Zooplankton from a North Western Mediterranean area as a model of metal transfer in a marine environment

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Table 1
Metals concentrations in marine water (mean values of the three sampling sites, μg L^{-1})

	0-3 m water depth			
	Spring	**Summer**	**Autumn**	**Winter**
Mn	0.62	0.61	0.41	1.85
Fe	1.21	2.37	1.27	20.26
Al	1.70	1.50	1.65	1.80
Co	2.10	1.45	0.02	0.03
Ni	6.20	10.60	0.26	0.35
Cu	0.36	1.37	0.39	0.34
Zn	4.18	11.43	3.69	2.26
Cd	0.02	0.02	0.02	0.02
Pb	0.49	1.45	2.30	0.80
	50 m water depth			
	spring	**summer**	**autumn**	**winter**
Mn	0.60	0.58	0.63	0.65
Fe	1.07	1.60	2.15	4.02
Al	1.25	1.30	2.00	2.61
Co	1.60	1.50	0.02	0.02
Ni	8.70	11.20	0.39	0.28
Cu	0.68	0.93	0.70	0.22
Zn	3.27	8.33	4.70	2.15
Cd	0.02	0.02	0.04	0.02
Pb	0.26	1.11	2.33	0.16
	100 m water depth			
	spring	**summer**	**autumn**	**winter**
Mn	0.46	0.35	0.45	0.49
Fe	0.78	0.75	1.07	0.72
Al	1.30	1.05	2.10	2.71
Co	1.10	1.65	0.02	0.02
Ni	9.10	10.40	0.27	0.28
Cu	1.54	0.23	0.34	0.41
Zn	3.30	3.52	0.56	3.12
Cd	0.02	0.02	0.03	0.02
Pb	1.34	2.03	0.50	1.20

Table 2
Metals concentrations in marine zooplankton (mg Kg^{-1})

	Spring	Summer	Autumn	Winter
	0-3 m water depth			
Al	81.00	62.30	122.79	174.14
As	0.23	0.26	0.41	0.75
Be	0.01	0.01	0.01	0.04
Cd	0.20	0.40	0.41	0.08
Co	0.06	0.08	0.21	0.13
Cr	0.83	0.39	1.61	1.50
Cu	1.90	3.88	14.31	3.72
Fe	109.15	91.03	138.45	354.42
Mn	1.22	1.27	3.26	2.57
Mo	0.15	0.27	0.18	0.11
Ni	1.10	0.88	1.76	1.10
Pb	1.27	1.42	3.46	1.01
Sb	0.10	0.08	0.11	0.02
Se	0.23	0.52	0.32	0.22
Sn	0.07	0.05	0.10	0.24
V	0.31	0.25	0.73	3.06
Zn	22.60	15.68	25.52	11.74
	50 m water depth			
Al	440.38	92.91	445.02	621.68
As	0.46	0.14	0.55	0.39
Be	0.02	0.01	0.03	0.01
Cd	0.08	0.05	0.19	0.07
Co	0.23	0.10	0.31	0.08
Cr	7.25	3.23	4.99	2.10
Cu	4.64	1.86	39.7	2.75
Fe	539.44	146.20	659.86	166.24
Mn	5.55	1.96	23.00	2.20
Mo	0.32	0.07	0.45	0.15

Ni	5.48	2.09	3.72	0.85
Pb	12.38	2.60	10.62	4.58
Sb	0.48	0.23	0.41	0.07
Se	0.18	0.12	0.25	0.18
Sn	0.42	0.07	0.86	0.29
V	0.89	0.26	1.28	0.79
Zn	132.33	16.14	51.36	24.85
		100 m water depth		
Al	1738.23	255.61	648.8	452.66
As	0.65	0.32	0.78	0.40
Be	0.03	0.01	0.04	0.02
Cd	0.16	0.06	0.16	0.08
Co	0.03	0.02	0.38	0.14
Cr	2.87	1.30	3.33	1.19
Cu	3.72	5.91	4.72	1.46
Fe	1741.76	453.45	675.7	320.85
Mn	8.82	5.46	29.85	4.43
Mo	0.16	0.06	0.22	0.05
Ni	2.04	1.23	2.75	1.03
Pb	10.92	10.70	12.02	2.37
Sb	0.59	0.18	0.39	0.06
Se	0.24	0.15	0.25	0.13
Sn	0.28	0.24	0.53	0.07
V	1.18	0.50	1.73	1.11
Zn	79.85	27.43	32.78	15.47

Table 3
Bioaccumulation factors (BAFs) for marine zooplankton

	spring	summer	autumn	winter
	0-3 m water depth			
Mn	1964	2069	7951	1389
Fe	90505	38389	109015	17493
Cu	5349	2837	36692	10941
Zn	5401	1372	6915	5194
Al	47	41	74	96
Ni	177	83	6769	3142
Co	28	56	10500	4333
Pb	2615	978	1506	1257
Cd	10050	22222	17521	3921
	50 m water depth			
Mn	9228	3382	36311	3373
Fe	502833	91209	307483	41373
Cu	6870	2002	57105	12298
Zn	40467	1696	10927	11547
Al	352	71	222	238
Ni	629	186	9627	3048
Co	143	66	11666	4210
Cd	4545	3067	4947	3153
Pb	47836	2345	4550	29134
	100 m water depth			
Mn	190004	15762	66718	9011
Fe	2245114	601153	629847	448239
Cu	2416	25518	13898	3604
Zn	24226	7799	58327	4951
Al	1337	243	308	167
Ni	224	118	10315	3642
Co	272	147	21839	7216
Cd	7920	3669	6400	4000
Pb	8168	5282	23811	1975

Table S1
Quantification limit (mg Kg^{-1}), reference material values (oyster tissues) and percentages of recovery.

Element	LOQ	SRM 1566b	% recovery
Al	0.010	197.2 ± 6.0	82
As	0.010	7.650.65±	103
Be	0.010	-	-
Cd	0.010	2.48±0.08	104
Ce	0.010	-	-
Co	0.010	0.371±0.009	99
Cu	0.010	71.6±1.6	105
Fe	0.010	205.8±6.8	104
Hg	0.034	0.0132±0.0013	102
La	0.010	-	-
Mn	0.010	18.5±0.2	95
Ni	0.010	1.04±0.09	98
Pb	0.010	0.308±0.009	98
Sb	0.010	-	-
Se	0.010	2.06± 0.15	117
V	0.010	0.0577 ± 0.023	102
Zn	0.010	1424±46	105

- These elements were not present in the certified material

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Abstract

We monitored the concentration of 21 trace elements in zooplankton samples collected in a Northwestern Mediterranean coastal ecosystem (Italy). In the last 20 years, this area has been the target of important anthropogenic impacts including maritime traffic and substantial industrial activities. Zooplankton contributes to the transfer of trace metals to higher trophic levels and constitute one of the recommended groups for the baseline studies of metals in the marine environment. The essential trace elements (As, Cu, Mn, Zn, Fe, Mo, Co, Cr, Se, Ni) and the nonessential trace elements (Al, Be, Cd, Pb, Sb, Sn, V) were generally found at concentrations of no concern in the analyzed zooplankton samples, but showed important variations between seasons and different water depths. The zooplankton was found to be a significant accumulator of metals, and bioaccumulation factors were in the range of 28 (Co) to 109015 (Fe) in marine surficial waters, with increasing values at increasing water depth. Zooplankton is a useful bioindicator to assess metal contamination and its impact in the marine environment.

Keywords: trace elements, zooplankton, Mediterranean Sea, bioaccumulation

Introduction

The Mediterranean Sea is characterized by a rich biodiversity, but the presence of chemical and mining industries in the majority of coastal areas threatens this ecosystem by producing significant amounts of chemical waste, of which trace metals constitute an important part (EEA, 2006; Lafaibre, 2008). Some of these trace metals, such as copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), and chromium (Cr) are essential for the metabolism of the organisms while others, like cadmium (Cd), lead (Pb), and mercury (Hg), are nonessential. All metals, essential or not, are toxic above a threshold bioavailability and can be considered as serious pollutants of aquatic environments because of their toxicity, persistence, and tendency to concentrate in organisms (Ikem and Egiebor, 2005).

Plankton is a determinant for metal transfer in marine food webs and trace metals are included in plankton biochemical cycles (Whitfield, 2001). Zooplankton is particularly critical to the functioning of ocean food webs because of their sheer abundance and vital ecosystem roles. Zooplankton is mainly composed of copepods, the most abundant animal taxon on the Earth (Schminke 2007); copepods are the major grazers in ocean food webs, providing the principal pathway for energy from primary producers to consumers at higher trophic levels (Richardson 2008; Fernández-Severini *et al*., 2013). Additionally, zooplankton play an important role in the biogeochemical cycling of trace metals in marine ecosystems. In fact, in surface pelagic waters, plankton can strongly affect the vertical transport of elements; biogenic particle flux accounts for a lot of the vertical flux and hence controls the residence times of particle-reactive elements in the sea (Fisher *et al*., 1991).

Metals may be adsorbed onto organic films or colloidal materials at the particle surface or by crossing the plankton cell (Fisher and Reinfelder, 1995). Adsorption of metals to plankton varies greatly in the first trophic levels. In general, plankton with higher surface: volume ratio has higher concentration factors, especially for non-reactive particles. Once aggregated or incorporated into

plankton, elements may be transferred along the web chain and transformed by successive organisms, either enhancing or alleviating their toxicity (Watras and Bloom, 1992).

Metals that are egested by sinking fecal pellets from zooplankton are exported out of the surface waters, enriching the deep-water dissolved-metal pool through re-mineralization and release (Fisher *et al*. 1991). Cellular metals regenerated in the dissolved state during grazing may be recycled many times and re-utilized by the phytoplankton community. In assessing environmental quality with respect to trace elements in seawater, the bioavailable fraction is of major importance as toxicity is dependent on the bioavailable exposure concentration (Kahle and Zauke, 2003). This bioavailable fraction can only be assessed by determining the amount of metals incorporated into organisms, which is the main goal in biomonitoring (Rainbow, 1993).

Because of their wide geographic distribution, trophic position, rapid turnover, huge biomass and high capacity to accumulate trace metals, zooplanktonic organisms can be used as biomonitors for trace metals in marine environments (Barka *et al*., 2001; Kahle and Zauke, 2003; Fang *et al*., 2006; Hsiao *et al*., 2011).

Coastal areas receive large amounts of contaminants introduced by domestic, industrial and agricultural activities, either directly, via rivers or through atmospheric deposition (Usero *et al*., 2005). Costal marine ecosystems are important for the fate of contaminants and are therefore worth studying.

Data regarding metal concentrations in zooplankton communities of Mediterranean coastal environments are very scarce and usually report just a few metals, for example, rare earth elements (Gulf of Lion, France; Strady *et al*., 2015); Cu, Pb, and Cd (Toulon Bay, France; Rossi and Jamet, 2008); Zn, Cu, and Cd (estuary of Var, France; Hardstedt–Romeo and Laumond, 1980). No previous studies have analyzed the concentrations of this many trace elements, reported here, in zooplankton from a Northwestern Mediterranean area, specifically from an Italian coastal region.

76 The study area is a highly productive coastal region characterized by remarkable commercial
77 maritime traffic and several industrial plants. Moreover, in the last twenty years, due to its peculiar
78 geomorphology, the area has been the target of important anthropogenic impacts, such as the
79 discharges of Leghorn harbor mud (1,873,000 m^3) contaminated by Cu, Zn and Pb (ARPAT, 2006).
80 Up until the 1990s, the chloralkali plant of Solvay (Rosignano) built in 1918, discharged about 8000
81 m^3 h^{-1} of industrial effluents directly into the sea (Balestri *et al*., 2004). Contamination by Hg has
82 been reported in areas close to this plant (Balestri *et al*., 2004; Lafabrie *et al*., 2007a, b). The Arno
83 River drains a wide inland area, transporting Al, Fe, Hg and other trace elements at high
84 concentrations towards the sea (Cortecci *et al*., 2009). In addition, three coastal towns (Pisa,
85 Leghorn and Cecina), discharge partially treated effluent into the rivers (Renzi *et al*., 2009).
86 Furthermore, this coastal area also experiences summer tourism, which leads to a substantial
87 increase in inhabitants. Therefore, municipal wastewater treatment plants show effluents
88 characterized by worsened water quality and an increase in the nutrient concentration of marine
89 water (Renzi *et al*., 2009).
90 The main objectives of the present study were:
91 i) to analyze the concentrations of aluminum (Al), antimony (Sb), arsenic (As), beryllium
92 (Be), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), iron (Fe), manganese
93 (Mn), mercury (Hg), molybdenum (Mo), lanthanum nickel (Ni), lead (Pb), copper (Cu),
94 selenium (Se), tin (Sn), thallium (Tl), vanadium (V) and zinc (Zn) in zooplankton
95 samples collected in all four seasons at three different depths.
96 ii) to verify if this marine ecosystem, which was heavily influenced in the past by
97 anthropogenic activities, is still compromised by the presence of high concentrations of
98 metals
99 iii) to evaluate the relevance of zooplankton (and in particular of copepods) as a metal bio-
100 indicator.

