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

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13

14 **Abstract**

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

30

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

33

34 **1. Introduction**

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

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

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

66 It is well known that bottom sediments can be used as valuable indicators of the impact of human
67 activity on the water bodies' quality, being they capable of accumulating both organic and
68 inorganic pollutants over time. Several studies have considered the role of sediments in
69 determining the fate of metals and organic compounds in seawater (Chapman D. 1996; Tomadin,
70 2000; Spagnoli et al., 2010; Mugnai et al. 2010; Lofu et al. 2016; Chapman et al. 2013; Mali et
71 al. 2016). Moreover, the impact of hydrodynamic processes on the spatial distribution of
72 pollutants in coastal areas has been addressed (Malcangio et al. 2017; Valentini et al. 2017; Mali
73 et al. 2017a, 2017b; 2018). Evaluation of contaminant concentration in marine sediments is a
74 major part of the assessment program for coastal area because it helps to define the hazard
75 degree, to predict the potential threat of pollutants, and to identify the allocation of pollution
76 sources (Morillo et al., 2004; 2008; Borja et al. 2008; Piva et al. 2011; Mali et al. 2017c). For
77 this reason, great efforts have been made to establish Sediment Quality Guidelines (SQGs), using
78 different calculation approaches (Chapman 1989; Del Valls et al. 1998; Wenning et al. 2005;
79 Long et al. 2006; Chapman 2001; Chapman 2007; Ritter et al. 2008; Piva et al. 2011; Regoli et
80 al., 2013, Gredilla et al. 2014; Zahra et al. 2014; Souza et al. 2016).

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

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

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

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

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

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

122

123 2. Materials and Methods

124 2.1. Hydrographical and geomorphological features of the study area

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

131 From a morphological point of view, according to the criteria defined in Apulia Coastal Regional
132 Plan (Piscitelli et al. 2011), the analyzed coastal track includes four natural Physiographic Units
133 (UPs), that constitute areas delimiting barriers against the longitudinal transport of solids (Figure
134 1a).

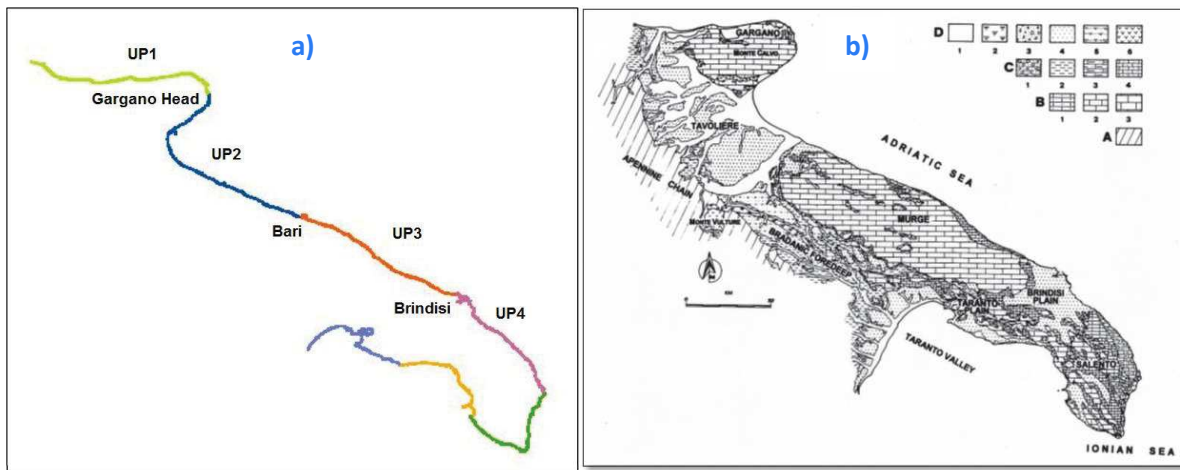
135 The northern UP (Physiographic Unit - UP1) extends from the Peschici-Vieste up to Head of
136 Gargano Promontory. It is characterized by generally high cliff rocky coast, with predominantly
137 calcareous sediments and siliceous calcareous rocks. In this track, there are widespread forms of
138 marine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

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

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

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

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



167
168 **Figure 1.** a) The Physiographic Unit (UPs) defined by the Regional Coastal Plan of Apulia
169 Region.; b) the Geological features of Apulia platform according to Piscitelli et al, 2011.

170

171 2.2. Data collection

172 As required by the Italian Legislation transposing at national level the WFD (D.Lgs. 152/2006;
173 D.M. 260/2010), the long-term monitoring programme for coastal area has been activated by
174 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 m². 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.



200

201 **Figure 2.** The sampling map; a) sediment sampling distribution and river estuaries location
 202 alongside coast, b) an inset showing a close-up of the two transects considered (01 and 02 for
 203 200/500 and 1750 m from the coastline respectively)

204

205 **2.4. Analytical methods**

206 Each sample was classified according to Shepard (1954) into four sections: gravel: >2 mm; sand:
 207 2–0.063 mm; silt: 0.063–0.002 mm; clay <0.002 mm. A set of ASTM sieves was used for the
 208 granulometric separation (Romano and Gabellini, 2001).

209 Trace metal concentrations were measured by inductively coupled plasma mass spectrometry
 210 (ICP/MS X Series Thermo Fisher Scientific) after sample mineralization by total acid digestion
 211 (HCl, HNO₃ and HF) (Pellegrini and Lucarotti, 2001). The < 63 μm fraction, dried at 105 °C,
 212 was used for the determination of metals in order to reduce the grain size effect. The detection
 213 limits (LODs) were calculated from 3 replicates of procedural blanks. The estimated LODs were
 214 equal to 1 ppb for all metals. Marine Sediment Reference Materials 2702 (Inorganics in Marine
 215 Sediment) were used to control the analysis quality: the agreement between the analytical results
 216 for the certified and measured values was satisfactory, with recoveries ranging from 80% to
 217 100% for all metals.

218 The total nitrogen amount (N_{tot}) was determined by an elemental analysis procedure with a
219 Perkin–Elmer 240B CHN Elemental Analyzer. The total phosphorus concentration (P_{tot}) was
220 determined by colorimetric titration using the molybdenum-blue method (Aspila et al., 1976).
221 Total organic carbon (TOC) was determined by an elemental analysis procedure with a Perkin–
222 Elmer 240B CHN Elemental Analyzer, after removal of carbonates by reaction with
223 hydrochloric acid (Giani, 2001).

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

238

239 **2.5. Statistical analyses**

240 Analysis of variance (ANOVA) was applied to perform the spatial and temporal distribution of
241 contaminants and hazard degree in sediment samples. One-way ANOVA was used to identify
242 the differences in the hazard levels in sediment due to their physicochemical properties and total
243 contents of pollutants based on the relevant physiographic unit (UP). PLS-DA was used to
244 optimize separation between samples belonging to different groups and to identify the main
245 contaminants controlling each UP and each Grain Size classes. The PLS-DA is aimed at
246 maximizing the covariance between the independent variables (in our case the UP and Grain
247 Size features), and the corresponding dependent variable Y (concentration of contaminants). On
248 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) | $I_{geo} = I_{g0} + \frac{C_n}{1.5 + B_n}$ 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) | $E.F. = \frac{\left(\frac{C_x}{B_x}\right)_{sample}}{\left(\frac{C_b}{B_b}\right)_{background}}$ 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) | $RAC = \frac{F1}{C_{tot}}$ 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) | $CF = \frac{C_{metal}}{C_b}$ 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) | $PLI = (CF1 \times CF2 \times CF3 \times \dots \times CFn)^{1/n}$ 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 $Ei = Ti \times CF$); Ti is the toxicity response factor and CF the Contamination Factor (see 4) | Hakanson. (1980) |

276

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

278 3. Results

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

285 3.1.1. Chemical Hazard Index for pollution degree assessment (HI_{ch})

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

- 294 - it is a speedy index for a comprehensive chemical pollution evaluation since it considers
295 only the main Priority Substances considered by Directive 2013/39/EU for water quality
296 purposes (six metals and six persistent organic pollutants) selected as indicator
297 contaminants controlling the chemical status of sediments;
- 298 - the modified Contamination Factor used to calculate the HI_{ch} , includes a "weighted"
299 value, introduced by Directive 2013/39/EU for water quality assessment. This value
300 ranges from 1 to 1.3, depending on whether the contaminant is not covered by the
301 Directive (weight = 1), or, on the contrary it is included in the list of "priority" substances
302 (weight = 1,1) or in the list of "dangerous and priority" substances (weight = 1,3), aiming
303 therefore to attribute to each pollutant a proportional relevance on the chemical status of
304 sediment according to their hazard potential.

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

$$CF_{ir} = \frac{C_{mi}}{C_{si}} \times W_o \quad (\text{Eq. 1})$$

307

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

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

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

320

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

322 3.1.2. Toxicological contribution to the comprehensive hazard evaluation

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

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

- 341 1. for each toxicological class defined by the Italian legislation normative (ICRAM 2009),
342 an arbitrary coefficient “k” was given, ranging from 1 to 2.5: $k = 1.0$ for Class A; $k=1.5$
343 for Class B; $k = 2$ for Class C; $k=2.5$ for Class D.
- 344 2. An “eco-toxicological weighted factor”, W_{oe} , was given also to each result, as function of:
 - 345 a) the severity of the effect “En”, intended as the severity of the biological damage
346 measured by the specific end-point considered: $En = 2.4$ was given for bioluminescence;
347 $En = 2.1$ was given for the inhibition of growth rate test; $En = 1.9$ was given for
348 development rate test);
 - 349 b) the type of exposure T (acute or short-term $T= 1$ while $T= 0.8$ for chronic or long-term
350 exposure);
 - 351 c) the type of test matrix M: $M=0.8$ when the elutriate phase was considered; $M=1$ when
352 the sediment or solid phase was considered as matrix for eco-toxicological tests.
 - 353 d) a representative weight given for the potential hormesis for each experimental type.
354 For the bioluminescence on solid phase and elutriate on *Vibro-fisheri* a
355 representativeness weight $W=0.25$ is defined; for the inhibition of algal growth rate on
356 elutriate a $W=0.10$ is considered, and for embryo-toxicity with *Paracentrotus Lividus* is
357 given $W=0.15$.

358 The above reported values given to elements constituting the weighting factor W_{oe}
359 comply with what proposed by Piva et al., (Piva et al. 2011). Actually, these are taken as
360 guidelines in the most recent Italian National Laws on Sediment Handling (D.Lgs.
361 172/2016 and 173/2016) for the integrated quality assessment of dredged sediments.

