



# Politecnico di Bari

Repository Istituzionale dei Prodotti della Ricerca del Politecnico di Bari

Combining chemometric tools for assessing hazard sources and factors acting simultaneously in contaminated areas. Case study: "Mar Piccolo" Taranto (South Italy)

This is a post print of the following article

*Original Citation:*

Combining chemometric tools for assessing hazard sources and factors acting simultaneously in contaminated areas. Case study: "Mar Piccolo" Taranto (South Italy) / Mali, Matilda; Dell'Anna, Maria Michela; Notarnicola, Michele; Damiani, Leonardo; Mastrorilli, Pietro. - In: CHEMOSPHERE. - ISSN 0045-6535. - 184:(2017), pp. 784-794. [10.1016/j.chemosphere.2017.06.028]

*Availability:*

This version is available at <http://hdl.handle.net/11589/108495> since: 2021-03-08

*Published version*

DOI:10.1016/j.chemosphere.2017.06.028

*Terms of use:*

(Article begins on next page)

1 **Combining chemometric tools for assessing hazard sources and factors acting**  
2 **simultaneously in contaminated areas. Case study: “Mar Piccolo” Taranto**  
3 **(South Italy)**

4 *Matilda Mali\**, *Maria Michela Dell’Anna*, *Michele Notarnicola*, *Leonardo Damiani*, *Piero*  
5 *Mastrorilli*

6  
7 DICATECh, Politecnico di Bari, via Orabona, 4 I-70125 Bari, Italy

8 \*Corresponding author e-mail addresses: [matilda.mali@poliba.it](mailto:matilda.mali@poliba.it) (M. Mali); tel:+39 080 5963666;  
9 fax: +39 080 5963611

10

11 **Abstract**

12 Almost all marine coastal ecosystems possess complex structural and dynamic characteristics,  
13 which are influenced by anthropogenic causes and natural processes as well. Revealing the impact  
14 of sources and factors controlling the spatial distributions of contaminants within highly polluted  
15 areas is a fundamental propaedeutic step of their quality evaluation. Combination of different  
16 pattern recognition techniques, applied to one of the most polluted Mediterranean coastal basin,  
17 resulted in a more reliable hazard assessment. PCA/CA and factorial ANOVA were exploited as  
18 complementary techniques for apprehending the impact of multi-sources and multi-factors acting  
19 simultaneously and leading to similarities or differences in the spatial contamination pattern, thus  
20 defining areas of most concern.

21

22 **Keywords.** Marine sediments, hazard assessment, chemometrics, PCA, factorial ANOVA,  
23 contamination pattern

24

25 **1. Introduction**

26 Coastal areas are the most impacted natural environments, being subjected to various forms of  
27 contamination. Thousands of pollutants can affect the quality of both water column and bottom  
28 sediments, triggering severe changes in marine ecosystem and posing high risks to its structure  
29 and function (Kennish et al. 2014). The continuous loading of multiple disturbances needs to be  
30 monitored and evaluated by means of accurate and complex hazard assessment procedures calling  
31 for multidisciplinary competences and knowledges. Moreover, advanced tools are needed in order

32 to extract the most relevant informations from the extensive amount of bio-geo-chemical data  
33 usually acquired in the characterization phase aimed at identifying the pollution pattern in the  
34 study area.

35 In recent years, the use of chemometrics has grown significantly, becoming a discipline well  
36 recognized for environmental evaluation of complex matrixes (Simeonov et al. 2002; 2003;  
37 Kowalkowski et al. 2006; Shrestha et al. 2007; Venugopal et al. 2009; Zhou et al. 2007; Varol et  
38 al. 2011; Dabioch et al. 2013; Mali et al. 2016; Mali et al. 2017a, Mali et al. 2017b). Pattern  
39 recognition techniques, either supervised or unsupervised, and generally all modern multivariate  
40 analyses, have been widely exploited for generating continuous spatial pollution maps at  
41 global/local scales and for identifying anthropogenic multi-sources (Einax et al., 1997; Gredilla et  
42 al. 2013; Hopke K.P., 2015). The main reason for this large use lies in the possibility to extract  
43 hidden informations from large data sets and the easy-way of graphically resolving relationships  
44 between multiple samples and variables. Nevertheless, identifying pollution sources is often not  
45 enough to describe the possible recovery trajectories of contaminants and, thus, to define the best  
46 remediation actions, especially in high-polluted areas where complicated interaction processes  
47 occur and a plethora of factors control the spatial pollution pattern (Clements et al. 2010).

48 Sea sediments are known to be very complex matrixes since they act as sink for all contaminants  
49 discharged in water column (Long. et al. 1995; 2006; Caeiro et al. 2005; Burton G.A. 2013,  
50 Chapman et al. 2013; Mali et al. 2015). The distribution of contaminants within sediments  
51 depends not only on anthropogenic and natural sources but also upon the textural characteristics,  
52 organic matter content, mineralogical composition and other factors. The evaluation of effects  
53 exerted by these factors is surely necessary, but getting insights into simultaneous interaction  
54 phenomena could enhance the robustness of the hazard estimation.

55 In this paper we present the application of two different statistical techniques to assess the hazard  
56 degree in a highly contaminated area. The chosen statistical techniques are Principal Component  
57 Analysis/Cluster Analysis (PCA/CA) and factorial Analysis of Variance (ANOVA). The  
58 combination of these complementary techniques is considered useful for emphasizing interaction  
59 effects between factors controlling pollution spectrum. Indeed, PCA/CA was applied in order to  
60 obtain information about the existing site-clusters with similar pollution character and to identify  
61 the most important discriminant contaminants (variables) within the same cluster. On the other  
62 side, ANOVA was used in order to assess the impact of some main factors, such as organic matter

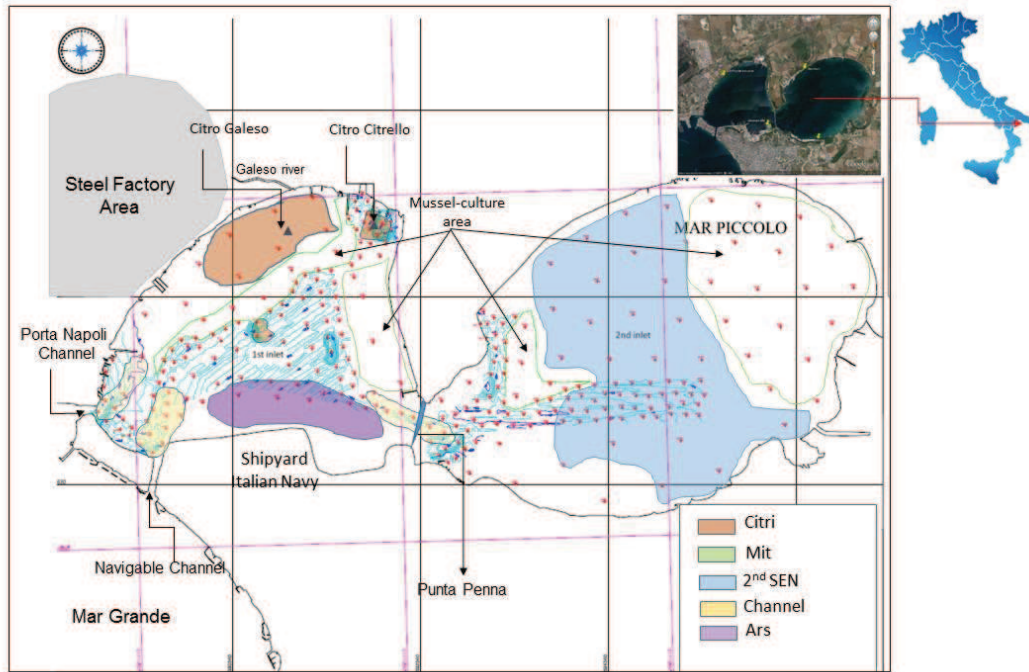
63 and textural sediment, when they act simultaneously. The combination of the two techniques were  
64 thus suggested as useful approach for a meaningful hazard assessment procedure.

65 As case study area we selected one of the most industrialized and contaminated zone in the  
66 Mediterranean: “Mar Piccolo”, a marine basin located in the Ionian Sea of Southern Italy near city  
67 of Taranto, a densely populated town subjected to a high environmental risk. Due to the  
68 extraordinary industrialization activity affecting the quality of soil and groundwater, Taranto and  
69 the whole catchment area were declared contaminated Site of National Interest (SIN). Continuous  
70 monitoring campaigns have been conducted in the area and the present work is based on the  
71 characterization campaign carried out by ISPRA, Italian Agency of Environment Protection of  
72 Coastal Areas and committed by the Environmental Ministry for the SIN Taranto area in 2010  
73 (ISPRA. 2010).