2. Materials and methods

2.1. Sampling area

The sampling area was located offshore from the Italian coast, at the border between the Northern Tyrrhenian Sea and the Ligurian Sea (Figure 1). Station 1 (43°29'40" N – 10°01'45" E), Station 2 (43°28'10" N, 10°01'55" E) and Station 3 (43°27'10" N, 10°03'00" E) were located at 12.5 nautical miles off the coast, over the continental shelf towards the strongly impacted neritic zone close to Solvay-Rosignano. The study area was in fact in the Ligurian Sea, within the "Cetacean Sanctuary" where the number of cetaceans is at least twice as high as anywhere else in the Mediterranean (Ambrose, 1999). The Ligurian sea is situated at the north east border of the Western Mediterranean and is connected to the southern basin (Tyrrhenian Sea) across the Corsica Channel. The major large-scale feature of the water dynamic of the Ligurian Sea is a cyclonic circulation that is active all year round, but more intense in winter than in summer, involving both deep and surface layers (Aliani *et al*., 2003). Climatic forcing can greatly change the intensity of fluxes, but the general pattern can be considered permanent (Molinero *et al*., 2005a, b). Southern waters flowing towards the Ligurian sea occur by means of two main currents running along each side of Northern Corsica. The West Corsica Current (WCC) runs along the western side of Corsica while the warm and salty Tyrrhenian current (TC) goes through the Corsica Channel (Artale *et al.,* 1994) (Figure S1). The two waters merge at the north of Corsica and flow together along the Ligurian coast towards the Gulf of Lions. The Tyrrhenian current permits warm species from the south to reach the Ligurian basin, passing through the Corsica Channel. The investigated sector is characterized by the large extension of the continental shelf and limited depth (100 m), even at remarkable distances from the coast (18 miles) (Chiocci and La Monica, 1996).

2.2. Sample collection

125 Zooplankton samples were collected during four expeditions in May, August, and November 2014
126 and February 2015. The same neritic areas were investigated across all seasons. The three sampling
127 stations were aligned along a transect parallel to the coast (12.5 NM offshore), as shown in Figure
128 1. Stations 1 – 3 (Figure 1) were located on the continental shelf above bottom depths ranging from
129 109 to 114 m. The entire water column was sampled by three hauls: one surface haul, and two
130 vertical hauls (5 – 50 m, 50 – 100 m depth, respectively); zooplankton samples were taken with a
131 WP-2 standard net, with a mesh size of 300 μm and a diameter of 60 cm. The horizontal sampling
132 time was approximately 15 min at a vessel cruising speed of 2 knots, while during the vertical
133 sampling the net was hauled at 0.7 m s^{-1}.
134 Each net was fitted with a flow meter (KC Denmark model 23.090) to measure the volume of water
135 filtered, which ranged from 14 to 422 m^3. The net hauls were consistently carried out at night to
136 minimize variability due to vertical migration. One whole sample of each net was divided into two
137 aliquots using a Folsom splitter immediately after sampling; one aliquot was used for estimating
138 biomass and analyzing zooplankton composition, and the second aliquot was immediately frozen at
139 -20 °C onboard for subsequent analysis of for trace element concentrations (Fang *et al*., 2014;
140 Fernandez de Puelles *et al*., 2014). Samples used for estimating biomass (mg of dry weight m^{-3})
141 were collected on pre-weighed glass fiber filters and heated at 60 °C for 24-36 h (Lovegrove, 1966).
142 Samples for composition analysis were fixed in 4% neutralized formaldehyde buffered with borax
143 and kept in the dark (Boltovskoy, 1981). Subsamples of mesozooplankton were obtained using a
144 Folsom Plankton Splitter, and at least two subsamples were counted completely to determine the
145 abundance, presented as ind. m^{-3}, of the main zooplankton groups (Boltovskoy, 1981). Considering
146 the quail-quantitative importance of copepods, the adults were identified to species level and
147 juvenile stages to genus level, wherever possible (Vives and Shmeleva, 2007, 2010). In addition to
148 the entire Mediterranean Sea, in the Ligurian and Tyrrhenian Seas, the bulk of copepod populations
149 are concentrated in the epipelagic layer, up to a depth of 100 m, with abundances decreasing sharply

thereafter (Mazzocchi *et al.*, 2007). Moreover, some aspects of biodiversity were evaluated by using the Shannon-Wiener index (H') (Shannon and Weaver, 1963), calculated on abundance data of adult copepods at the four sampling seasons.

Seawater samples for total dissolved trace metal analysis were collected at depths of 1, 50 and 100 m using 5 L Niskin bottles and stored in a cool box until subjected to filtration. All samples were kept refrigerated before analysis.

2.3. Detection of trace elements

2.3.1 Zooplankton

The zooplankton samples were placed in a small nylon sieve and thoroughly rinsed with Milli-Q water to remove salts. Samples were divided into two sub-samples, one for Hg quantification with a Direct Mercury Analyzer (DMA-80 Analyzer from Milestone, Shelton, CT, USA) and the other one for detecting all the other metals by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS Xseries II, Thermo Scientific, Bremen, Germany). The Direct Mercury Analyzer performs thermal decomposition, catalytic reduction, amalgamation, desorption and atomic absorption spectroscopy without having to pre-treat the samples. Between 0.05 g and 0.1 g of samples were directly weighed on graphite shuttles and processed for Hg content.

Determination of Al, Sb, As, Be, Cd, Ce, Co, Cr, Cu, Fe, Mn, Mo, La, Pb, Ni, Se, Sn, Tl, V and Zn, was performed after wet digestion using acids and oxidants (HNO_3 and H_2O_2) of the highest quality grade (Suprapure). In this case, between 1.5 and 2.0 g of samples were subjected to microwave digestion (microwave oven ETHOS 1 from Milestone, Shelton, CT, USA) with 7 mL of HNO_3 (70% v/v) and 1.5 mL of H_2O_2 (30% v/v). Ultrapure water was added to samples to reach a final weight of 50 g (Arium611VF system from Sartorius Stedim Italy S.p.A., Antella - Bagno a Ripoli, FI, Italy). Multi-elemental determination was performed by ICP-MS after daily optimization of instrumental parameters and using an external standard calibration curve; Rhodium and Germanium were used as internal standards. Analytical performances were verified by processing Certified

175 Reference Materials (Oyster Tissue -SRM 1566b from the National Institute of Standard and
176 Technology), along with blank reagents in each analytical session. The limit of quantification
177 (LOQ) for each element, the reference material values and the percentages of recovery obtained are
178 shown in Table S1.

179 *2.3.2 Seawaters*

180 The direct introduction of seawater to a Mass Spectrometer can cause a number of serious
181 problems. The high content of dissolved solids (approximately 3.5%) in seawater can suppress
182 analyte signals due to inter-element interferences, and clog the interface sampler and skimmer
183 cones. Therefore, a chelating polymer resin, the SPR-IDA Reagent (Suspended Particulate Reagent
184 – Iminodiacetate, by Cetac Technologies, Omaha, USA) was used for pre concentration/ matrix
185 elimination of seawater. The reagent consists of 10 micron-diameter polymer beads derivatized with
186 the chelating agent iminodiacetate. A 15 mL sample of seawater was directly added to a pre-cleaned
187 15 mL volume polypropylene centrifuge tube. A 100 µL aliquot of a 10% suspension of SPR-IDA
188 reagent beads was then pipetted directly into the sample. Tubes were covered with parafilm and the
189 contents were mixed thoroughly. Samples were then spiked with 0.5 µg/L yttrium, which functions
190 as an internal standard, helping to correct for any volume differences in the blanks, samples, and
191 spiked samples. High-purity ammonium hydroxide (NH_4OH, 29%) was added in two steps (25 µL +
192 20 µL) to adjust the pH to approximately 8. The SPR-IDA beads were then allowed to settle for
193 approximately 1 hour. Samples were then placed in a centrifuge and spun at 2000 rpm for 10 min.
194 The supernatant liquid was then carefully poured off to minimize any loss of beads. The beads were
195 mostly compacted at the bottom of the tube. A solution of deionized water, adjusted to pH 8 with
196 high purity NH_4OH, was then added to the 15 mL mark of the sample tube and the contents were
197 mixed. The beads were again allowed to settle, centrifuged, and the resulting supernatant liquid
198 carefully poured off and discarded. A 0.5 mL aliquot of 7% v/v absolute high-purity nitric acid

199 (Suprapure) was then added to the bead residue to extract any bound metal ions. The extract was
200 then diluted to 3 mL with deionized water and analyzed by ICP-MS. The following metals were
201 then quantified in seawater: Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn.

202 *2.4 Bioaccumulation factors (BAFs*)

203 The bioaccumulation factor (BAF) is the ratio of a chemical concentration in an organism to the
204 concentration in water. BAFs were estimated for Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn, the same
205 elements that were quantified both in seawaters and in plankton.

206 All metal concentrations in seawater are reported in μg L^{-1}, while in zooplankton, the values are
207 expressed in mg kg^{-1}. For estimation of BAFs, the metal levels in zooplankton were converted in μg
208 kg^{-1}.

209 2.5 *Merging data from the different stations*

210 Data were tested for normality and equality of variance. The mean zooplankton abundance and
211 biomass from each sampling station at the same season were compared using the *Kruskal-Wallis*
212 *test*, to determine if it was acceptable to combine these datasets. Statistical analyses were carried out
213 using the R software, Version 3.1.2 (R Core Team, 2014), $p < 0.05$ was considered as statistically
214 significant.

215 There were no significant differences between the three stations for each season (*Kruskal-Wallis* X^2
216 $= 0.024$, d$f = 2$, $p = 0.988$). Based on these estimates, it was decided that it was acceptable to merge
217 the datasets from the three sampling stations (Figure 1), and thereby enable concise and
218 comprehensible representations of the results.

219 **3. Results and discussion**

220 *3.1. Zooplankton communities*

221 The zooplankton communities of the three sampling sites (Figure 1) showed very comparable
222 compositions, so we presented their mean values (Figure 2) to facilitate data discussion.

223 The analyzed zooplankton presented the highest biomass value in winter (mean values over areas
224 19.1 mg m^{-3}), followed by autumn (16.2 mg m^{-3}), spring (4.6 mg m^{-3}) and summer (1.07 mg m^{-3}).
225 Differences between seasons were evident for the most abundant groups of mesozooplankton, with
226 copepods being the most abundant group (69% in spring, 56% in summer, 77% in autumn and 82%,
227 in winter) (Figure 3), being indicative of the general pattern of zooplankton annual distribution. In
228 addition, 14 zooplankton taxa were found but only 8 (>1%) were considered to be important in the
229 mesozooplankton community. Cladocerans followed copepods in abundance (7% and 14%, in
230 spring and summer, respectively). This group, along with thaliaceans showed similar abundances
231 during both seasons. Misydacea/Euphausiacea were more abundant in autumn and winter (6% and
232 9%, respectively) than in spring and summer, when they decreased drastically (to just 1%). Instead,
233 the chaetognaths showed the highest values in autumn and spring (5% and 4%, respectively). Other
234 groups, such as pteropods (5%) and siphonophores (4%) were more abundant in summer than in the
235 cold seasons. Finally, meroplankton larvae, mainly represented by gastropoda and decapod larvae,
236 were relatively abundant, but only during the summer (3%).

237 *3.2. Abundance and diversity of copepods*

238 More than 130 copepod species belonging to 29 families and 52 genera were recorded during the
239 four surveys. The greatest number of species was found in spring (66), whereas the lowest number
240 (50) was found in autumn. Richness species (d) and species diversity (H') indices (Figure 3a, b)
241 showed similar spatial trends throughout the study area, with the exception of autumn, where d
242 reaches the minimum value. The seasonal trend of diversity, calculated on the integrated water
243 column at a depth of 5 – 100 m, showed the lowest Shannon-Wiener index values (range: 1.93-
244 2.59) in February, corresponding to the maximum of abundance, and the highest values in summer
245 (range: 3.056-3.063). The highest richness species was recorded during the summer (range: 8.098-
246 8.514), in which there was the lowest copepod abundance (range: 6.319-7.319).

247 *Clausocalanus*, *Oithona* and *Acartia* were the most common genera found throughout the year
248 (48%, 20%, and 13%, respectively). The abundance of copepods found at all stations during the
249 February expedition ranged from 614 to 2361 ind./m^3, and was significantly higher than during
250 November (141.54 and 694.56 ind./m^3)
251 In spring 2014, the genera *Clausocalanus* (mainly *Clausocalanus pergens* and *Clausocalanus*
252 *arcuicornis*) and *Centropages typicus* clearly dominated the biomass of the copepods in the water
253 off the Tuscan coast. Indeed, in the superficial hauls the percentages of *Clausocalanus* spp. and *C.*
254 *typicus* were 32.96% and 30.21%, respectively. Moreover, *Farranula rostrata*, rarely found in the 5
255 – 50 m and 50 –100 m layers, were abundant on the surface (14.51%). The dominant cladoceran
256 species was *Evadne spinifera* (118.04 ind.m^{-3} ± 41.8).
257 During summer 2014, throughout the whole water column, the maximum distribution of the
258 copepods *Clausocalanus arcuicornis*, *Clausocalanus furcatus*, *Temora stylifera* and *Nannocalanus*
259 *minor* was 61.77%, with a peak of 78.05% in the 50 – 100 m layer. *Penilia avirostris* (205.8 ind. m^{-3}
260 ± 76.2) dominated among the cladocerans, followed by *Pseudoevadne tergestina* and *Evadne*
261 *spinifera*.
262 *Clausocalanus paululus*, *Clausocalanus furcatus*, *Calocalanus styliremis* and *Oithona plumifera*
263 were the most common species in autumn 2014, constituting 46% of the copepod biomass (with a
264 peak abundance of 71.58% in the 5 – 50 m layer).
265 In winter 2015, when the maximum abundance of copepods was observed, copepods such as
266 *Acartia negligens*, *Clausocalanus lividus*, *Clausocalanus mastigophorus* and *Oithona decipiens*
267 were predominant (39.24%), although some other larger copepods, such as *Centropages typicus*,
268 *Calanus helgolandicus* and *Temora stylifera* were also highly abundant, particularly in the 5 – 50 m
269 layer.
270 During all seasons the dominant copepods species are herbivorous.
271 *3.2 Trace elements in seawater*