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

$$\begin{aligned}
 364 \quad & Woe_i = En_i \times T_i \times M_i \times W_i \\
 & HI_{tox} = \sum_{i=1}^j (k_i \times Woe_i) \\
 365 \quad & \hspace{20em} \text{(Eq. 3)}
 \end{aligned}$$

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

369 **3.1.3. Comprehensive pollution Index (cPLI)**

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

$$\begin{aligned}
 373 \quad & cPLI = HI_{ch} * HI_{tox} \quad \text{(Eq. 4)}
 \end{aligned}$$

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

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

379 **3.2. Potential ecological Risk Index for comprehensive pollution degree**

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

$$\begin{aligned}
 383 \quad & CE_i = T_i \times CF_{ir} \quad \text{(Eq. 5)}
 \end{aligned}$$

$$\begin{aligned}
 384 \quad & cRI = \sum_{i=1}^n (T_i \times CF_{ir}) \quad \text{(Eq. 6)}
 \end{aligned}$$

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

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

- 394 1. the formulae for calculation of each pollutant toxic factor requirement, T_i , were those
395 defined by Hakanson, therefore the T_i values are: $T_{Hg} = 80$; $T_{Cd} = 30$; $T_{As} = 10$, $T_{Pb} = 5$;
396 $T_{Ni} = T_{Cr} = 2$; $T_{PCB} = 40$.
- 397 2. the value of T_i of PCB was selected as representative for the other lipophilic toxic
398 substances considered in this study. Indeed, being the organic pollutants included in the
399 same list of Priority substances, the value $T_i = 40$ given for PCB is considered as
400 appropriate also for BaP (Benzo(a)Pyrene), BbF (Benzo(b)Floranthene), BghiP
401 (Benzo[ghi]perylene), BkF (Benzo(k)Floranthene), InD (Indeno, 1.2.3 c.d.pyrene).
- 402 3. the evaluation of Risk Index (cRI) and Pollution index (cPLI) includes only the priority
403 Substances given by European Decree (39/2008/CE) for water bodies quality namely 6
404 metals/metalloids and 6 organic pollutants (Sum of for congeners of PCB and 5 high
405 molecular PAHs). Therefore, the discussion about the hazard risk evaluation is referred to
406 the presence of these contaminants (As, Hg, Cd, Pb, Cr, Ni, Sum of PCB, BaP, BbF,
407 BghiP, BkF, InD). Nevertheless, being these contaminants considered by the Water
408 Framework Directive as the main “weighted” pollutants to be monitored in the long term
409 programs for water quality, the assessment results can also be extended to other
410 contaminants having the same behavior in coastal ecosystem;
- 411 4. since some physico-chemical properties of the surficial sediments are very important,
412 especially when the sensitivity of different marine basins was considered, we used the
413 organic content (TOC) and grain size distribution as necessary co-parameters in the
414 calculation of Risk Indexes (see the discussion below).

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

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

| <i>cPLI</i> | <i>Comprehensive Pollution Hazard degree as (HI_{ch}:HI_{10c})</i> | <i>cRI</i> | <i>Potential ecological risk degree as $\sum E_i \cdot T_i$</i> |
|-----------------------|--|-------------|--|
| <i>cPLI</i> <1.9 | Low hazard degree | cRI<95 | Low risk |
| 1< <i>cPLI</i> <2.8 | Moderate hazard degree | 95<cRI<190 | Moderately risk |
| 2.8< <i>cPLI</i> <6.5 | Considerable Hazard degree | 190<cRI<380 | Considerable risk |
| <i>cPLI</i> >6.5 | High hazard degree | cRI>380 | Very high risk |

419

420 3.3. Comparative assessment of different hazard indexes

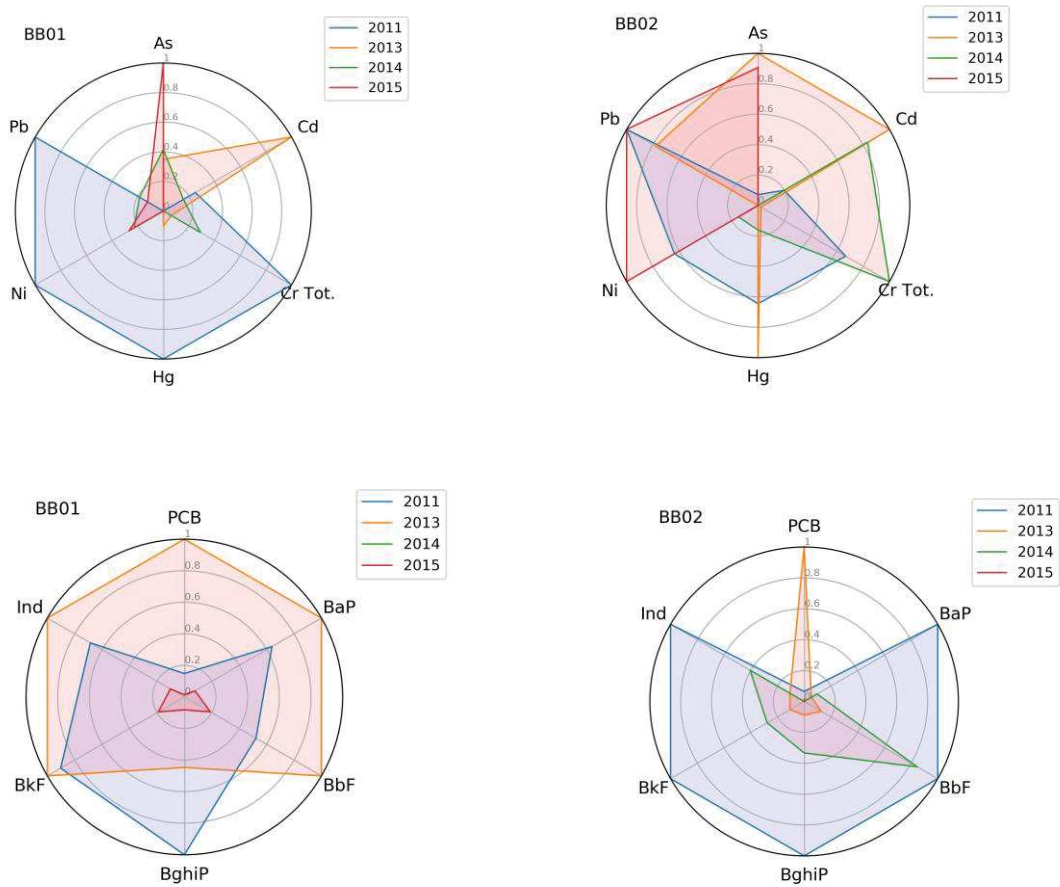
421 We compared the results obtained with cPLI and cRI with those achieved with the cumulative
 422 index c-NWAC (SWRCB, 2006; Mali et al., 2016) and mean ERM quotient (mERMq),
 423 respectively. In particular, c-NWAC considered different classes of contaminants (heavy metals
 424 and organic pollutants)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

427 4. Discussion of results

428 4.1 Chemical contamination factors

429 The basic statistical parameters and the measured concentrations of pollutants are shown in
 430 **Table 3.** The distribution of the metal and organic pollutant concentrations during different years
 431 are illustrated through radial graphs as reported Figure 3. Comparing the concentration of
 432 contaminants with the Environmental Standard Values (simple contamination factor, CF_i)
 433 revealed that As, Cd and Ni are the pollutants of most concern, followed by Hg, BaP, BkF since
 434 for all of them CF_i>1. The respective hazard degree for these pollutants indicates hazard levels
 435 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_1 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.

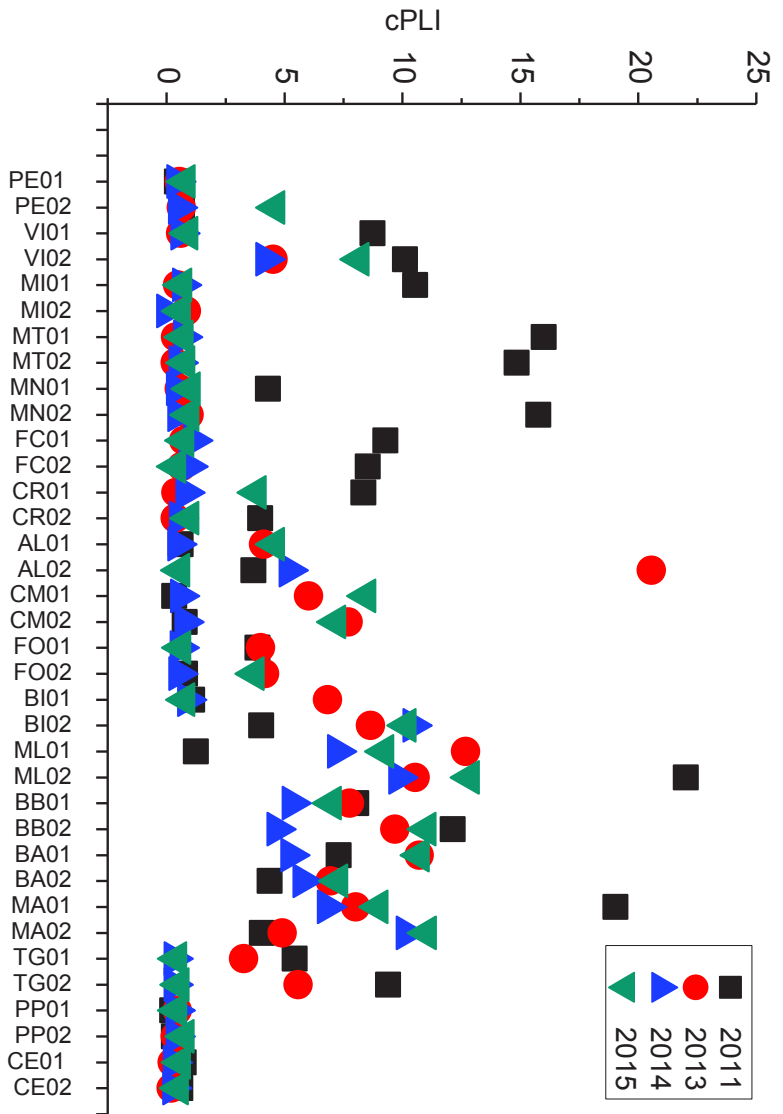
450

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.