## 74 **2. Design and methods**

### 75 **2.1 Study area and sampling programs**

76 Taranto’s Mar Piccolo is a semi-enclosed basin with a surface area of approximately 21 km<sup>2</sup> and a  
77 maximum depth of 13 m. It is divided into two inlets and it is connected to Mar Grande and to  
78 Taranto Gulf (Ionian Sea) through two channels, one artificial named Navigable Channel and the  
79 other one natural called Porta Napoli’s Channel. In this study, the relative large channel  
80 connecting the two inlets is called Punta Penna Channel, like the bridge name over the channel.  
81 (Figure 1). A peculiar characteristic of Mar Piccolo is the presence of several submarine  
82 freshwater springs called “citri” (Caroppo and Cardellicchio, 1995) which contribute to freshwater  
83 inputs together with small karstic rivers. The two most significant “citri” are located within the  
84 first inlet and are called citro-Galeso and citro-Citrelo. The area is characterized by a low water  
85 exchange and hypoxic conditions as consequence of oxygen consumption by microbial  
86 degradation due to large inputs of organic matter (from wastewater discharges, mussel farming  
87 and agricultural soil drainage) which also contribute to an excess of nutrients in the system  
88 (Umgiesser et al. 2007). Previous studies refer also high sulphide concentrations in the bottom  
89 sediments as a result of anaerobic degradation of organic matter (Cardellicchio et al. 2006).



90

91 **Figure 1.** Sampling map within two inlets of Mar Piccolo. The figure reports the location of the  
 92 main anthropogenic sources and freshwater springs within the two inlets.

93 Many anthropogenic activities load on the first inlet, affecting adversely the quality of the basin.  
 94 The most relevant ones are: intensive mussel cultures and fishery facilities, an abandoned shipyard  
 95 (built at the beginning of the twentieth century) and a very large Navy port (Buccolieri et al.  
 96 2006). Furthermore, urban discharges and agricultural freshwater are transported within Mar  
 97 Piccolo basin through a number of pipes and karstic rivers. Finally, nearby the first inlet one of the  
 98 most important European steel factory is still active. Although nowadays some pollution sources  
 99 are no longer active, the quality of the area is strongly compromised (Bellucci et al. 2016).

100 A sampling grid matrix on regular basis was defined according national guidelines (ICRAM–  
 101 APAT (2007)) including 266 samples collected within the two inlets at different depth levels as  
 102 shown in Figure 1.

## 103 2.2 Chemical analysis

104 For the purpose of the present study the following specific variables were considered: *i)* 11 metals  
 105 and metalloids (As, Cd, Cu, Cr, Hg, Ni, Pb, V, Zn, Al and Fe); *ii)* Sum of Polychlorinated  
 106 Biphenyls (PCB), sum of Polycyclic Aromatic Hydrocarbons (PAHs) and sum of Total  
 107 HydroCarbons (THC), the latter split into the following two sub-groups: sum of Light molecular

108 Total HydroCarbons LTHC ( $C < 12$ ) and sum of High molecular Total HydroCarbons, HTHC  
109 ( $C > 12$ ); *iii*) main physico-chemical parameters, i.e. grain size (in terms of % of coarse, % of sand,  
110 % of mud, % of clay), water content, Total Organic Carbon (TOC), Total Nitrogen ( $N_{tot}$ ) and Total  
111 Phosphorous ( $P_{tot}$ ). The TOC/ $N_{tot}$  ratio was also included in the database being a useful indicator  
112 of the organic matter provenance (Mayer 1993; Meyers 2003; Gao et al. 2012). For the 266  
113 sampling points, sediments were collected at five different depths (0.10 cm; 10-30 cm; 30-50 cm;  
114 100-120 cm; 180-200 cm; 280-300 cm), for a total of 1021 samples. [The analytical procedures for  
115 each contaminant's class are reported in Table S1.](#)

## 116 2.3 Statistical analysis

117 Two software were utilized for statistical analysis: Soft Independent Modeling of Class Analogy  
118 (SIMCA) 10.2 for PCA/CA analyses and STATISTICA 10.0 for two-way factorial ANOVA and  
119 for normality-test of the raw and log-transformed data. The SIMCA software was used due to its  
120 very intuitive graphical interphase. Two-way ANOVA was preferred with respect to the three  
121 ANOVA in order to reduce the risk of getting false positive effects with increasing the  
122 independent factors (McKillup, 2012). [The Type II ANOVA model was chosen because the levels  
123 assigned to main independent factors were randomly selected. In addition the model was  
124 considered suitable for the purpose of the manuscript \(\*vide infra\*\).](#)

## 125 3. Statistical treatment and results

### 126 3.1 Preliminary data elaboration

127 All multivariate analyses are sensitive to outliers and to non-normality of data sets (Reimann and  
128 Filzmoser, 2000; Reimann et al. 2005; Micò et al. 2006; Filzmoser et al. 2009; Gałuszka et al.  
129 2015). Usually environmental data are not normally distributed, but their logarithmically  
130 transformed values can follow a normal distribution (Leardi et al. 2000; Rostron & Ramsey 2012).  
131 Prior to performing multivariate analyses, grain size and/or geochemical normalization is also  
132 recommended (Covelli and Fontolan, 1997; Ho et al. 2012). In the present study, we normalized  
133 metal concentrations using Al as proxy (Horowitz, 1997; Mali et al. 2015), before checking the  
134 normality features of all variables. [The results of normality tests are presented in Appendix A,  
135 while the basic statistical parameters for the whole dataset \(1021x24\) are reported in Table S2.](#)

136 In order to enhance the performance of the statistical analyses, additional variables were defined,  
137 grouped as “spatial” and “qualitative” variables. The “spatial” variables include “Depth” and

138 “Basin-type”. “Depth” is referred to the depth of core-sampling and three classes were thus  
139 defined: “surficial” (S) including samples collected at 0-10cm; 20-30cm and 30-50 cm,  
140 “intermediate” (I) including samples collected at 100-120 cm, and “deep” (P) including samples  
141 collected at 180-200 and 280-300 cm. “Basin-type” distinguishes sample zones influenced by  
142 different neighboring industrial activities and human pressure loading. Six “Basin-type” classes  
143 were defined (Figure 1): 1) “*channel*” indicates those samples located at the entrance of the three  
144 existing channels; 2) “*citro*” indicates samples located around citro-Galeso and citro-Citrello  
145 freshwater fluxes and in the zone where the Galeso river flows; 3) “Ars” refers to samples located  
146 nearby Navy area; 4) “Mit” belongs to all core-samples located in the mussel-culture activity; 5)  
147 “2<sup>nd</sup> Sen” refers to samples sited within the second inlet; 6) “In” indicates all remaining samples  
148 not included in previous groups.

149 It is well known that contaminant (especially trace elements) concentrations within sediments are  
150 strongly affected by textural characteristics, organic matter content, mineralogical composition  
151 and depositional environment. The generally accepted opinion is that the smaller the size of  
152 sediment fraction, the larger the amount of trace elements and pollutants bound to this fraction. In  
153 particular, trace elements are mainly present in the clay/mud particles with grain size smaller than  
154 0.063 mm diameter. For the importance of the granulometric distribution, an additional variable  
155 named “Grain size class” was included and three classes were defined: the finest fraction  
156 including samples with fraction  $\theta < 63 \mu\text{m}$  more than 55%; the coarse/sand fraction including  
157 samples with  $\theta > 63 \mu\text{m}$  more than 55%; and the **mix** fraction accounting for the remaining cases.

158 Organic matter content (in terms of TOC) and nutrients (in terms of  $P_{\text{tot}}$  and  $N_{\text{tot}}$ ) play an  
159 important role in the marine chemistry (van Nugteren, 2009). The distribution of TOC within  
160 sediments in Mar Piccolo spans an unusually large range (from 0.13 up to 8.10 %<sub>w</sub>) even more  
161 evident within different depths. For this reason, we included TOC concentration as an additional  
162 “qualitative” variable, defining four different range-concentrations: “low”, “medium”, “high” and  
163 “very high” for TOC concentration ranging from 0-1 %<sub>w</sub>, 1-3 %<sub>w</sub>, 3-5 %<sub>w</sub> and  $> 5$  %<sub>w</sub>,  
164 respectively.

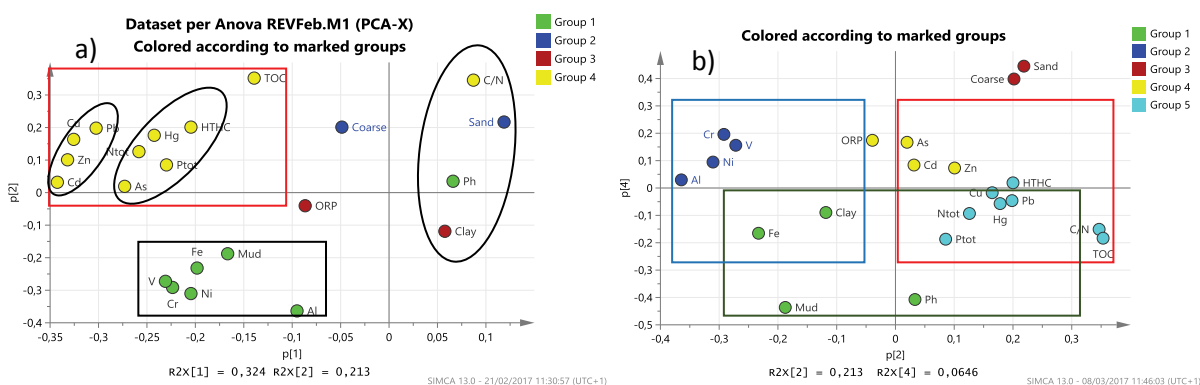
### 165 **3.2 Spatial Distribution of Contaminants through PCA/CA analyses**

166 A model with four principal components (PCs) accounting for a cumulative variance explained of  
167 70% was considered. Despite additional elaborations (such as the exclusion of “spike”  
168 concentrations and variables with negligible influence on PCs), the cumulative variance explained

169 by the first four PCs, remained almost unchanged. We presume that this circumstance is related to  
 170 the occurrence of too many simultaneous and multi-source effects, each of them marginally  
 171 affecting contamination pattern. Therefore, in order to evaluate how the unexplained variance  
 172 could affect the results obtained, we decided to perform cluster analysis (CA) considering both the  
 173 whole dataset and data coming from the selected PCs. The clusters obtained by both approaches  
 174 were then compared (*vide infra*).

