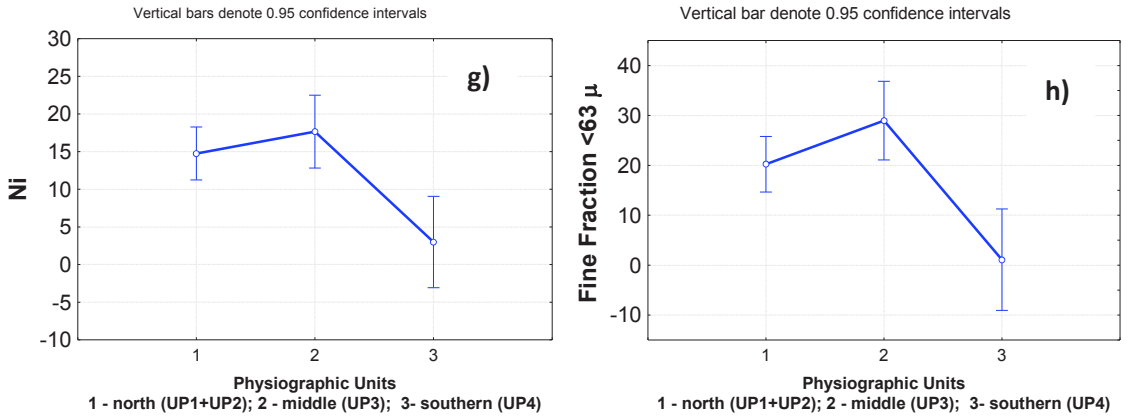
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Vertical bars denote 0.95 confidence intervals
Pb
e)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

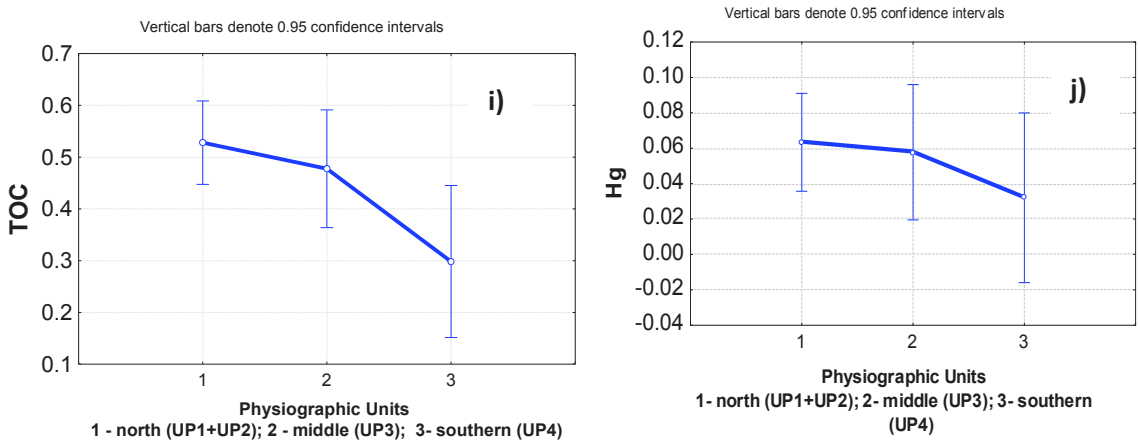

Vertical bars denote 0.95 confidence intervals
Cr Tot
f)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

527

528




529




530 **Figure 5.** Concentration of As (a), Cd (b), BkF (c), PCB (d), Pb (e), $Cr_{tot}$ (f), Ni (g), Fine fraction
531 (h), TOC (i) and Hg (j) in the investigated Physiographic Units.

532 As to UP4, all contaminants show low concentration levels, except for total Nitrogen ($N_{tot}$) that
533 reaches very high concentration levels. The analyses of the distribution of $N_{tot}$ during the years
534 within different UPs by means of factorial ANOVA (Figure 6) showed that the highest
535 concentration of total nitrogen was registered during 2013. This suggests that the presence of the
536 stream of "Canale Reale" (Royal Channel) (near sites TG1 and TG2) might cause the spike
537 concentration verified during 2013. In addition, the long-term monitoring of the excess of
538 nutrients demonstrates also the slow capacity of the ecosystem to naturally absorb the $N_{tot}$
539 contamination, as shown by the persisting of contamination for at least two years after the 2013.

540

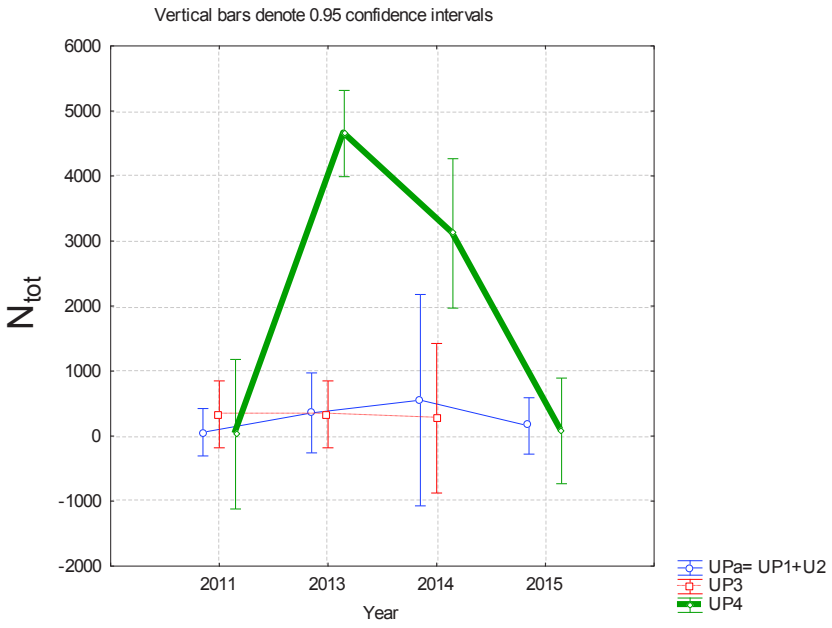


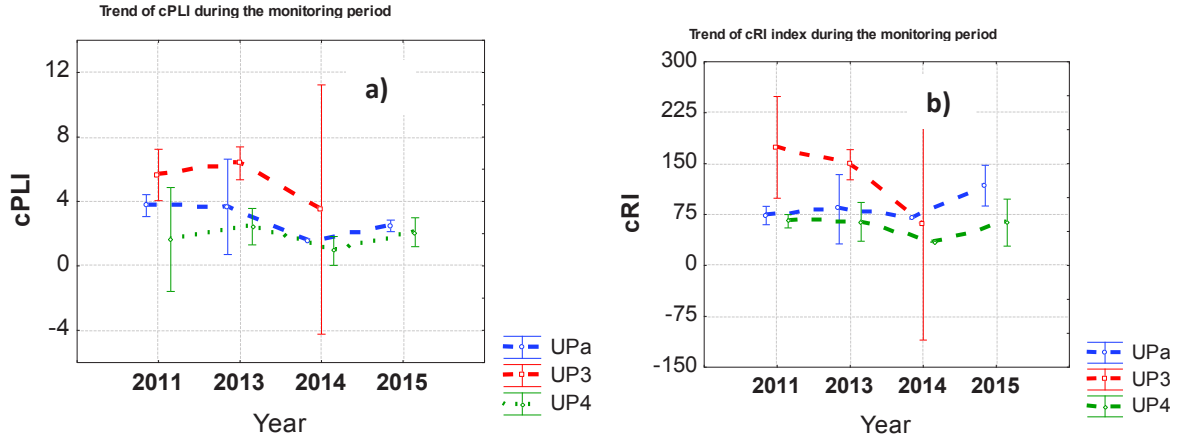

**Figure 6.** Distribution of total Nitrogen ($N_{tot}$), (calculated as unweighted mean) at different years and different UPs, performed by two-way ANOVA considering the year, as independent Factor A (Year including four classes: 2011 – 2013 -2014 -2015) and the three UPs considered (UPa=UP1+UP2) reported in blue, UP3 (in red) and UP4 (in green) as independent Factor B.

The one-way ANOVA was performed also to understand the influence of grain size in contamination degree, considering thus the content of Fine Fraction (∅ < 63 μm) as independent factor. Two classes were comprised in this case: Class A which includes samples with a content of finest sediment less than 50%; Class B which includes samples with fine fraction content more than 50%; Nevertheless, in this case the variance of contaminant concentration in the sediments within the two classes of grain size, demonstrated that there is no significant difference between the concentration of all six organic pollutants considered. The differences resulted significant only for three metals: $Cr_{tot}$ (F=19.48 p=0.000), Ni (F=30.24, p=0.000) and Pb (F=18.69; p=0.000) and for nutrients TOC (F=15.55, p=0.000), $P_{tot}$ (F=9.44 p=0.002). This circumstance confirms the importance of grain size (textural features) and organic content as carriers for lithogenic trace elements (Loring D.H. 1991; Covelli and Fontolan, 1997; Mayer, L.M., 1993; Dung et al. 2013; Mali et al. 2015; 2017c).

In order to understand the spatial and temporal trend of Pollution index (cPLI) and Risk Index (cRI) we performed two-way factorial ANOVA considering the Physiographic Unit as

561 independent factor A, and the different years as factor B (Figure 7). Also in this case the
562 differences resulted significant (except for the UP3) at $p<0.0001$. It was found that the average
563 values of the Risk Index for the UPa, which include both UP1+UP2 (countersigned in blue in the
564 graphs) and UP4 (green) indicate "low" ecological risk degree, while the Risk Index values for
565 the UP3 resulted the highest registered in the whole coastal track. A more marked difference
566 among the three UPs is observed by the analysis of the cPLI values that confirmed that the most
567 polluted sites belong to UP3 and the less polluted ones are those of UP4. In addition, cPLI
568 analysis revealed that $UP_a$ and UP4 areas, both less polluted than UP3, do not share the same
569 level of risk, being the $UP_a$ of higher concern respect to UP4. ANOVA tests confirmed also the
570 descending trend for hazard/risk revealed from 2011 to 2014 within the three UPs and the
571 increasing trend from 2014-2015 for the northern UPs and UP4.[1] It is necessary to highlight the
572 high extension of vertical bars at 0.95 confidence level of the mean values of the cPLI and cRI,
573 that demonstrates a high variability of the hazard level registered during 2014, indicating the
574 persistence of hot-spot sites within the UP3 (countersigned in red) during the monitoring period.

575




576

577

578 **Figure 7**. The cPLI (a) and cRI (b) trend in the sediments samples during the monitoring period
579 within the three UPs according factorial two-way ANOVA considering as Factor A the UP
580 displayed in the ordinate with three UP classes (UPa =UP1+UP2), UP3 and UP4) and, as factor
581 B, the Years displayed in abscissa, with four classes ("2011", "2013", "2014" and "2015").

[1] The mean values for UP3 did not present significant differences during 2015, therefore, the mean results of the dependent variables for this UP in 2015 was not calculated



b)

582 **4.2.2 PLS-DA responses**

583 Multivariate analyses, both supervised and unsupervised, are largely utilized in environmental
584 studies (Otto M. 1998; Kowalkowski et al. 2006; Astel et al. 2008). These methods allow to
585 extract the main orthogonal contributions (principal components) explaining most of the variance
586 of the dataset facilitating an overview of the environmental status of a given area scrutinizing
587 only the main responsible contaminants. This study was completed by performing a Partial Least
588 Squares Discriminant Analysis (PLS-DA) to identify clusters of contaminants that can
589 successfully discriminate the classes considered which, in our case, are the physiographic units.
590 PLS-DA method, which is a combination of PLS regression (PLSR) with discrimination rules
591 designed for classification (Ballabio & Consonni, 2013), helps to identify PLS components
592 which uncover the main covariation pattern within and between data matrices *X* and *Y*.