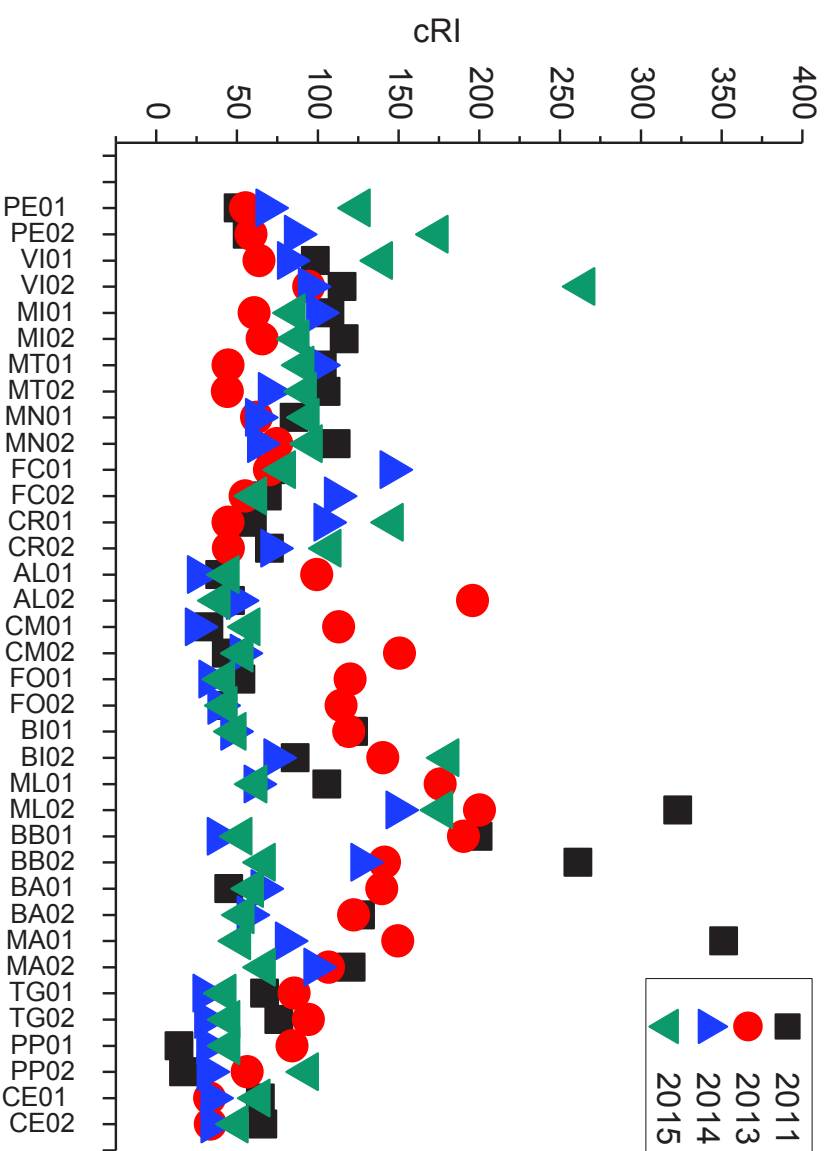
| Year | 2011 | | | | 2013 | | | | 2014 | | | | 2015 | | | |
|-------------------------------------|-------|------|-------|--------|--------|-------|-------|-------|-------|--------|-------|---------|-------|--------|-------|--------|
| | MEAN | MIN | MAX | S.D. | MEAN | MIN | MAX | S.D. | MEAN | MIN | 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 (<i>Pelite</i>) (%) | 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.



473



474

475

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
$$\text{Cd} > \text{Hg} > \text{BkF} > \text{As} > \text{BaP} > \text{BbF} > \text{BhgiP} > \text{InP} > \text{Pb} > \text{Ni} > \text{Cr} > \text{PCB}$$

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).

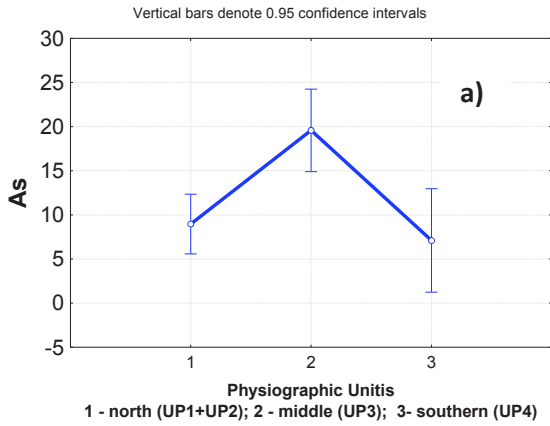
496 **4.2.1 ANOVA responses**

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

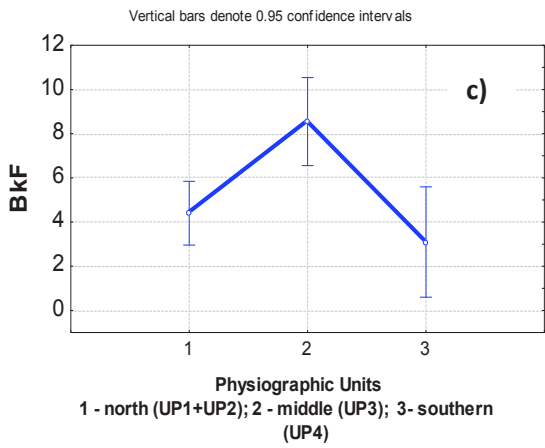
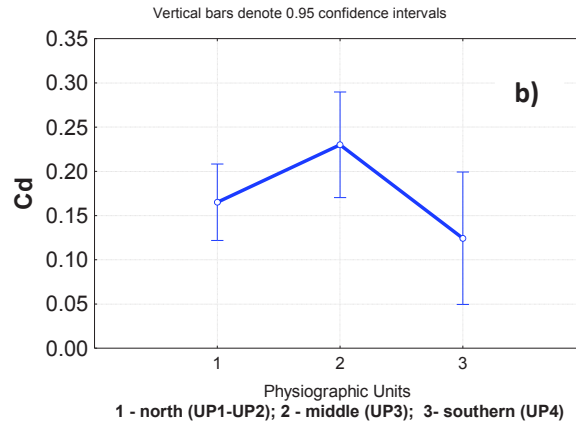
505 evaluates the variance of contaminant concentration in sediments within each class characterized
506 by an independent factor (in our case the belonging to one of the UPs). Outputs of the ANOVA
507 analyses are the degrees of freedom (df), sums of squares (SS), mean squares (MS) and the F
508 value 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 $\mu_{UPa} = \mu_{UP3} = \mu_{UP4}$ (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.

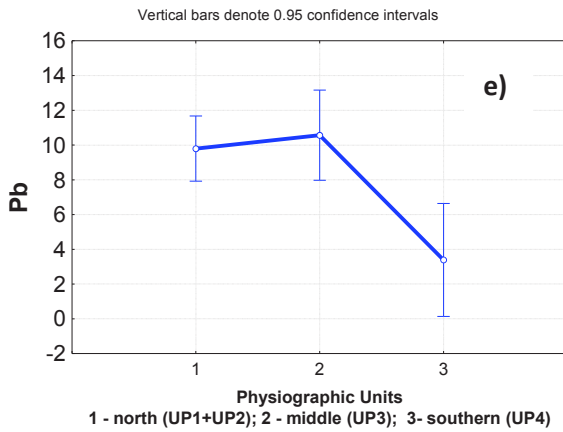
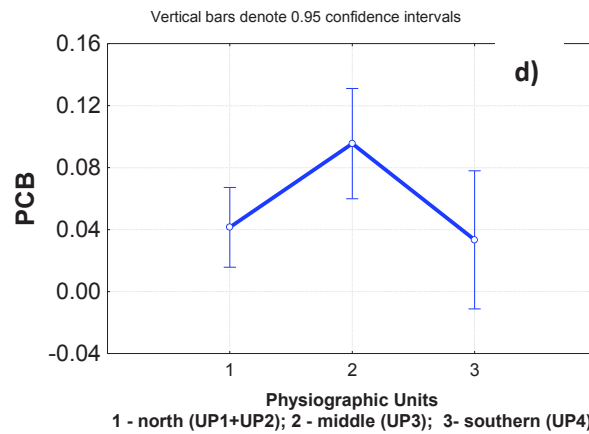
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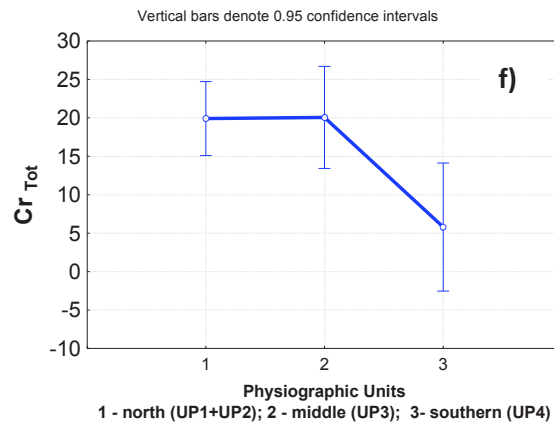
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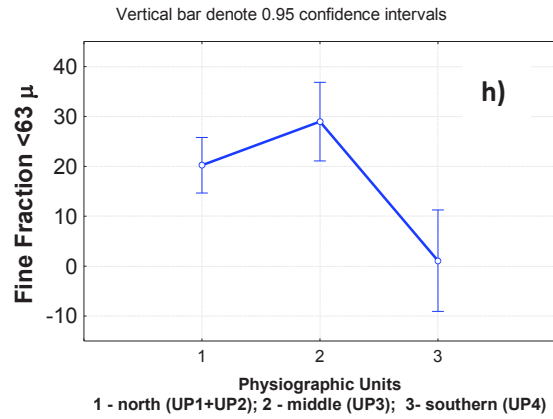
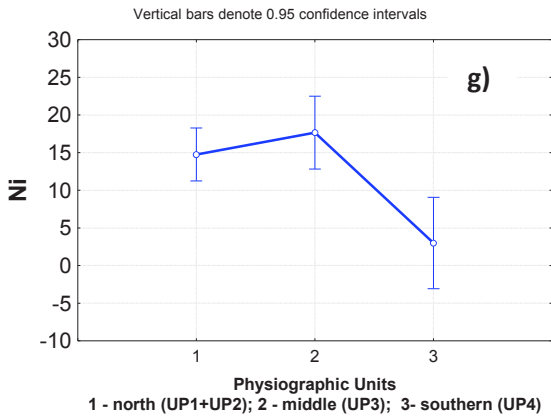