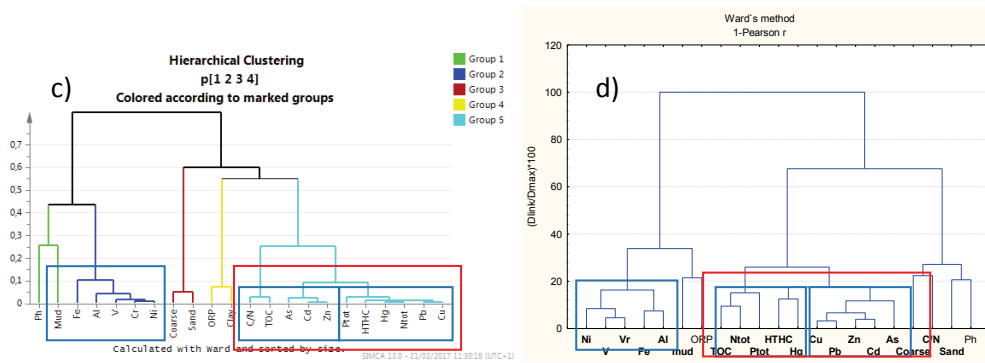
175 Inspection of loadings PCs projections evidenced different variable groups (Figure 2). In PC1/PC2  
 176 space (Figure 2a), elevated negative loadings on PC1 are marked for four metals grouped in a  
 177 cluster: Cd, Zn, Cu and Pb. A second cluster, displaying less negative PC1 loadings and  
 178 constituted by two toxic elements, As and Hg, is also clearly visible. This cluster resulted highly  
 179 correlated with nutrients ( $N_{tot}$ ,  $P_{tot}$ ) and with HTHC. Both clusters fall in the same PC1/PC2 plane  
 180 and show a positive correlation with TOC, even if the latter displays higher positive loadings on  
 181 PC2. The obtained results suggest different distribution fate (partially affected by organic matter)  
 182 and similar anthropogenic sources for contaminants grouped in the two clusters.

183 Indeed, all metals in both clusters are sulphophile elements, that is those elements which show a  
 184 high affinity to sulfur, forming insoluble sulfides. However, differently from Cu, Zn, Cd and Pb,  
 185 As and Hg are also subjected to easy methylation (facilitated by high levels of TOC and nutrients)  
 186 and therefore are more mobile in the sediment phases with respect to Pb, Cu, Zn and Cd. Similar  
 187 conclusions were drawn by Chakraborty et al. (2015) and (Liu et al. 2013). In addition, it was  
 188 demonstrated that the strong affinity of As and Hg for iron and manganese (hydr)oxides controls  
 189 their sequestration and their bioavailability in sediments and aquifers, in strong relation to  
 190 microbial activity and physico-chemical conditions (Burnol et al. 2011).



191





192

193

194

195

**Figure 2.** PCA loading plots for: a) PC1/PC2, b) PC2/PC4, c) partial hierarchical clustering calculated with Ward's method considering four PCs; d) hierarchical clustering calculated with Ward's method considering the whole dataset. \*C/N stands for TOC/N<sub>tot</sub>

196

197

198

199

200

201

202

203

204

205

206

As to the pollution sources, dumping of contaminated material and untreated terrestrial discharges can be considered possible causes affecting Cd, Cu, Pb, Zn, As, Hg contents (the variables displaying positive loadings on PC2), as suggested by previous studies in the area (Cardellicchio et al. 2007; Petronio et al. 2012). Nevertheless, these metals resulted to be the main elements contained in dust emission from steel factories (EC Remus et al. 2013, Arruti et al. 2010, Dore et al. 2014). For this reason, we can reasonably assume that metal-reached atmospheric input should be an important contamination source of these metals, being the study area located very near to a steel factory. As far as Pb and Hg are concerned, both metals could derive also from oil spilling and ship-fuel losses. The presence of "Navy" in the area can contribute to fuel losses from military ships during maneuvering (Mali et al. 2017b) and thus can explain the association of Pb and Hg with HTHC.

207

208

209

210

211

212

213

214

215

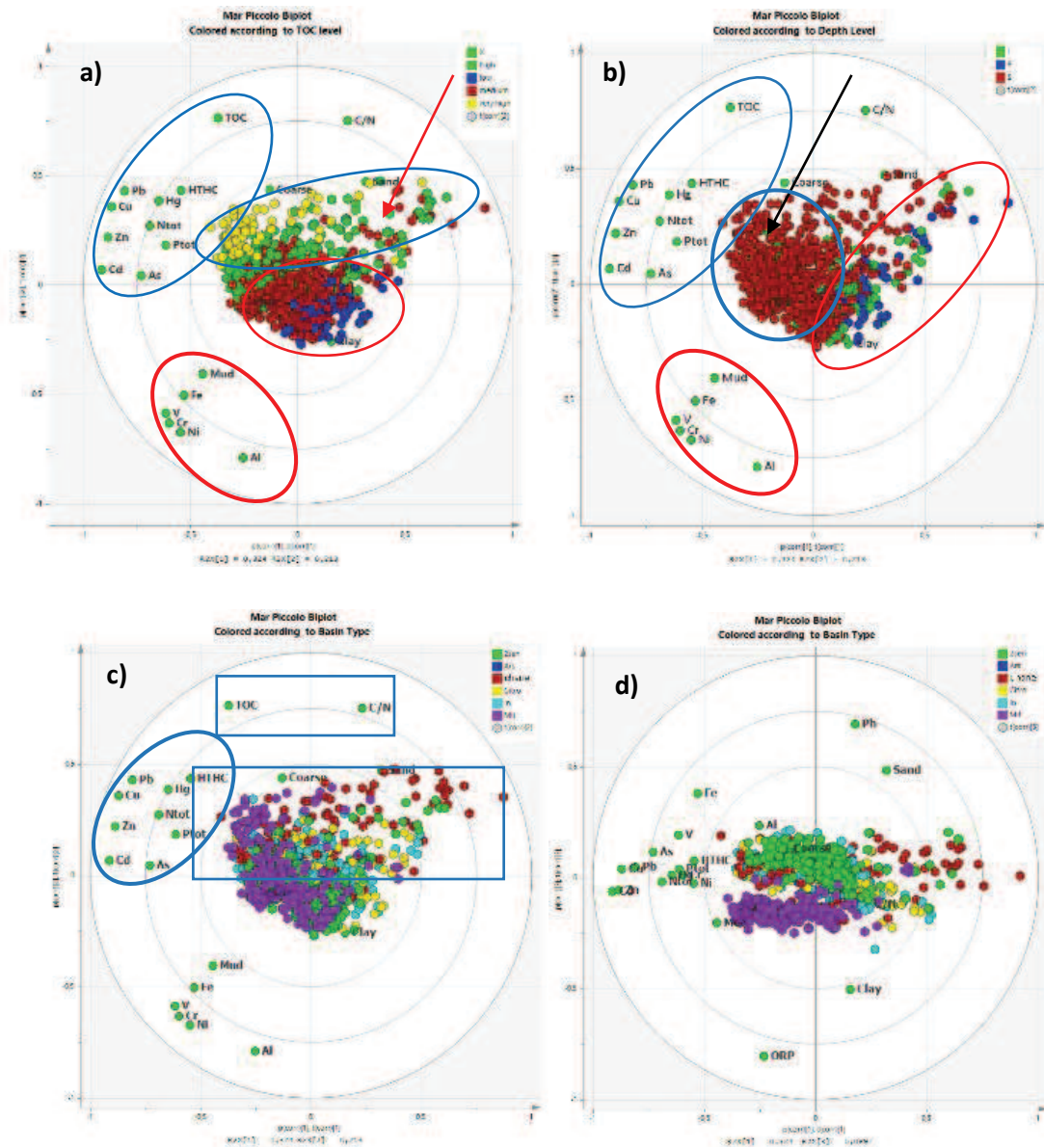
Continuing analyzing of PC2 negative loadings (Figure 2a), it is apparent another metal cluster constituted by V, Ni, Cr and Fe, strongly correlated with Al and mud fraction ( $4\mu\text{m} < \text{mud} < 63\mu\text{m}$ ) and in contrast trend with respect to Cd, Zn, Cu, Pb, As, Hg and HTHC. Both circumstances suggest that the presence of V, Ni, Cr and Fe might be attributed to natural origin. Probably, the geochemical composition of the parent rocks which generated Mar Piccolo sediments, could be the main factor controlling the variability of V, Ni, Cr and Fe. In fact, statistically significant positive correlations (R) were found between these metals and the finest grain size sediments (Table S4), supporting our hypothesis of their enrichment predominantly due to sedimentary processes (Dung et al. 2013; Lofu et al. 2016; Mali et al. 2015; Mali et al. 2016).