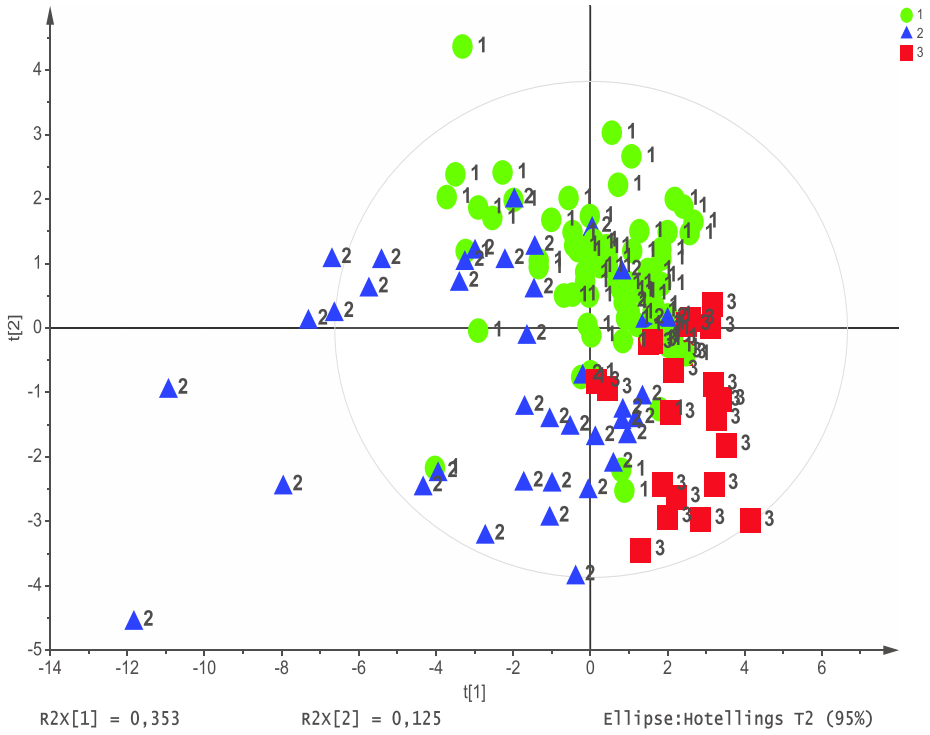
593 A model with four PLS components was considered, covering 75% of the total co-variance, as
594 shown in Figure 8. The validation success rate (SR) achieved for the classification is 81.25%, a
595 good result in environmental studies. The Score and Loading plots graphs for PLS1/PLS2 are
596 reported in the Figure 8. (Figure S1 shows plots for other 4 PLS components; Table S5 report the
597 miscellaneous classes of PLSDA).

598 Inspection of PLS1/PLS2 plot indicates that samples belonging to $UP_a$ are correlated to the
599 cluster of contaminants constituted by $Cr_{tot}$, Ni, Pb, Cd, that resulted highly associated with TOC
600 and fine fraction (named "*pelite"* in the plot). This circumstance indicates the occurrence of
601 terrigenous contribution of sediments coming from inland hydrographic waterways, especially
602 from different rivers discharges (Cervaro (VI), Candelaro (CR) and Ofanto (FO)). The
603 resuspension of fine sediments within watercourses leads to the transportation of fine particles of
604 terrigenous origin with the water flows (Fostner, 1984; Hancock, 2001). Indeed, the transported
605 particles erode the surfaces over which they pass and contributing to the breakdown of
606 continental rocks, nourishing the coastal sediment ad influencing their mineralogical and
607 geochemical composition (Mali et al. 2015; 2016). ). In addition, it is observed that Hg follows
608 the TOC trend. The results confirm the important role of organic matter in controlling mercury
609 (Hg) distribution and the terrestrial origin of organic matter in marine sediments. (Chakraborty et
610 al. 2015).

611 As to the second physiographic unit UP3, which covers the middle part of the Apulia coast and
612 includes highly populated areas such as Bisceglie (BB), Molfetta (MI), and Bari (BA), the

associated cluster of contaminants includes nearly all organic pollutants plus arsenic. In this UP the highest values for cRI and cPLI values were registered. More important, the highest relationship among main contaminants characterizing UP3 and the two indexes cRI and cPLI was found. This confirms that the main contribution in the deterioration of the quality of coastal water is caused by human activity.

In UP4, total nitrogen $N_{tot}$ constitute the main differentiating variables. The presence of the mouth of the stream "Canale Reale" (PP and TG) discharge, a channel that during its course crosses different municipalities (Francavilla Fontana, Oria, Latiano, Mesagne, Brindisi and Carovigno) potentially polluted with fertilizers from the agricultural campaign and other un-authorized discharges, can be held as the cause for the association of this contaminant with the hazard profile of the UP4.



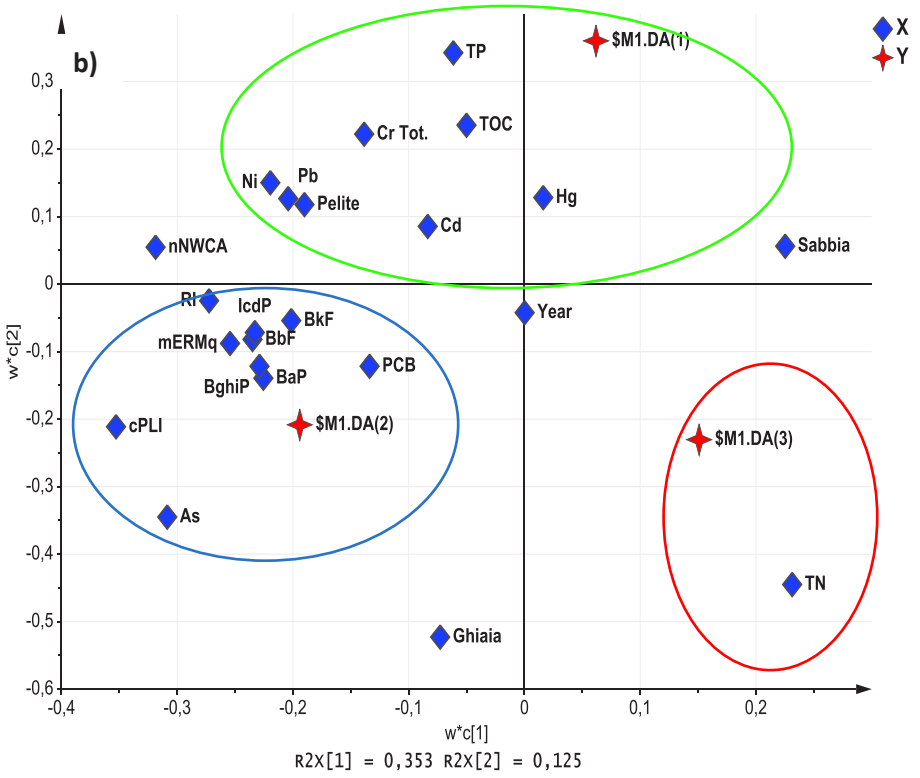




**Figure 8.** PLS score plot (a) and Loading plots (b) for PLS1/PLS2. The different Physiographic Units are countersigned with the numbers 1 for the north (UPa=UP1+UP2); with number 2 the middle UP3 and with number 3 the southern UP4.

### 4.3 Comparative analyses of cPLI and cRI with previous cumulative indexes

Aiming at validating the efficiency of the proposed indexes, a correlation between cRI and cPLI with two other cumulative indexes reported in literature was performed. The cRI was compared with the mean mERMq quotient (mERMq, Long at al. 2006), given that mERMq index individuates sediment sites with high probability of toxicity. The obtained square correlation resulted significant, as indicated by the values of the correlation coefficients $R^2$= 0.684, $r$=0.824, $p < 0.005$ (Figure S2). The cPLI was compared with the c_NWAC, being both indices related to the cumulative synergic effect of co-presence of contaminants of different classes. Also in this case the correlation coefficients resulted positive even if less significant ($R^2$= 0.469, $r$=0.65 $p$<0.000). The slightly worst correlation of the cNWAC with cPLI can be explained with the fact that the cPLI includes also an unpredictable toxic contribution $HI_{toc}$ (by the toxicological response of the bioassay tests performed) that was not considered in the previously calculated cNWAC.

These results support the reliability of the two performed indexes in providing practical and speedy tools for individuating hot spots within the coastal area and comprehensively evaluation of the hazard degree.

## 5 Conclusions

Given that the quality of sediments is an indicator of marine-water pollution status, the proposed pollution indexes cPLI and cRI, based on weighted relevance of some contaminant indicators controlling the pollution status of the sea sediment, support a reliable assessment of the contamination trend within the marine-coastal area of Apulia Region. The coastal tracks of most concern resulted those close to metropolitan cities, confirming that the main contribution in the deterioration of the marine coasts arises from human activity. In addition, an important role of the inland hydrographic network was recognized, which caused a contamination of terrigenous origin (river charges, rainfall waters, etc.) by transferring organic pollutants and fertilizers from the agricultural campaign to the marine coastal water bodies.

The combination of cPLI and cRI with multivariate analyses resulted to be crucial for extracting essential information from large datasets generated by the long-term monitoring and furnished a quick way to interpret data stemming from complex systems. Indeed, while on one side, the

678 long-term monitoring offered useful information on anthropogenic and natural changes occurring
679 over time, the multivariate analyses supported identification of contaminant of most concern,
680 facilitated the prediction of the contamination trend and identified the factors responsible for
681 such trend, distinguishing between the contribution weight given by the natural and
682 anthropogenic ones.

683 Finally, the proposed strategy can directly improve the reliability of Hazard Assessment
684 Procedure reducing costs and time and helping an efficient orienting of future environmental
685 monitoring of coastal area.

686

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690

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# Long-Term Monitoring Programs to Assess Environmental Pressures on Coastal Area: Weighted Indexes and Statistical Elaboration as Handy Tools for Decision-Makers

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## Abstract

Data generated by long-term monitoring programs for coastal areas are intricate and require advanced tools able to identify the factors, natural or anthropogenic, responsible for the observed quality status. In the present study, data stemming from a 5-year monitoring programme of the Apulian coast were utilized for validating a speedy and comprehensive approach to assess the environmental quality of the marine-coastal area. Selecting 12 indicator contaminants controlling the pollution degree of the bottom sediments and attributing to each of them a weighted relevance according to their hazard potential, two multimetric indexes were calculated, helping to establish how and to what extent the selected contaminants could affect the achievement of good chemical and ecological status of coastal area. The relationships between calculated hazard degree values and the main natural factors loading on the study area were addressed through multivariate analyses. The variability of hazard degree values over time was explained by means of combined use of multivariate analyses and multimetric indexes, affording a handy method that allows to differentiate the role of natural factors, such as hydrodynamic and morphological features of the coastal track versus that of anthropogenic pressures. The combined approach adopted supports a reliable hazard assessment at long-term period and at a large spatial scale.

**Keywords**: coastal ecosystem, SQGs, multimetric indexes, long-term monitoring, multivariate analyses, hazard assessment.

## 1. Introduction

Marine ecosystems are extremely exposed to numerous disturbances that associated with the rapid development of industrial and urban activities, cause severe anthropogenic impacts produced by chronic or acute uncontrolled sources of pollution (Rombouts et al. 2013). In addition, natural factors, such as land inputs by hydrographic networks and morphological features of coastal area can often dramatically emphasize or mitigate the adverse effects that the pollution can cause to marine life due to their synergistic combinations with anthropogenic pressures. The main threats resulting by such pressures is the increase of the concentration of trace metals and persistent organic pollutants, as well as excess of nutrients that can cause deleterious effects on marine equilibria. This problem occurs especially in densely populated coastal areas, where land use is intensified and storm-water run-off, effluent discharges from industry and sewage treatment plants are constantly pressing.

In this context, the long-term monitoring program for marine-coastal environment can play a fundamental and irreplaceable role in studying current and future impacts. Analyses of the complex data set generated by such programs with appropriate tool can support distinguishing between impacts from natural factors and those caused by anthropogenic changes and fluctuations occurring over time.(Navarrete et al. 2010; Lohner & Dixon, 2013). A long-term monitoring program can support understanding of the vulnerability of marine habitats and, at the same time, can constitute useful tool for decision makers in designing efficient marine protection strategies at regional level. Introduction of the Water Framework Directive (WFD, 2000/60/EU), containing the broadest ranging and the most specific measure programme for water bodies, constitutes a legislative support for achieving this goal. Article 8 of the Directive emphasized the need of implementing comprehensive programs for the continuous monitoring of water bodies status, suggesting three levels of monitoring systems: *i)* surveillance monitoring; *ii)* operational monitoring and *iii)* investigative monitoring. All EU Member States, in compliance with WFD, have indeed enacted specific transposition laws to activate monitoring programs, delegating the Regional competent authorities for local application.

However, the long-term monitoring of marine-coastal water bodies is a very complex task due to unstable nature of coastal ecosystem affected by unpredictable point and/or diffuse pressures that, combined with hydro-morphological changes in water, can affect the ecological and chemical status of coastal ecosystem. This calls for reliable searching and identification of the main factors influencing the water quality.

It is well known that bottom sediments can be used as valuable indicators of the impact of human activity on the water bodies' quality, being they capable of accumulating both organic and inorganic pollutants over time. Several studies have considered the role of sediments in determining the fate of metals and organic compounds in seawater (Chapman D. 1996; Tomadin, 2000; Spagnoli et al., 2010; Mugnai et al. 2010; Lofu et al. 2016; Chapman et al. 2013; Mali et al. 2016). Moreover, the impact of hydrodynamic processes on the spatial distribution of pollutants in coastal areas has been addressed (Malcangio et al. 2017; Valentini et al. 2017; Mali et al. 2017a, 2017b; 2018). Evaluation of contaminant concentration in marine sediments is a major part of the assessment program for coastal area because it helps to define the hazard degree, to predict the potential threat of pollutants, and to identify the allocation of pollution sources (Morillo et al., 2004; 2008; Borja et al. 2008; Piva et al. 2011; Mali et al. 2017c). For this reason, great efforts have been made to establish Sediment Quality Guidelines (SQGs), using different calculation approaches (Chapman 1989; Del Valls et al. 1998; Wenning et al. 2005; Long et al. 2006; Chapman 2001; Chapman 2007; Ritter et al. 2008; Piva et al. 2011; Regoli et al., 2013, Gredilla et al. 2014; Zahra et al. 2014; Souza et al. 2016).