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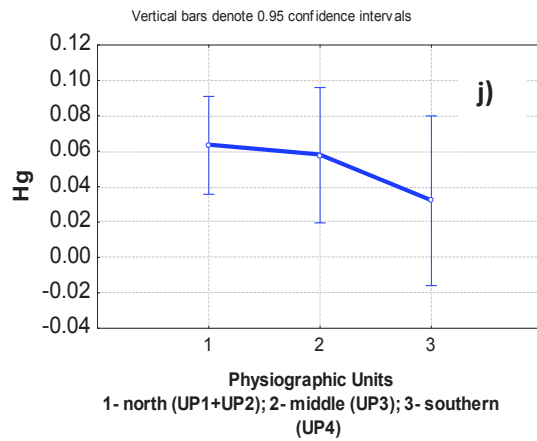
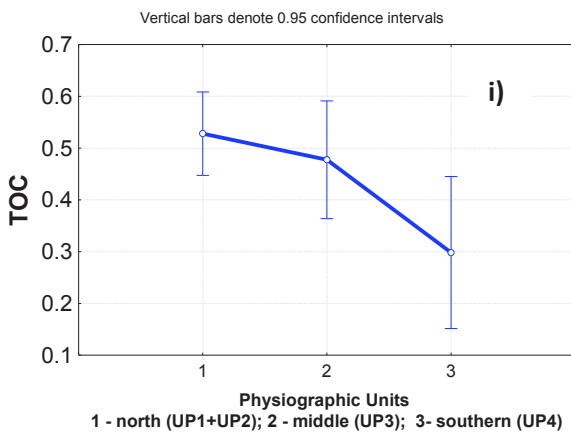


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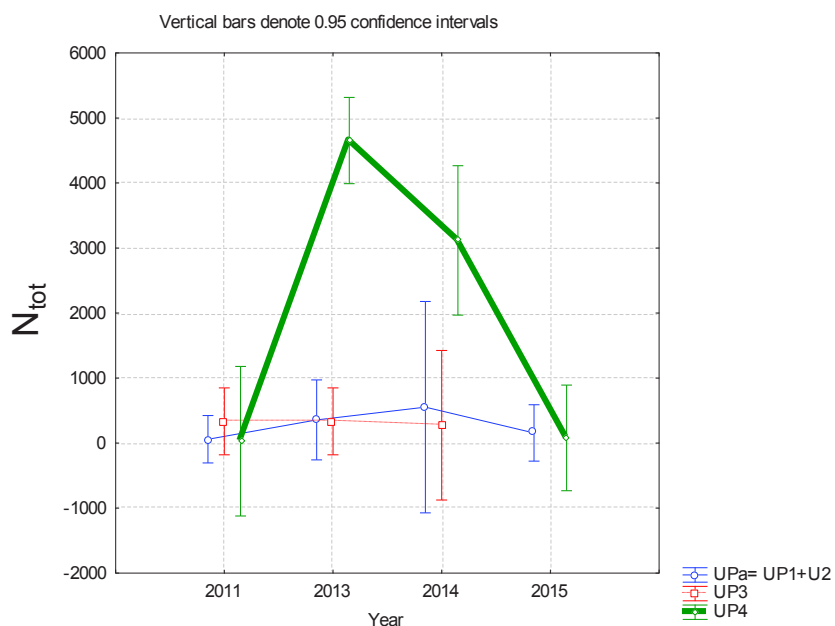


529

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



541

542 **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.

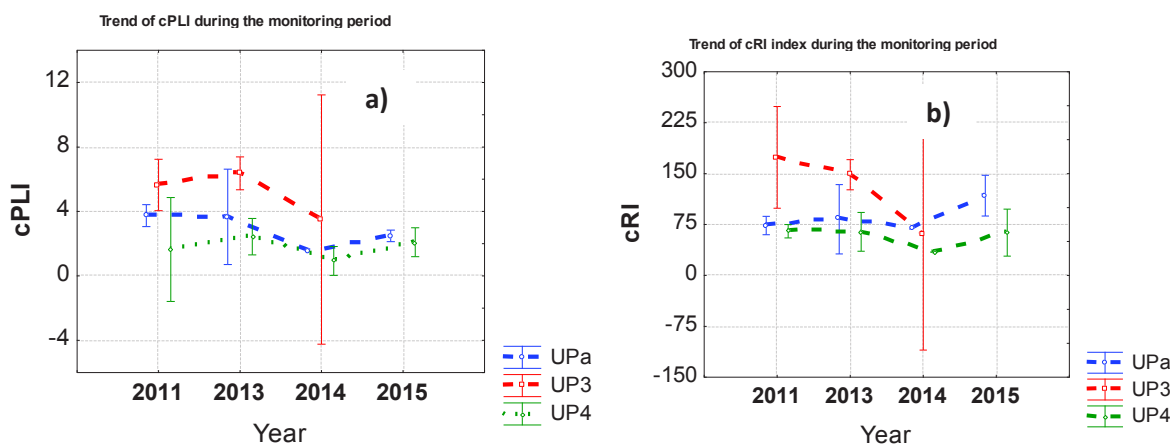
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547 The one-way ANOVA was performed also to understand the influence of grain size in
 548 contamination degree, considering thus the content of Fine Fraction ($\varnothing < 63 \mu\text{m}$) as independent
 549 factor. Two classes were comprised in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

559 In order to understand the spatial and temporal trend of Pollution index (cPLI) and Risk Index
 560 (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 UP_a, 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 (UP_a =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

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 and 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).

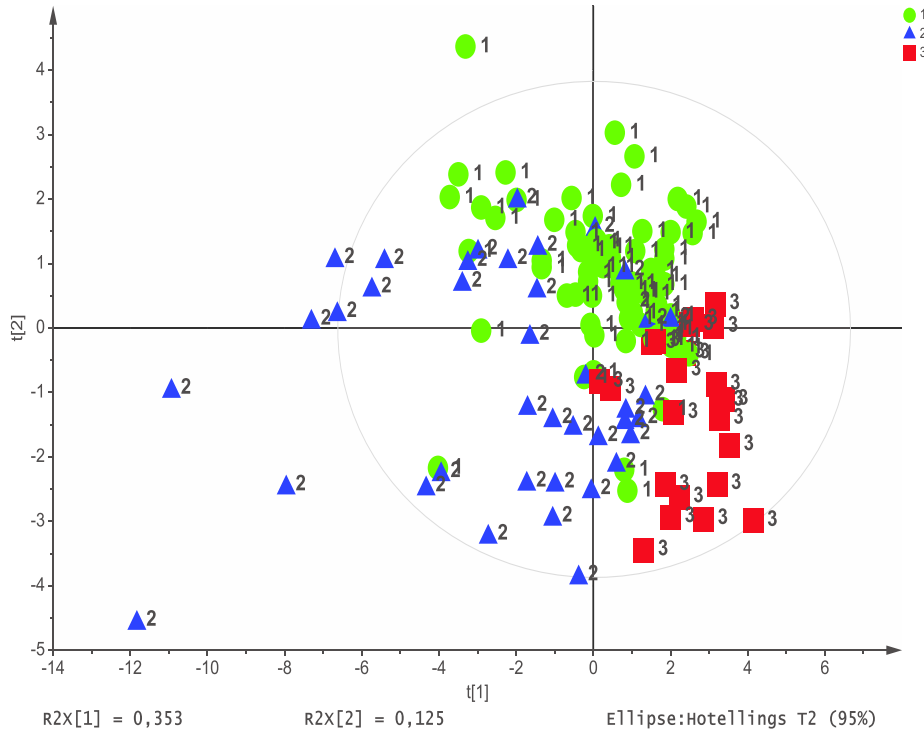
611 As to the second physiographic unit UP₃, which covers the middle part of the Apulia coast and
612 includes highly populated areas such as Bisceglie (BB), Molfetta (MI), and Bari (BA), the

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

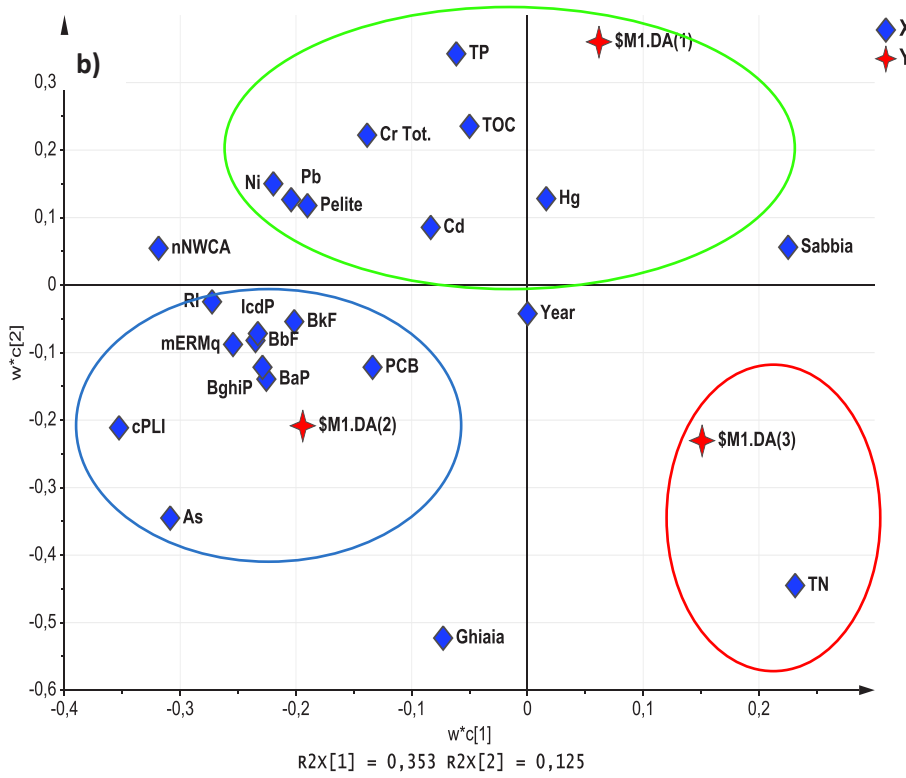
618 In UP4, total nitrogen N_{tot} constitute the main differentiating variables. The presence of the
619 mouth of the stream “Canale Reale” (PP and TG) discharge, a channel that during its course
620 crosses different municipalities (Francavilla Fontana, Oria, Latiano, Mesagne, Brindisi and
621 Carovigno)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

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

648 **4.3 Comparative analyses of cPLI and cRI with previous cumulative indexes**

649 Aiming at validating the efficiency of the proposed indexes, a correlation between cRI and cPLI
650 with two other cumulative indexes reported in literature was performed. The cRI was compared
651 with the mean mERMq quotient (mERMq, Long et al. 2006), given that mERMq index
652 individuates sediment sites with high probability of toxicity. The obtained square correlation
653 resulted significant, as indicated by the values of the correlation coefficients $R^2= 0.684$, $r=0.824$,
654 $p < 0.005$ (Figure S2). The cPLI was compared with the c_NWAC, being both indices related to
655 the cumulative synergic effect of co-presence of contaminants of different classes. Also in this
656 case the correlation coefficients resulted positive even if less significant ($R^2= 0.469$, $r=0.65$
657 $p<0.000$). The slightly worst correlation of the cNWAC with cPLI can be explained with the fact
658 that the cPLI includes also an unpredictable toxic contribution HI_{toc} (by the toxicological
659 response of the bioassay tests performed) that was not considered in the previously calculated
660 cNWAC.