272 The three sampling sites (Figure 1) showed very comparable concentrations, so we presented their
273 mean values (Table 1) to facilitate data discussion.
274 The temporal and spatial distributions of dissolved Mn, Fe, Cu, Zn, Al, Ni Co, Cd and Pb are shown
275 in Figure 4. In surface water, the average metal concentrations were in the following order:
276 Zn>Ni>Fe>Al>Pb>Co>Mn>Cu>Cd, while at 50 m of water depth, the order was
277 Ni>Zn>Fe>Al>Pb>Co> Cu>Mn> Cd and at 100 m water depth, the order was Ni> Zn> Al>Pb>
278 Fe>Co>Mn> Cu>Cd.
279 Cd concentrations ranged from 0.02 to 0.04 μg L^{-1} and did not show significant variations between
280 seasons or water depths. Pb was in the range of 0.16 to 2.33 μg L^{-1} with slight variations between
281 seasons (the highest values in autumn) and water depths. Zn, Ni and Fe were the nutrient trace
282 elements with the highest concentrations in seawater, and their levels seemed to be strongly
283 influenced by the seasons. In fact, the highest levels of Zn and Ni were recorded in summer, both in
284 surficial water and at 50 m water depth, while the highest concentration of Fe was in winter. Cu
285 concentrations ranged from 0.22 to 1.54 μg L^{-1} and did not show variations between water depths
286 (Table 1); the highest Cu concentration was registered in summer (Figure 4). Co concentrations
287 ranged from 0.02 to 1.65 μg L^{-1} and Mn from 0.35 to 1.84 μg L^{-1}, both elements had slight
288 variations between water depths and seasons (Figure 4, Table 1). Al concentration was relatively
289 low (1.50-1.80 μg L^{-1}) and relatively constant in the analyzed waters, in line with previous findings
290 in the Mediterranean Sea (Caschetto and Wollast, 1979).
291 Trace metals in coastal waters are usually higher than concentrations in the open ocean, owing to
292 metal inputs from continental sources, such as ground water and coastal sediments (Sunda, 2012). In
293 the Mediterranean Sea, nutrient-like metals usually exhibit surface concentrations higher than those
294 of the Pacific and Atlantic Oceans and their vertical profiles are more or less homogenous, differing
295 from the distribution observed in the open ocean, where there are depleted surface layers and
296 increasing concentrations with depth (Voutsinou-Taliadouri *et al*., 2000). This peculiarity is due to

297 limited recycling of nutrients in this semi-closed sea and to trace metal-enriched source waters
298 derived from rivers. The concentrations we detected in seawater were comparable or lower than
299 those recently detected in the Mediterranean Sea (e.g. Safaa, 2015; Ebling and Landing, 2015).
300 However, measuring metal concentrations in water does not provide information on the risks posed
301 by metal bioaccumulation or biomagnification (Ricart *et al*., 2010; Maceda-Veiga *et al*., 2013).

302 *3.2 Trace elements in zooplankton, nonessential elements*

303 *3.2.1 Cadmium and lead*

304 In marine zooplankton, Cd and Pb levels ranged from 0.05 to 0.41 mg kg^{-1} and from 1.01 to 12.38
305 mg kg^{-1}, respectively (Figure 5, Table 2). It is well known that the distribution of Cd in seawater is
306 regulated by marine biogeochemical processes, such as uptake by phytoplankton in surface waters,
307 consequential decomposition of organic matter produced, and demineralization in deep waters
308 (Paimpillil *et al*., 2010). Since Cd has no significant physiological role, it is probably adsorbed on
309 the surface of zooplanktonic debris or fecal pellets during its transportation to bottom waters
310 (Kremling and Pohl, 1989). Pb is known to form colloids in seawater that could be adsorbed onto
311 planktonic debris (Paimpillil *et al*., 2010). Pb concentrations in zooplankton increased with
312 increasing water depths, while Cd appeared to be uninfluenced by this parameter (Table 2). In
313 comparison with other findings in other marine environments of the world, such as Taiwan, India,
314 and Argentina, (Fang *et al*., 2006; Pempkoviak *et al*., 2006; Fernandez-Severini *et al*., 2013) Cd
315 concentrations were lower, but of the same magnitude as those reported in the Seine estuary
316 (France) by Miramand and coauthors (2001). Pb levels registered in zooplankton were comparable
317 or lower with previous findings in Mediterranean coastal areas (Rossi and Jamet, 2008). The
318 highest Pb concentrations in zooplankton were registered in autumn, at all the three water depths
319 considered (Table 2).

320 *3.2.2 Aluminum and vanadium*

To our knowledge, these two nonessential elements have not been previously investigated in zooplankton from the Mediterranean Sea. Despite some reports of a role of Al in physiological processes, there is no clear evidence that it plays an essential function in organisms (Pérez-Granados and Vaquero 2002). In surface seawater, Al remains for just 4 weeks to 4 years (Orians and Bruland, 1986). Processes removing Al from seawater include passive scavenging into particles (Vink and Measures, 2001), and biological absorption is the predominant mechanism of biological scavenging of dissolved Al (Li *et al*., 2013). Al levels in zooplankton ranged from 62.30 to 1738.23 mg kg^{-1} and increased with increasing water depths, with the highest Al concentrations registered in spring (Figure 5, Table 2).

Vanadium enters the aquatic system through multiple routes, including release of flying ash and through run-off from naturally V-rich soils, irrigated areas and industrial plants (Mackey *et al*., 1996). V concentrations were low in zooplankton, ranging from 0.25 to 1.73 mg kg^{-1}, and as for Al, concentrations increased with water depth (Table 2).

3.2.3 Antimony, beryllium, tin

Antimony is found at very low levels throughout the environment (ATSDR, 1992), usually < 1 mg kg^{-1} in soil and water. Data on Sb concentrations in marine environments are very scarce, but in marine plants, especially algae, results are generally in the range of 0.1 to 0.2 mg kg^{-1} (Filella *et al*., 2007). In zooplankton, we found Sb concentrations ranging from 0.02 to 0.59 mg kg^{-1}, with the lowest levels in winter and the highest in spring (Table 2).

Beryllium occurs in rocks and minerals at concentrations ranging from 0.038 to 11.4 mg kg^{-1} (Drury *et al.,* 1978). In most natural waters, the majority of Be is adsorbed to suspended matter or in the sediment, rather than dissolved. Be is not significantly bioconcentrated from water by aquatic species (US EPA, 1980). According to the environmental low levels, Be was under the LOQ in

344 marine zooplankton collected in surficial water and at 50 m depth, while detectable levels were
345 found at 100 m depth (0.02 to 0.04 mg kg^{-1}).
346 Tin and its compounds are significant and controversial chemicals in the environment, and are
347 generally considered as being relatively immobile in food chains. Coastal regions are hot spots for
348 environmental contamination by organotin compounds due to their proximity to harbor areas, as
349 well as to industrial and domestic points of effluent discharge (WHO, 2005). Sn concentrations
350 were found to be low in the study area, but always detectable in zooplankton, ranging from 0.05 to
351 0.42 mg kg^{-1}, with the lowest levels in surficial samples and in the summer (Figure 6, Table 2). We
352 were unable to find any literature on Sb, V and Sn in zooplankton, but the detected levels can be
353 considered low and of no concern.
354 Moreover, the nonessential elements Hg and Tl and the rare earth elements La and Ce were below
355 the LOQ (0.037 mg kg^{-1}, Hg; 0.010 mg kg^{-1}, La, Ce and Tl) of the analytical methods.
356 *3.3 Trace elements in zooplankton, essential elements*
357 *3.3.1 Copper, manganese, iron and zinc*
358 Some marine organisms are known to concentrate significant amounts of copper in seawater, such
359 as crustaceans that require this element as a component of enzymes and haemocyanin (Paimpillil *et*
360 *al*., 2010). Zooplankton, and especially copepods, do not express haemocyanin, and the requirement
361 of Cu is thus restricted to enzyme activity, which are important for life-history processes, such as
362 egg production and growth (Paimpillil *et al*., 2010). Cu levels in the analyzed zooplankton ranged
363 from 1.90 to 39.70 mg kg^{-1} (Figure 6, Table 2), the highest values were registered in autumn, and
364 increased with water depth. These levels were in accordance with those registered from other
365 marine environments of the world in areas of low anthropogenic impact (Fernandèz-Severini *et al*.,
366 2013).
367 Manganese is a naturally occurring metal in seawater and can be significantly bioconcentrated by
368 aquatic biota at lower trophic levels (WHO, 2004). In zooplankton samples, this element ranged

369 from 1.22 to 29.85 mg kg^{-1} and like Cu, the highest values were registered in autumn, and were
370 influenced by water depth (Figure 6, Table 2). The values were one order of magnitude lower than
371 those recorded in more contaminated marine areas, e.g. in the Bay of Bengal (Paimpillil *et al*.,
372 2010), in the northern cost of Taiwan (Hsiao *et al*., 2011), and in the Gulf of Gdansk (Fialkowski *et*
373 *al*., 2003).
374 Iron is an essential trace element for the biological requirements of marine plankton, and was the
375 element with the highest concentration of the essential elements analyzed. In fact, Fe levels reached
376 levels of 354.42 mg kg^{-1} in surficial water and the Fe concentration was strongly influenced by
377 water depth (Figure 6), reaching levels of 1741.76 mg kg^{-1} at 100 m of depth.
378 These three essential elements- copper, manganese, and iron- showed nutrient-like vertical
379 distributions, being depleted in surface waters due to uptake by the biota and increased in
380 concentration with increasing depths as a result of the remineralization of sinking organic matter.
381 Zinc is another essential element, and in tissues of aquatic organisms, Zn levels usually greatly
382 exceed those required for normal metabolism; much of the excess is bound to macromolecules or
383 present as insoluble metal inclusions in tissues (Eisler, 1993). The zinc levels in zooplankton
384 reflected the occurrence of zinc in marine environment and in fact, this element is concentrated
385 more effectively than copper. High accumulation of Zn in zooplankton may be due to its co-
386 precipitation with calcium carbonate (Paimpillil *et al*., 2010). Diet is the most significant source of
387 Zn to aquatic organisms and is significantly more important than Zn uptake from seawater (Eisler,
388 1993). The concentration of Zn in zooplankton was in the range of 14.47 to 132.33 mg kg^{-1}, much
389 lower than values recorded in contaminated coastal areas (Paimpillil *et al*., 2010).
390 *3.3.2 Arsenic, chromium and selenium*
391 Arsenic is a metalloid which can be toxic in its inorganic form, and innocuous in some organic
392 forms such as arsenobetaine. Studies have suggested that inorganic arsenic is an essential dietary
393 nutrient; although its physiological role has not been clearly defined, it has been suggested that

394 arsenic could play a role in methionine metabolism (Uthus, 2003). Water contains mainly inorganic
395 As, and aquatic organisms accumulate, retain, and transform arsenic species inside their bodies
396 when exposed to it through their diet and other sources such as water, soil, particles (Sharma and
397 Sohn, 2009). As biomagnification is inconsistent, and total As accumulation usually decreased by
398 one order of magnitude with each trophic level (Maher et al., 2011). Our findings have shown that
399 As levels in zooplankton were low, considering that concentrations reported were usually between
400 0.2 to 24.4 mg kg^{-1} (Rahman *et al.*, 2012) and we found values from 0.14 to 0.78 mg kg^{-1}.

401 In the open sea, chromium is involved in biogeochemical cycles, with biologically mediated Cr
402 removal in the surface layers and elevated Cr levels in deeper waters because of mobilization of Cr
403 upon breakdown of sinking biogenic particles (Campbell and Yeats, 1981). Published data for Cr
404 content in zooplankton are very scarce, but the concentrations we found between 0.39 to 7.25 mg
405 kg^{-1} (the highest values in autumn) were much lower than those recorded for Cr in polluted marine
406 ecosystems, such as the northern cost of Taiwan (Hsiao *et al*., 2011) and west coast of India
407 (Kadam *et al*., 2015).

408 Selenium has been an element of concern in aquatic environments for many years due to its
409 potential toxicity (Purkerson *et al*., 2003). The lower trophic levels, such as phytoplankton and
410 bacteria bioaccumulate dissolved Se, and higher trophic levels such as zooplankton and fish
411 accumulate Se through ingestion (Kehrig *et al*. 2009). It has been reported that little or no
412 bioaccumulation of Se occurs along a marine food chain from algae to herbivores and carnivores
413 (Schneider *et al*., 2015). We found Se concentrations ranging from 0.12 to 0.52 mg kg^{-1} in
414 zooplankton, levels which were not influenced by seasons or water depth (Figure 6, Table 2). These
415 concentrations were one order of magnitude lower than those detected in other marine areas
416 (Purkerson *et al*., 2003).

417 *3.3.3 Cobalt, molybdenum and nickel*

418 Cobalt is a particle-reactive metal and is scavenged by suspended particulate matter in coastal areas
419 (Paimpillil *et al*., 2010). We found Co concentrations ranging from 0.06 to 0.38 mg kg^{-1}, with the
420 highest levels recorded in autumn.
421 The element molybdenum is found in all living organisms and is considered an essential or
422 beneficial micronutrient. Concentrations < 2.00 mg kg^{-1} were recorded in marine zooplankton
423 (Eisler, 1989). In the analyzed samples, Mo content was very low and ranged from 0.06 to 0.32 mg
424 kg^{-1}. Similarly to Co, the highest values of Mo were registered in autumn.
425 Nickel is a required nutritionally for many eukaryotic and prokaryotic organisms and occurs in
426 aquatic systems as soluble salts adsorbed on clay particles or organic matter (detritus, algae,
427 bacteria), or associated with organic particles, such as humic and fulvic acids and proteins
428 (Thomson, 1982). Absorption processes may be reversed leading to the release of nickel from the
429 sediment; there is no biomagnification of Ni along the food chain, at least in aquatic ecosystems,
430 (WHO 1991). In the analyzed samples, Ni ranged from 0.85 to 5.48 mg kg^{-1} (Table 2),
431 corresponding to the mean of 4 mg kg^{-1} found in marine compartments (Eisler, 1988).
432 Aquatic organisms have developed different strategies to deal with metals; the tissue concentrations
433 are more or less under homeostatic control for essential elements such as copper or zinc, whereas
434 the nonessential do not appear to be regulated very strongly (e.g., cadmium or lead). The role of
435 essential trace metals, such as iron, copper and zinc in the growth of marine zooplankton still need
436 to be deeply investigated, but being essential for life, the most common types of plankton contained
437 a regular number of trace metals in their chemical make-up, such as zinc, iron, and copper
438 (Paimpillil *et al*., 2010). Trace element concentrations in marine zooplankton are in a state of
439 dynamic equilibrium, depending on both uptake and elimination processes occurring
440 simultaneously. In fact, regulation occur by matching metal excretion to metal uptake so that the
441 internal body concentration of the metal remains constant, or by storing part of the metal in a

442 physiologically inactive pool. Marine organisms employ both strategies and in many cases effective
443 regulation and/or detoxification is achieved by combining the two methods (CIESM, 2002).