Among the principal approaches, we cite the index method and the model index method. The index method refers to substituting the actual pollutant concentrations into the mathematical formula to get the pollution indices: comparison of such indices with the corresponding assessment criteria thus gives the pollution degree. On the other hand, the model index method assesses metal pollutions by constructing very complex mathematical models. These models have some advantages than index method when processing the fuzzy boundary effect, but they require a lot of mathematical functions and cumbersome operations, which limits their applications. Thus, the index method is the most preferred one, especially for decision makers that need easy-to use tools for evaluating sediment quality.

In this paper, a handy method for evaluating the pollution degree in marine-coastal sediments is proposed as an integrative approach that can be considered a middle ground between index and

model index methods. The pollution degree assessment by the proposed index was indeed associated with multivariate analyses as wide-ranging tools for interpreting complex data generated by long term monitoring programs.

As case study, marine sediments of Apulia Region coast were investigated, using data obtained by a 5-year monitoring programme activated by the Italian Ministerial Decree (MD) n. 260/2010 Ministerial Decree, the national law transposing the Water Framework Directive (2000/60/EU) at Italian level. The proposed index is a modified version of the environmental Pollution Index (PLI, Tomlison et al. 1984) that in the new proposed version (cPLI) includes two new elements: a Chemical Hazard Index ($HI_{ch}$), that takes into account a revised Contamination Factor ($CF_{ir}$) and an Eco Toxicological Hazard Contribution ($HI_{tox}$), derived by bioassay responses.

The MD 260/2010 has defined Environmental Quality Standards for water bodies, used also for quality sediments in water-coastal bodies. Therefore, using the monitoring data and standards established in compliance with this MD, we calculated the hazard index considering only some the priority substances given by Directive 2013/39/EU (2013/39/EU) as principal contaminants in the field of water policy. We selected 12 contaminants as toxic indicators (6 metals and metalloids and 6 persistent organic pollutants), controlling the pollution status of the coastal waters and we tried to establish how these contaminants could affect the achievement of good ecological status of coastal marine water, attributing to each of them a weighted relevance on the sediment quality, according to their hazard potential. Furthermore, we calculated the relationships between the hazard index values and the main natural factors, such as hydrodynamic and morphological features of the studied coastal track, by using supervised multivariate statistical elaboration to get insight the contamination variability over time and to understand the role of natural and anthropogenic factors.

The novelty of this approach consists in reading the complexity of long-term monitoring program data by using Multivariate Statistical Elaboration and by performing handy multimetric and comprehensive indexes that consider weighted contribution of indicator contaminants measuring their chemical and eco-toxicological impact in the hazard degree. The idea is to propose a speedy, cheap and comprehensive approach for hazard assessment at long-term period and at a large spatial scale capable to improve the objectivity in defining the responsible contaminants within hot spots areas.

## 2. Materials and Methods

### 2.1. Hydrographical and geomorphological features of the study area

The southern Adriatic coastline investigated is extended on 370 km length, from Peschici (PE) in the Gargano promontory up to the Nature Reserve "Le Cesine" (CE) in Salento Peninsula. The coast is composed mainly of micritic and calcarenitic limestone and sands (Spagnoli et al. 2010; Caldara et al. 2013). The sediments contain mainly marine-derived carbonate as well as terrigenous fractions indicating highly heterogeneous composition dictated by different provenance and complex transport processes.

From a morphological point of view, according to the criteria defined in Apulia Coastal Regional Plan (Piscitelli at al. 2011), the analyzed coastal track includes four natural Physiographic Units (UPs), that constitute areas delimiting barriers against the longitudinal transport of solids (Figure 1a).

The northern UP (Physiographic Unit - UP1) extends from the Peschici-Vieste up to Head of Gargano Promontory. It is characterized by generally high cliff rocky coast, with predominantly calcareous sediments and siliceous calcareous rocks. In this track, there are widespread forms of marine and karstic erosion accompanied by cavities and caves.

The UP2 extends from the Head of Gargano Promontory (near Vieste), including the whole Gargano Gulf, heading until near Bari city coast. This coastal track is very heterogeneous with different morphological profiles due to the marine abrasion that acts in a selective form in different areas. Terrigenous filling material, transported to river estuaries affect the marine sediment composition. Different rivers characterized by torrential regime such as Candelaro, Cervaro, Carapelle, contribute with sandy silt sediments originated from the disintegration of limestone and siliceous calcareous rocks that make up the nearby hills inland. Furthermore, the UP2 includes the Ofanto River, one of the main watercourses of Apulia Region that, with its 170 km long, is the most significant river flowing into the Southern Adriatic Sea. Its hydraulic regime is not always constant, with discharges concentrated during the autumn-inter period.

The UP3 extends from the northern coast of the metropolitan city of Bari (BA) heading southward to Monopoli Beach (MA). The first track is constituted by a low sandy coast characterized by sandy beach rock and strongly diagenized limestone strips belonging to different sea terraces. The coastal tract near the metropolitan city of Bari is heavily modified by

large-scale artificial burying work and only short coastal tracks are preserved from human intervention. Several blades and small rivers of seasonal flow regime are also present in this area. The last track (among Polignano and Monopoli) is characterized by a high coast rocky that can end with a vertical wall (cliff) or with a sloping profile. In the first case they are associated with the presence of caves and with evidence of erosion phenomena.

The last physiographic unit investigated, the southern UP4, extents from Torre Guaceto (TG), getting through the coast of Brindisi city, until the Natural Protected Reserve Area, denominated "Le Cesine". The UP4 have two rivers that nourish with terrigenous sediment the coast (Great River and Small River). There are also several barrier works on watercourses. It is worth mentioning the Dam on clay on the Cillarese stream for the supply of water resources for industrial uses and the presence of Royal Channel that flows through different inland municipalities, enriching its watercourses with inland contribution. UP4 ends with sandy stretches often accompanied by the presence of marshes and retrodunal lakes (Fontanelle, Alimini) shallow beaches and dune beds with dunes even 10 m high.

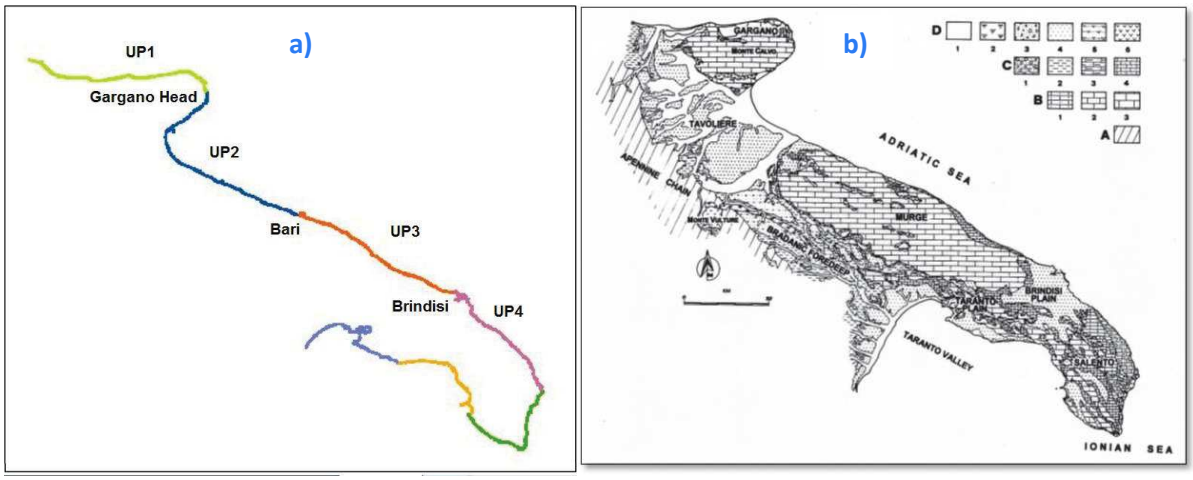



**Figure 1.** a) The Physiographic Unit (UPs) defined by the Regional Coastal Plan of Apulia Region.; b) the Geological features of Apulia platform according to Piscitelli et al, 2011.

### 2.2. Data collection

As required by the Italian Legislation transposing at national level the WFD (D.Lgs. 152/2006; D.M. 260/2010), the long-term monitoring programme for coastal area has been activated by Apulia Region and realized by the Regional Agency for Environment Prevention and Protection

(ARPA Puglia) starting from March 2010 and continued, on an annual basis, until April 2015. Within this five-year temporal span, two types of monitoring programs were carried out: *i)* surveillance monitoring (April 2010- March 2011); *ii)* operational monitoring (April 2012-March 2013; April 2013 - March 2014 and March 2014 – April 2015). According to the Italian D.M.260/2010, Regional Monitoring Programme includes Water Courses, Lakes/Reservoir Waters, Transitional Waters and Marine-Coastal Waters. The present work was focused only on Marine-Coastal Water Bodies (MCWB). The quality assessment of this category includes analyses of water column, sediments and biota. To the purpose of the present paper, only sediment analyses were considered.

### 2.3. Sampling strategy and sediment sample handling

A total of 144 surface sediment samples, collected from 70 coastal sites belonging to 35 marine transects were selected. These transects belong to two different distances from the coast: 200/500 m and 1750 m, respectively. The location of the sampling sites with details of their longitudes and latitudes are reported in Supporting Information (Table S1), while the sampling site distribution is reported in Figure 2. Sampling sites are selected as representative of the corresponding Apulian MCWB, as identified according to the Italian Ministerial Decree n. 131/2008 taking into account the local morphological and hydrodynamic features as well as the anthropogenic pressures loading.

The surficial sediment samples were taken with a "van Veen" bucket having a sampling surface of 0.10 $m^2$. In each station, three sampling buckets, corresponding to three replicates, were collected. Adequate clean plastic jars with Teflon coated lids were used for storage and transport of the samples to the laboratory conserved at 4 °C. Once in laboratory, the collected sediment samples were freeze-dried, gently ground in an agate mortar trying to not alter the grain size features, then passed through a 0.5 mm mesh sieve to remove debris and pebbles, and finally stored at −20 °C prior to analysis.

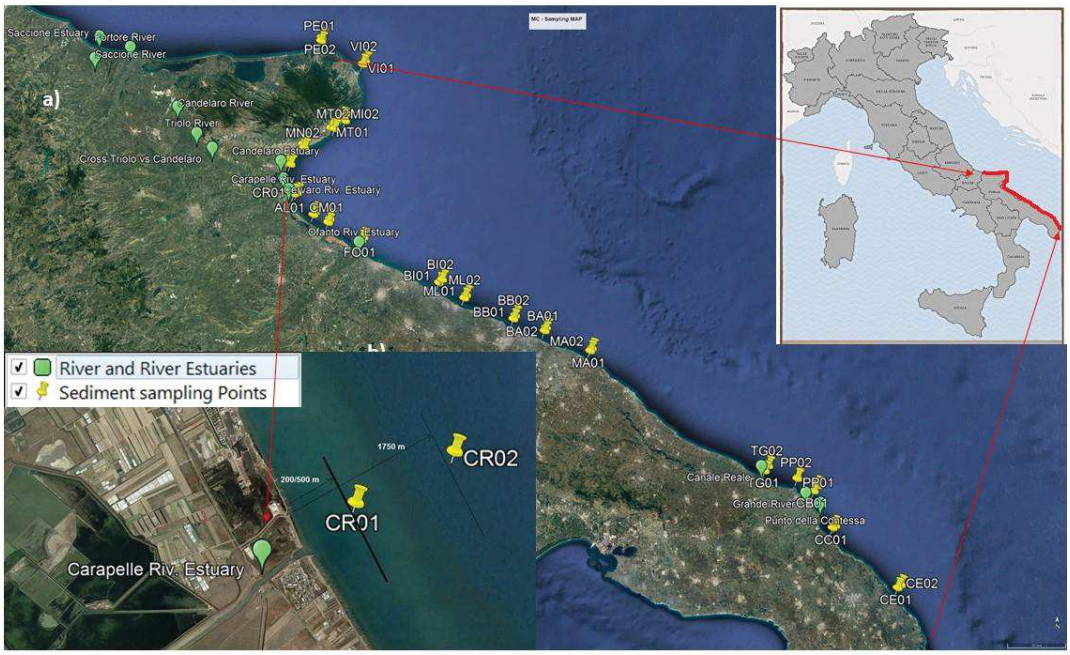



**Figure 2.** The sampling map; a) sediment sampling distribution and river estuaries location alongside coast, b) an inset showing a close-up of the two transects considered (01 and 02 for 200/500 and 1750 m from the coastline respectively)

### 2.4. Analytical methods

Each sample was classified according to Shepard (1954) into four sections: gravel: >2 mm; sand: 2–0.063 mm; silt: 0.063–0.002 mm; clay <0.002 mm. A set of ASTM sieves was used for the granulometric separation (Romano and Gabellini, 2001).