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

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665 **5 Conclusions**

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

675 The combination of cPLI and cRI with multivariate analyses resulted to be crucial for extracting
676 essential information from large datasets generated by the long-term monitoring and furnished a
677 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.

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690

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

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13

14 **Abstract**

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

30

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

33

34 1. Introduction

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

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

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

66 It is well known that bottom sediments can be used as valuable indicators of the impact of human
67 activity on the water bodies' quality, being they capable of accumulating both organic and
68 inorganic pollutants over time. Several studies have considered the role of sediments in
69 determining the fate of metals and organic compounds in seawater (Chapman D. 1996; Tomadin,
70 2000; Spagnoli et al., 2010; Mugnai et al. 2010; Lofu et al. 2016; Chapman et al. 2013; Mali et
71 al. 2016). Moreover, the impact of hydrodynamic processes on the spatial distribution of
72 pollutants in coastal areas has been addressed (Malcangio et al. 2017; Valentini et al. 2017; Mali
73 et al. 2017a, 2017b; 2018). Evaluation of contaminant concentration in marine sediments is a
74 major part of the assessment program for coastal area because it helps to define the hazard
75 degree, to predict the potential threat of pollutants, and to identify the allocation of pollution
76 sources (Morillo et al., 2004; 2008; Borja et al. 2008; Piva et al. 2011; Mali et al. 2017c). For
77 this reason, great efforts have been made to establish Sediment Quality Guidelines (SQGs), using
78 different calculation approaches (Chapman 1989; Del Valls et al. 1998; Wenning et al. 2005;
79 Long et al. 2006; Chapman 2001; Chapman 2007; Ritter et al. 2008; Piva et al. 2011; Regoli et
80 al., 2013, Gredilla et al. 2014; Zahra et al. 2014; Souza et al. 2016).

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

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

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

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

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

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

122

123 2. Materials and Methods

124 2.1. Hydrographical and geomorphological features of the study area

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

131 From a morphological point of view, according to the criteria defined in Apulia Coastal Regional
132 Plan (Piscitelli et al. 2011), the analyzed coastal track includes four natural Physiographic Units
133 (UPs), that constitute areas delimiting barriers against the longitudinal transport of solids (Figure
134 1a).

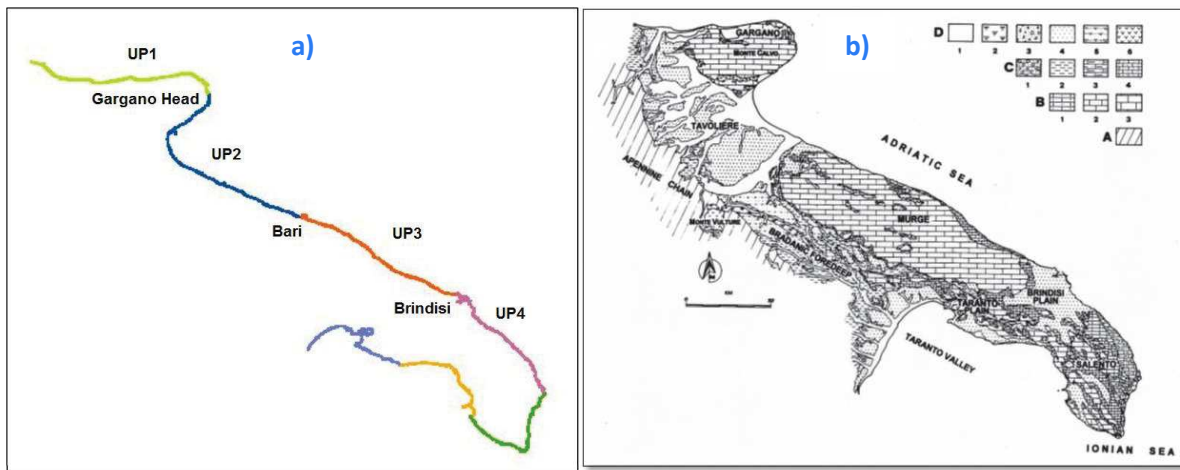
135 The northern UP (Physiographic Unit - UP1) extends from the Peschici-Vieste up to Head of
136 Gargano Promontory. It is characterized by generally high cliff rocky coast, with predominantly
137 calcareous sediments and siliceous calcareous rocks. In this track, there are widespread forms of
138 marine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

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

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

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

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



167
168 **Figure 1.** a) The Physiographic Unit (UPs) defined by the Regional Coastal Plan of Apulia
169 Region.; b) the Geological features of Apulia platform according to Piscitelli et al, 2011.

170

171 2.2. Data collection

172 As required by the Italian Legislation transposing at national level the WFD (D.Lgs. 152/2006;
173 D.M. 260/2010), the long-term monitoring programme for coastal area has been activated by
174 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 m². 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.

218 The total nitrogen amount (N_{tot}) was determined by an elemental analysis procedure with a
219 Perkin–Elmer 240B CHN Elemental Analyzer. The total phosphorus concentration (P_{tot}) was
220 determined by colorimetric titration using the molybdenum-blue method (Aspila et al., 1976).
221 Total organic carbon (TOC) was determined by an elemental analysis procedure with a Perkin–
222 Elmer 240B CHN Elemental Analyzer, after removal of carbonates by reaction with
223 hydrochloric acid (Giani, 2001).

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

238

239 **2.5. Statistical analyses**

240 Analysis of variance (ANOVA) was applied to perform the spatial and temporal distribution of
241 contaminants and hazard degree in sediment samples. One-way ANOVA was used to identify
242 the differences in the hazard levels in sediment due to their physicochemical properties and total
243 contents of pollutants based on the relevant physiographic unit (UP). PLS-DA was used to
244 optimize separation between samples belonging to different groups and to identify the main
245 contaminants controlling each UP and each Grain Size classes. The PLS-DA is aimed at
246 maximizing the covariance between the independent variables (in our case the UP and Grain
247 Size features), and the corresponding dependent variable Y (concentration of contaminants). On
248 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; Desaulles
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) | $I_{geo} = I_{g0} + \frac{C_n}{1.5 + B_n}$ 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) | $E.F. = \frac{\left(\frac{C_x}{B_x}\right)_{sample}}{\left(\frac{C_b}{B_b}\right)_{background}}$ 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) | $RAC = \frac{F1}{C_{tot}}$ 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) | $CF = \frac{C_{metal}}{C_b}$ 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) | $PLI = (CF1 \times CF2 \times CF3 \times \dots \times CFn)^{1/n}$ 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 $Ei = Ti \times CF$); Ti is the toxicity response factor and CF the Contamination Factor (see 4) | Hakanson. (1980) |

276

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

278 3. Results

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

285 3.1.1. Chemical Hazard Index for pollution degree assessment (HI_{ch})

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

- 294 - it is a speedy index for a comprehensive chemical pollution evaluation since it considers
295 only the main Priority Substances considered by Directive 2013/39/EU for water quality
296 purposes (six metals and six persistent organic pollutants) selected as indicator
297 contaminants controlling the chemical status of sediments;
- 298 - the modified Contamination Factor used to calculate the HI_{ch} , includes a "weighted"
299 value, introduced by Directive 2013/39/EU for water quality assessment. This value
300 ranges from 1 to 1.3, depending on whether the contaminant is not covered by the
301 Directive (weight = 1), or, on the contrary it is included in the list of "priority" substances
302 (weight = 1,1) or in the list of "dangerous and priority" substances (weight = 1,3), aiming
303 therefore to attribute to each pollutant a proportional relevance on the chemical status of
304 sediment according to their hazard potential.

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

$$CF_{ir} = \frac{C_{mi}}{C_{si}} \times W_0 \quad (\text{Eq. 1})$$

307

308 where C_{mi} is the concentration of the contaminant “ i ” determined within sediment samples and
 309 expressed in ppm ds, C_{si} is the Environmental Quality Standard value defined for the given
 310 pollutant by the MD 260/2010 within WFD, and W_0 is the weighting factor attributed to each
 311 pollutant. The revised CF_{ir} calculated in this way considers the contamination factor of the single
 312 element: when $CF_{ir} > 1$, the substance is considered as contaminating or enriched; when $CF_{ir} \leq 1$,
 313 the substance cannot be considered as contaminating or (anthropogenically or naturally)
 314 enriched.

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

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

320

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

322 3.1.2. Toxicological contribution to the comprehensive hazard evaluation

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

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

- 341 1. for each toxicological class defined by the Italian legislation normative (ICRAM 2009),
342 an arbitrary coefficient “k” was given, ranging from 1 to 2.5: $k = 1.0$ for Class A; $k=1.5$
343 for Class B; $k = 2$ for Class C; $k=2.5$ for Class D.
- 344 2. An “eco-toxicological weighted factor”, W_{oe} , was given also to each result, as function of:
 - 345 a) the severity of the effect “En”, intended as the severity of the biological damage
346 measured by the specific end-point considered: $En = 2.4$ was given for bioluminescence;
347 $En = 2.1$ was given for the inhibition of growth rate test; $En = 1.9$ was given for
348 development rate test);
 - 349 b) the type of exposure T (acute or short-term $T= 1$ while $T= 0.8$ for chronic or long-term
350 exposure);
 - 351 c) the type of test matrix M: $M=0.8$ when the elutriate phase was considered; $M=1$ when
352 the sediment or solid phase was considered as matrix for eco-toxicological tests.
 - 353 d) a representative weight given for the potential hormesis for each experimental type.
354 For the bioluminescence on solid phase and elutriate on *Vibro-fisher* a
355 representativeness weight $W=0.25$ is defined; for the inhibition of algal growth rate on
356 elutriate a $W=0.10$ is considered, and for embryo-toxicity with *Paracentrotus Lividus* is
357 given $W=0.15$.

358 The above reported values given to elements constituting the weighting factor W_{oe}
359 comply with what proposed by Piva et al., (Piva et al. 2011). Actually, these are taken as
360 guidelines in the most recent Italian National Laws on Sediment Handling (D.Lgs.
361 172/2016 and 173/2016) for the integrated quality assessment of dredged sediments.