444 Earlier investigations on the vertical transport of trace metals by zooplankton suggested that greater
445 availability of food in the surface layers resulted in a more rapid breakdown of zooplankton, and
446 thus less time was available for adsorption of metals from the water by the exoskeleton (Martin,
447 1970). It was suggested that more of these elements were adsorbed to copepod exoskeletons at
448 greater depths because food-dependent molting rates were lower and more time was available for
449 elemental adsorption to take place (Martin, 1970). Consequently, the adsorption of dissolved metals
450 to copepod exoskeletons constitutes an important pathway of trace metal accumulation in deep
451 seawaters (Martin 1970; Sick and Baptist 1979). In our study, increases in the concentrations of
452 trace elements, such as Al, Pb, Cu, Fe, Mn were found in the samples from greater depths,
453 according to the previous findings of Martin (1970).

454 *3.3.4 Bioaccumulation factor* (*BAF)*

455 Bioaccumulation of naturally occurring substances occurs along a continuum of exposure, and trace
456 amounts of metals, both essential and nonessential, can be found in all biota (Mc Geer *et al.*, 2003)
457 The bioaccumulation factor (BAF) is the ratio of a chemical concentration in an organism to the
458 concentration in water; the concentration in the aquatic organism results from all possible routes of
459 exposure, such as dietary absorption and transport across the respiratory surface (Gobas and
460 Morrison, 2000). The metals considered in our study for estimation of BAFs were Al, Cd, Co, Cu,
461 Fe, Mn, Ni, Pb, and Zn (Table 3). Given the wide range of concentrations considered in the BAF
462 dataset, values were converted to log scale to aid visual comparisons (Figure 7).

463

464 The data shown in Table 3 and Figure 7 confirm the high potential of the zooplankton as
465 bioaccumulators of metals. The average BAF in zooplankton from surficial waters are in the
466 following order Fe> Cd> Cu>Zn>Co>Mn>Ni>Pb>Al. At increasing water depths, an increase in

467 BAFs for the essential elements Fe, Cu, Zn, and Mn was observed, while BAFs were fairly constant
468 for Ni and Co. The three nonessential elements Al, Pb and Cd showed a different trend in
469 bioaccumulation, as Al and Pb bioaccumulation increased with water depth while Cd BAF
470 decreased (Figure S2).

471 Bioaccumulation of trace elements in zooplankton could be due to low rates of efflux and relatively
472 high rates of assimilation efficiency, as the zooplankton may be slow to metabolize or excrete the
473 metals, the elements may bioaccumulate within the organism (Fernandéz-Severini *et al.*, 2013).
474 Many aquatic invertebrates show accumulation patterns for most trace metals that include some
475 storage of accumulated metal in its detoxified form somewhere in the body (Rainbow, 2002). Trace
476 elements typically have an affinity for sulphur and nitrogen (Nieboer and Richardson, 1980), and
477 proteins are made up of amino acids, most of which contain sulphur and/or nitrogen, and there are
478 therefore numerous potential binding sites for trace metals within cells (Rainbow, 1997). It should
479 be considered that toxicity is related to a threshold concentration of metabolically-available metal
480 and not to the total metal concentration in an organism.

481 Even if the BAFs for most of the analyzed elements reveal the high potential of marine zooplankton
482 as a bioaccumulator, many processes are involved in the bioaccumulation of metals in zooplankton,
483 such as bioavailability, the amount of dissolved metal uptake, the physiological efficiency of the
484 organism to excrete metals, as well as on the feeding rate and prey availability (Rainbow, 1997).

485 **Conclusions**

486 The study area which, a decade ago, was heavily influenced by metals derived from industrial
487 activities does not presently show metal levels of particular concern for the aquatic ecosystem,
488 probably due to the reduced anthropogenic inflows and to an improvement of industrial wastewater
489 treatments. Mesozooplankton biomass, species composition and biodiversity values measured in the
490 present study are similar to previous reports in areas with a negligible metal pollution and

anthropogenic impact, for example, in the Mallorca channel (Fernández de Puelles *et al*., 2003, 2007, 2014), the Tyrrhenian Sea (Brugnano *et al*., 2012), the Ligurian Sea (Razouls and Kouwenberg, 1993) or in the Sicily channel (Brugnano *et al*., 2010). However, the most abundant copepods, such as *Clausocalanus* and *Oithona*, showed a widespread distribution in the Mediterranean sea, which may indicate a tolerant characteristic within different hydrographic conditions (Gaudy, 1985) and their ability to adapt to fluctuating environmental states (Mazzocchi and Ribera d'Alcala, 1995). In seawater, the trace element concentrations were comparable or lower than those recently detected in the Mediterranean Sea, while the examined zooplankton showed a great ability to accumulate concentrations of metals by several thousand times, compared to concentrations detected in marine water, in particular the essential elements Fe, Cu, Zn, Co and Mn and the nonessential element Cd. No unusual patterns in the taxonomic composition and abundance of the zooplankton were found, but the abundance of the zooplankton taxa varied according to the season of the year, and as such the metal concentrations in these organisms were related to the seasons. In fact, for many trace elements (Al, As, Cr, Cu, Fe, Mn, Pb, Ni, V and Zn) the highest metal concentrations were detected in winter and autumn, the seasons with the highest biomass values, with a predominance of copepods.

Monitoring programs are mainly designed to discern spatial and temporal patterns in contaminant concentrations in the environment, and bio indicators are organisms that can be used to provide information on the variation of pollutants over time and space. Accumulated metal concentrations in zooplankton provide a great deal of information of applied relevance in terms of the geographical and temporal variation in the bioavailabilities of essential and nonessential trace elements in the environment. Due to its wide occurrence, abundant species and sensitive responses, zooplankton has shown to play a key role as a highly suitable candidate bio indicator in the bio monitoring of metal in marine ecosystem.

515 This study has provided a novel insight into metal distribution in zooplankton, which are the main
516 food constituents of most fish of the coastal waters, and thus play an important role in the transfer
517 of metals through the food chain.

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524
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527

528 **References**

529 Agency for Toxic Substances and Disease Registry (ATSDR), 1992. Toxicological Profile for
530 Antimony and compounds. U.S. Public Health Service, U.S. Department of Health and Human
531 Services, Atlanta, GA.

532 ARPAT, 2006. Progetto Interregionale IIIA Sardegna-Corsica-Toscana Moniqua: monitoraggio
533 della qualità delle acque e dell'ambiente marino. pp.1-133.
534
535 Aliani, S., Griffa, A., Molcard, A., 2003. Floating debris in the Ligurian Sea, northwestern
536 Mediterranean. Mar. Pollut. Bull. 46, 1142-1149.

537 Ambrose, P., 1999. Cetacean sanctuary in the Mediterranean Sea. Mar. Pollut. Bull. 38 (9), 748.

538 Artale, V., Astraldi, M., Buffoni, G., Gasparini, G.P., 1994. Seasonal variability of the gyre-scale
539 circulation in the Northern Tyrrhenian Sea. J. Geophys. Res. 99 (C7), 14127-14137.

540 Balestri, E., Benedetti-Cecchi, L., Lardicci, L., 2004. Variability in patterns of growth and
541 morphology of *Posidonia oceanica* exposed to urban and industrial wastes: contrasts with two
542 reference locations. J. Exp. Mar. Biol. Ecol. 308: 1-21.

543 Barka, S., Pavillon, J. F., Amiard, J. C., 2001. Influence of different essential and non-essential
544 metals on MTLP levels in the copepod *Tigriopus brevicornis*. Comp. Biochem. Physiol. 128C,
545 479–493.
546
547 Boltovskoy, D., 1981. Atlas del Atlàntico sudoccidental y métodos de trabajo con el zooplankton
548 marino. In: Boltovskoy, D. (Eds.), INIDEP, Argentina pp.1-935.
549

550 Brugnano, C., Bergamasco, A., Granata, A., Guglielmo, L., Zagami, G., 2010. Spatial distribution
551 and community structure of copepods in a central Mediterranean key region (Egadi Islands – Sicily
552 Channel). J. Mar. Syst. 81, 312-322.
553
554 Brugnano, C., Granata, A., Guglielmo, L., Zagami, G., 2012. Spring diel vertical distribution and
555 community structure of copepod abundance and diversity in the open Central Tyrrhenian Sea
556 (Western Mediterranean). J. Mar. Syst. 105-108, 207-220.
557
558 Campbell, J.A., Yeats, P.A., 1981. Dissolved chromium in the Northwest Atlantic Ocean. Earth
559 Planet. Sci. Lett. 53, 427-433.
560
561 Caschetto, S., Wollast, R., 1979. Vertical distribution of dissolved aluminium in the Mediterranean
562 Sea. Mar. Chem. 7(2), 141–155.

563 Chiocci, F. L., La Monica, G. B., 1996. Analisi sismostratigrafica della piattaforma continentale, In:
564 Il Mare del Lazio – Elementi di oceanografia fisica e chimica, biologia e geologia marina, clima
565 meteomarino, dinamica dei sedimenti ed apporti continentale. Regione Lazio. Tip. Borgia. Roma,
566 pp. 40-61.

567 CIESM, 2002. Metal and radionuclides bioaccumulation in marine organisms, In: CIESM
568 Workshop Monographs n°19, Monaco <www.ciesm.org/publications/Ancona02.pdf
569
570 Cortecci, G., Boschetti T., Dinelli, E., Cidu, R., Podda, F., Doveri, M., 2009. Geochemistry of trace
571 elements in surface waters of the Arno River basin, northern Tuscany, Italy. Appl. Geochem. 24,
572 1005-1022.
573
574 Drury, J.S., Shriner, C.R., Lewis, E.B., Towill, L.E., Hammons, A.S., 1978. Reviews of the
575 environmental effects of pollutants: VI. Beryllium. Prepared under IAG-D5-0403 by Oak Ridge
576 National Laboratory, Union Carbide Corp., Oak Ridge, TN (EPA 600/1-78-028; NTIS PB-290966).
577
578 Ebling, A.M., Landing, W.M., 2015. Sampling and analysis of the sea surface microlayer for
579 dissolved and particulate trace elements. Mar. Chem. *In press,*
580 http://dx.doi.org/10.1016/j.marchem.2015.03.012.
581
582 Eisler, R. 1988. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. In: Cont. Haz.
583 Rev., U.S. Department of the Interior, Fish and Wildlife Service (Eds.), Report 34, pp.1-95.
584
585 Eisler, R. 1989. Molybdenum hazards to fish, wildlife, and invertebrates: a synoptic review. In:
586 Cont. Haz. Rev., U.S. Department of the Interior, Fish and Wildlife Service (Eds.), Report 85, pp.
587 1-19.
588
589 Eisler, R. 1993. Zinc Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review, In: Cont.
590 Haz. Rev., U.S. Department of the Interior, Fish and Wildlife Service (Eds.), Report 26, pp. 1-126.
591
592 European Environment Agency (EEA), 2006. Priority issues in the Mediterranean environment.
593 EEA Report N°4, pp. 1-92.
594
595 Fang, T. H., Hwang, J. S., Hsiao, S. H., Chen, H. Y., 2006. Trace metals in seawater and copepods
596 in the ocean outfall area off the northern Taiwan coast. Mar. Env. Res. 61, 224–243.

Fang, T. H., Hsiao, S.H., Nan, F.H., 2014. Nineteen trace elements in marine copepods collected from the coastal waters off northeastern Taiwan. Cont. Shelf Res. 91, 70-81.

Fernández de Puelles, M. L., Gras, D., Hernández de León, S., 2003. Annual cycle of zooplankton. Biomass, abundance and species composition in the neritic areas of the Balearic Sea, Western Mediterranean. P.S.Z.N. Mar. Ecol. 24 (2), 123-129.

Fernández de Puelles, M. L., Alemany, F., Jansá, J., 2007. Zooplankton time-series in the Balearic Sea (Western Mediterranean): variability during the decade 1994-2003. Prog. Oceanogr. 74, 329-354.

Fernández de Puelles, M. L., Macias, V., Vicente, L., Molinero, J. C., 2014. Seasonal spatial pattern and community structure of zooplankton in waters off the Baleares archipelago (Central Western Mediterranean). J. Mar. Syst. 138, 82-94.

Fernández-Severini, M.D., Hoffmeyer, M.S., Marcovecchio, J. E., 2013. Heavy metals concentrations in zooplankton and suspended particulate matter in a southwestern Atlantic temperate estuary (Argentina). Env. Monit. Assess. 185(2), 1495-1513.

Fialkowski, W., Rainbow, P. S., Smith, B. D., Zmudzinski, L., 2003. Seasonal variation in trace metal concentrations in three talitrid amphipods from the Gulf of Gdansk, Poland. J. Exp. Mar. Biol. Ecol. 288, 81-93

Filella, M., Belzile, N., Lett, M.C., 2007. Antimony in the environment: a review focused on natural waters. III. Microbiota relevant interactions. Earth Sci. Rev. 80, 195–217.