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, HNO3 and HF) (Pellegrini and Lucarotti, 2001). The < 63 μm fraction, dried at 105 °C, was used for the determination of metals in order to reduce the grain size effect. The detection limits (LODs) were calculated from 3 replicates of procedural blanks. The estimated LODs were equal to 1 ppb for all metals. Marine Sediment Reference Materials 2702 (Inorganics in Marine Sediment) were used to control the analysis quality: the agreement between the analytical results for the certified and measured values was satisfactory, with recoveries ranging from 80% to 100% for all metals.

The total nitrogen amount ($N_{tot}$) was determined by an elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer. 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 an elemental analysis procedure with a Perkin–Elmer 240B CHN Elemental Analyzer, after removal of carbonates by reaction with hydrochloric acid (Giani, 2001).

As for the five high molecular weight Polycyclic Aromatic Hydrocarbons (PAHs) congeners considered in this study, benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), Benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP); indeno[1,2,3-cd] (Ind) the analytical methods performed is according Ausili (Ausili 2000) that foreseen extraction with cyclohexane/methanol mixture and determination in High Pressure Liquid Chromatograph. The sum of Polychlorinated biphenyls (PCBs) congeners (28, 47, 99, 100, 153, 154) considered in this study was determined by the Cicero et al. method (Cicero et al. 2000) through extraction in acetone/petroleum solvent followed by analysis by Gas Chromatograph equipped with an Electron Capture Detector. Ecotoxicological tests (Microtox® SPT with Vibrio Fisheri, fertilization success bioassay with sea urchin Paracentrotus lividus, inhibition of a marine algal growth with *Dunaliella tertiolecta* were performed following procedures defined by Azur Environmental 1994, Onorati and Mecozzi, 2004; Lera et al. 2006; ISO 10253, 2006. All analyses were carried out in the ARPA Puglia (Apulian Regional Agency for Environmental Protection) laboratories.

### 2.5. Statistical analyses

Analysis of variance (ANOVA) was applied to perform the spatial and temporal distribution of contaminants and hazard degree in sediment samples. One-way ANOVA was used to identify the differences in the hazard levels in sediment due to their physicochemical properties and total contents of pollutants based on the relevant physiographic unit (UP). PLS-DA was used to optimize separation between samples belonging to different groups and to identify the main contaminants controlling each UP and each Grain Size classes. The PLS-DA is aimed at maximizing the covariance between the independent variables (in our case the UP and Grain Size features), and the corresponding dependent variable Y (concentration of contaminants). On the other side, Spearman correlation analysis was performed to identify the relationships between

249 the detected sediment properties, contaminant concentrations and hazard index values.
250 Differences were considered statistically significant only for that cases in which the $p < 0.05$.

251 Two different software were utilized for Statistical Analyses: Soft Independent Modeling of
252 Class Analogy (SIMCA) 10.2 for PLS_DA analyses and STATISTICA 10.0 for One way
253 ANOVA and Factorial ANOVA, Correlation Analyses and for normality-test of the raw and log-
254 transformed data. The SIMCA software was selected for its intuitive graphical interphase
255 allowing to extract the main information describing dataset variability, while the non-parametric
256 approaches of STATISTICA were selected as the most powerful tests in case of environmental
257 data for which distribution assumptions are violated due to the presence of outliers or to non-
258 normal distributions.

259 **2.6. Assessment of sediment contamination**

260 All Sediment Quality Guidelines (SQGs) developed in literature and dealing with environmental
261 concerns, provide a simple comparative mean for assessing the risk of contamination in an
262 aquatic ecosystem (Macdonald et al., 2000), mainly by comparing the chemical concentration of
263 individual pollutants with their corresponding limit concentrations. The most exploited and
264 internationally accepted indexes are those used for trace metal pollution assessment (Table 1),
265 widely used by researchers in environmental science (Salomon and Foster 1984; Zhang and Liu,
266 2002; Dassenakis et al. 2003; Spagnoli et al. 2008; Rath et al. 2009; Varol et al. 2011; Desaules
267 et al. 2012; Banu et al. 2013; Mali et al. 2015; Maanan et al. 2015, Zhang et al. 2017). The main
268 limitation of these indexes consists in not considering the potentiality of the combination effects
269 due to the coexistence of different contaminants (organic and inorganic) accumulated in the
270 sediments (Birch et al. 2018). These limitations become more dramatic when dealing with
271 complex matrix, such as marine sediments, affected by "matrix effect" that calls for
272 comprehensive pollution assessment able to consider simultaneously *i)* the level of
273 contamination; *ii)* the combination effects of different groups of contaminants (heavy metals,
274 organic pollutants and nutrients) and *iii)* the occurrence of natural factors that can emphasize
275 such effects.

| Multimetrix Index | Formula | Reference |
|---|---|---|
| **1. $I_{geo}$ (Geoaccumulation Index)** | $Igeo = Ig0 + \frac{Cn}{1.5 + Bn}$ where $C_n$ and $B_n$ are metal concentration in sediment sample and in the reference material, respectively. | Muller G., (1969) |
| **2. E.F. (Enrichment Factor)** | $E.F. = \frac{\left(\frac{Cx}{Bx}\right)sample}{\left(\frac{Cb}{Bb}\right)background}$ where $C_n$ and $B_n$ are metal concentration in sediment sample and in the reference material, respectively | Muller et al. (1974); |
| **3. RAC (Risk Assessment Code)** | $RAC = \frac{F1}{Ctot}$ where F1 is the percentage of metal fraction extracted by $CH_3COOH$ 0.11 M solution with respect to the metal total concentrations. | Perin et al. (1985); |
| **4. CF (Contamination factor)** | $CF = \frac{Cmetal}{Cb}$ where $C_{metal}$ is the metal concentration determined in the sample and $C_b$ is the reference values (according to the specific SQG considered) | Muller, (1979); |
| **5. PLI (Pollution load index)** | $PLI = (CF1xCF2xCF3x \ldots CFn)^{1/n}$ where CF Contamination Factor for each metal (see 4.) | Tomlinson et al. (1980); |
| **6. RI (Risk Index)** | $RI = \sum_{i=1}^{k}(Ei)$ where Ei is the ecological risk index for given metal (according $Ei = Ti\,x\,CF$); Ti is the toxicity response factor and CF the Contamination Factor (see 4) | Hakanson. (1980) |

276

277 **Table 1.** Sediment quality guidelines used globally.

## 3. Results

In this study, a modified Pollution Index (cPLI) is proposed, based on two levels of evidence: a Chemical Hazard Index ($HI_{ch}$), calculated through a revised Contamination Factor ($CF_{ir}$), that takes into account the chemical hazard and an Eco toxicological Hazard Contribution ($HI_{tox}$) derived by bioassay responses. The responses of cPLI index were associated with the results obtained by a new Ecological Risks (RI) to provide a comprehensive evaluation of chemical and ecological status of marine coastal area investigated.

### 3.1.1. Chemical Hazard Index for pollution degree assessment ($HI_{ch}$)

It is known that conventional contamination factor ($CF_i$), called also single-factor pollution index, can be obtained by dividing each contaminant concentration by a baseline value defined for each of them. As mentioned above, the Contamination Factor cannot reflect the whole pollution degree deriving comprehensively from various pollutants and is only applicable to a single factor pollution assessment. However, it can be the basis of environmental quality standards, as in our case. Our Chemical Hazard Index ($HI_{ch}$) takes as baseline values the environmental quality standards established by MD 260/2010 for coastal water quality. The novelty of this index ($HI_{ch)}$ stands in two aspects:

- it is a speedy index for a comprehensive chemical pollution evaluation since it considers only the main Priority Substances considered by Directive 2013/39/EU for water quality purposes (six metals and six persistent organic pollutants) selected as indicator contaminants controlling the chemical status of sediments;

- the modified Contamination Factor used to calculate the $HI_{ch}$, includes a "weighted" value, introduced by Directive 2013/39/EU for water quality assessment. This value ranges from 1 to 1.3, depending on whether the contaminant is not covered by the Directive (weight = 1), or, on the contrary it is included in the list of "priority" substances (weight = 1,1) or in the list of "dangerous and priority" substances (weight = 1,3), aiming therefore to attribute to each pollutant a proportional relevance on the chemical status of sediment according to their hazard potential.

Thus, the modified Contamination Factor ($CF_i$) for a single pollutant is calculated according to the formula (Eq.1):

$$CF_{ir} = \frac{C_{mi}}{C_{si}} \; x \; W_0 \qquad \text{(Eq. 1)}$$

where $C_{mi}$ is the concentration of the contaminant "*i*" determined within sediment samples and expressed in ppm ds, $C_{si}$ is the Environmental Quality Standard value defined for the given pollutant by the MD 260/2010 within WFD, and $W_o$ is the weighting factor attributed to each pollutant. The revised $CF_{ir}$ calculated in this way considers the contamination factor of the single element: when $CF_{ir}>1$, the substance is considered as contaminating or enriched; when $CF_{ir}\leq 1_i$, the substance cannot be considered as contaminating or (anthropogenically or naturally) enriched.

Subsequently, the specific chemical contamination degree ($HI_{ch}$) was obtained following Bebianno method for quality assessment of harbor sediments (Bebianno et al. 2015). The $HI_{ch}$ calculated according to Eq 2, is based on the average of all the $CF_{ir} \leq 1$, added of the sum of the $CF_{ir}$ of all contaminants displaying a $CF_{ir} > 1$ (aiming at stressing the contribution of the elements considered contaminants or anthropogenically enriched). In formula:

$$HI_{ch} = \sum_{j=1}^{k} \frac{(CF_{ir} \leq 1)}{k} + \sum_{i=1}^{n} (CF_{ir} > 1) \qquad \text{(Eq. 2)}$$

*k* is the number of $CF_{ir} < 1$ and *n* the number of $CF_{ir} >1$.

### 3.1.2. Toxicological contribution to the comprehensive hazard evaluation

The eco-toxicological results foreseen by MD 260/2010 were exploited for defining the toxicological contribution to the comprehensive Pollution Index. To this purpose, 144 sediment samples, were subjected to a bioassay battery of three biological indicators: bioluminescence of *Vibrio Fishery*, (in both elutriate and solid phase), inhibition of a marine algal growth (in our case *Dunaliella tertiolecta*, determined on the elutriate phase) and embryo-toxicity test with echinoderm *Paracentotus lividus*. The eco-toxicological results and the eco-toxicological classification according to the national legislation (expressed in EC20 and/or EC50 results) are given in Table S2 and Table S3, respectively. There are four toxic classes degree: Classes A that indicates "no toxic" response; Classes B for "moderately" toxic; Classes C for "high toxic" and classes D for "severe toxic" response.

It is known that each bio-indicator has a different pollutant sensitivity. The eco-toxicological results do not correspond to the chemical hazard levels, since the total concentration of contaminants is not the unique factor contributing to the toxicity response (Burton 2002; Wenning 2005). Many other factors need to be considered, most of them unpredictable. Therefore, in order to attribute to each sample results an eco-toxicological contribution to the comprehensive hazard evaluation according to the bioassay responses, we calculated a Toxic Hazard Coefficient ($HI_{tox}$) utilizing an approach similar to that adopted for chemical hazard degree, consisting in the following steps:

1. for each toxicological class defined by the Italian legislation normative (ICRAM 2009), an arbitrary coefficient "k" was given, ranging from 1 to 2.5: k = 1.0 for Class A; k=1.5 for Class B; k = 2 for Class C; k=2.5 for Class D.

2. An "eco-toxicological weighted factor", $W_{oe,}$ was given also to each result, as function of:

   a) the severity of the effect "En", intended as the severity of the biological damage measured by the specific end-point considered: En = 2.4 was given for bioluminescence; En = 2.1 was given for the inhibition of growth rate test; En = 1.9 was given for development rate test);

   b) the type of exposure T (acute or short-term T= 1 while T= 0.8 for chronic or long-term exposure);

   c) the type of test matrix M: M=0.8 when the elutriate phase was considered; M=1 when the sediment or solid phase was considered as matrix for eco-toxicological tests.

   d) a representative weight given for the potential hormesis for each experimental type. For the bioluminescence on solid phase and elutriate on Vibro-fisheri a representativeness weight W=0.25 is defined; for the inhibition of algal growth rate on elutriate a W=0.10 is considered, and for embryo-toxicity with *Paracentrotus Lividus* is given W=0.15.

   The above reported values given to elements constituting the weighting factor $W_{oe}$ comply with what proposed by Piva et al., (Piva et al. 2011). Actually, these are taken as guidelines in the most recent Italian National Laws on Sediment Handling (D.Lgs. 172/2016 and 173/2016) for the integrated quality assessment of dredged sediments.