216 Interesting data derived from PC2/PC4 space plot (Figure 2b), where some differences among  
217 anthropogenic enriched metals are emphasized. On the negative PC4 plan, Hg and Pb (and Cu in  
218 less extent) resulted associated with nutrients ( $N_{tot}$  and  $P_{tot}$ ) and TOC. All these variables display  
219 positive correlation with Fe, fine sediments (mud and clay) and pH. On the contrary, As, Cd and  
220 Zn are displayed on the positive PC4 plan as well as lithogenic metals.

221 We propose that the observed dissimilarities between these two groups (Hg, Pb and Cu group with  
222 respect to the As, Cd and Zn one) might be caused by different affinity of these metal for different  
223 phases (ion-exchangeable, carbonate, organic, iron-oxy-hydroxide, sulphides etc.) (Calloy et al.,  
224 2002; Bucolieri et al. 2006). Hg, Pb and Cu are probable bound to the more mobile phases with  
225 respect to As, Cd and Zn. This hypothesis is supported by previous studies: Calace et al. (2008)  
226 found that Pb in Mar Piccolo is present mainly as ion-exchangeable species or bound to carbonate  
227 while Hg and Cu result predominantly associated to organic matter. Cardellicchio et al. 2006  
228 found that, except for Hg and Pb, metals such as Zn, Cd and As in Mar Piccolo result to be present  
229 mainly as insoluble sulphides encouraged by high sulphide concentrations and negative redox  
230 (ORP) values.

231 In order to reveal the hidden sample clusters (scores) and to identify the discriminant contaminants  
232 (variables-loadings) for each cluster, we considered meaningful the analysis of biplot graphs  
233 colored according to “TOC levels”, “Depth” and “Basin Type” (Figure 3 a,b,c,d).

234 Analysis of the PC1/PC2 biplot colored according to TOC levels (Figure 3a), reveals that almost  
235 all samples containing high and very high TOC concentration (yellow and green) resulted  
236 associated with anthropogenically influenced metals (As, Cd, Cu, Hg, Zn, Pb) and display positive  
237 relationship with TOC and (Corg/N). On the contrary, samples containing low and medium TOC  
238 concentrations (blue and red) resulted associated with lithogenic metals (V, Cr, Ni, Al and Fe),  
239 following positive correlation with the finest sediment grain size (mud and clay). In particular, the  
240 samples with low TOC concentration (blue) are strongly associated with clay. Exceptions were  
241 observed for some medium TOC samples (red arrow) that display the same trend of high and very  
242 high TOC levels. The circumstance suggests that probably in these samples anthropogenic  
243 contamination could take place and/or synergistic effects of TOC with other variables might  
244 control their contamination pattern.



245

246

247 **Figure 3.** Biplot PC1/PC2, a) colored according “TOC level”, b) colored according Depth, c)  
 248 colored according “basin type”, d) Biplot PC1/PC3 colored according to “basin type”

249 The biplot graph colored according to “depth” (Figure 3b) points out that almost all superficial (S)  
 250 sediments are associated with anthropogenic-influenced metals Pb-Cu-Zn-Cd-As-Hg and HTHC,  
 251  $N_{tot}$ ,  $P_{tot}$ , while the majority of intermedium (I) and deep (P) samples are associated with two grain  
 252 size phases (sand and clay). Also in this case some exceptions are detected: few I samples (green)  
 253 show the same trend of the superficial ones (indicated with black arrow, Figure 3b), suggesting an  
 254 anthropogenic metal contamination also in the intermedium layer for these sites. These  
 255 exceptional cases belong all to “channels” and “Citro” basin type areas (Figure S1a,b). For this  
 256 reason, we decided to study the biplot PC1/PC2 projection colored according to “Basin type”

257 (Figure 3c). The graph confirms that almost all samples coming from Channels (red) and the  
258 predominance of samples from “Citro” (yellow) are affected by contamination, irrespective of the  
259 depth. We can reasonably assume that these samples are more subjected to dynamic conditions,  
260 *i.e.* high hydrodynamic circulation and bio-perturbation phenomena, that might promote an active  
261 vertical transport of metal contaminants versus the deepest layers (Pascali et al. 2015; Mali et al.  
262 2017b). In addition, it is evident that the superficial layer of “channel” and “Citro” resulted more  
263 affected by terrestrial organic matter (high values of  $C_{org}/N$  ratio) (Figure S1c) than by metals  
264 suggesting that the provenance of organic matter in sediments might control the distribution of  
265 contaminants (Calace et al. 2008).

266 The biplot projection of PC1/PC3 (Figure 3d) shows evidence of another interesting factor:  
267 samples located in the mussel (Mit) culture activity (violet) resulted well separated from the rest of  
268 basin groups. Indeed, Mit-samples are characterized by low pH values and increasing ORP (less  
269 negative values), suggesting probable metal leaching processes from sediment to water column  
270 (Miao et al. 2006; Malviya & Chaudhary, 2006), although high concentrations of Cd, Hg and Zn  
271 persist in these sediments, as shown by the variable loading of PC1/PC3.

272 Finally, Cluster Analysis (CA) considering the whole dataset was carried out, using Ward’s  
273 method. By comparing the cluster dendrograms (Figure 2d) with cluster obtained by analyzing  
274 only 70 % of information of four PCs (Figure 2c), it is apparent that the clusters found in both CA  
275 and PCA are almost the same (the only difference is constituted by As that belongs to Cd, Cu, Pb,  
276 and Zn cluster in CA). This evidence corroborates our conclusions based on the selected PCA  
277 model.

### 278 3.3 ANOVA analysis

279 PCA elaborations discussed so far allowed understanding the influence of several factors on the  
280 contamination pattern. Nonetheless, when the hazard assessment is required, the simultaneous  
281 effect, synergistic or antagonist, of different loading factors needs to be clarified. We decided to  
282 combine PCA elaboration with the factorial ANOVA in order to determine the factors that most  
283 significantly control the chemical compositions of marine sediments and to gain insights into their  
284 possible synergistic effects. The ANOVA procedure is detailed in Appendix B. The independent  
285 factors selected for ANOVA discussed in the present paper are: organic matter content, expressed  
286 in terms of TOC range concentration (TOC level), grain size distribution and sampling depth,  
287 whose classes are described in the section 3.1. We performed the two-way ANOVA considering

288 two different pairs of independent factors (A, B, AxB), *i.e.* TOC Level *and* Depth, Gran Size *and*  
 289 TOC level and Grain Size *and* Depth.

290 The results of two-way ANOVA (3x4) considering Depth as factor A and TOC level as factor B,  
 291 are shown in Table 1, reporting the values of variance ratios (F), probabilities ( $p$ ), and effect size  
 292 ( $\eta^2$ ). Usually, when the estimated probability ( $p$ ) is lower than 0.01, the considered independent  
 293 factor makes a significant difference in the contaminant concentration. In our case, almost all  
 294 factors resulted statistically significant ( $p < 0.01$ ). This is because a statistical test with a large  
 295 amount of input data ( $n = 1021$ ) is highly sensitive to very small differences. Therefore, we  
 296 decided to calculate also the partial effects given by  $\eta^2$  to form a plausible estimation of  $p$  value  
 297 irrespective of sample number. Magnitudes of  $\eta^2 < 0.01$ ,  $0.01 \leq \eta^2 < 0.05$ ,  $0.05 \leq \eta^2 < 0.10$ , and  $\eta^2$   
 298  $\geq 0.10$  represent no effect, a small effect, an intermediate effect, and a significant effect,  
 299 respectively (Ohta et al. 2015). In the present study, we assume that each factor with  $\eta^2 \geq 0.05$  has  
 300 a *significant effect* on the contamination patterns of the sediments. On the other hand, the  
 301 interaction effect (AxB) refers to the influence of a factor on the other one, helping thus in  
 302 quantifying how and to what extent both A and B factors synergistically affect the concentration  
 303 of contaminants. We assume synergistic interaction effects *insignificant*, *significant* and *dominant*  
 304 for  $\eta^2_{(axb)} < 0.01$ ,  $< 0.01 \eta^2_{(axb)} < 0.05$  and  $\eta^2_{(axb)} > 0.05$ , respectively. This estimation is conform to the  
 305 main purpose of the ANOVA elaboration, aiming at understanding the causes of synergistic  
 306 effects and not at explaining the 100% variability of the contaminant concentration (our dependent  
 307 variables) (Shaw & Mitchell-Olds 1993; Ohta et al. 2015).