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

$$\begin{aligned}
 364 \quad & Woe_i = En_i \times T_i \times M_i \times W_i \\
 & HI_{tox} = \sum_{i=1}^j (k_i \times Woe_i) \\
 365 \quad & \hspace{20em} \text{(Eq. 3)}
 \end{aligned}$$

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

369 3.1.3. Comprehensive pollution Index (cPLI)

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

$$373 \quad cPLI = HI_{ch} * HI_{tox} \quad \text{(Eq. 4)}$$

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

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

379 3.2. Potential ecological Risk Index for comprehensive pollution degree

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

$$383 \quad CE_i = T_i \times CF_{ir} \quad \text{(Eq. 5)}$$

$$384 \quad cRI = \sum_{i=1}^n (T_i \times CF_{ir}) \quad \text{(Eq. 6)}$$

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

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

- 394 1. the formulae for calculation of each pollutant toxic factor requirement, T_i , were those
395 defined by Hakanson, therefore the T_i values are: $T_{Hg} = 80$; $T_{Cd} = 30$; $T_{As} = 10$, $T_{Pb} = 5$;
396 $T_{Ni} = T_{Cr} = 2$; $T_{PCB} = 40$.
- 397 2. the value of T_i of PCB was selected as representative for the other lipophilic toxic
398 substances considered in this study. Indeed, being the organic pollutants included in the
399 same list of Priority substances, the value $T_i = 40$ given for PCB is considered as
400 appropriate also for BaP (Benzo(a)Pyrene), BbF (Benzo(b)Floranthene), BghiP
401 (Benzo[ghi]perylene), BkF (Benzo(k)Floranthene), InD (Indeno, 1.2.3 c.d.pyrene).
- 402 3. the evaluation of Risk Index (cRI) and Pollution index (cPLI) includes only the priority
403 Substances given by European Decree (39/2008/CE) for water bodies quality namely 6
404 metals/metalloids and 6 organic pollutants (Sum of for congeners of PCB and 5 high
405 molecular PAHs). Therefore, the discussion about the hazard risk evaluation is referred to
406 the presence of these contaminants (As, Hg, Cd, Pb, Cr, Ni, Sum of PCB, BaP, BbF,
407 BghiP, BkF, InD). Nevertheless, being these contaminants considered by the Water
408 Framework Directive as the main “weighted” pollutants to be monitored in the long term
409 programs for water quality, the assessment results can also be extended to other
410 contaminants having the same behavior in coastal ecosystem;
- 411 4. since some physico-chemical properties of the surficial sediments are very important,
412 especially when the sensitivity of different marine basins was considered, we used the
413 organic content (TOC) and grain size distribution as necessary co-parameters in the
414 calculation of Risk Indexes (see the discussion below).

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

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

| <i>cPLI</i> | <i>Comprehensive Pollution Hazard degree as (HI_{ch}:HI_{10c})</i> | <i>cRI</i> | <i>Potential ecological risk degree as $\sum E_i \cdot T_i$</i> |
|-----------------------|--|-------------|--|
| <i>cPLI</i> <1.9 | Low hazard degree | cRI<95 | Low risk |
| 1< <i>cPLI</i> <2.8 | Moderate hazard degree | 95<cRI<190 | Moderately risk |
| 2.8< <i>cPLI</i> <6.5 | Considerable Hazard degree | 190<cRI<380 | Considerable risk |
| <i>cPLI</i> >6.5 | High hazard degree | cRI>380 | Very high risk |

419

420 3.3. Comparative assessment of different hazard indexes

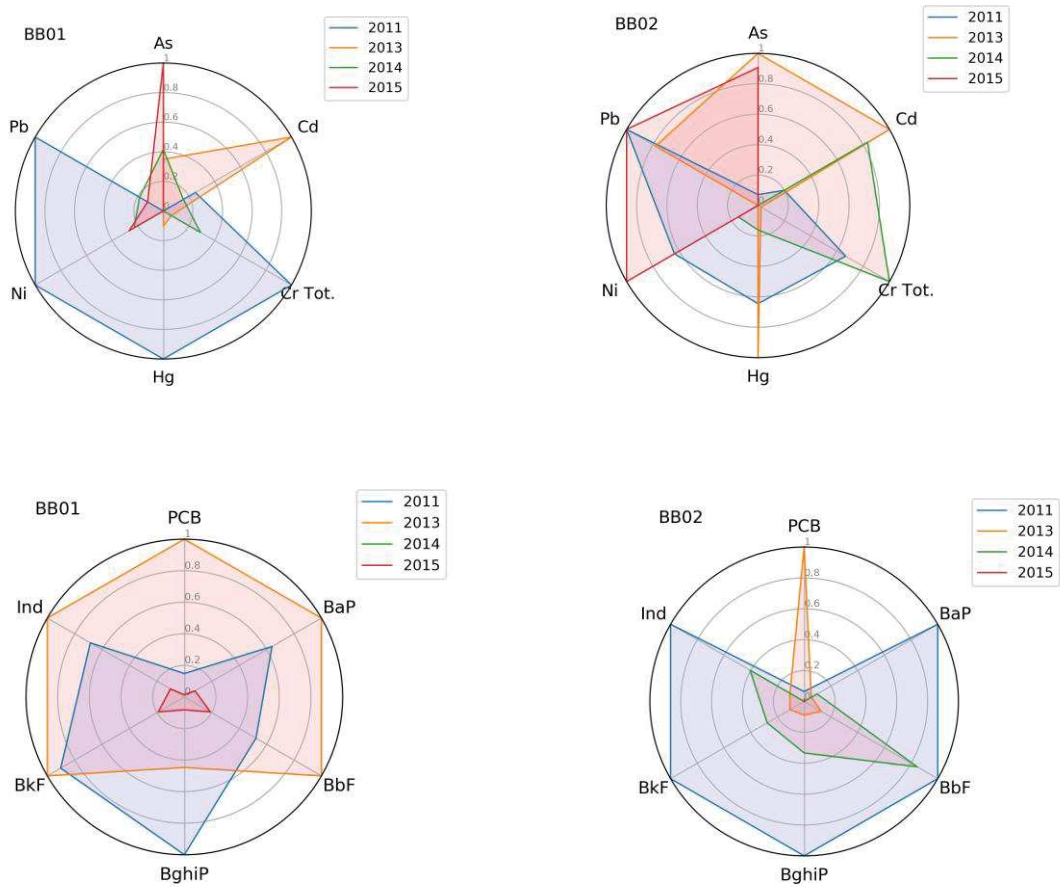
421 We compared the results obtained with cPLI and cRI with those achieved with the cumulative
 422 index c-NWAC (SWRCB, 2006; Mali et al., 2016) and mean ERM quotient (mERMq),
 423 respectively. In particular, c-NWAC considered different classes of contaminants (heavy metals
 424 and organic pollutants)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

427 4. Discussion of results

428 4.1 Chemical contamination factors

429 The basic statistical parameters and the measured concentrations of pollutants are shown in
 430 **Table 3.** The distribution of the metal and organic pollutant concentrations during different years
 431 are illustrated through radial graphs as reported Figure 3. Comparing the concentration of
 432 contaminants with the Environmental Standard Values (simple contamination factor, CF_i)
 433 revealed that As, Cd and Ni are the pollutants of most concern, followed by Hg, BaP, BkF since
 434 for all of them CF_i>1. The respective hazard degree for these pollutants indicates hazard levels
 435 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_1 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

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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.

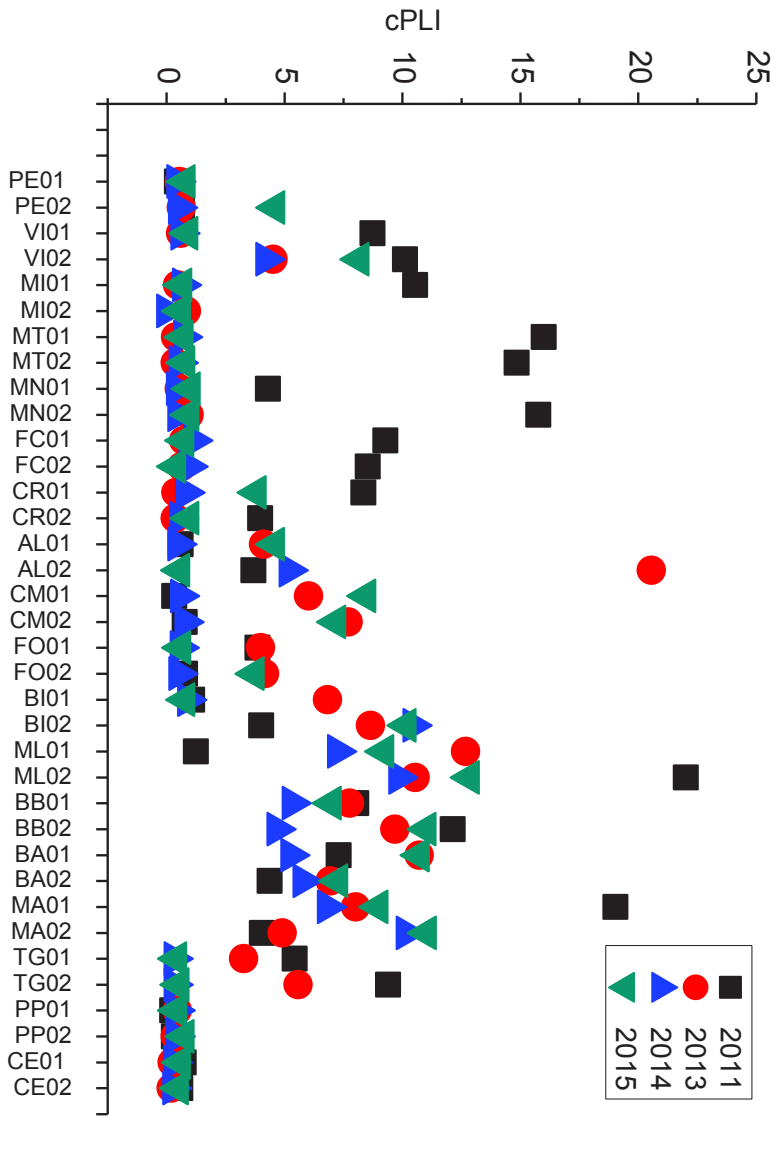
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451 **Table 3.** Basic Statistic Data of the concentration of contaminants during investigated years. The concentration of N_{tot} , P_{tot} are expressed in mg/kg
 452 d.s.; the concentration of As, Cd, Cr_{tot} , Hg, Ni, Pb are expressed in ppm;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