3. With the above information, the $HI_{tox}$ contribution was calculated according to the following linear formula:

$$Woe_i = En_i \, x \, T_i \, x \, M_i x \, W_i$$

$$HI_{tox} = \sum_{i=1}^{j} (k_i \, x \, Woe_i) \quad \text{(Eq. 3)}$$

where, $k_i$ is the coefficient given to each of the four-classes. *En*, *T* and *M* and *W* are the weighting factor values given to the severity effects (En), type of exposure (T) , type of text matrix (W) and the environmental representative of the tested Matrix (M).

### 3.1.3. Comprehensive pollution Index (cPLI)

With the abovementioned results, the comprehensive modified Pollution Index (cPLI) can now be calculated by considering chemical characterization ($HI_{ch}$) and toxicological contribution ($HI_{tox}$), as Levels of Evidence according to the formula:

$$cPLI = HI_{ch} * HI_{tox} \quad \text{(Eq. 4)}$$

where $HI_{ch}$ is the chemical hazard Index of Eq 2 and $HI_{tox}$ the Toxicological Coefficient defined by Eq. 3.

The comprehensive cPLI is conceived to measure marine environmental impact integrating chemical and eco-toxicological results, being therefore a practical, reliable and predictive tool for assessing sediment quality.

## 3.2. Potential ecological Risk Index for comprehensive pollution degree

Aiming at assessing the ecological risk degree and comparing its estimation ability with those of the cPLI, a revised ecological Risk Index (cRI) was implemented using formulae reported in Eq.5 and Eq. 6, modifying what originally proposed by Hakanson (Hakanson, L. 1980).

$$cE_i = T_i \, x \, CF_{ir} \quad \text{(Eq. 5)}$$

$$cRI = \sum_{i=1}^{n} (T_i \, x \, CF_{ir}) \quad \text{(Eq. 6)}$$

where $cE_i$ is the individual risk for each contaminant, $CF_{ir}$ is the revised contamination factor that considers the weighted factor for each contaminant, $T_i$ is the toxic factor requirement on the individual contaminant defined by Hakanson. (Hakanson, 1980) and $n$ is the number of the pollutants considered in the calculation of the potential ecological Risk Index. The cRI calculated according to this approach allows to assess the pollution status in sediment, combining the environment effects with ecological toxicity, providing thus a better evaluation of the potential risk of contamination with the index level.

Some consideration should be made before discussing the reliability of the hazard assessment according to the cPLI and cRI indexes:

1. the formulae for calculation of each pollutant toxic factor requirement, $T_{i,}$ were those defined by Hakanson, therefore the Ti values are: $T_{Hg}$ = 80; $T_{Cd}$ = 30; $T_{As}$ = 10, $T_{Pb}$ = 5; $T_{Ni}$ = $T_{Cr}$ =2; $T_{PCB}$ = 40.

2. the value of $T_i$ of PCB was selected as representative for the other lipophilic toxic substances considered in this study. Indeed, being the organic pollutants included in the same list of Priority substances, the value $T_i$ = 40 given for PCB is considered as appropriate also for BaP (Benzo(a)Pyrene), BbF (Benzo(b)Floranthene), BghiP (Benzo[ghi]perylene) , BkF (Benzo(k)Floranthene), InD (Indeno, 1.2.3 c.d.pyrene).

3. the evaluation of Risk Index (cRI) and Pollution index (cPLI) includes only the priority Substances given by European Decree (39/2008/CE) for water bodies quality namely 6 metals/metalloids and 6 organic pollutants (Sum of for congeners of PCB and 5 high molecular PAHs). Therefore, the discussion about the hazard risk evaluation is referred to the presence of these contaminants (As, Hg, Cd, Pb, Cr, Ni, Sum of PCB, BaP, BbF, BghiP, BkF, InD). Nevertheless, being these contaminants considered by the Water Framework Directive as the main "weighted" pollutants to be monitored in the long term programs for water quality, the assessment results can also be extended to other contaminants having the same behavior in coastal ecosystem;

4. since some physico-chemical properties of the surficial sediments are very important, especially when the sensitivity of different marine basins was considered, we used the organic content (TOC) and grain size distribution as necessary co-parameters in the calculation of Risk Indexes (see the discussion below).

Table 2 includes the grades of potential pollution degree and potential ecological index for environment.

**Table 2.** Potential pollution degree of cPLI and cRI according Tomlinson et al. 1980 and Hakanson, L. (1980) respectively, proposed in Maanan et al. 2015.

| ***cPLI*** | ***Comprehensive Pollution Hazard degree as*** $(HI_{ch} \cdot HI_{toc})$ | ***cRI*** | ***Potential ecological risk degree as*** $\Sigma Ei \cdot Ti$ |
|---|---|---|---|
| *cPLI<1.9* | Low hazard degree | cRI<95 | Low risk |
| *1<cPLI<2.8* | Moderate hazard degree | 95<cRI<190 | Moderately risk |
| *2.8<cPLI<6.5* | Considerable Hazard degree | 190<cRI<380 | Considerable risk |
| *cPLI>6.5* | High hazard degree | cRI>380 | Very high risk |

### 3.3. Comparative assessment of different hazard indexes

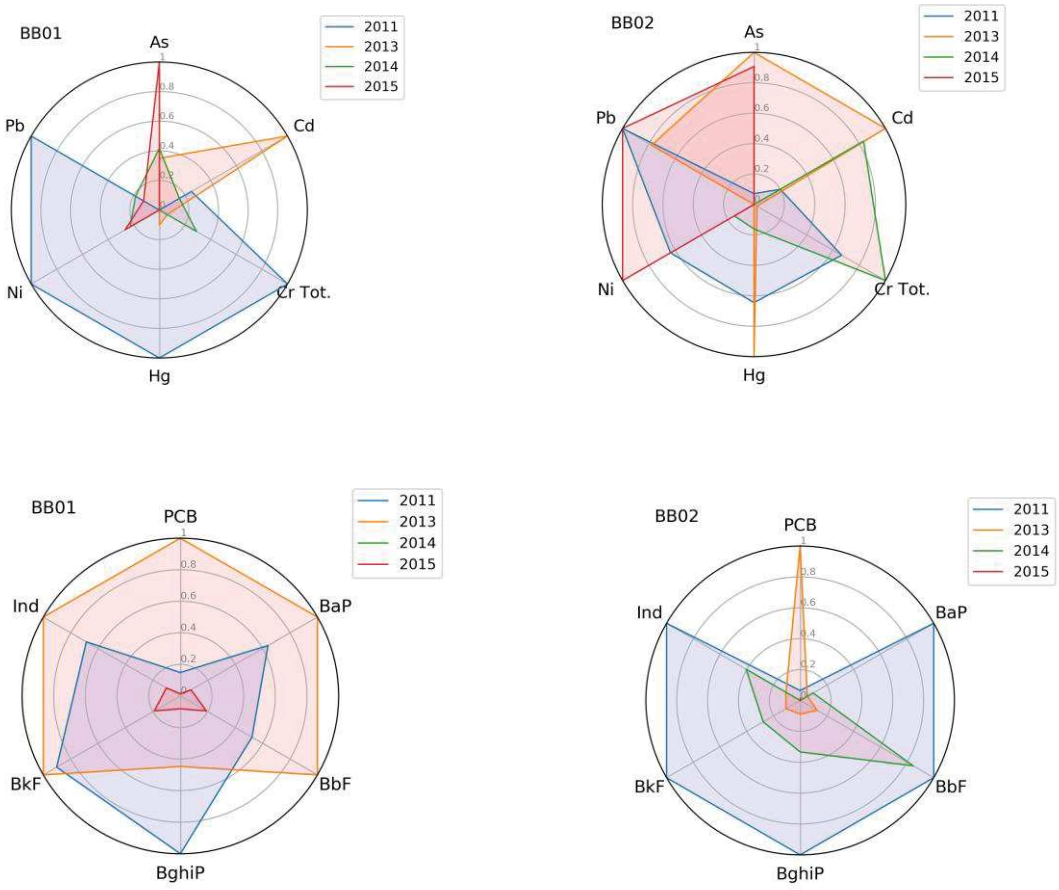
We compared the results obtained with cPLI and cRI with those achieved with the cumulative index c-NWAC (SWRCB, 2006; Mali et al., 2016) and mean ERM quotient (mERMq), respectively. In particular, c-NWAC considered different classes of contaminants (heavy metals and organic pollutants) while mERMq has been applied to determine the possible biological effect of contaminant toxic groups (Long et al. 2006). The hazard degree classifications of the two indexes are reported in Table S4.

## 4. Discussion of results

### 4.1 Chemical contamination factors

The basic statistical parameters and the measured concentrations of pollutants are shown in **Table 3**. The distribution of the metal and organic pollutant concentrations during different years are illustrated through radial graphs as reported Figure 3. Comparing the concentration of contaminants with the Environmental Standard Values (simple contamination factor, $CF_i$) revealed that As, Cd and Ni are the pollutants of most concern, followed by Hg, BaP, BkF since for all of them CFi>1. The respective hazard degree for these pollutants indicates hazard levels ranging from “moderate” to “high”. In detail, 28% and 6% of samples display respectively

436 "moderate" and "high" hazard degree for Arsenic. The contamination factor of Cd shows "low"
437 to "moderate" hazard degree for 18% of sediment samples, while the $CF_i$ of Ni reaches "low" to
438 "moderate" hazard degree in 10% of samples. As to Hg, even if for only two specific sites, the
439 hazard degree resulted that of the most concern, with value of $CF_{Hg}$=8 in ML site in 2013 and
440 $CF_{Hg}$= 2 in VL site in 2014, indicating a "severe" hazard degree in these points. As to the organic
441 pollutants, only for BaP and BkF and in some specific cases, "low" or "moderate" hazard degree
442 was registered (respectively for 2% and 3% of the samples). For the sum of PCB and other
443 organic contaminants, no risk level was observed.




444
445
446

447 **Figure 3.** The distribution of the normalized concentration of six metals and six organic
448 pollutant considered, determined within sediments of one of the UP3 sites, named Bari Balice
449 (BB01; BB02), during the monitoring period.

450

451 **Table 3.** Basic Statistic Data of the concentration of contaminants during investigated years. The concentration of $N_{tot}$, $P_{tot}$ are expressed in mg/kg

452 d.s.; the concentration of As, Cd, $Cr_{tot}$, Hg, Ni, Pb are expressed in ppm; the concentration of organic contaminants are expressed in ppb.