Type II	A=Depth B= TOC (3x4)								
	F			p			$\eta^2$		
	A	B	AxB	A	B	AxB	A	B	AxB
Ph	4.00	24.2	1.50	0.02	0.00	0.17	0.01	0.07	0.01
W	25.1	28.3	2.90	0.00	0.00	0.01	0.04	0.07	0.01
ORP	0.30	28.4	1.40	0.74	0.00	0.22	0.00	0.08	0.01
C/N	12.0	197	5.43	0.00	0.00	0.00	0.01	0.36	0.02
Al	18064	79.1	2.41	0.00	0.00	0.03	0.03	0.18	0.01
As	182	2.48	12.0	0.00	0.06	0.00	0.25	0.01	0.06
Cd	126	45.0	10.2	0.00	0.00	0.00	0.17	0.09	0.05
Cr	26.1	33.5	3.30	0.00	0.00	0.00	0.04	0.09	0.02
Fe	29.6	18.7	5.64	0.00	0.00	0.00	0.05	0.05	0.03
Hg	73.9	59.4	4.40	0.00	0.00	0.00	0.11	0.13	0.02
Ni	15.0	47.3	5.30	0.00	0.00	0.00	0.02	0.12	0.03
Pb	138	123	5.20	0.00	0.00	0.00	0.16	0.22	0.02
Cu	146	101	14.0	0.00	0.00	0.00	0.17	0.18	0.05
V	41.1	22.6	1.89	0.00	0.00	0.08	0.07	0.06	0.01
Zn	133	59.5	7.94	0.00	0.00	0.00	0.18	0.12	0.03
Ptot	18.1	52.2	3.73	0.00	0.00	0.00	0.03	0.13	0.02
Ntot	18.7	156	4.16	0.00	0.00	0.00	0.02	0.30	0.02

TOC	3.45	1951	0.69	0.03	0.00	0.66	0.00	0.85	0.00
HTHC	27.9	73.3	4.86	0.00	0.00	0.00	0.04	0.17	0.02

308

309 **Table 1.** The variance ratios (F), probabilities ( $p$ ), and partial effect size ( $\eta^2$ ) due to each main  
310 factor (factor A: Depth), (factor B: TOC level), and the interaction effect (factor A×B) of two-way  
311 ANOVA 3x4 - Type II)

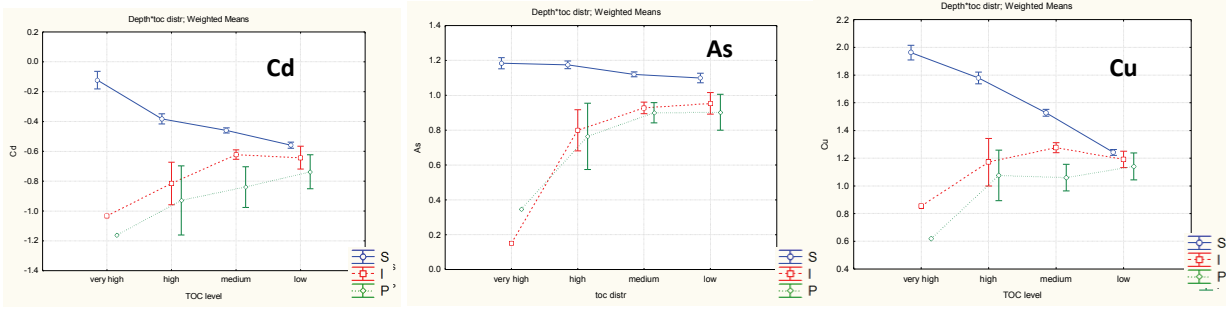
312 The two-way model reveals that sampling depth (Factor A) strongly influences the concentration  
313 of all metals, while TOC level (Factor B) has a dominant influence on the contamination  
314 distribution of almost all metals except for As ( $\eta^2 = 0.01$ ,  $p > 0.01$ ). Furthermore, the interaction  
315 effects of both factors ( $\eta^2_{A \times B}$ ) resulted important for all metals but the weight of interaction effect  
316 is different for two metal-groups. For As, Cd and Cu the interaction effect is *dominant* ( $\eta^2 > 0.05$ ),  
317 while it resulted *significant* ( $\eta^2 > 0.01$ ) for the other metals (Cr, Fe, Ni, Pb, Zn, Hg), nutrients and  
318 HTHC. In order to facilitate the interpretation of data and to get insights into the interaction  
319 effects, the results of two way ANOVA are graphically reported in [Figure 4](#).

320 Inspection into the graphs reveals that the concentrations trend of Cd, Zn, Cu, Pb, As and Hg  
321 within superficial layers (blue line) differs substantially from the trend determined in the  
322 intermedium and deep levels (green and red lines, respectively): in superficial samples the higher  
323 the TOC content the higher the metal concentration while in intermedium (I) and deep (P) layers,  
324 the higher the TOC content the lower the metal concentration (Figure 4). The circumstance  
325 suggests a different source of TOC within different layers. TOC of superficial layers seems to  
326 derive from organo-metallic or organic contaminants (like HTHC), while TOC of the deeper  
327 layers seems to be not containing metal-binding substances, for instance unreactive humin or  
328 degraded organic matter.

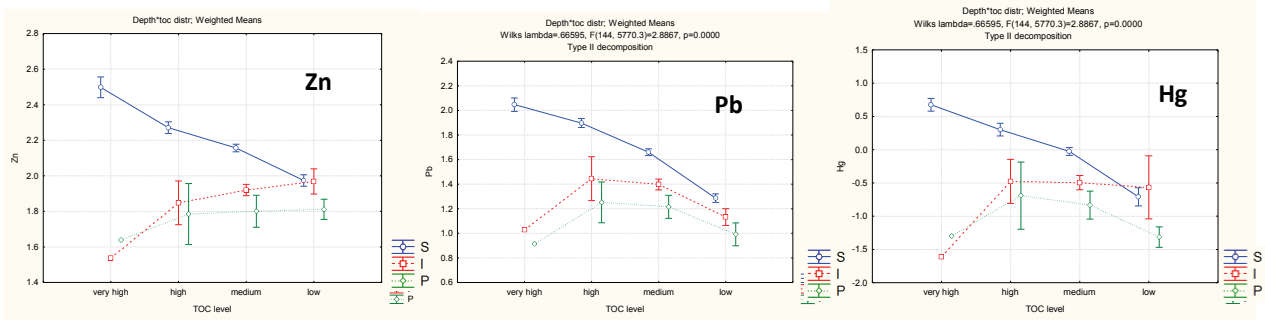
329 The concentrations of nutrients ( $N_{tot}$  and  $P_{tot}$ ) and HTHC within samples of superficial layers (S)  
330 follow the same trend displayed by Cd, Zn, Cu, Pb, As and Hg, *i.e.* they increase when TOC level  
331 increases. This trend is in line with Kenney study (Kenney et al., 2002), that reported an  
332 enhancement of primary productivity when nutrient supply increases, which in turn leads to an  
333 increase of TOC.



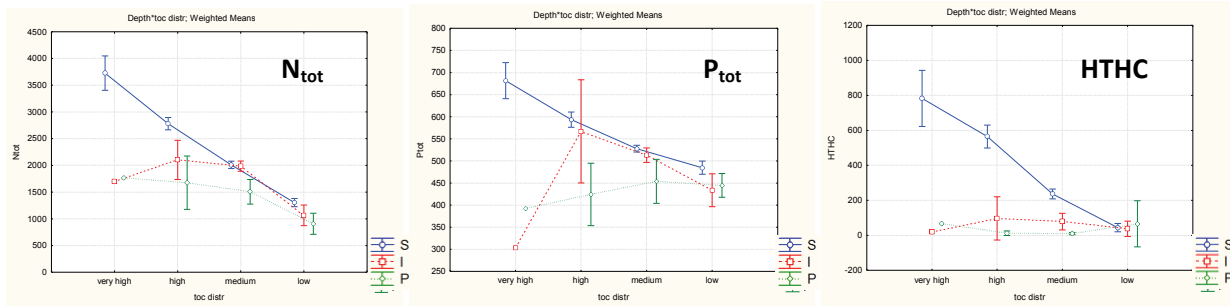
334



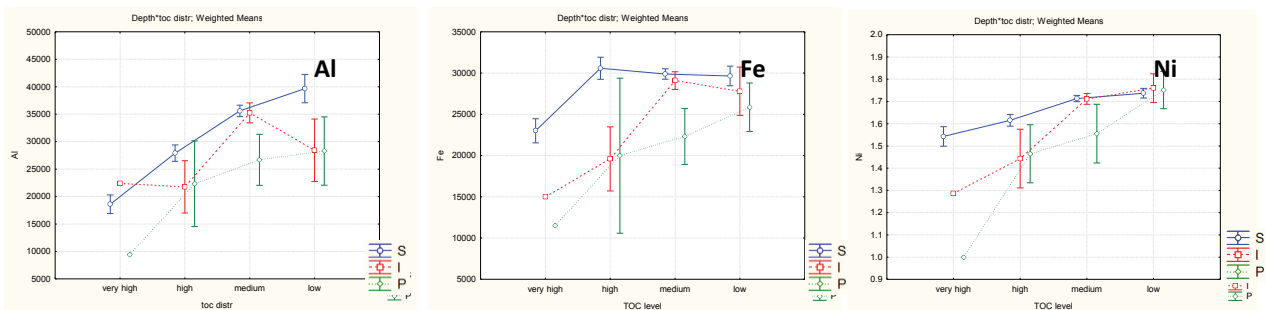
335



336



337



338 **Figure 4.** Mean concentrations (calculated as weighted means) of Cd, As, Cu, Zn, Pb, Hg, N<sub>tot</sub>,  
 339 P<sub>tot</sub>, HTHC, Al, Fe and Ni according to two main effects, Depth (Factor A in ordinate, with three  
 340 classes, superficial (S), intermedium (I) and deep (P)) and TOC concentration level (Factor B, in  
 341 abscissa, with four classes, “low”, “medium”, “high” and “very high”) (F, p,  $\eta^2$  are reported in  
 342 Table 1).