Fisher, N. S., Nolan, C. V., Fowler, S. W., 1991. Assimilation of metals in marine copepods and its biogeochemical implications. Mar. Ecol. Progr. Ser. 71, 37–43.

Fisher, N.S., Reinfelder, J.R., 1995. The trophic transfer of metals in marine systems, in Tessier, A., Turner, D.R. (Eds.), Metal speciation and bioavailability in aquatic systems. Wiley, J & Sons, New York, pp.363-406.

Gaudy, R., 1985. Features and peculiarities of the zooplankton community from the western Mediterranean. In: Moraitou Apostolopoulou, M. & V. Kiortsis (Eds.), Mediterranean Marine Ecosystem. Plenum press, New York, USA, pp. 279-302.

Gobas, F.A.P.C., Morrison, H.A., 2000. Bioconcentration and biomagnification in the aquatic environment, In: Boethling, R.S., Mackay, D. (Eds.), Handbook of Property Estimation Methods for Chemicals, Lewis Publishers, Boca Raton, FL, pp. 189–231.

Hardstedt-Roméo, M., Laumond, F., 1980. Zinc, copper and cadmium in zooplankton from the N.W. Mediterranean. Mar. Pollut. Bull. 11, 133-138.

Hsiao, S., Hwang, J., Fang, T., 2011. Copepod species and their trace metal contents in coastal northern Taiwan. Mar. Syst. 88, 232–238.

Ikem, A., Egiebor, N.O., 2005. Assessment of trace elements in canned fishes (Mackerel, Tuna, Salmon, Sardines and Herrings) marketed in Georgia and Alabama (United States of America). J. Food Compos. Anal. 18,771–787.

Kadam, S. S., Tiwari, L. R., 2015. Trace metal analysis in zooplankton from Dandi creek -west coast of India. Int. J. Sci. Res. Publ. 5(1), 1-10.

Kahle, J., Zauke, G.P., 2003. Trace metals in Antarctic copepods from the Weddell Sea (Antarctica). Chemosphere 51,409–17.

Kehrig, do A., Seixas, T.G., Palermo, E.A., Baêta, A.P., Castelo-Branco, C.W., Malm, O., Moreira, I., 2009. The relationships between mercury and selenium in plankton and fish from a tropical food web. Environ. Sci. Pollut. Res. Int. 16(1), 10-24.

Kremling, K., Pohl, C., 1989. Studies on the spatial and seasonal variability of dissolved cadmium, copper and nickel in northeast Atlantic surface waters. Mar. Chem. 27: 43-60.

Lafabrie, C., Pergent-Martini, C., Pergent, G., 2008. Metal contamination of *Posidonia oceanica* meadows along the Corsican coastline (Mediterranean). Environ. Pollut. 151 (1), 262–268.

Lafabrie, C., Pergent, G., Kantin, R., Pergent-Martini, C., Gonzalez, J.L., 2007a. Trace metals assessment in water, sediment, mussel and seagrass species – Validation of the use of *Posidonia oceanica* as a metal biomonitor. Chemosphere 68 (11), 2033-2039.

Lafabrie, C., Pergent, G., Pergent-Martini, C., Capiomont, A., 2007b. *Posidonia oceanica*: a tracer of past mercury contamination. Environmental pollution, 148 (2): 688-692.

Li, F. M., Ren, J. L., Yan, L., Liu, S., Liu, C., Zhou, F., Zhang, J., 2013. The biogeochemical behavior of dissolved aluminum in the southern Yellow Sea: Influence of the spring phytoplankton bloom. Chin. Sci. Bull. 58, 238-248.

Lovegrove, T., 1996. The determination of the dry weight of plankton and the effect of various factors of the values obtained. In: Barnes, R. (Ed.), Some Contemporary Studies in Marine Science. Allen G., Unwin, London, pp. 462-467.

Maceda-Veiga, A., Monroy, M., Navarro, E., Viscor, E., de Sostoa, A., 2013. Metal concentrations and pathological responses of wild native fish exposed to sewage discharge in a Mediterranean river. Sci. Total Environ. 449, 9–19.

Mackey, E.A., Becker, P.R., Demiralp, R,, Greenberg, R.R., Koster, B.J., Wise, S.A., 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. Arch. Environ. Contam. Toxicol. 30, 503–512.

Maher, W.A., Foster, S.D., Taylor, A.M., Krikow, F., Duncan, E.G., Chariton, A.A., 2011. Arsenic distribution and species in two *Zostera capricorni* seagrass ecosystems, New South Wales, Australia. Environ. Chem. 8, 9–18.

Martin, J.H., 1970. The possible transport of trace metals via moulted copepod exoskeletons. Limnol. Oceanogr. 15,756-761.

692 Mazzocchi, M.G., Christou, E.D., Di Capua, I., Fernàndez de Puelles, M.L., Fonda-Umani, S.,
693 Molinero, J.C., Nival, P., Siokou-Frangou, I., 2007. Temporal variability of *Centropages typicus* in
694 the Mediterranean Sea over seasonal-to-decadal scales. Prog. Oceanogr. 72, 214-232.

695 Mazzocchi , M.G., Ribera d'Alcala, M., 1995. Recurrent patterns in zooplankton structure and
696 succession in a variable coastal environment. ICES J. Mar. Sci. 52, 679-691.

697 Mc Geer, J.C., Brix, K.V., Skeaff, J.M., Deforest, D.K., Brigham, S.I., Adams, W.J., Green, A.,
698 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals:
699 implications for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem.
700 22 (5), 1017–1037.

701 Miramand, P., Guyot, T., Rybarczyk, H., Elkaim, B., Mouny, P., Dauvin, J.C., Bessineton, C.,
702 2001. Contamination of the biological compartment in the Seine estuary by Cd, Cu, Pb, and Zn.
703 Estuaries 24(6), 1056-1065.

704 Molinero, J.C., Ibanez, F., Nival, P., 2005a. North Atlantic climate and northwestern plankton
705 variability. Limnol. Oceanogr. 50 (4), 1213-1220.

706 Molinero, J.C., Ibanez, F., Souissi, S., Chifflet, M., Nival, P., 2005b. Phenological changes in the
707 Northwestern Mediterranean copepods *Centropages typicus* and *Temora stylifera* linked to climate
708 forcing. Oecologia 145, 640-649.

709 Nieboer, E., Richardson, D.H.S., 1980. The replacement of the non-descript term 'heavy metals' by
710 a biologically and chemically significant classification of metal ions. Env. Poll. 1, 3–26.
711
712 Orians, K. J., Bruland, K. W., 1986. The biogeochemistry of aluminum in the Pacific Ocean. Earth
713 Planet. Sci. Lett., 78,397–410.
714
715 Paimpillil, J.S., Thresiamma, J., Rejomon G., Gerson, V.J., 2010. Metals in Coastal Zooplanktons
716 - A Coastal Living Resource Hazard. Ind. Geol. Congr. 199-207.
717
718 Pempkowiak, J., Walkusz-Miotk, J., Beldowski, J., Walkusz, W., 2006. Heavy metals in
719 zooplankton from the Southern Baltic. Chemosphere 62, 1697–1708.
720
721 Perez-Granados, A.M., Vaquero, M.P., 2002. Silicon, aluminum, arsenic and lithium: Essentiality
722 and human health implications. J. Nutr. Health Aging 6, 154-162.

723 Purkerson, D.G., Doblin, M.A., Bollens, S.M., Luoma, S.N., Cutter, G.A., 2003. Selenium in San
724 Francisco Bay zooplankton: potential effects of hydrodynamics and food web interactions.
725 Estuaries 26, 956–969.
726
727 Rahman, M.A., Hasegawa, H., Lim, R.P., 2012. Bioaccumulation, Biotransformation and Trophic
728 Transfer of Arsenic in the Aquatic Food Chain. Environ. Res. 116, 118-135.
729
730 Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what?
731 Environ. Pollut. 120, 497-452.
732
733 Rainbow, P.S., 1997. Trace metal accumulation in marine invertebrates: marine biology or marine
734 chemistry? J. Mar. Biol. Ass. UK 77, 195–210.

Rainbow, P.S., 1993. The significance of trace metal concentration in marine invertebrates, In: Dallinger, R., Rainbow, P.S. (Eds.), Ecotoxicology of metals in invertebrates. Lewis Publishers, Boca Raton, USA, pp.4-23.

Razouls, C., Kouwenberg, J., 1993. Spatial distribution and seasonal variation of mesozooplankton biomass in the Gulf of Lions. *Oceanol. Acta*, 16: 393-402.

Renzi, M., Perra, G., Lobianco, A., Mari, E., Guerranti, C., Specchiulli, A., Pepi, M., Focardi, S., 2009. Environmental quality assessment of the marine reserve of the Tuscan Archipelago, Central Tyrrhenian Sea (Italy). Chem. Ecol. 26, 299-317.

Ricart, M., Guasch, H., Barceló´, D., Brix, R., Conceicaõ, M.H., Geiszinger, A., 2010. Primary and complex stressors in polluted Mediterranean rivers: pesticide effects on biological communities. J. Hydrol. 383, 52–61.

Richardson, A. J., 2008. In Hot Water: Zooplankton and Climate Change. ICES J. Mar. Sci. 65, 279-295.

Rossi, N., Jamet, J.L., 2008. *In situ* heavy metals (copper, lead and cadmium) in different plankton compartments and suspended particulate matter in two coupled Mediterranean coastal ecosystems (Toulon Bay, France). Mar. Poll. Bull. 56, 1862–1870.

Safaa A. A. G., 2015. Trace metals in seawater, sediments and some fish species from Marsa Matrouh Beaches in northwestern Mediterranean coast, Egypt. Egypt. J. Aquat. Res. 41(2), 145–154.

Schminke, H. K., 2007. Entomology for the copepodologist. J. Plankton Res. 29, 149-162.

Schneider, L., Maher, W.A., Potts, J., Taylor, A.M., Batley, G.E., Krikowa, F., Chariton, A.A., Gruber, B. 2015. Modeling food web structure and selenium biomagnification in Lake Macquarie, New South Wales, Australia, using stable carbon and nitrogen isotopes. Environ. Toxicol. Chem. 34 (3), 608–617.

Shannon, C.E., Weaver, W., 1963. The mathematical theory of communication. University of Illinois Press. (Eds.), Urbana, pp. 1-117.

Sharma, V.K., Sohn, M., 2009. Aquatic arsenic: Toxicity, speciation, transforma- tions, and remediation. Environ. Int. 35, 743–759.

Sick, L.V., G.J. Baptist 1979. Cadmium incorporation by the marine copepod *Pseudodiaptomus coronatus*. Limnol. Oceanogr. 24(3), 453-462.

Strady, E., Kim, I., Radakovitch, O., Kim, G., 2015. Rare earth element distributions and fractionation in plankton from the northwestern Mediterranean Sea. Chemosphere, 119, 72–82.

Sunda, W.G., 2012. Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Front. Microbiol. 3, 1-22.

Thomson, A.J., 1982. Proteins containing nickel. Nature 298, 602-603.

US EPA, 1980. Ambient water quality criteria for beryllium. Washington, DC, US Environmental Protection Agency, Division of Water Planning and Standards, EPA Report 440/580024.

Usero, J., Morillo, J., Garcia, I., 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. Chemosphere 59, 1175-1181.

Uthus, E.O., 2003. Arsenic essentiality: A role affecting methionine metabolism, J. Trace Elem. Exp. Med. 16 (4), 345–355.

Vink, S., Measures C. I., 2001. The role of dust deposition in determining surface water distributions of Al and Fe in the South West Atlantic. Deep-Sea Res. 48, 2787–2809.

Vives, F., Shmeleva, A., 2007. Crustacea. Copépodos marinos I. Calanoida. In: Ramos, M.A., (Eds.), Fauna Ibérica, Museo National de Ciencias Naturales CSIC, Madrid, pp.1-1152.

Vives, F., Shmeleva, A., 2010. Crustacea. Copépodos marinos II. Non Calanoida, In: Ramos, M.A., (Eds.), Fauna Ibérica, Museo National de Ciencias Naturales CSIC, Madrid, pp. 1-486.

Voutsinou-Taliadouri, F., Zeri, C., Moriki, A., 2000. Distribution and transfer of trace metals in the Aegean Seawater (Easter Mediterranean Basin). Med. Mar. Sci. 1(2), 5-30.