| Year | 2011 | | | | 2013 | | | | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *MEAN* | *MIN* | *MAX* | *S.D.* | *MEAN* | *MIN* | *MAX* | *S.D.* | *MEAN* | *MIN* | *MAX* | *S.D.* | *MEAN* | *MIN* | *MAX* | *S.D.* |
| TOC | 542 | 100 | 1290 | 339.36 | 491 | 70 | 1470 | 387 | 475 | 100 | 1700 | 398.84 | 394 | 100 | 1300 | 353.71 |
| Coarse (%) | 3 | 0 | 25.2 | 6.06 | 5 | 0 | 42.1 | 10 | 6 | 0 | 32 | 10.13 | 6 | 0 | 33 | 10 |
| Sand (%) | 67 | 4.17 | 100 | 30.20 | 74 | 17 | 100 | 25 | 78 | 3.5 | 100 | 23.29 | 83 | 21.9 | 100 | 21 |
| Fine Fraction (*Pelite*) (%) | 30 | 0 | 95.83 | 31.71 | 20 | 0 | 83 | 25 | 16 | 0.0001 | 94.5 | 24.39 | 12 | 0.0001 | 78.2 | 21 |
| TOC (%) | 0.542 | 0.1 | 1.29 | 0.34 | 0 | 0.07 | 1.47 | 0 | 0.475 | 0.1 | 1.7 | 0.40 | 0.394 | 0.1 | 1.3 | 0.35 |
| $N_{tot}$ | 187 | 5 | 638 | 214 | 1067 | 15 | 9362 | 1980 | 1085 | 150 | 5250 | 1442.49 | 140 | 39 | 535 | 128 |
| $P_{tot}$ | 606 | 88 | 2700 | 521 | 281.16 | 22 | 1718 | 305 | 460 | 17.2 | 1381 | 361.86 | 225 | 37 | 650 | 151 |
| As | 10.37 | 2.05 | 19.86 | 4.88 | 15.36 | 1.63 | 69.9 | 16 | 7.30 | 0.1 | 23 | 7.27 | 12.46 | 0.30 | 40.00 | 11.28 |
| Cd | 0.17 | 0.05 | 0.43 | 0.13 | 0.25 | 0.02 | 0.67 | 0.17 | 0.14 | 0.025 | 0.3 | 0.08 | 0.08 | 0.03 | 0.20 | 0.04 |
| Cr Tot. | 23.86 | 3.06 | 70.46 | 18.62 | 12.68 | 1.69 | 46.4 | 10.73 | 14.21 | 0.90 | 57.00 | 12.86 | 9.96 | 2.04 | 34.29 | 8.30 |
| Hg | 0.04 | 0.00 | 0.26 | 0.06 | 0.02 | 0.005 | 0.1 | 0.02 | 0.11 | 0.01 | 2.40 | 0.40 | 0.07 | 0.00 | 0.60 | 0.12 |
| Ni | 19.05 | 2.15 | 42.65 | 12.85 | 10.54 | 0.255 | 46.5 | 10.26 | 7.65 | 0.20 | 30.00 | 7.71 | 9.28 | 1.10 | 30.66 | 7.43 |
| Pb | 10.65 | 0.55 | 31.44 | 7.53 | 6.07 | 1.15 | 18.99 | 4.30 | 6.28 | 2.00 | 15.00 | 3.55 | 8.41 | 1.90 | 23.20 | 5.15 |
| Sum PCB (28-47-99-100-153-154) | 0.06 | 0.01 | 0.19 | 0.04 | 0.08 | 0.01 | 0.67 | 0.15 | 0.05 | 0.02 | 0.08 | 0.03 | 0.02 | 0.01 | 0.10 | 0.02 |
| benzo(a)pyrene | 9.45 | 0.26 | 62.00 | 14.46 | 5.82 | 2.50 | 26.30 | 4.07 | 3.63 | 0.50 | 17.00 | 2.99 | 4.61 | 2.50 | 15.50 | 3.46 |
| benzo(b)fluoranthene | 5.95 | 0.17 | 39.00 | 7.64 | 5.41 | 2.50 | 11.30 | 2.26 | 4.22 | 0.50 | 19.00 | 4.06 | 4.59 | 2.50 | 21.16 | 4.06 |
| benzo(ghi)perylene | 8.08 | 0.17 | 41.50 | 9.74 | 6.25 | 2.50 | 13.50 | 3.14 | 3.64 | 0.50 | 12.00 | 2.59 | 4.43 | 2.50 | 13.22 | 2.75 |
| benzo(k)fluoranthene | 6.22 | 0.24 | 34.40 | 7.53 | 4.93 | 2.50 | 11.40 | 1.81 | 3.15 | 0.00 | 9.00 | 2.34 | 5.27 | 2.50 | 28.82 | 5.90 |
| indeno(1,2,3-cd)pyrene | 6.68 | 0.28 | 33.40 | 7.59 | 6.06 | 2.50 | 20.19 | 3.53 | 3.46 | 0.50 | 12.00 | 2.52 | 4.10 | 2.50 | 10.90 | 2.20 |

Considering the "weighted" contamination factors ($CF_{ir}$), the situation become more critical. For As, being the $W_0$ as high as 1.1, the percentage of samples with "moderate" risk passed from 28 to 34%, increasing the number of samples with "high" hazard degree that reach 7% of total samples. The same consideration for Cd, having a $W_0$=1.3, for which the percentage of samples presenting "low "and "moderate" increase substantially, passing from 10% to 23%. For Ni ($W_0$=1.1) the samples reaching the "low to moderate" risk pass from 10% to 22%. Also the number of samples having "high" hazard degree for Hg increased as did the percentage of samples with "moderate" degree due to BaP and BkF ($W_0$=1.3).

The comprehensive pollution risk calculated according to cPLI, indicates that almost all sites were interested by different pollution potential, starting from low, moderate and severe degree, as reported in Figure 4. The highest cPLI values were observed near the Molfetta coastal track (at ML01 site with cPLI=22) alongside UP3, while the lowest value of 0.18 registered in CE02 (Cerano coastal track) in the southern UP4. The Pollution degree varies also during the different years considered. The temporal hazard distribution along years indicates the persistence of hot-spot area, especially those located near river estuaries (CR and FO, respectively close to Candelaro river estuary and Ofanto river Estuary) and near the highly populated centers, (ML, BB, BA, respectively for Molfetta, Barletta-Bisceglie and Bari coasts). By analyzing the nutrient distribution, it is apparent that the sites close to coastal cities registered also high concentration of $N_{tot}$, $P_{tot}$, TOC, probably due to the municipality wastewater discharges and run-off from agriculture areas, which are widely occurring in the area.

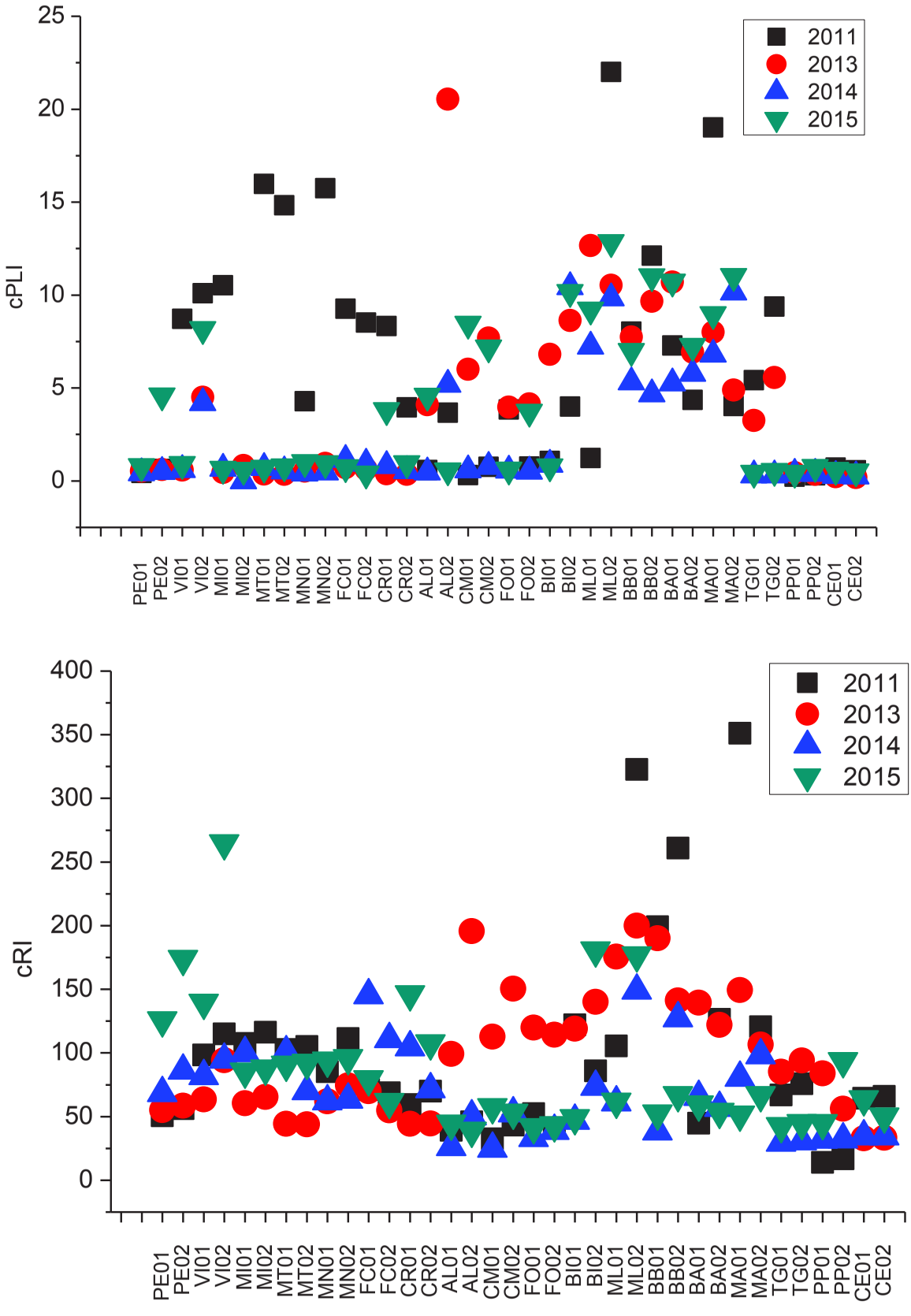



**Figure 4**. cPLI (a) and cRI (b) values in sediment samples during the monitored years.

476 Results deriving by the analyses of Risk Factor (cRI) values, confirm the pollution assessment
477 made according to cPLI. Nevertheless, the comparison pointed out the most severe evaluation
478 made by cPLI that considers both levels of evidence (chemical and toxicological) with respect to
479 cRI that takes into account the toxicological contribution, suggesting a greater objectivity of the
480 comprehensive pollution assessment (cPLI) based on chemical and toxic potential.

481 As to the single contaminants, analyzing the $E_i$ values for the individual ecological risk, it was
482 found that Cd, Hg and BkF result the contaminants of most concern, demonstrating the severe
483 toxicity associated to such contaminants. Nevertheless, it needs to be specified that for Hg only
484 some samples registered a high ecological risk. In general, the order of risks for the contaminant
485 analyzed through individual $E_i$, is the following:

486 Cd>Hg>BkF>As>BaP>BbF>BhgiP>InP>Pb>Ni>Cr>PCB

487 showing the highest individual ecological risk for Cd, Hg, BkF and As and the lowest ones for
488 Pb, Ni, Cr and sum of 5 congeners of PCB.

489

490 **4.2 Spatial and temporal contamination trend by ANOVA and PCA/PLS**

491 The spatial and temporal contamination trend was assessed through the combination of two
492 statistical elaboration, PCA/CA and ANOVA that are considered two complementary techniques
493 for apprehending the impact of multi-sources and multi-factors acting simultaneously in the
494 spatial contamination pattern. The importance of the combination of these two techniques was
495 previously explained (Mali et al. 2017c).

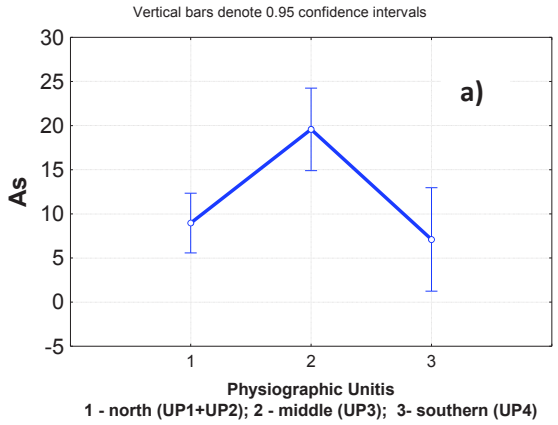
496 **4.2.1 ANOVA responses**

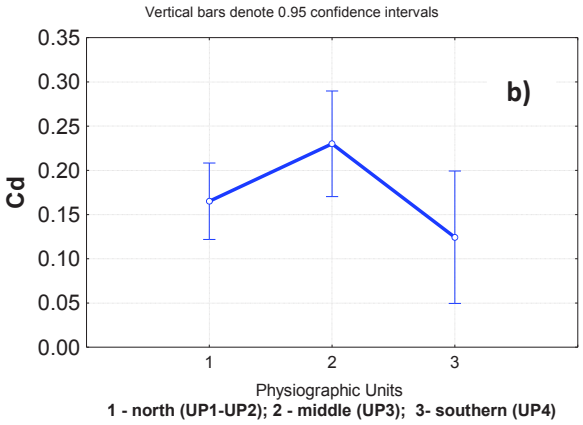
497 Plotting the cPLI and cRI values during different years and within different physiographic units
498 (Figure 3a, b) shows a variable trend of the hazard and ecological risk, that seems to decrease on
499 going from 2011 to 2014, when it starts to increase. The trend confirms also the persistence of
500 some hot-spot areas during years. Aiming at analyzing more deeply these differences, trying to
501 understand the influence of different hydrodynamic and morphological features of the coastal
502 area in the contaminant trend, one-way analysis of variance (ANOVA) was performed.
503 Homogeneity of variance was tested by Levene's Test, and post-hoc comparisons (Tukey HSD
504 test) were applied to discriminate between the means of values. Indeed, the one-way ANOVA

505 evaluates the variance of contaminant concentration in sediments within each class characterized
506 by an independent factor (in our case the belonging to one of the UPs). Outputs of the ANOVA
507 analyses are the degrees of freedom (df), sums of squares (SS), mean squares (MS) and the F
508 value for each independent variable considered. The F-value is the ratio between the mean of a
509 dependent variable (in our case the concentration of contaminants or hazard and risk level)
510 within each class of independent factor (UP) with the mean of the same variable in all dataset.
511 The F value is usually associated with p-value that measures the goodness/power with which the
512 analyses can verify or reject the null hypotheses, namely, that there is no difference between the
513 mean variable within each level of UP: thus the mean variable is μUPa= μUP3= μUP4 (where
514 $UP_a$=UP1+UP2). An estimated probability (p) lower than 0.01 and F>1 means that the
515 independent factor selected makes a significant difference in the variable (*i.e.* contaminant
516 concentration).