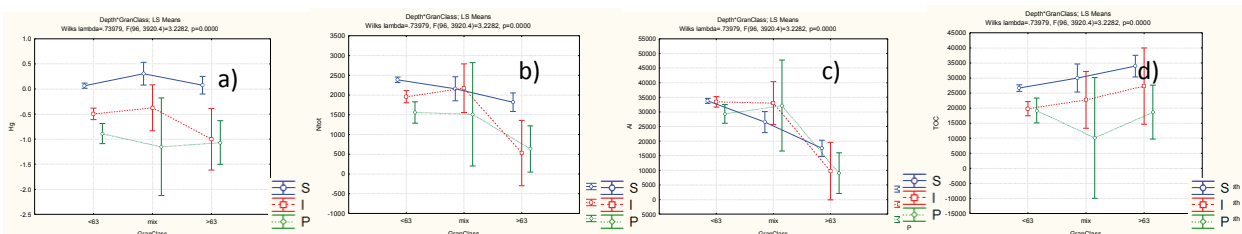
343 Nevertheless, the distribution trend of N<sub>tot</sub> and P<sub>tot</sub> vs TOC is different for the three depth layers. In  
 344 the intermedium layer (red line), concentrations of N<sub>tot</sub> and P<sub>tot</sub> increase when TOC increases from  
 345 “low” to “high” level, but they decay immediately when TOC passes from “high” to “very high”

346 level. On the other hand, for the P level (green line), the increase of TOC corresponds to a  
 347 decrease of nutrients. This circumstance reinforces the hypothesis of different nature of organic  
 348 matter in the different layers and suggests also that synergistic effects may occur in sediments of  
 349 intermedium layer when the TOC level become high (more than 3%).

350 As to HTHC, their concentration in the intermedium and deep layers does not change with  
 351 increasing of TOC, indicating almost a surficial sediment contamination by HTHC.

352 Analyzing two-way ANOVA graphs for lithogenic metals Cr, Ni, V, Fe and Al, it is apparent that  
 353 an increase of TOC goes with a depletion of metal concentration irrespective to depth, except for  
 354 the intermedium layer (red line), in which metal concentrations increase when TOC passes from  
 355 “low” to “medium” level. Also in this case, the intermedium layer present an irregular pattern.

356 Concerning the role of the granulometry in the distribution of contaminants within different depth  
 357 layers, two-way ANOVA considering as independent variables the Grain Size (Factor A) and  
 358 Depth (Factor B) was carried out. Both factors influence the concentration of metals as indicated  
 359 by high  $\eta^2$  values for almost all metals (Table S3), while their interaction effects resulted to be  
 360 minor significant with respect to the interaction of TOC *with* Depth. Inspection of graphs (Figure  
 361 S2) reveals that anthropogenically influenced metals (As, Cd, Cu, Pb, Hg and Zn) display higher  
 362 concentration in the surficial layers (blue lines) with respect to the deepest layers (red and green  
 363 lines) irrespective to the grain size. Clear exceptions are again evident for samples in intermedium  
 364 layer (red lines) (in Figure 5a Hg distribution is shown as representative of the anthropogenically-  
 365 influenced metal cluster), that display an increase of metal concentrations passing from finest  
 366 (<63 $\mu\text{m}$ ) to mixed grain size. This anomalous trend is followed also by  $N_{\text{tot}}$  (Figure 5b) and all  
 367 lithogenic metals such as Al (Figure 5c), that display increasing concentrations with increasing of  
 368 grain size from “fine” to “mix”. On the contrary, the amount of TOC (Figure 5d) unexpectedly  
 369 decreases with the decrease of the grain size.



370  
 371 **Figure 5.** Mean concentrations of Hg (a),  $N_{\text{tot}}$  (b), Al (c) and TOC (d) in different Grain Size  
 372 classes (Factor A, represented in ordinate with three classes  $\theta < 63\mu\text{m}$ , mix,  $\theta > 63\mu\text{m}$ ) and different



373 depth (Factor B, reported in abscise with three classes, surficial (S), intermedium (I) and deep (P)),  
374 obtained from the two-way ANOVA (3x3). (F,p,  $\eta^2$  are reported in Table S3).

375 It is clear that the intermedium layer represents a discontinuity point between the surficial and  
376 deepest levels and we can put forward the following hypothesis:

- 377 i. sediments with “mixed grain size” in the intermedium layer (I) can be interested by  
378 contamination of both metals and nutrients coming probably by channels that causes the  
379 anomalous contamination pattern of this layer (see PCA biplot graphs 3);
- 380 ii. synergistic effects of organic matter and grain size can occur, especially considering salt  
381 water medium. In fact, Koltyar et al. (Koltyar et al. 1996) found that concentration of salinity  
382 above 0.5 % in sediments with relevant organic matter acts as a coagulant of fine particles,  
383 promoting a fast aggregating process. In the salt water of Mar Piccolo, increase of fine  
384 particles coupled with certain levels of organic matter (from 3-5%), could drive to  
385 aggregation process of finest sediment, that turn in higher concentration of metals in these  
386 samples.
- 387 iii. in the deepest layer (P), fluxes of un-reactive and nitrogen-poor organic matter in sediments  
388 with  $>63\mu\text{m}$ , might be present. The depletion of *all* metal contents (including lithogenic ones)  
389 with increase of quartz-rich phases (sand) suggests that a dilution effect might occur (Hedges  
390 et al. 1984; 1995). However, quartz dilution effects can only partially control the metal  
391 distribution in our case, as revealed by the negative, but not statistically significant R values  
392 found for the sand fraction vs lithogenic metals correlation ( $R < -0.50$ , Table S4) (Bern R.C.  
393 2009).

394 In order to verify the advanced hypothesis of coupling effects of TOC with Grain Size, we  
395 completed the statistical analysis performing two way ANOVA (TOC *and* Grain Size). The values  
396 of Wilks lambda=0.83, F (90, 5602.1)=2.0568,  $\eta^2=0.33$  confirmed significant main effects of both  
397 TOC and Grain Size (Table S5), revealing that TOC concentration exerts more dominant effects  
398 with respect to the Grain Size. [Inspection of graphs for each contaminant, reported in Appendix C,](#)  
399 [confirms the odd metal pattern in samples with “mixed” grain size and “medium” TOC](#)  
400 [concentration.](#) Consequently, we can reasonable suppose that the unusually high metal  
401 concentrations found in the samples with “mixed” grain size and “medium to high” TOC level are  
402 probable due to the synergistic effects of grain size, organic matter and salinity as previously

403 reported (Koltyar et al. 1996). Further studies are still necessary to better understand the effect of  
404 salinity as independent factor.

## 405 **Conclusion**

406 This study demonstrates that chemometric approach is an advantage for assessing and modeling  
407 contamination patterns of highly contaminated areas on a large scale, and thus could contribute to  
408 effective monitoring their quality. The spatial distribution of contaminants within Mar Piccolo,  
409 analyzed using two complementary chemometric techniques, PCA/CA and factorial ANOVA,  
410 reflected the complex contamination pattern, arisen from the superposition of natural and  
411 anthropogenic processes and by multiple factors acting simultaneously on a local scale.

412 PCA/CA technique gave the following informations:

- 413 i. Contaminants can be divided in two clusters. The first one contains all sulphophilic metals (As,  
414 Cd, Cu, Hg, Pb and Zn),  $N_{tot}$ ,  $P_{tot}$  and organic pollutants (HTHC). All of them resulted highly  
415 associated with TOC. Within the group, dissimilar fates are also distinguishable: Cd, Cu, Pb  
416 and Zn resulted differently influenced by absorption/desorption mechanisms onto iron-  
417 sulphides or iron-oxyhydroxides with respect to As and Hg that, on the contrary, resulted  
418 strongly influenced by alkylation phenomena promoted by TOC. Furthermore, slight  
419 differences were noticed for Cd, Zn and Hg, which resulted highly concentrated in the organic  
420 matter-reached sediments of the mussel culture area despite the low pH conditions here  
421 determined. Relationship with textural sediment phases seems to influence the  
422 mobilization/immobilization mechanisms of these elements. The second cluster includes  
423 lithogenic elements (Cr, V, Fe, Al and Ni) originated by parent rocks of Mar Piccolo  
424 sediments, thus not anthropogenically enriched.
- 425 ii. The areas of most concern resulted to be: those close to “Navy” characterized by high  
426 concentration of toxic metals, those exposed to natural waterways and channels when a  
427 vertical transport of contaminants versus the deepest layers occur due to high hydrodynamic  
428 condition and due to high organic matter contents, and, finally, the areas hosting the mussel  
429 activity plants characterized by high organic matter and low pH values, thus subjected to  
430 leaching phenomena.
- 431 iii. The depth interested by high contamination either by metals and by organic compounds seems  
432 to be the surficial one (up to 50 cm), while the distribution of contaminants within the deepest

433 layers resulted influenced by different processes and factors acting simultaneously  
434 (absorption/desorption phenomena, quartz or organic matter dilution processes etc.).

435 On the other hand, ANOVA analyses pointed out the main factors controlling the contaminant  
436 pattern that can be summarized as following:

- 437 - The nature and origin of organic matter within different depth layers
- 438 - The rates of dilution by terrigenous sediments, being the principal diluents quartz and/or  
439 organic matter coming with material flow through channels;
- 440 - The grain size fractions and aggregation phenomena promoted in salt water;
- 441 - The original composition/mineralogy of the sediment parent rocks.

442 The combination of both techniques revealed that the intermedium layer represents a discontinuity  
443 point in terms of contamination trend between the surficial and the deepest levels related to  
444 synergistic effects of independent factors such as TOC and Grain Size. Nevertheless, TOC  
445 concentration resulted to be dominant conditioning factor with respect to Grain Size.