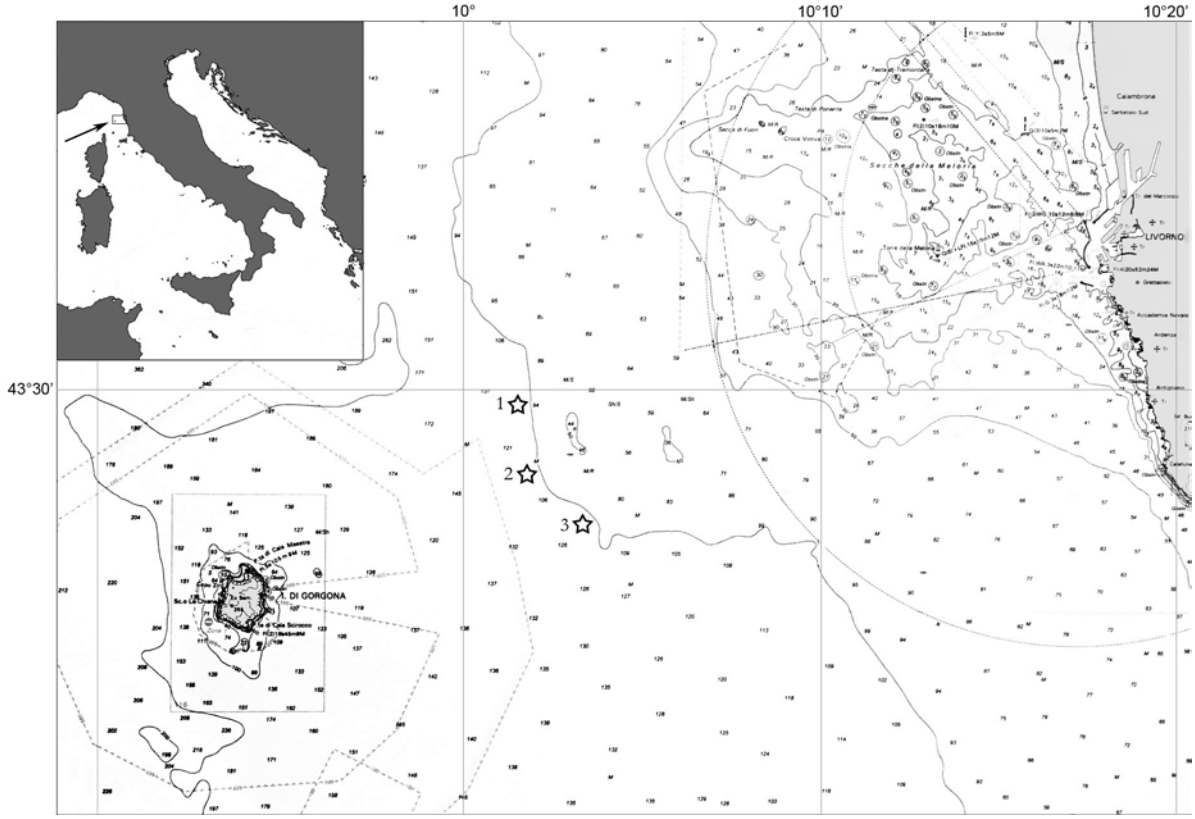
Watras, C.J., Bloom, N.S., 1992. Mercury and methylmercury in individual zooplankton – implications for bioaccumulation. Limn. Oceanogr. 37(6), 1313-1318.

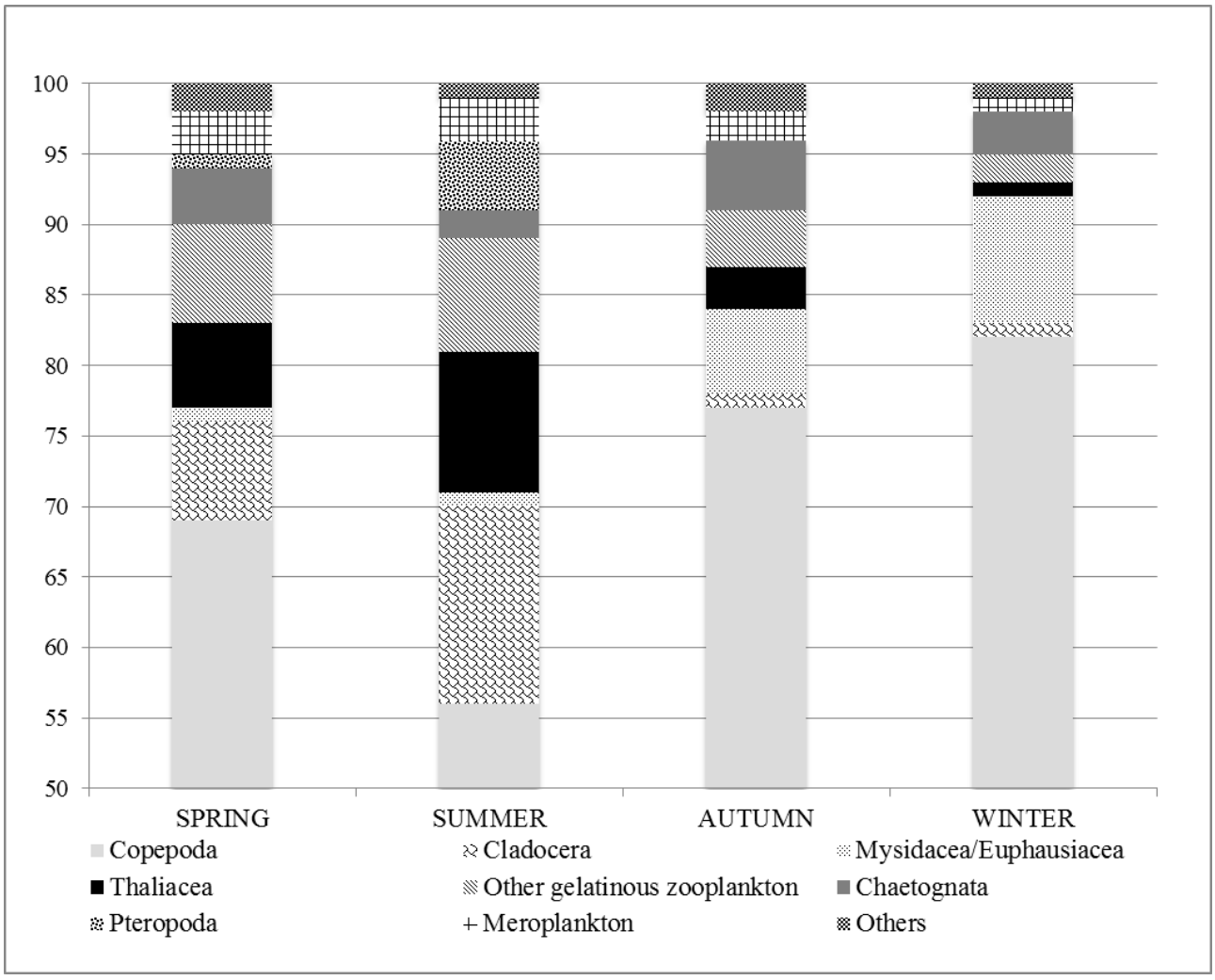
Whitfield, M., 2001. Interactions between phytoplankton and trace metals in the ocean. Adv. Mar. Biol. 41, 1–128.

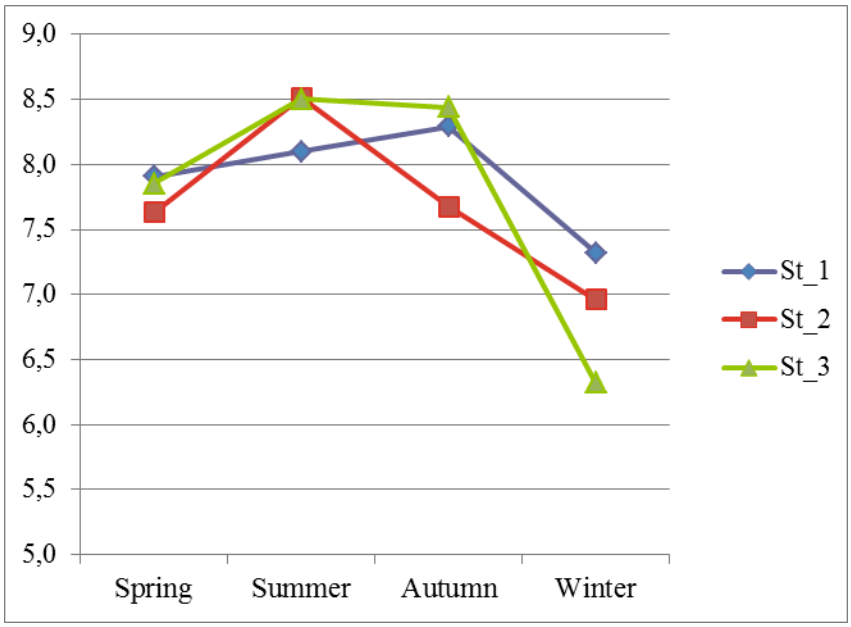
World Health Organization (WHO), 1991. Nickel and its compounds: environmental aspects. Howe, P.D., Malcolm, H.M., Dobson, S., Centre for Ecology & Hydrology, Monks Wood, United Kingdom.

World Health Organization (WHO), 2004. Manganese and its compounds: environmental aspects. Howe, P.D., Malcolm, H.M., Dobson, S., Centre for Ecology & Hydrology, Monks Wood, United Kingdom.

World Health Organization (WHO), 2005. Tin and inorganic tin compounds. P.D. Howe, Malcolm, H.M., Dobson S., Centre for Ecology & Hydrology, Monks Wood, United Kingdom.


10°
10°10'
10°20'
43°30'
1
2
3
I. DI GORGONA
LIVORNO
Secche della Meloria
Calambrone


100
95
90
85
80
75
70
65
60
55
50
SPRING
SUMMER
AUTUMN
WINTER
Copepoda
Cladocera
Mysidacea/Euphausiacea
Thaliacea
Other gelatinous zooplankton
Chaetognata
Pteropoda
Meroplankton
Others


9,0
8,5
8,0
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6,5
6,0
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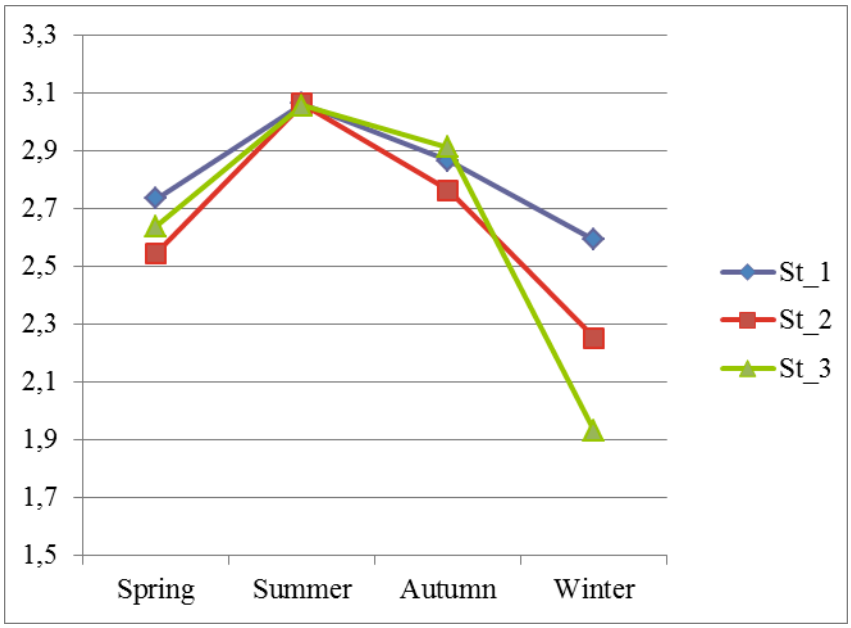
5,0
Spring
Summer
Autumn
Winter
St_1
St_2
St_3


3,3
3,1
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Spring
Summer
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Winter
St_1
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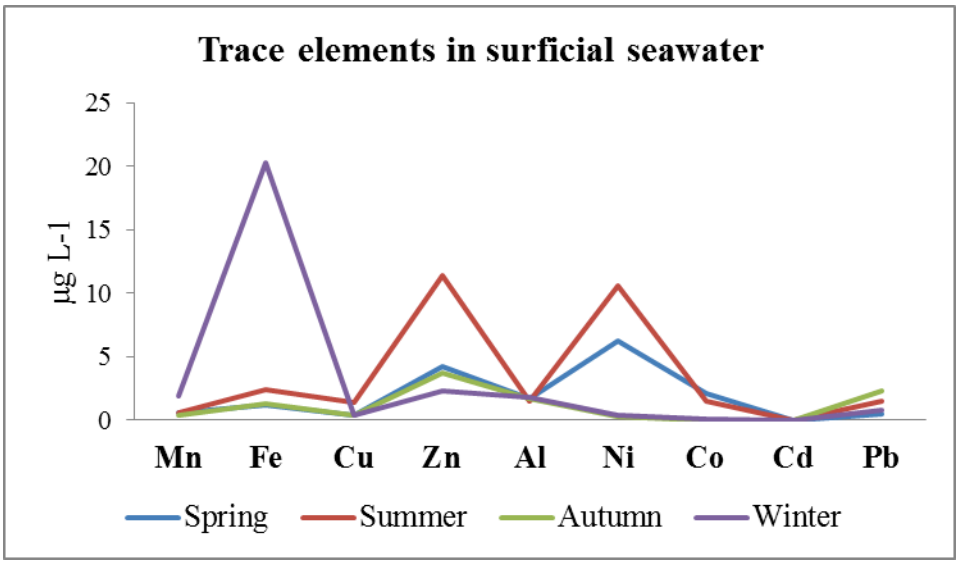
St_3