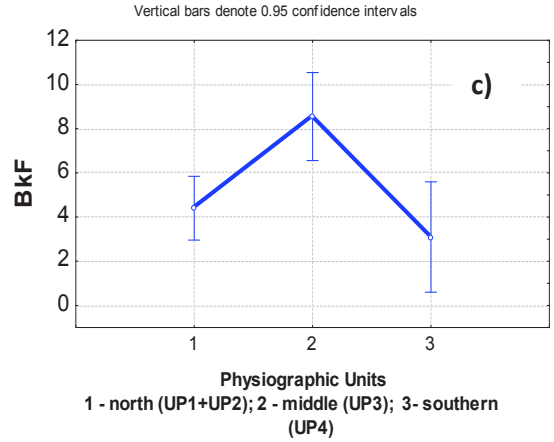
517 The ANOVA results confirmed the significant differences within the physiographic units
518 investigated, both in terms of cluster of prevalent contaminants for each UP and in terms of
519 average of contaminant concentration and hazard levels within each UP. As shown in Figure 5
520 a÷i, As, Cd and almost all the organic pollutants are the most relevant contaminants for UP3, in
521 which they display high levels of average concentration with respect to the northern coastal
522 track, $UP_a$ ($UP_a$=UP1+UP2) and to the southern UP (UP4). In addition, $UP_a$ and UP3, display
523 similar concentration trend of Ni, Cr and Pb, contrary to what happened for UP4.

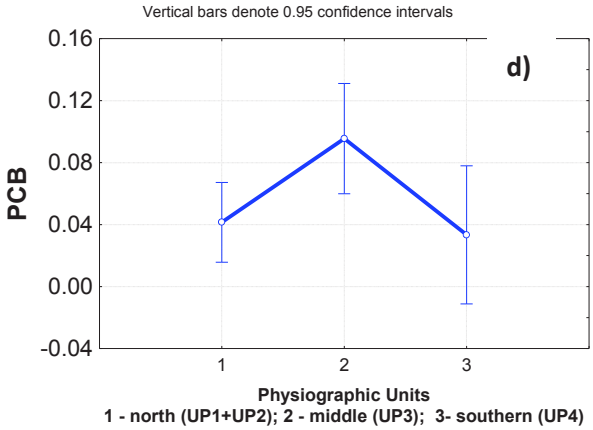
524


Vertical bars denote 0.95 confidence intervals
As
a)
Physiographic Unitis
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

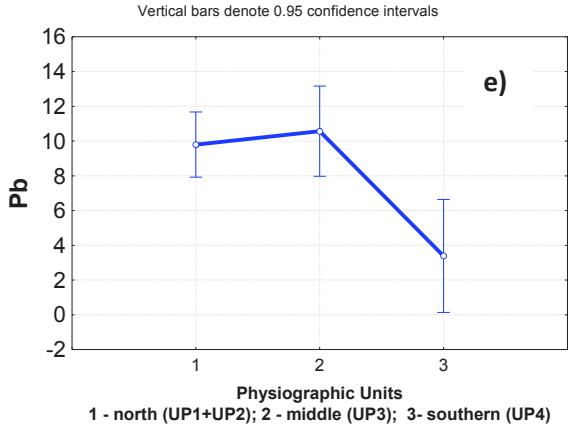

Vertical bars denote 0.95 confidence intervals
Cd
b)
Physiographic Units
1 - north (UP1-UP2); 2 - middle (UP3); 3- southern (UP4)

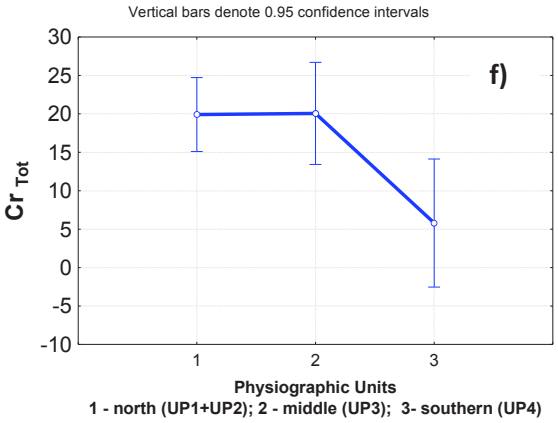
525


Vertical bars denote 0.95 confidence intervals
BkF
c)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)


Vertical bars denote 0.95 confidence intervals
PCB
d)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

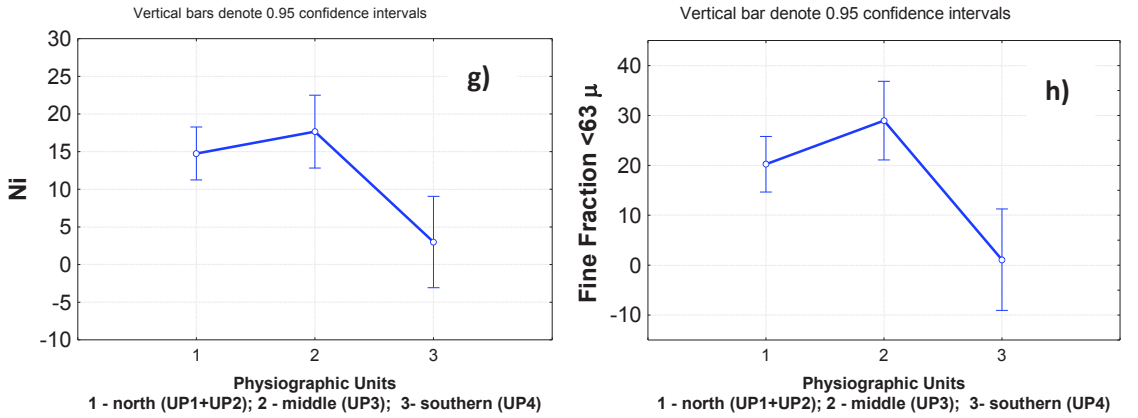
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Vertical bars denote 0.95 confidence intervals
Pb
e)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

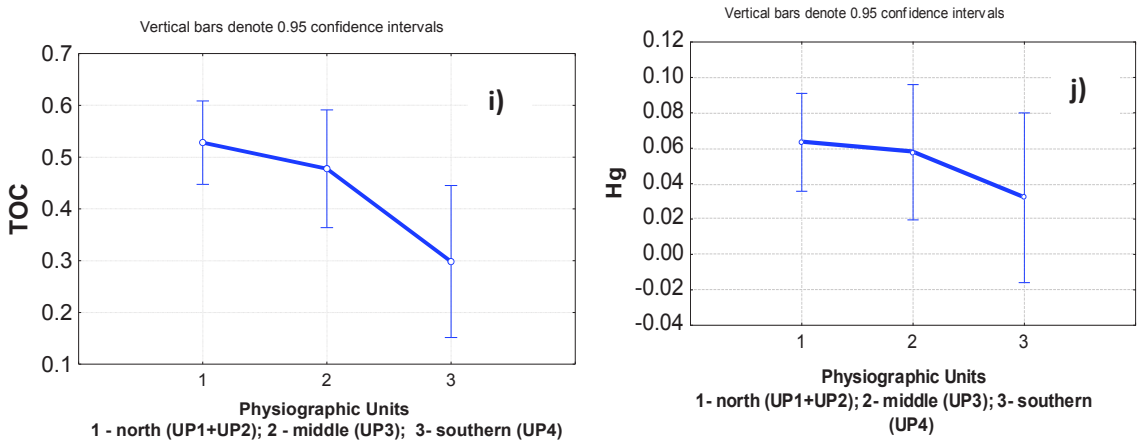

Vertical bars denote 0.95 confidence intervals
Cr Tot
f)
Physiographic Units
1 - north (UP1+UP2); 2 - middle (UP3); 3- southern (UP4)

527

528




529




530 **Figure 5.** Concentration of As (a), Cd (b), BkF (c), PCB (d), Pb (e), $Cr_{tot}$ (f), Ni (g), Fine fraction
531 (h), TOC (i) and Hg (j) in the investigated Physiographic Units.

532 As to UP4, all contaminants show low concentration levels, except for total Nitrogen ($N_{tot}$) that
533 reaches very high concentration levels. The analyses of the distribution of $N_{tot}$ during the years
534 within different UPs by means of factorial ANOVA (Figure 6) showed that the highest
535 concentration of total nitrogen was registered during 2013. This suggests that the presence of the
536 stream of "Canale Reale" (Royal Channel) (near sites TG1 and TG2) might cause the spike
537 concentration verified during 2013. In addition, the long-term monitoring of the excess of
538 nutrients demonstrates also the slow capacity of the ecosystem to naturally absorb the $N_{tot}$
539 contamination, as shown by the persisting of contamination for at least two years after the 2013.

540

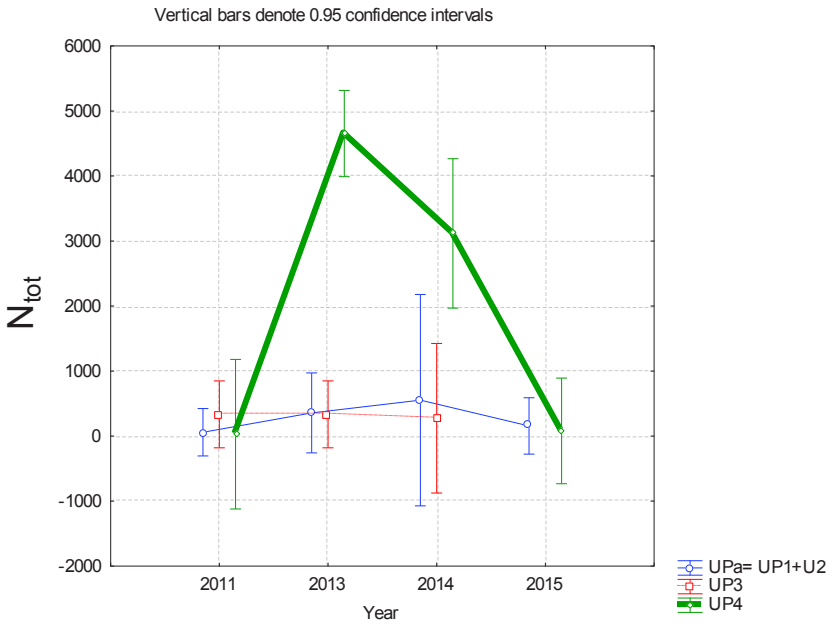


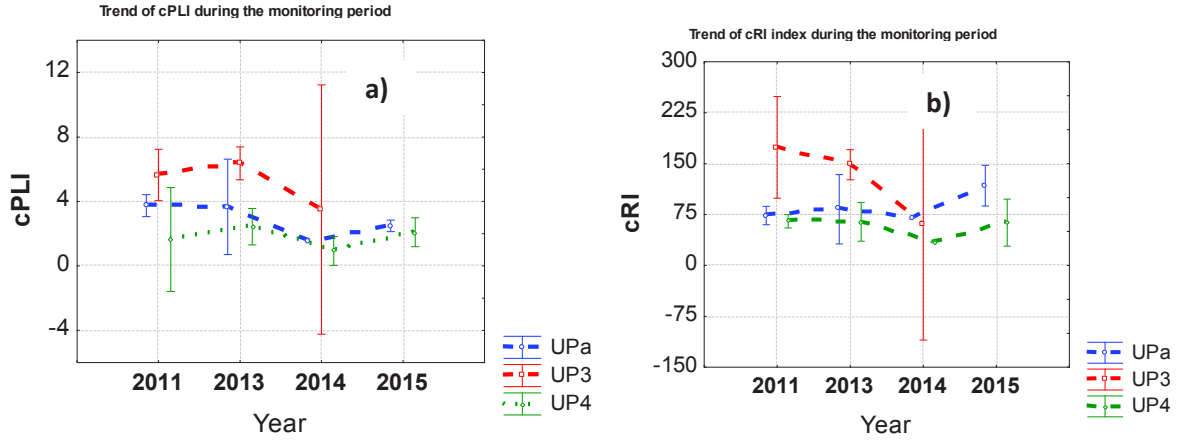

**Figure 6.** Distribution of total Nitrogen ($N_{tot}$), (calculated as unweighted mean) at different years and different UPs, performed by two-way ANOVA considering the year, as independent Factor A (Year including four classes: 2011 – 2013 -2014 -2015) and the three UPs considered (UPa=UP1+UP2) reported in blue, UP3 (in red) and UP4 (in green) as independent Factor B.

The one-way ANOVA was performed also to understand the influence of grain size in contamination degree, considering thus the content of Fine Fraction (∅ < 63 μm) as independent factor. Two classes were comprised in this case: Class A which includes samples with a content of finest sediment less than 50%; Class B which includes samples with fine fraction content more than 50%; Nevertheless, in this case the variance of contaminant concentration in the sediments within the two classes of grain size, demonstrated that there is no significant difference between the concentration of all six organic pollutants considered. The differences resulted significant only for three metals: $Cr_{tot}$ (F=19.48 p=0.000), Ni (F=30.24, p=0.000) and Pb (F=18.69; p=0.000) and for nutrients TOC (F=15.55, p=0.000), $P_{tot}$ (F=9.44 p=0.002). This circumstance confirms the importance of grain size (textural features) and organic content as carriers for lithogenic trace elements (Loring D.H. 1991; Covelli and Fontolan, 1997; Mayer, L.M., 1993; Dung et al. 2013; Mali et al. 2015; 2017c).

