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- 1 Combining chemometric tools for assessing hazard sources and factors acting
- 2 simultaneously in contaminated areas. Case study: "Mar Piccolo" Taranto
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- 11 Abstract

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

21

22 Keywords. Marine sediments, hazard assessment, chemometrics, PCA, factorial ANOVA,

- 23 contamination pattern
- 24

25 1. Introduction

Coastal areas are the most impacted natural environments, being subjected to various forms of contamination. Thousands of pollutants can affect the quality of both water column and bottom sediments, triggering severe changes in marine ecosystem and posing high risks to its structure and function (Kennish et al. 2014). The continuous loading of multiple disturbances needs to be monitored and evaluated by means of accurate and complex hazard assessment procedures calling for multidisciplinary competences and knowledges. Moreover, advanced tools are needed in order to extract the most relevant informations from the extensive amount of bio-geo-chemical data usually acquired in the characterization phase aimed at identifying the pollution pattern in the study area.

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

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

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

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

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

74 2. Design and methods

75 2.1 Study area and sampling programs

Taranto's Mar Piccolo is a semi-enclosed basin with a surface area of approximately 21 km² and a 76 maximum depth of 13 m. It is divided into two inlets and it is connected to Mar Grande and to 77 Taranto Gulf (Ionian Sea) through two channels, one artificial named Navigable Channel and the 78 other one natural called Porta Napoli's Channel. In this study, the relative large channel 79 connecting the two inlets is called Punta Penna Channel, like the bridge name over the channel. 80 (Figure 1). A peculiar characteristic of Mar Piccolo is the presence of several submarine 81 freshwater springs called "citri" (Caroppo and Cardellicchio, 1995) which contribute to freshwater 82 inputs together with small karstic rivers. The two most significant "citri" are located within the 83 first inlet and are called citro-Galeso and citro-Citrello. The area is characterized by a low water 84 exchange and hypoxic conditions as consequence of oxygen consumption by microbial 85 degradation due to large inputs of organic matter (from wastewater discharges, mussel farming 86 87 and agricultural soil drainage) which also contribute to an excess of nutrients in the system (Umgiesser et al. 2007). Previous studies refer also high sulphide concentrations in the bottom 88 sediments as a result of anaerobic degradation of organic matter (Cardellicchio et al. 2006). 89



90

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

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

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

103 **2.2 Chemical analysis**

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

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

116 2.3 Statistical analysis

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

125 **3. Statistical treatment and results**

126 3.1 Preliminary data elaboration

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

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

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

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

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

3.2 Spatial Distribution of Contaminants through PCA/CA analyses

A model with four principal components (PCs) accounting for a cumulative variance explained of 70% was considered. Despite additional elaborations (such as the exclusion of "spike" concentrations and variables with negligible influence on PCs), the cumulative variance explained by the first four PCs, remained almost unchanged. We presume that this circumstance is related to the occurrence of too many simultaneous and multi-source effects, each of them marginally affecting contamination pattern. Therefore, in order to evaluate how the unexplained variance could affect the results obtained, we decided to perform cluster analysis (CA) considering both the whole dataset and data coming from the selected PCs. The clusters obtained by both approaches were then compared (*vide infra*).

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

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



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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_{tot}

196 As to the pollution sources, damping of contaminated material and untreated terrestrial discharges can be considered possible causes affecting Cd, Cu, Pb, Zn, As, Hg contents (the variables 197 displaying positive loadings on PC2), as suggested by previous studies in the area (Cardellicchio 198 et al. 2007; Petronio et al. 2012). Nevertheless, these metals resulted to be the main elements 199 contained in dust emission from steel factories (EC Remus et al. 2013, Arruti et al. 2010, Dore et 200 al.2014). For this reason, we can reasonably assume that metal-reached atmospheric input should 201 be an important contamination source of these metals, being the study area located very near to a 202 steel factory. As far as Pb and Hg are concerned, both metals could derive also from oil spilling 203 and ship-fuel losses. The presence of "Navy" in the area can contribute to fuel losses from military 204 ships during maneuvering (Mali et al. 2017b) and thus can explain the association of Pb and Hg 205 with HTHC. 206

Continuing analyzing of PC2 negative loadings (Figure 2a), it is apparent another metal cluster 207 constituted by V, Ni, Cr and Fe, strongly correlated with Al and mud fraction ($4\mu m < mud <$ 208 63µm) and in contrast trend with respect to Cd, Zn, Cu, Pb, As, Hg and HTHC. Both 209 circumstances suggest that the presence of V, Ni, Cr and Fe might be attributed to natural origin. 210 Probably, the geochemical composition of the parent rocks which generated Mar Piccolo 211 sediments, could be the main factor controlling the variability of V, Ni, Cr and Fe. In fact, 212 statistically significant positive correlations (R) were found between these metals and the finest 213 grain size sediments (Table S4), supporting our hypothesis of their enrichment predominantly due 214 to sedimentary processes (Dung et al. 2013; Lofu et al. 2016; Mali et al. 2015; Mali et al. 2016). 215

Interesting data derived from PC2/PC4 space plot (Figure 2b), where some differences among anthropogenic enriched metals are emphasized. On the negative PC4 plan, Hg and Pb (and Cu in less extent) resulted associated with nutrients (N_{tot} and P_{tot}) and TOC. All these variables display positive correlation with Fe, fine sediments (mud and clay) and pH. On the contrary, As, Cd and 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 respect to the As, Cd and Zn one) might be caused by different affinity of these metal for different 222 phases (ion-exchangeable, carbonate, organic, iron-oxy-hydroxide, sulphides etc.) (Calloy et al., 223 2002; Bucolieri et al. 2006). Hg, Pb and Cu are probable bound to the more mobile phases with 224 respect to As, Cd and Zn. This hypothesis is supported by previous studies: Calace et al. (2008) 225 found that Pb in Mar Piccolo is present mainly as ion-exchangeable species or bound to carbonate 226 while Hg and Cu result predominantly associated to organic matter. Cardellicchio et al. 2006 227 found that, except for Hg and Pb, metals such as Zn, Cd and As in Mar Piccolo result to be present 228 229 mainly as insoluble sulphides encouraged by high sulphide concentrations and negative redox (ORP) values. 230