Trace elements in surficial seawater
μg L-1
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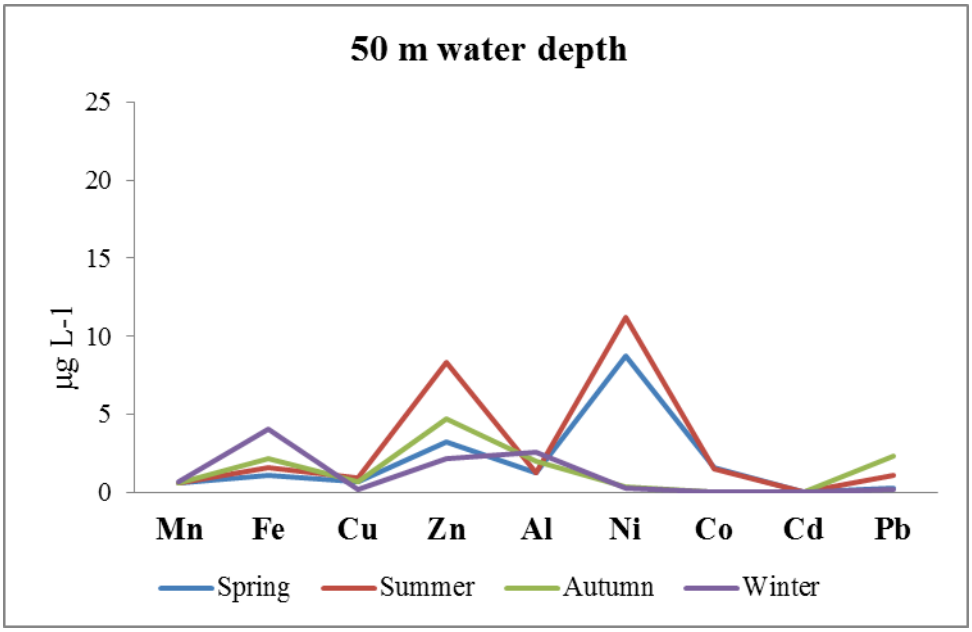
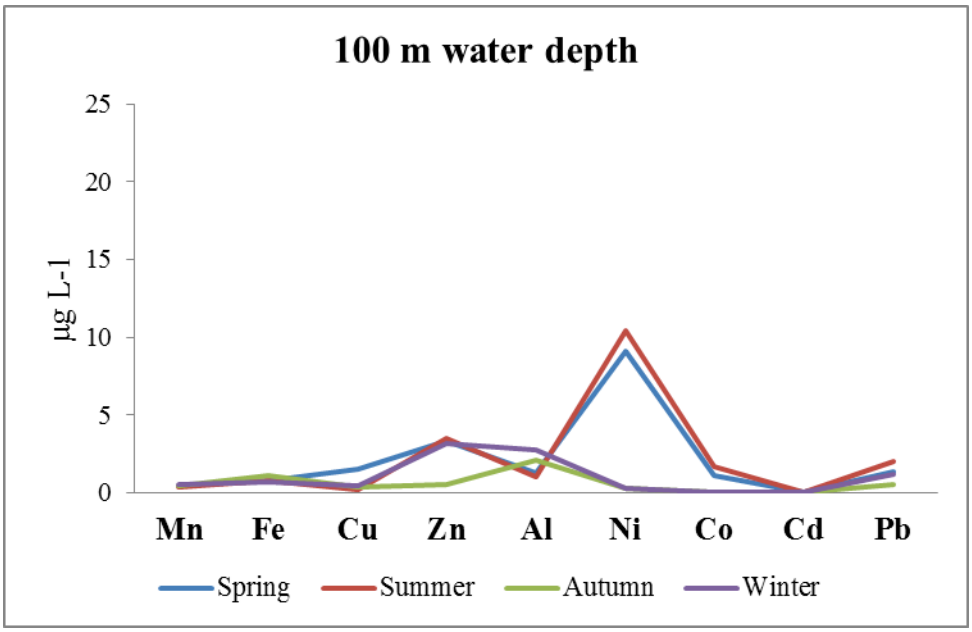
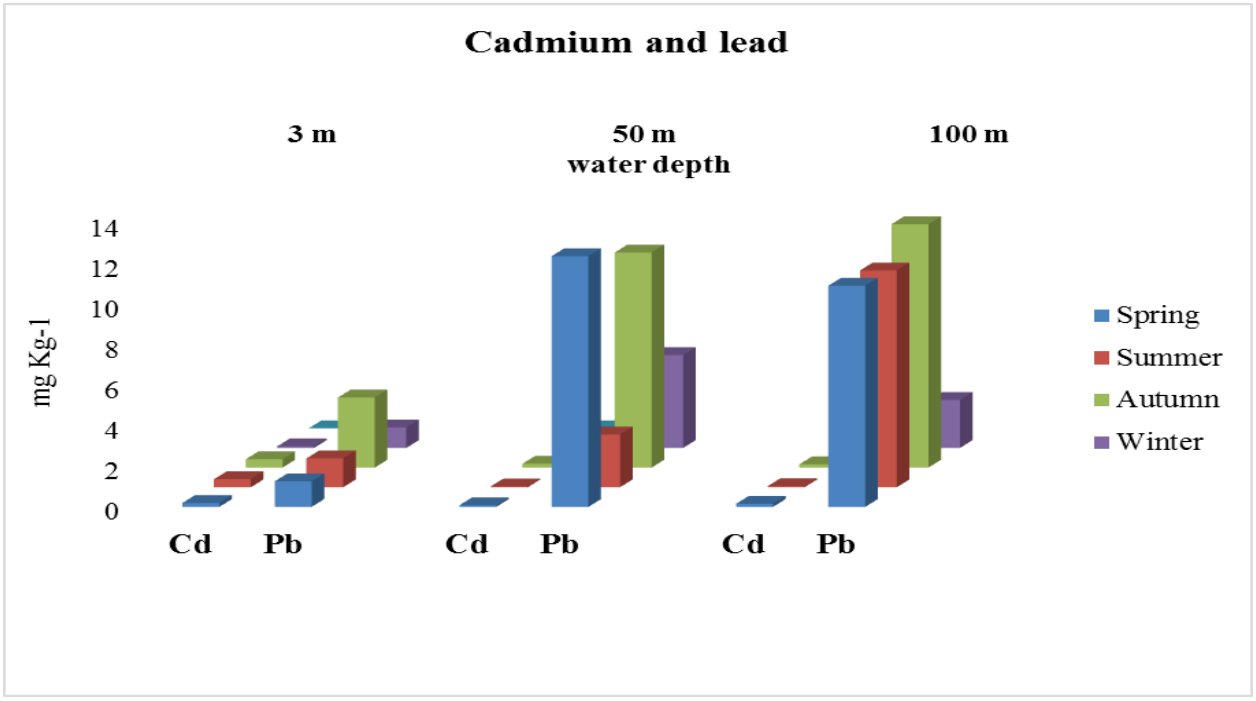
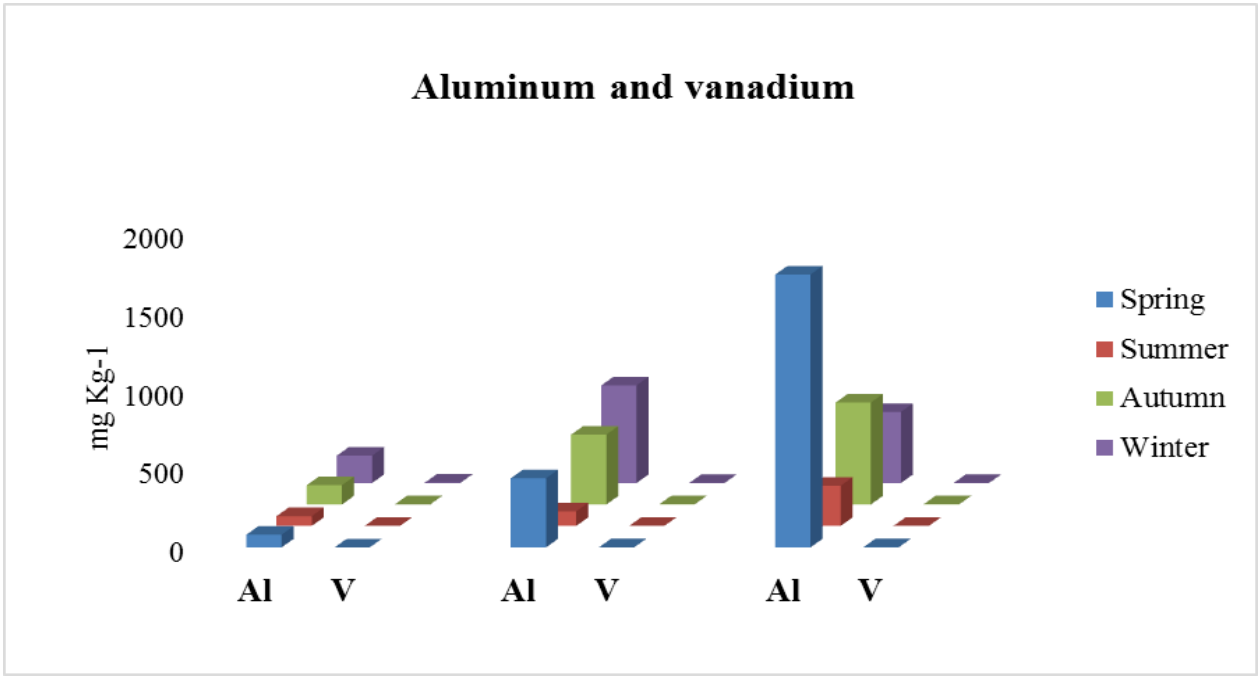
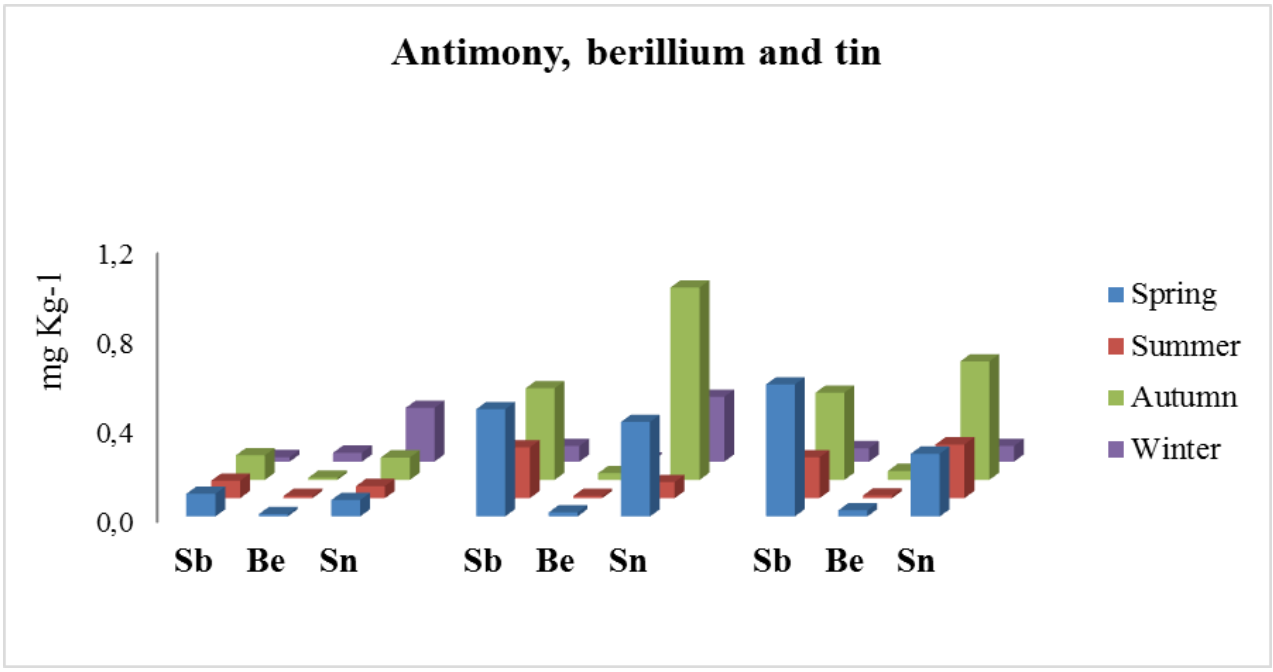
20
15
10
5
0
Mn
Fe
Cu
Zn
Al
Ni
Co
Cd
Pb
Spring
Summer
Autumn
Winter


50 m water depth
μg L-1
25
20
15
10
5
0
Mn
Fe
Cu
Zn
Al
Ni
Co
Cd
Pb
Spring
Summer
Autumn
Winter


100 m water depth
μg L-1
25
20
15
10
5
0
Mn
Fe
Cu
Zn
Al
Ni
Co
Cd
Pb
Spring
Summer
Autumn
Winter


Cadmium and lead
3 m
50 m
water depth
100 m
mg Kg-1
14
12
10
8
6
4
2
0
Cd
Pb
Cd
Pb
Cd
Pb
Spring
Summer
Autumn
Winter


Aluminum and vanadium
mg Kg-1
2000
1500
1000
500
0
Al
V
Al
V
Al
V
Spring
Summer
Autumn
Winter