446 The evaluation of future scenarios and associated environmental risks in the study area need  
447 investigation of conjunction effects of salinity and pH on contamination pattern.

448 The analyses show the importance of a spatially representative overview given by PCA-  
449 CA/ANOVA to infer the historical anthropogenic source loading on the area and new factors  
450 affecting areas of most concern, making more complex the dynamic of the whole ecosystems.

#### 451 **Acknowledgments.**

452 The authors thank ISPRA for data available. Furthermore, the authors thanks Prof. Federica  
453 Cotecchia and Prof. Claudia Vitone for helpful discussions and Claudia Labianca for preliminary  
454 elaborations.

455

#### 456 **References**

457 Arruti A, Fernández-Olmo I, Irabien A. Evaluation of the contribution of local sources to trace  
458 metals levels in urban PM2.5 and PM10 in the Cantabria region (Northern Spain) *J Environ Monit*  
459 2010;12(7):1451–1458

460 Bellucci, L.G., Cassin, D., Giuliani, S. Botter M., Zonta R. Sediment pollution and dynamic in the  
461 Mar Piccolo of Taranto (southern Italy): insights from bottom sediment traps and surficial  
462 sediments. *Environ Sci Pollut Res* (2016) 23: 12554. doi:10.1007/s11356-016-6738-6

463 Birch G.G. 2017. Determination of sediment metal background concentrations and enrichment in  
464 marine environments – A critical review. *Sci. Tot. Environ.* 580; 813–831

465 Buccolieri A, Buccolieri G, Cardellicchio N, Dell’Atti A, Di Leo A, Maci A (2006) Heavy metals  
466 in marine sediments of Taranto Gulf (Ionian Sea, Southern Italy). *Mar Chem.* 99:227–235.  
467 doi:10.1016/j. marchem.2005.09.009

468 Burnol A., Harris-Hellal J., Bèny, C., Bourdoiseau, J.A., Crouzet, C. 2011. Comparison of arsenic  
469 and mercury release linked to iron and sulfur biogeo-chemical processes. *European Geosciences*  
470 *Union General Assembly 2011, Apr 2011, Vienna, Austria.* Online version: [https://hal-  
471 brgm.archives-ouvertes.fr/hal-00581251v2.](https://hal-brgm.archives-ouvertes.fr/hal-00581251v2)

472 Burton, G.A., 2013. Assessing sediment toxicity: past, present and future. *Environ. Toxicol.*  
473 *Chem.* 32 (7), 1438–1440.

474 Caeiro, S., Cost, M.H., Ramos, T.B., Ferandes, F., Silveira, N., Coimbra, A., Medeiros, G.,  
475 Painho, M., 2005. Assessing heavy metal contamination in Sado Estuary sediment: an index  
476 analysis approach. *Ecol. Indic.* 5, 151–169.

477 Calace N., Cardellicchio N., Ciardullo S., Petronio BM., Pietrantonio M., Pietroletti M. 2008.  
478 Metal distribution in marine sediments of the Mar Piccolo in Taranto (Ionian Sea, Southern Italy).  
479 *Toxicol Environ Chem* 90:549–564.

480 Calloy, S., O’Day, P. A., Esser, B., & Randall, S. (2002). *Speciation and Fate of Trace Metals in*  
481 *Estuarine Sediments under Reduced and Oxidized Conditions, Seaplane Lagoon, Alameda Naval*  
482 *Air Station (USA).* U.S. Department of Energy Office of Scientific and Technical Information,  
483 P.O. Box 62 Oak Ridge. <http://www.llnl.gov/tid/lof>.

484 Cardellicchio N, Buccolieri A, Di Leo A, Spada L (2006) Heavy Metals in Marine Sediments  
485 From the Mar Piccolo of Taranto (Ionian Sea, Southern Italy). *Ann. Chim.* 96 (11-12); 727-741.

486 Caroppo C, Cardellicchio N (1995) Preliminary study on phytoplankton communities of Mar  
487 Piccolo in Taranto (Jonian Sea). *Oebalia* 21:61–76.

488 Chakraborty P., Sarkar A., Vudamala K. Naik R. Nath N. B. 2015. Organic matter-A key factor in  
489 controlling mercury distribution in estuarine sediment. *Mar. Chem.*, vol.173; 2015; 302-309

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

492 Clements H.W., Vieira N.M.M, and Church E.S. 2010. Quantifying restoration success and  
493 recovery in a metal-polluted stream: a 17-year assessment of physicochemical and biological  
494 responses. *Journal of Applied Ecology* Vol. 47, No. 4 (August 2010), pp. 899-910

495 Covelli, S., Fontonlan, G., 1997. Application of a normalization procedure in determining regional  
496 geochemical baselines. *Environ. Geol* 30, 34-45.

497 Dabioch, M., Kita, A., Zerzucha, P. 2013. Assessment of elemental contamination in the bottom  
498 sediments from a dam reservoir using a sequential extraction technique and chemometric analysis.  
499 *Cent.Eur.J.Chem.* (2013) 11: 1981. doi:10.2478/s11532-013-0334-0

500 De Pascalis F, Petrizzo A, Grezzo M, Lorenzetti G, Manfè G, Alabiso G, Zaggia L., 2015.  
501 Estuarine circulation in the Taranto Seas. *Environ Sci Pollut Res.* 23 (13); 12515–12534  
502 doi:10.1007/s11356-015-5389;

503 Doré A. J., Hallsvarthy S. McDonald A.G., Werner M. Kryza M. Abbot J. Nemitz E. Dorè J. CH.  
504 Malcolm H. Vieno M. Reis S. 2014. Quantifying missing annual emission sources of heavy metals  
505 in the United Kingdom with an atmospheric transport model.  
506 <http://dx.doi.org/10.1016/j.scitotenv.2014.02.001>.

507 Dung, T.T.T., 2013. Cappuyns V., Swennen R., Phung N. K., *Rev. Environ. Sci. Biotechnology*  
508 12, 335-353.

509 EC IPPC, Best Available Techniques Reference (BAT) Document for Iron and Steel Production,  
510 Remus, R., Miguel A. Aguado-Monsonet. Serge Roudier, Luis Delgado Sancho. Dec. 2013.  
511 Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control)

512 Einax, J.W. Zwanzinger, H.W. Geiss. S. 1997 *Chemometrics in Environmental Chemistry* BCH,  
513 Weinham, Germany (1997)

514 Filzmoser P, Hron K, Reimann C. 2009. Univariate statistical analysis of environmental  
515 (compositional) data: problems and possibilities. *Sci Total Environ.* 2009 15; 407(23):6100-8.

516 Gałuszka A, Migaszewski ZM, Dołęgowska S, Michalik A, Duczmal-Czernikiewicz 2015. A.  
517 Geochemical background of potentially toxic trace elements in soils of the historic copper mining

518 area: a case study from Miedzianka Mt., Holy Cross Mountains, south-Central Poland. *Environ.*  
519 *Earth Sciences*. V. 74; 4589–4605. doi: 10.1007/s12665-015-4395-6.

520 Gao X, Yang Y, Wang C. 2012. Geochemistry of organic carbon and nitrogen in surface  
521 sediments of coastal Bohai Bay inferred from their ratios and stable isotopic signatures.  
522 *Mar.Poll.Bull.*; 64; 1148–1155.

523 Gredilla, A., Fdez-Ortiz de Vallejuelo, S., de Diego, A., Madariaga, J.M., Amigo, J.M. 2013.  
524 Unsupervised pattern-recognition techniques to investigate metal pollution in estuaries. *TrAC -*  
525 *Trends in Analytical Chemistry*; 46, pp. 59-69.

526 Hedges J & Stern J.H.. 1984. Carbon and nitrogen determinations of carbonate-containing solids.  
527 *Limnol. Oceanogr.* 29: 657-663.

528 Hedges, J. I., & R. G. Keil (1995), Sedimentary organic matter preservation: an assessment and  
529 speculative synthesis, *Mar. Chem.*, 49, 81–115.

530 Ho, H.H., Swennen R, Cappuys V, Vassilieva E, Tran TV. 2012. Necessity of normalization to  
531 aluminum to assess the contamination by heavy metal sand arsenic in sediments near Haiphong  
532 Harbor, Vientam. *J. Asian Earth. Sci.* 56, 229-239..

533 Hopke F.K. 2015. Chemometrics applied to environmental systems. *Chemometrics and Intelligent*  
534 *Laboratory Systems*; 149; 205–214

535 Horowitz, A.J., (1991). *A Primer on sediment-trace element chemistry* (pp. 1–136). Chelsea:  
536 Lewis Publishers

537 ICRAM–APAT (2007) *Manuale per la movimentazione di sedimenti marini. Manual for the*  
538 *handling of marine sediments.*

539 INES-EPER. (2006). Register of the National Institute of Emission and their sources integrated by  
540 European Pollutant Emission Register.

541 ISPRA 2010. Evaluation of characterization results for the identification of appropriate actions for  
542 remediation of site of national interest of Taranto. Technical Report August 2010, 90

543 Jorgensen B. 1996. Material flux in the sediment. In: Jorgensen B. and Richardson K. (Eds.)  
544 *Coastal and estuarine studies. American Geophysical Union.* p: 115-135.