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

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





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

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

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

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

278 3.3 ANOVA analysis

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

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

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

	A=Depth B= TOC $(3x4)$										
Type	TT Dept.	п. <u>р.</u> 100	(541)								
ll l	F			p			n2				
	А	В	AxB	А	B	AxB	А	В	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	187	156	4 16	0.00	0.00	0.00	0.02	0.30	0.02		

тос	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

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

The two-way model reveals that sampling depth (Factor A) strongly influences the concentration 312 of all metals, while TOC level (Factor B) has a dominant influence on the contamination 313 distribution of almost all metals except for As ($\eta^2 = 0.01$, p>0.01). Furthermore, the interaction 314 effects of both factors (η^2_{AxB}) resulted important for all metals but the weight of interaction effect 315 is different for two metal-groups. For As, Cd and Cu the interaction effect is *dominant* ($\eta^2 > 0.05$), 316 while it resulted significant (n²>0.01) for the other metals (Cr, Fe, Ni, Pb, Zn, Hg), nutrients and 317 HTHC. In order to facilitate the interpretation of data and to get insights into the interaction 318 effects, the results of two way ANOVA are graphically reported in Figure 4. 319

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

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



Figure 4. Mean concentrations (calculated as weighted means) of Cd, As, Cu, Zn, Pb, Hg, Ntot, Ptot, HTHC, Al, Fe and Ni according to two main effects, Depth (Factor A in ordinate, with three classes, superficial (S), intermedium (I) and deep (P)) and TOC concentration level (Factor B, in abscissa, with four classes, "low", "medium", "high" and "very high") (F, p, η^2 are reported in Table 1).

Nevertheless, the distribution trend of N_{tot} and P_{tot} *vs* TOC is different for the three depth layers. In
the intermedium layer (red line), concentrations of N_{tot} and P_{tot} increase when TOC increases from
"low" to "high" level, but they decay immediately when TOC passes from "high" to "very high"

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

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

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



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

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

375 It is clear that the intermedium layer represents a discontinuity point between the surficial and376 deepest levels and we can put forward the following hypothesis:

i. sediments with "mixed grain size" in the intermedium layer (I) can be interested by
contamination of both metals and nutrients coming probably by channels that causes the
anomalous contamination pattern of this layer (see PCA biplot graphs 3);

ii. synergistic effects of organic matter and grain size can occur, especially considering salt
water medium. In fact, Koltyar et al. (Koltyar et al. 1996) found that concentration of salinity
above 0.5 % in sediments with relevant organic matter acts as a coagulant of fine particles,
promoting a fast aggregating process. In the salt water of Mar Piccolo, increase of fine
particles coupled with certain levels of organic matter (from 3-5%), could drive to
aggregation process of finest sediment, that turn in higher concentration of metals in these
samples.

iii. in the deepest layer (P), fluxes of un-reactive and nitrogen-poor organic matter in sediments
with >63µm, might be present. The depletion of *all* metal contents (including lithogenic ones)
with increase of quartz-rich phases (sand) suggests that a dilution effect might occur (Hedges
et al. 1984; 1995). However, quartz dilution effects can only partially control the metal
distribution in our case, as revealed by the negative, but not statistically significant R values
found for the sand fraction *vs* lithogenic metals correlation (R<-0.50, Table S4) (Bern R.C.
2009).

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

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

405 Conclusion

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

412 PCA/CA technique gave the following informations:

i. Contaminants can be divided in two clusters. The first one contains all sulphofilic metals (As, 413 Cd, Cu, Hg, Pb and Zn), N_{tot}, P_{tot} and organic pollutants (HTHC). All of them resulted highly 414 associated with TOC. Within the group, dissimilar fates are also distinguishable: Cd, Cu, Pb 415 and Zn resulted differently influenced by absorption/desorption mechanisms onto iron-416 sulphides or iron-oxyhydroxides with respect to As and Hg that, on the contrary, resulted 417 strongly influenced by alkylation phenomena promoted by TOC. Furthermore, slight 418 differences were noticed for Cd, Zn and Hg, which resulted highly concentrated in the organic 419 matter-reached sediments of the mussel culture area despite the low pH conditions here 420 determined. Relationship with textural sediment phases seems to influence the 421 mobilization/immobilization mechanisms of these elements. The second cluster includes 422 lithogenic elements (Cr, V, Fe, Al and Ni) originated by parent rocks of Mar Piccolo 423 sediments, thus not anthropogenically enriched. 424

ii. The areas of most concern resulted to be: those close to "Navy" characterized by high
concentration of toxic metals, those exposed to natural waterways and channels when a
vertical transport of contaminants versus the deepest layers occur due to high hydrodynamic
condition and due to high organic matter contents, and, finally, the areas hosting the mussel
activity plants characterized by high organic matter and low pH values, thus subjected to
leaching phenomena.

431 iii. The depth interested by high contamination either by metals and by organic compounds seems432 to be the surficial one (up to 50 cm), while the distribution of contaminants within the deepest

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

435 On the other hand, ANOVA analyses pointed out the main factors controlling the contaminant436 pattern that can be summarized as following:

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

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

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

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

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