Antimony, berillium and tin
mg Kg-1
1,2
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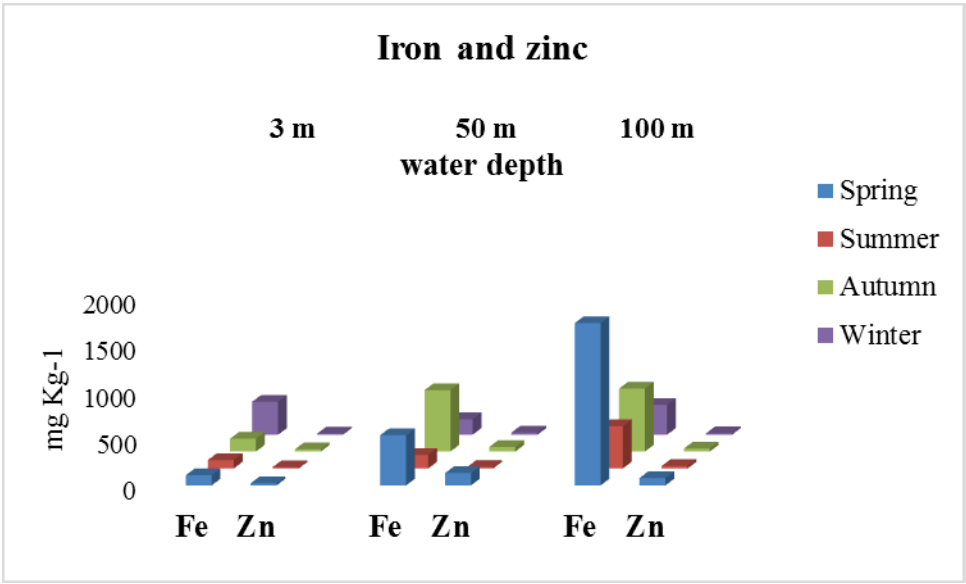
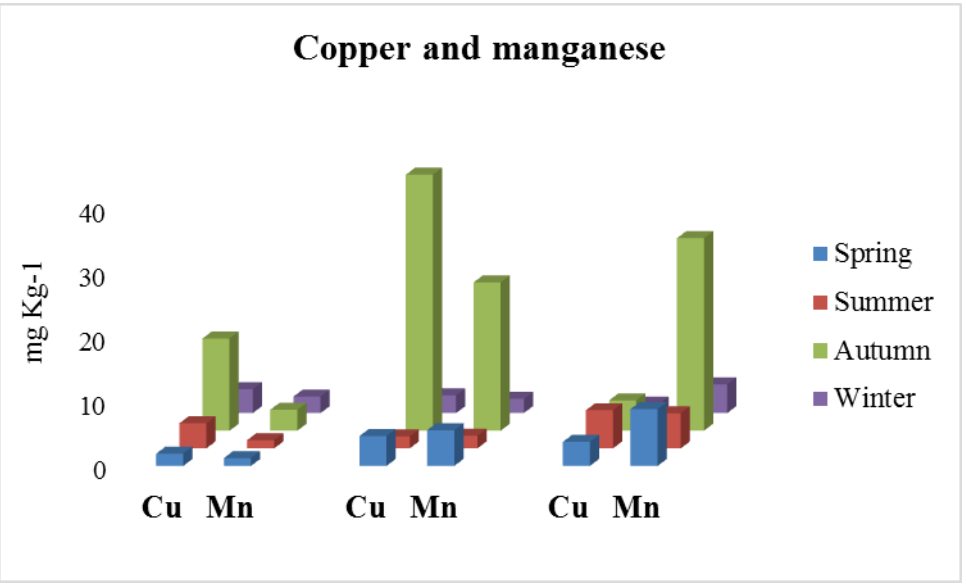
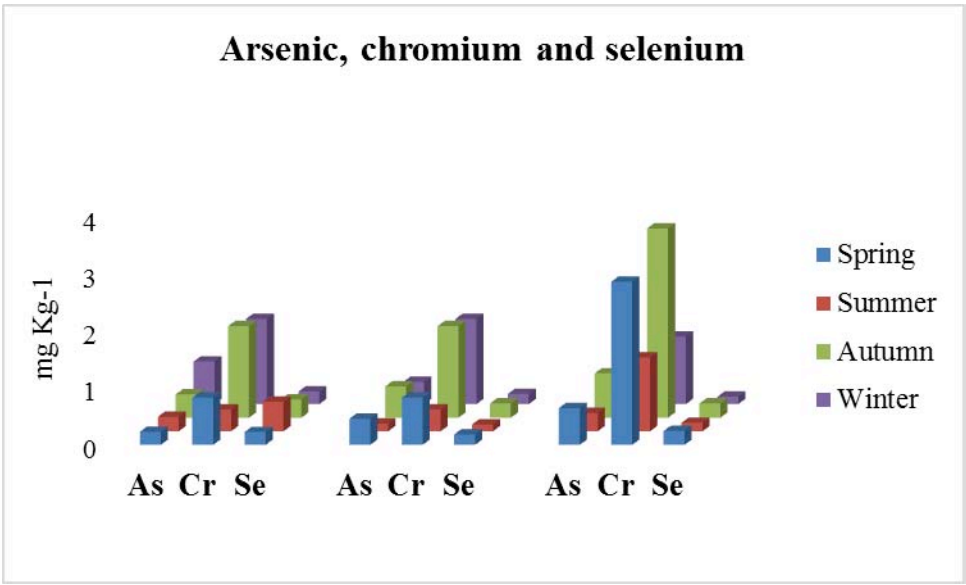
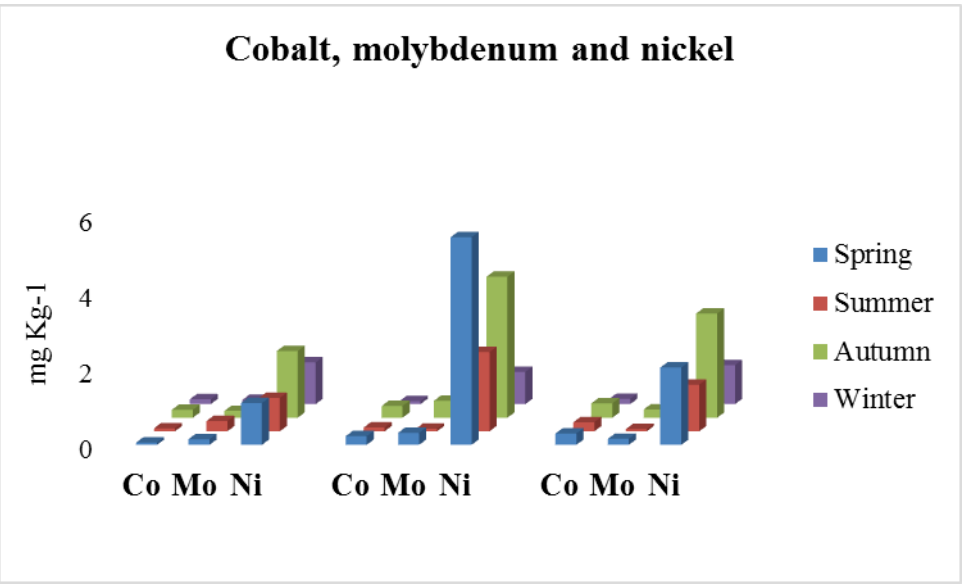
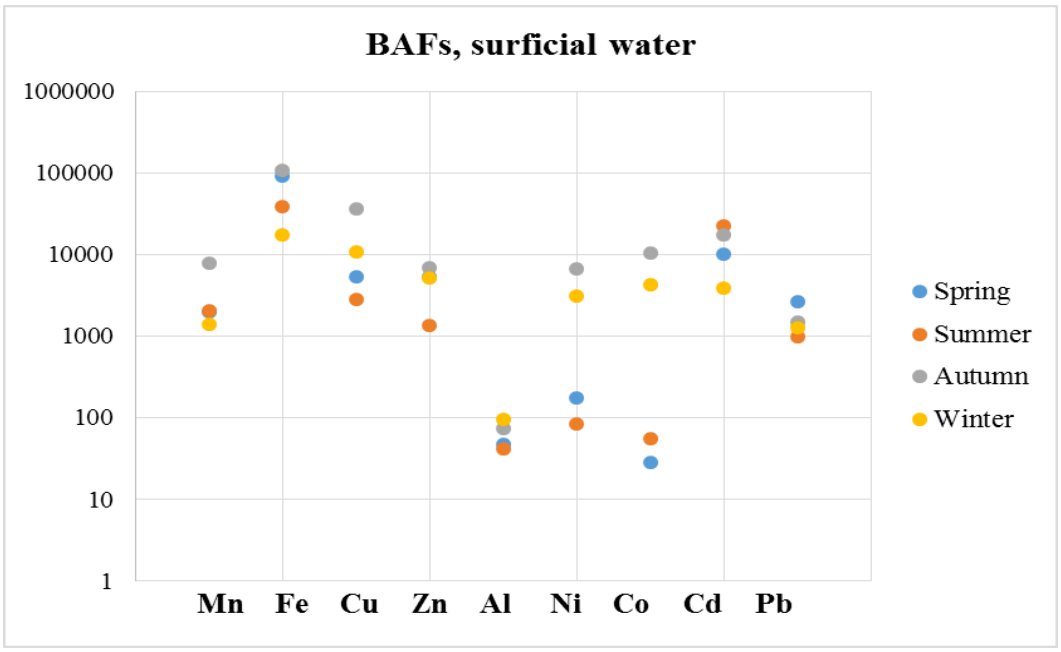
0,4
0,0
Sb
Be
Sn
Sb
Be
Sn
Sb
Be
Sn
Spring
Summer
Autumn
Winter

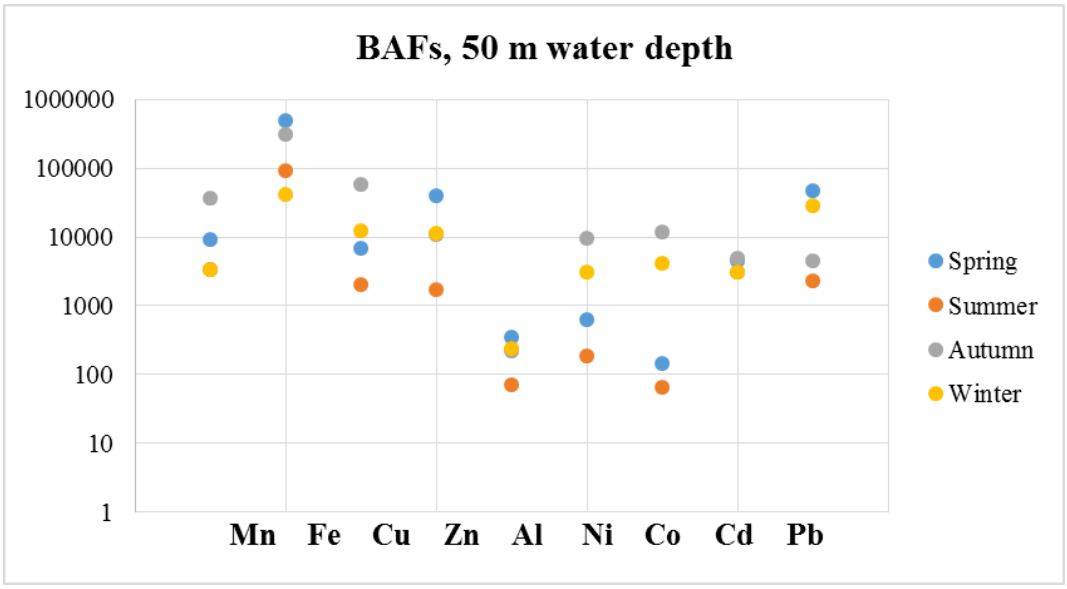

Iron and zinc
3 m
50 m
100 m
water depth
mg Kg-1
2000
1500
1000
500
0
Fe Zn
Fe Zn
Fe Zn
Spring
Summer
Autumn
Winter


Copper and manganese
mg Kg-1
40
30
20
10
0
Cu Mn
Cu Mn
Cu Mn
Spring
Summer
Autumn
Winter


Arsenic, chromium and selenium
mg Kg-1
4
3
2
1
0
As Cr Se
As Cr Se
As Cr Se
Spring
Summer
Autumn
Winter


Cobalt, molybdenum and nickel
mg Kg-1
6
4
2
0
Co Mo Ni
Co Mo Ni
Co Mo Ni
Spring
Summer
Autumn
Winter


BAFs, surficial water
1000000
100000
10000
1000
100
10
1
Mn
Fe
Cu
Zn
Al
Ni
Co
Cd
Pb
Spring
Summer
Autumn
Winter


BAFs, 50 m water depth
1000000
100000
10000
1000
100
10
1
Mn
Fe
Cu
Zn
Al
Ni
Co
Cd
Pb
Spring
Summer
Autumn
Winter

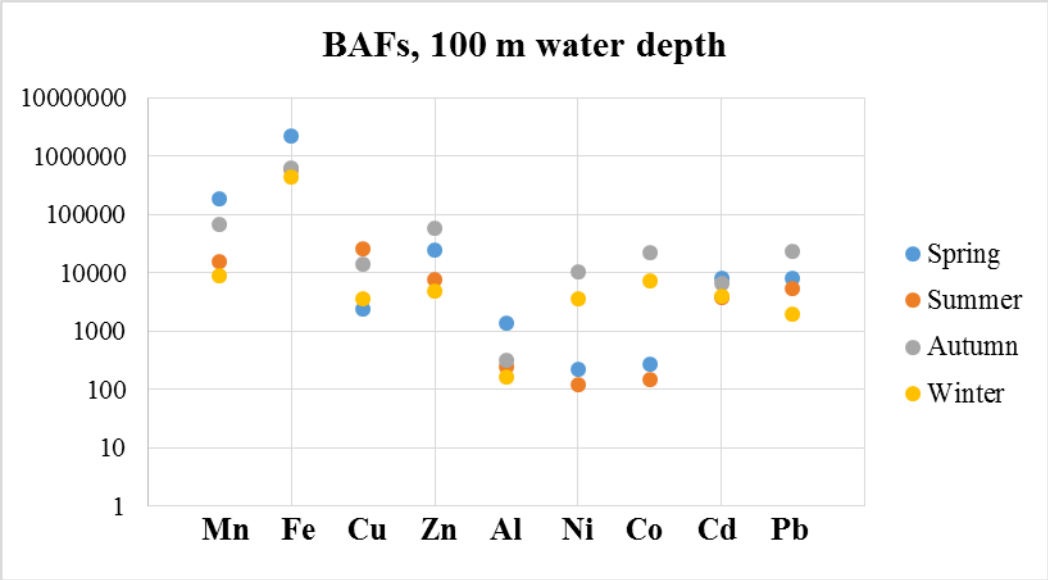

BAFs, 100 m water depth
10000000
1000000
100000
10000
1000
100
10
1
Mn
Fe
Cu
Zn
Al
Ni
Co
Cd
Pb
Spring
Summer
Autumn
Winter

Figure captions

Figure 1

Ligurian Sea (Western Mediterranean): study area and location of sampling station.

Figure S1

Map of the western Mediterranean indicating the study area and mean circulation patterns: NC, Northern Current; WCC, Western Corsica Current; ECC, Eastern Corsica Current.

Figure S2

Trends in BAFs zooplankton of Mediterranean Sea

Figure 2

Zooplankton community composition (biomass) by taxonomic group and season fo the samples from 2014 to 2015.

Figure 3

Seasonal copepods community at every sampling station: a) species richness (d) and b) Shannon – Wiener index H' (log*e*), for each season.

Figure 4

Metals levels ($\mu g\ L^{-1}$) in seawater at different water depths and seasons

Figure 5

Nonessential elements in marine zooplankton

Figure 6

Essential trace elements in marine zooplankton

Figure 7

Bioaccumulation factors (BAFs) for marine zooplankton at different water depths