In order to understand the spatial and temporal trend of Pollution index (cPLI) and Risk Index (cRI) we performed two-way factorial ANOVA considering the Physiographic Unit as

561 independent factor A, and the different years as factor B (Figure 7). Also in this case the
562 differences resulted significant (except for the UP3) at $p<0.0001$. It was found that the average
563 values of the Risk Index for the UPa, which include both UP1+UP2 (countersigned in blue in the
564 graphs) and UP4 (green) indicate "low" ecological risk degree, while the Risk Index values for
565 the UP3 resulted the highest registered in the whole coastal track. A more marked difference
566 among the three UPs is observed by the analysis of the cPLI values that confirmed that the most
567 polluted sites belong to UP3 and the less polluted ones are those of UP4. In addition, cPLI
568 analysis revealed that $UP_a$ and UP4 areas, both less polluted than UP3, do not share the same
569 level of risk, being the $UP_a$ of higher concern respect to UP4. ANOVA tests confirmed also the
570 descending trend for hazard/risk revealed from 2011 to 2014 within the three UPs and the
571 increasing trend from 2014-2015 for the northern UPs and UP4.[1] It is necessary to highlight the
572 high extension of vertical bars at 0.95 confidence level of the mean values of the cPLI and cRI,
573 that demonstrates a high variability of the hazard level registered during 2014, indicating the
574 persistence of hot-spot sites within the UP3 (countersigned in red) during the monitoring period.

575




576

577

578 **Figure 7**. The cPLI (a) and cRI (b) trend in the sediments samples during the monitoring period
579 within the three UPs according factorial two-way ANOVA considering as Factor A the UP
580 displayed in the ordinate with three UP classes (UPa =UP1+UP2), UP3 and UP4) and, as factor
581 B, the Years displayed in abscissa, with four classes ("2011", "2013", "2014" and "2015").

[1] The mean values for UP3 did not present significant differences during 2015, therefore, the mean results of the dependent variables for this UP in 2015 was not calculated



b)

582 **4.2.2 PLS-DA responses**

583 Multivariate analyses, both supervised and unsupervised, are largely utilized in environmental
584 studies (Otto M. 1998; Kowalkowski et al. 2006; Astel et al. 2008). These methods allow to
585 extract the main orthogonal contributions (principal components) explaining most of the variance
586 of the dataset facilitating an overview of the environmental status of a given area scrutinizing
587 only the main responsible contaminants. This study was completed by performing a Partial Least
588 Squares Discriminant Analysis (PLS-DA) to identify clusters of contaminants that can
589 successfully discriminate the classes considered which, in our case, are the physiographic units.
590 PLS-DA method, which is a combination of PLS regression (PLSR) with discrimination rules
591 designed for classification (Ballabio & Consonni, 2013), helps to identify PLS components
592 which uncover the main covariation pattern within and between data matrices *X* and *Y*.

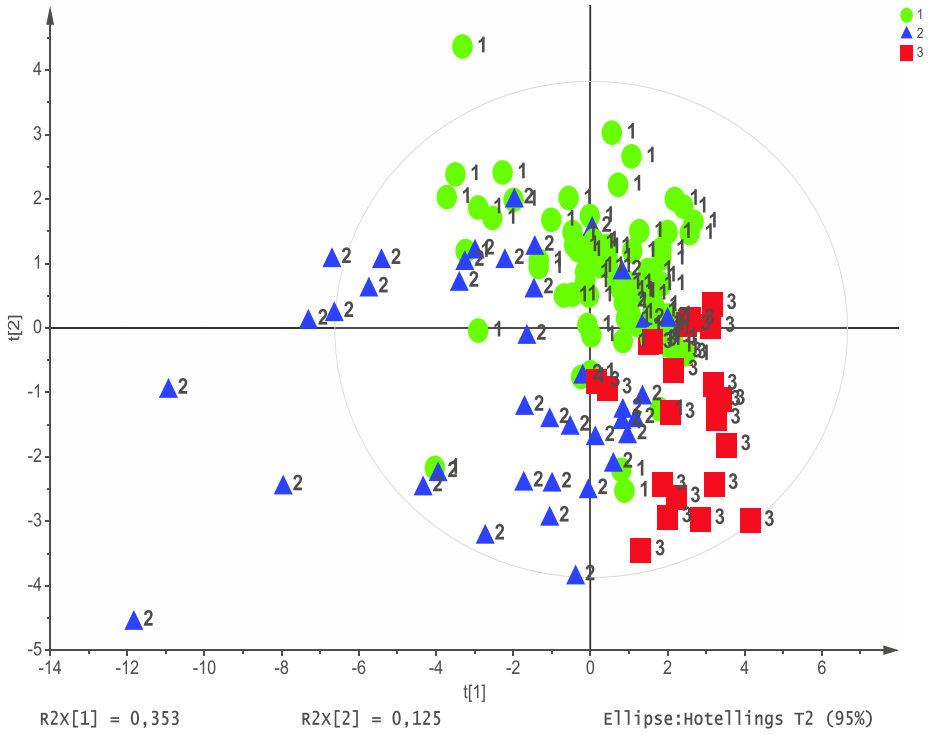
593 A model with four PLS components was considered, covering 75% of the total co-variance, as
594 shown in Figure 8. The validation success rate (SR) achieved for the classification is 81.25%, a
595 good result in environmental studies. The Score and Loading plots graphs for PLS1/PLS2 are
596 reported in the Figure 8. (Figure S1 shows plots for other 4 PLS components; Table S5 report the
597 miscellaneous classes of PLSDA).

598 Inspection of PLS1/PLS2 plot indicates that samples belonging to $UP_a$ are correlated to the
599 cluster of contaminants constituted by $Cr_{tot}$, Ni, Pb, Cd, that resulted highly associated with TOC
600 and fine fraction (named "*pelite"* in the plot). This circumstance indicates the occurrence of
601 terrigenous contribution of sediments coming from inland hydrographic waterways, especially
602 from different rivers discharges (Cervaro (VI), Candelaro (CR) and Ofanto (FO)). The
603 resuspension of fine sediments within watercourses leads to the transportation of fine particles of
604 terrigenous origin with the water flows (Fostner, 1984; Hancock, 2001). Indeed, the transported
605 particles erode the surfaces over which they pass and contributing to the breakdown of
606 continental rocks, nourishing the coastal sediment ad influencing their mineralogical and
607 geochemical composition (Mali et al. 2015; 2016). ). In addition, it is observed that Hg follows
608 the TOC trend. The results confirm the important role of organic matter in controlling mercury
609 (Hg) distribution and the terrestrial origin of organic matter in marine sediments. (Chakraborty et
610 al. 2015).

611 As to the second physiographic unit UP3, which covers the middle part of the Apulia coast and
612 includes highly populated areas such as Bisceglie (BB), Molfetta (MI), and Bari (BA), the

associated cluster of contaminants includes nearly all organic pollutants plus arsenic. In this UP the highest values for cRI and cPLI values were registered. More important, the highest relationship among main contaminants characterizing UP3 and the two indexes cRI and cPLI was found. This confirms that the main contribution in the deterioration of the quality of coastal water is caused by human activity.

In UP4, total nitrogen $N_{tot}$ constitute the main differentiating variables. The presence of the mouth of the stream "Canale Reale" (PP and TG) discharge, a channel that during its course crosses different municipalities (Francavilla Fontana, Oria, Latiano, Mesagne, Brindisi and Carovigno) potentially polluted with fertilizers from the agricultural campaign and other un-authorized discharges, can be held as the cause for the association of this contaminant with the hazard profile of the UP4.



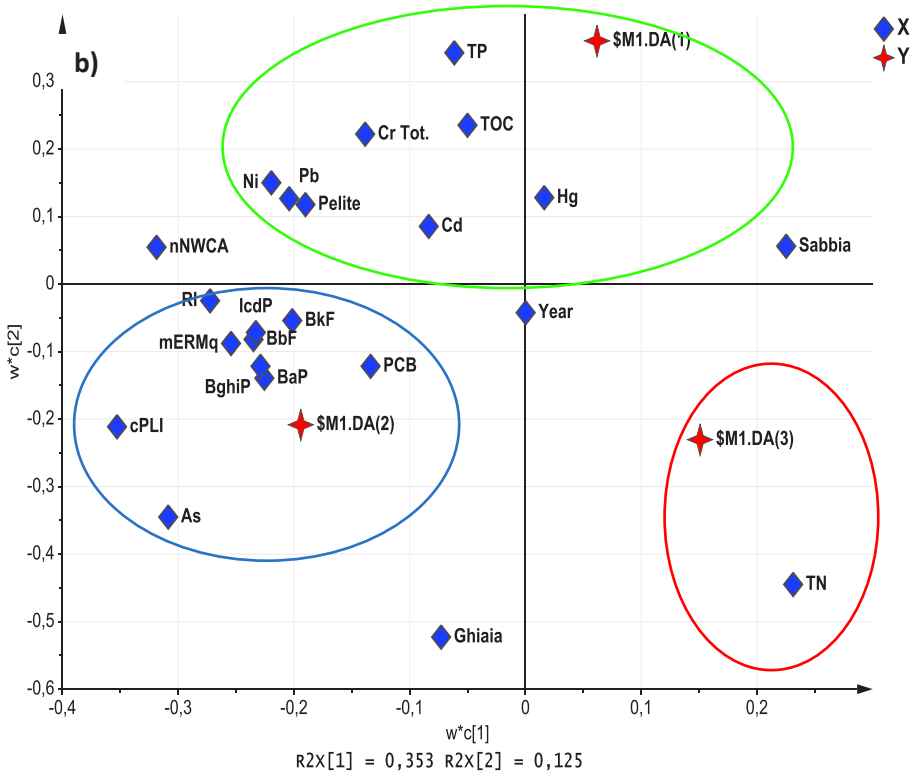




**Figure 8.** PLS score plot (a) and Loading plots (b) for PLS1/PLS2. The different Physiographic Units are countersigned with the numbers 1 for the north (UPa=UP1+UP2); with number 2 the middle UP3 and with number 3 the southern UP4.

### 4.3 Comparative analyses of cPLI and cRI with previous cumulative indexes

Aiming at validating the efficiency of the proposed indexes, a correlation between cRI and cPLI with two other cumulative indexes reported in literature was performed. The cRI was compared with the mean mERMq quotient (mERMq, Long at al. 2006), given that mERMq index individuates sediment sites with high probability of toxicity. The obtained square correlation resulted significant, as indicated by the values of the correlation coefficients $R^2$= 0.684, $r$=0.824, $p < 0.005$ (Figure S2). The cPLI was compared with the c_NWAC, being both indices related to the cumulative synergic effect of co-presence of contaminants of different classes. Also in this case the correlation coefficients resulted positive even if less significant ($R^2$= 0.469, $r$=0.65 $p$<0.000). The slightly worst correlation of the cNWAC with cPLI can be explained with the fact that the cPLI includes also an unpredictable toxic contribution $HI_{toc}$ (by the toxicological response of the bioassay tests performed) that was not considered in the previously calculated cNWAC.

These results support the reliability of the two performed indexes in providing practical and speedy tools for individuating hot spots within the coastal area and comprehensively evaluation of the hazard degree.

## 5 Conclusions

Given that the quality of sediments is an indicator of marine-water pollution status, the proposed pollution indexes cPLI and cRI, based on weighted relevance of some contaminant indicators controlling the pollution status of the sea sediment, support a reliable assessment of the contamination trend within the marine-coastal area of Apulia Region. The coastal tracks of most concern resulted those close to metropolitan cities, confirming that the main contribution in the deterioration of the marine coasts arises from human activity. In addition, an important role of the inland hydrographic network was recognized, which caused a contamination of terrigenous origin (river charges, rainfall waters, etc.) by transferring organic pollutants and fertilizers from the agricultural campaign to the marine coastal water bodies.

The combination of cPLI and cRI with multivariate analyses resulted to be crucial for extracting essential information from large datasets generated by the long-term monitoring and furnished a quick way to interpret data stemming from complex systems. Indeed, while on one side, the

long-term monitoring offered useful information on anthropogenic and natural changes occurring over time, the multivariate analyses supported identification of contaminant of most concern, facilitated the prediction of the contamination trend and identified the factors responsible for such trend, distinguishing between the contribution weight given by the natural and anthropogenic ones.

Finally, the proposed strategy can directly improve the reliability of Hazard Assessment Procedure reducing costs and time and helping an efficient orienting of future environmental monitoring of coastal area.

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