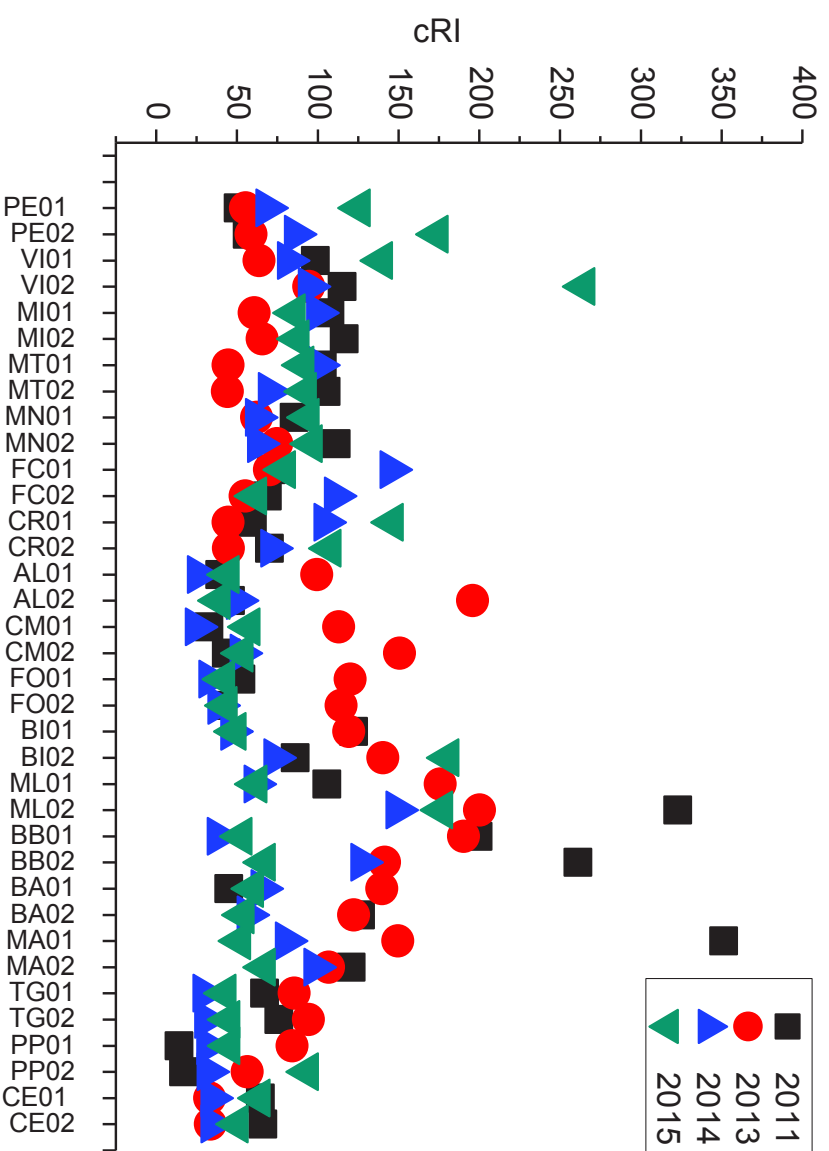
| Year | 2011 | | | | 2013 | | | | 2014 | | | | 2015 | | | |
|-------------------------------------|-------|------|-------|--------|--------|-------|-------|-------|-------|--------|-------|---------|-------|--------|-------|--------|
| | MEAN | MIN | MAX | S.D. | MEAN | MIN | MAX | S.D. | MEAN | MIN | 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 (<i>Pelite</i>) (%) | 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.



473



474

475

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
$$\text{Cd} > \text{Hg} > \text{BkF} > \text{As} > \text{BaP} > \text{BbF} > \text{BhgiP} > \text{InP} > \text{Pb} > \text{Ni} > \text{Cr} > \text{PCB}$$

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).

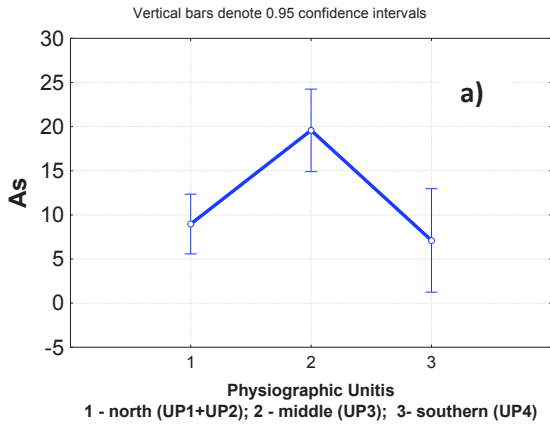
496 **4.2.1 ANOVA responses**

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

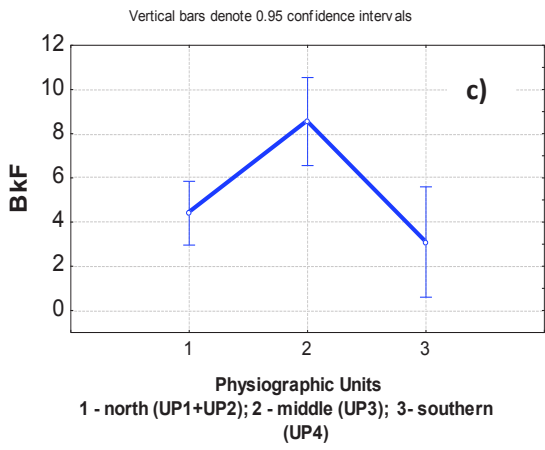
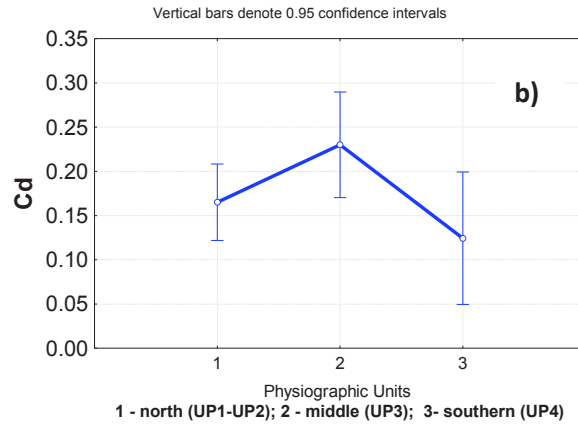
505 evaluates the variance of contaminant concentration in sediments within each class characterized
506 by an independent factor (in our case the belonging to one of the UPs). Outputs of the ANOVA
507 analyses are the degrees of freedom (df), sums of squares (SS), mean squares (MS) and the F
508 value 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 $\mu_{UPa} = \mu_{UP3} = \mu_{UP4}$ (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.

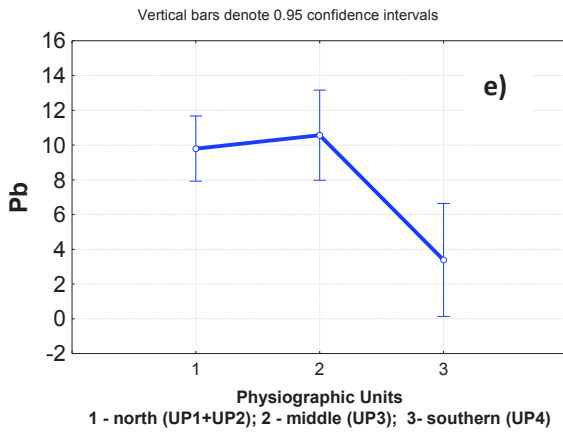
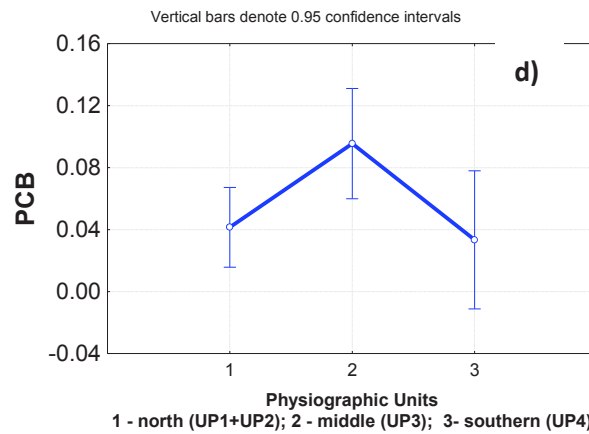
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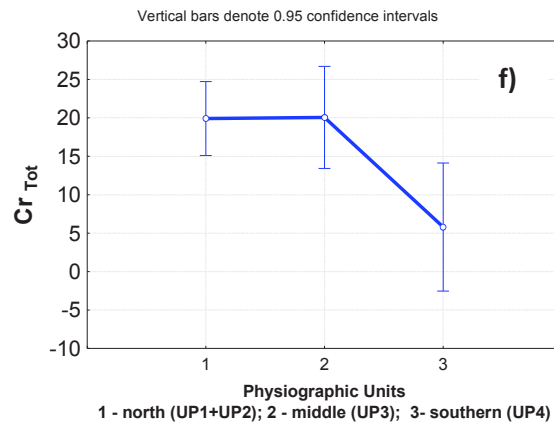
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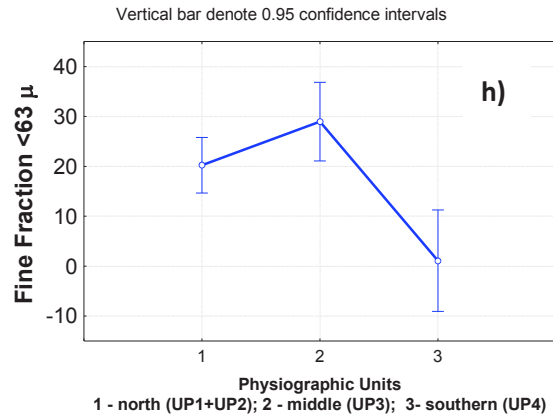
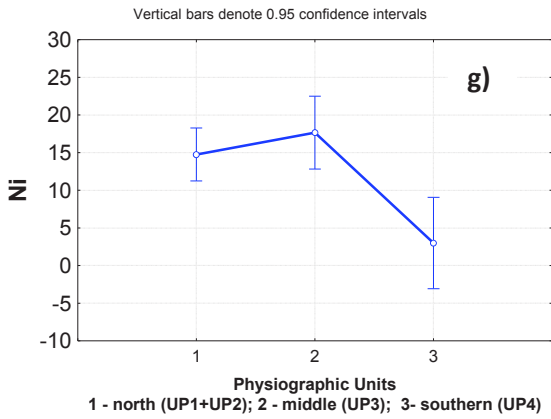


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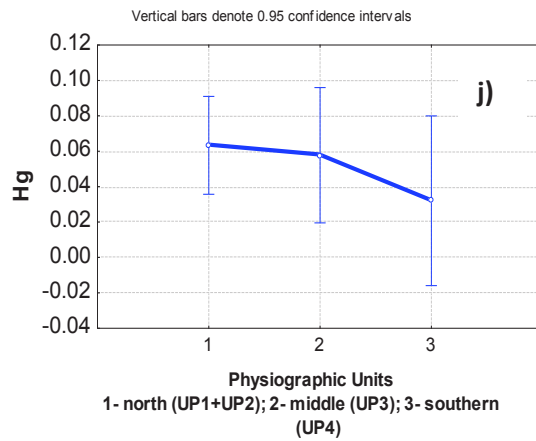
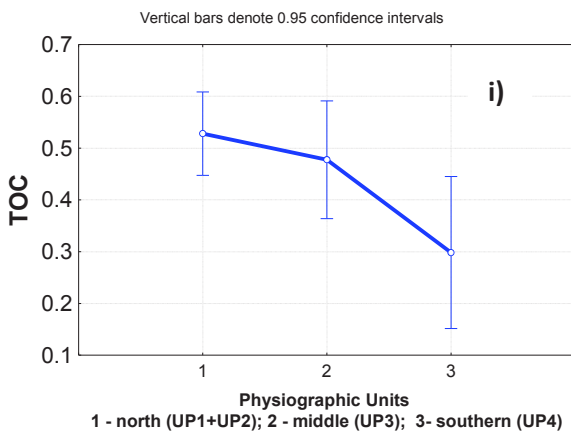