545 Kenney WF, Waters MN, Schelske CL, Brenner M (2002) Sediments records of phosphorus-  
546 driven shifts to phytoplankton dominance in shallow Florida lakes. *J Paleolimnol* 27:367–377

547 Kennish M.J., Brush M.J., Moore K.A. 2014. Drivers of Change in Shallow Coastal Photic  
548 Systems: An Introduction to a Special Issue *Estuaries and Coasts*; 37 (Suppl 1):S3–S19.

549 Kotlyar L.S., Sparks B.D. & Schutte R. (1996) Effect of salt concentration on aggregation of  
550 colloidal clays. *Clays Clay Miner.* 44, 121-131.

551 Kowalkowski T, Zbytniewski R, Szpejna J, Buszewski B. Application of chemometrics in river  
552 water classification. *Water Res* 2006;40:744-52.

553 Leardi R. Armanino C. Lanteri, S. Alberotanza L. J. *Chemom.*, 2000. Three-mode principal  
554 component analysis of monitoring data from Venice lagoon. *J. Chemometrics.* 14(3) 187-195

555 Liu C.W. & Lai C.C., Chen Y.Y.. 2013. Hydrogeochemical and mineralogical investigations of  
556 arsenic- and humic substance-enriched aquifers. *Journal of Hydrology* 498:59-75 DOI:  
557 10.1016/j.jhydrol.2013.06.017;

558 Lofù, A., Mastrorilli, P., Dell'Anna, M.M., Mali, M., Sisto, R., Vignola, R., 2016. Iron (II)  
559 modified natural zeolites for hexavalent chromium removal from contaminated water. *Arch.*  
560 *Environ. Protect.* 42 (1); 35-40.

561 Long, E.R. Macdonald, D.D. Smith, S.L. Calder F.D., 1995. Incidence of adverse biological  
562 effects with ranges of chemical concentrations in marine and estuarine sediments *Environ.*  
563 *Manag.*, 19 (1995), pp. 81–97

564 Long, E.R., Ingersoll, C.G., MacDonald D.D., 2006. Calculation and uses of mean sediment  
565 quality guideline quotients: a critical review. *Environ. Sci. Technol.*, 40 (2006), pp. 1726–1736

566 Mali, M., Dell'Anna, M.M., Mastrorilli, M.M., Damiani L., Piccinni F.A.; 2017a. Assessment and  
567 source identification of pollution risk for touristic ports: Heavy metals and polycyclic aromatic  
568 hydrocarbons in sediments of 4 marinas of the Apulia region (Italy). *Mar. Poll. Bull.* 114 (2); 768–  
569 777;

570 Mali, M., De Serio F., Dell'Anna MM., Mastrorilli P., Damiani L., Mossa M., 2017b. Enhancing  
571 the performance of hazard indexes in assessing hot spots of harbour areas by considering  
572 hydrodynamic parameters. *Ecological Indicators*, 73; 38–45.

573 Mali, M., Dell'Anna, M.M., Mastrorilli, P., Gredilla, A., Fdez-Ortiz De Vallejuelo, S. 2016.  
574 Identification of hot spots within harbour sediments through a new cumulative hazard index. Case  
575 study: Port of Bari, Italy 2016. *Ecological Indicators*; 2554; 548-556;

576 Mali, M.M. Dell'Anna, P. Mastrorilli, L. Damiani, N. Ungaro, C. Belviso, S. Fiore. 2015. Are  
577 conventional statistical techniques exhaustive for defining metal background concentrations in  
578 harbour sediments? A case study: the coastal area of Bari (southeast Italy); *Chemosphere*, 138;  
579 708–717

580 Malviya R, & Chaudhary R., 2006. Leaching behavior and immobilization of heavy metals in  
581 solidified/stabilized products. *J Hazard Mater* 137:207–217;

582 Marchand, C., Lalliet Verges, E., Baltzer, F., Alberic, P., Cossa, D., & Baillif, P. 2006. Heavy  
583 Metals Distribution in Mangrove Sediments along the Mobile Coastline of French Guiana. *Marine*  
584 *Chem.* 98, 1-17.

585 Mayer, L.M., 1993, Organic matter at the sediment–water interface, in Engel, M.H., Macko, S.A.,  
586 (eds), *Organic Geochemistry –principles and applications*: New York, Plenum Press, 171–184

587 Mayer, L.M., 1994. Surface area control of organic carbon accumulation in continental shelf  
588 sediments. *Geochim. Cosmochim. Acta* 58, 1271–1284;

589 McKillup. 2012. *Statistics explained. An introductory guide for life scientists* Cambridge  
590 University Press. Ch. 13 two-factor analysis of variance.

591 Meyers PA. 2003. Applications of organic geochemistry of paleolimnological reconstructions: a  
592 summary of examples from the Laurentian Great Lakes. *Org. Geochem.* 34:261–289

593 Micò C, Recantala L, Peris M, Sanchez J. 2006. Assessing heavy metal sources in agricultural  
594 soils of an European Mediterranean area by multivariate analysis. *Chemosphere*; 65 (5); 863–87  
595 <http://dx.doi.org/10.1016/j.chemosphere.2006.03.016>

596 Ministry Decree 468/01. Programma nazionale di bonifica e ripristino ambientale. Ministero  
597 dell'ambiente e della tutela del territorio. *Gazzetta Ufficiale*, January 16th 2002, Supplemento  
598 Ordinario n. 10. In Italian.

599 Ohta, A., Imai, N., Terashima, S., Tachibana, Y. 2005. Application of multi-element statistical  
600 analysis for regional geochemical mapping in central Japan. *Appl. Geochem.* 20, 1017–1037



601 Ohta, A., Imai, N., Terashima, S., Tachibana, Y., Ikehara, K. Katayama, H. 2015. Elemental  
602 distribution of surface sediments around oki trough including adjacent terrestrial area: Strong  
603 impact of Japan Sea Proper Water on silty and clayey sediments. *Bull. Geol. Surv. Japan* V. 66,  
604 81-101.

605 Petronio B.M., Cardellicchio N., Calace N., Pietroletti M., Pietrantonio M., Caliandro L., Maienza  
606 A. 2012. Spatial and temporal heavy metal concentration (Cu, Pb, Zn, Hg, Fe, Mn, Ni) in  
607 sediments of the Mar Piccolo in Taranto (Ionian Sea, Italy). *Water Air Soil Poll.* 223:863–875.  
608 doi:10.1007/s11270-011-0908-4

609 Reimann C, Filzmoser P, Garrett RG, Dutter R. *Statistical data analysis explained. 2008 Applied*  
610 *environmental statistics with R.* Chichester, UK: Wiley; 2008.

611 Reimann, C., Filzmoeser, P., Garret, R.G., 2005. Background and threshold: critical comparison  
612 of methods of determination. *Sci. Total Environ.* 346, 1-16.

613 Reimann, C., Filzmoser, P., 2000. Normal and lognormal data distribution in geochemistry: death  
614 of a myth. Consequences for the statistical treatment of geochemical and environmental data.  
615 *Environ. Geol.* 39, 1001–1014;

616 Rostron P, Ramsey MH. 2012. Cost effective, robust estimation of measurement uncertainty from  
617 sampling using unbalanced ANOVA. *Accred. Qual. Assur.* (2012) 17:7–14

618 Shaw, R.G.; Mitchell-Olds, T. Anova for unbalanced data: An overview. *Ecology* 1993, 74,  
619 1638-1645. (55)

620 Shrestha S, Kazama F. Assessment of surface water quality using multivariate statistical  
621 techniques: a case study of the Fuji River Basin, Japan. *Environ Modell Softw* 2007;22:464-75

622 Simeonov V, Stratis JA, Samara C, 2003. Assessment of the surface water quality in Northern  
623 Greece. *Water Res.* 37; 4119-24;

624 Simeonov, V. Einax, J.A. Stanimirova I and Kraft J. 2002 “Environmetric modeling and  
625 interpretation of river water monitoring data”, *Anal Bioanal Chem* (2002) 374, pp. 898–905, DOI  
626 10.1007/s00216-002-1559-5;

627 Stanimirova I, Simeonov V. 2005 Modeling of environmental four-way data from air quality  
628 control - *Chemometrics and Intelligent Laboratory Systems* 77 (2005) 115 – 121

- 629 Umgiesser G, Scroccaro I, Alabiso G (2007) Mass exchange mechanisms in the Taranto Sea,  
630 *Transit. Waters Bull* 2:59–71.
- 631 Van Nugteren P., Moodley L., Brummer GJ, Heip C.H.R., Herman P.M.J, Middelburg J.J., 2009.  
632 Seafloor ecosystem functioning: the importance of organic matter priming. *Mar Biol.* 2009;  
633 156(11): 2277–2287. doi: 10.1007/s00227-009-1255-5
- 634 Varol, M., 2011. Assessment of heavy metal contamination in sediments of the Tigris River  
635 (Turkey) using pollution indices and multivariate statistical techniques. *J. Hazard. Mater.* 195,  
636 355– 364;
- 637 Venugopal T, Giridharan L, Jayaprakash M. 2009. Application of chemometric analysis for  
638 identifying pollution sources: a case study on the River Adyar, India. *Marine Freshwater Res.* V.  
639 60:1254-64;
- 640 Zhou F., Guo H.C., Liu Y., Jiang Y.M.. 2007. Chemometrics data analysis of marine water quality  
641 and source identification in Southern Hong Kong. *Mar Pollut Bull* 2007;54:745-56.