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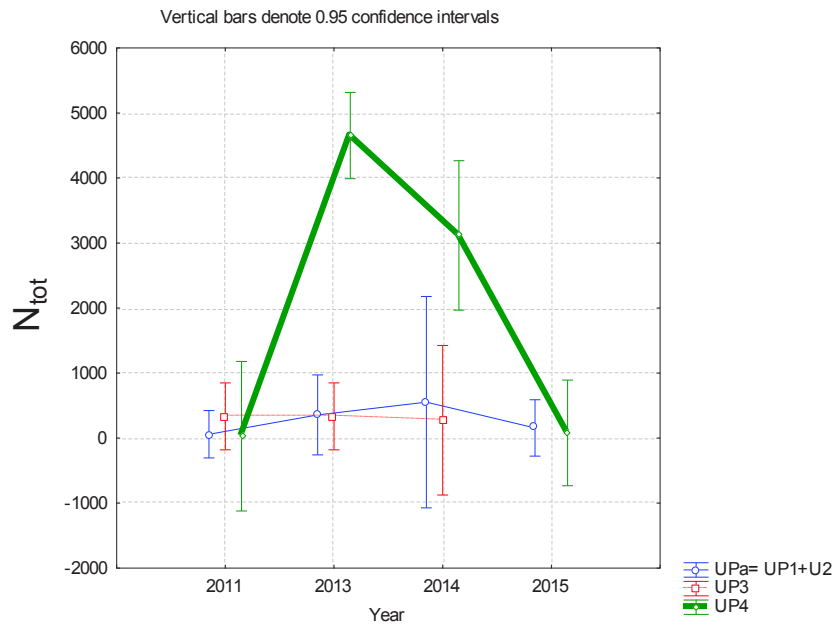


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530 **Figure 5.** Concentration of As (a), Cd (b), BkF (c), PCB (d), Pb (e), Cr_{tot} (f), Ni (g), Fine fraction
531 (h), TOC (i) and Hg (j) in the investigated Physiographic Units.

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



541

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

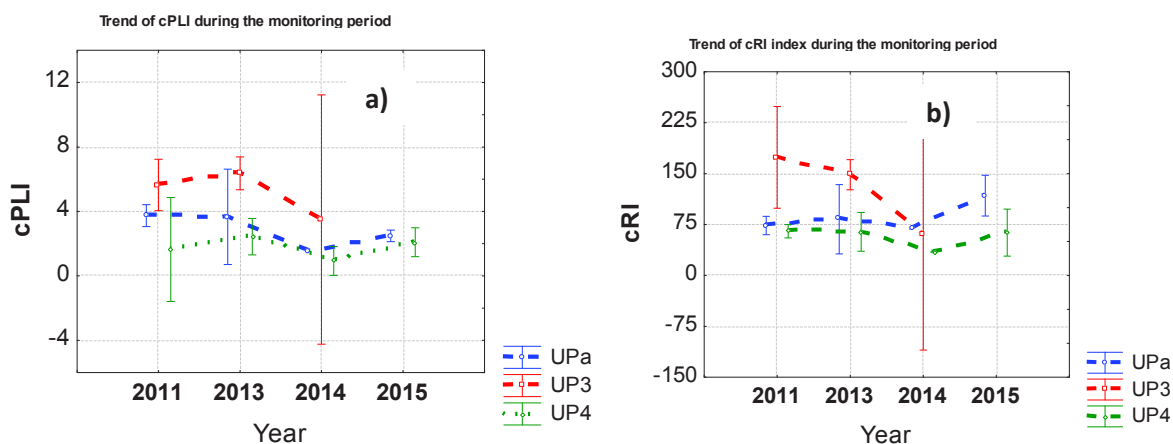
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547 The one-way ANOVA was performed also to understand the influence of grain size in
 548 contamination degree, considering thus the content of Fine Fraction ($\varnothing < 63 \mu m$) as independent
 549 factor. Two classes were comprised in this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

559 In order to understand the spatial and temporal trend of Pollution index (cPLI) and Risk Index
 560 (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 UP_a, 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 (UP_a =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

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 and 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

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

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

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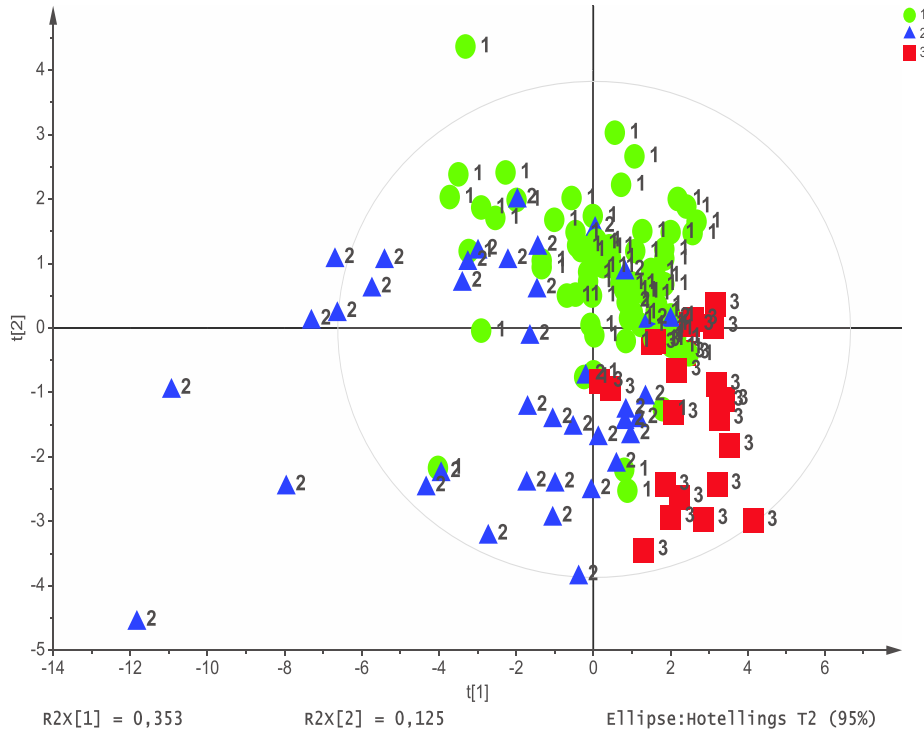
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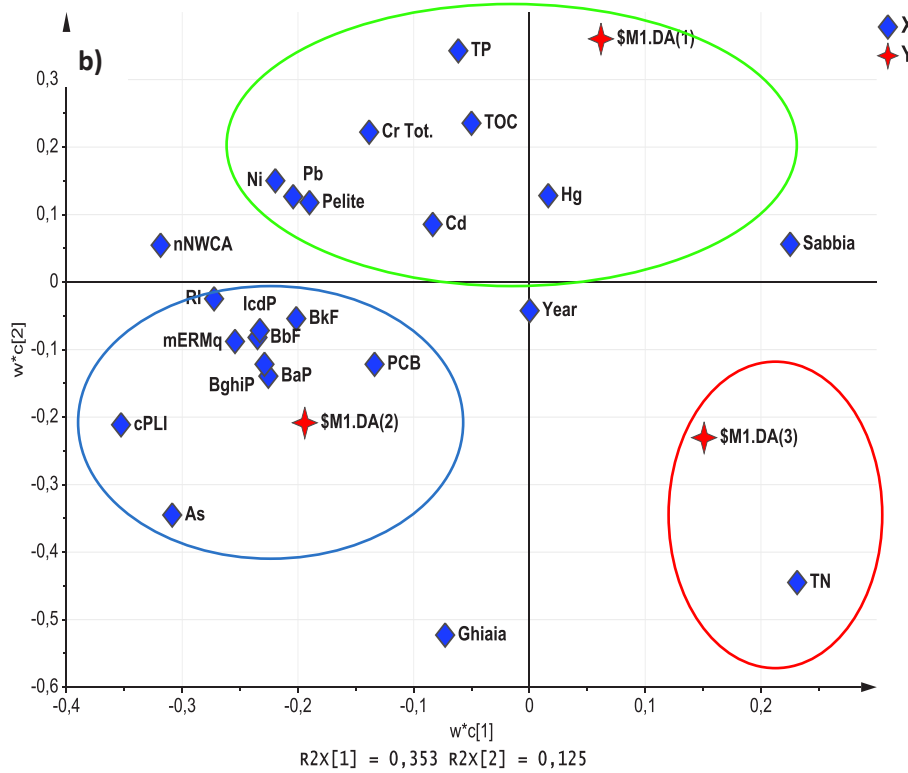
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Figure 8. PLS score plot (a) and Loading plots (b) for PLS1/PLS2. The different Physiographic Units are countersigned with the numbers 1 for the north (UPa=UP1+UP2); with number 2 the middle UP3 and with number 3 the southern UP4.

648 **4.3 Comparative analyses of cPLI and cRI with previous cumulative indexes**

649 Aiming at validating the efficiency of the proposed indexes, a correlation between cRI and cPLI
650 with two other cumulative indexes reported in literature was performed. The cRI was compared
651 with the mean mERMq quotient (mERMq, Long et al. 2006), given that mERMq index
652 individuates sediment sites with high probability of toxicity. The obtained square correlation
653 resulted significant, as indicated by the values of the correlation coefficients $R^2= 0.684$, $r=0.824$,
654 $p < 0.005$ (Figure S2). The cPLI was compared with the c_NWAC, being both indices related to
655 the cumulative synergic effect of co-presence of contaminants of different classes. Also in this
656 case the correlation coefficients resulted positive even if less significant ($R^2= 0.469$, $r=0.65$
657 $p<0.000$). The slightly worst correlation of the cNWAC with cPLI can be explained with the fact
658 that the cPLI includes also an unpredictable toxic contribution HI_{toe} (by the toxicological
659 response of the bioassay tests performed) that was not considered in the previously calculated
660 cNWAC.

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

664

665 **5 Conclusions**

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

675 The combination of cPLI and cRI with multivariate analyses resulted to be crucial for extracting
676 essential information from large datasets generated by the long-term monitoring and furnished a
677 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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