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### ► To cite this version:

Sandrine Chifflet, Nicolas Briant, Rémi Freydier, Daniel Araùjo, Marianne Quéméneur, et al.. Isotopic compositions of copper and zinc in plankton from the Mediterranean Sea (MERITE-HIPPOCAMPE campaign): Tracing trophic transfer and geogenic inputs. *Marine Pollution Bulletin*, 2022, 185 (Part A), pp.114315. 10.1016/j.marpolbul.2022.114315. hal-03865956

**HAL Id: hal-03865956**

**<https://hal.science/hal-03865956>**

Submitted on 22 Nov 2022

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1 **Isotopic compositions of Copper and Zinc in plankton from the Mediterranean Sea**  
2 **(MERITE-HIPPOCAMPE campaign): tracing trophic transfer and geogenic inputs**

3

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16 For submission to Marine Pollution Bulletin Special issue “*Plankton and Contaminants in the*  
17 *Mediterranean Sea: Biological pump and interactions from regional to global approaches*”  
18 as a full-length research paper

19

20 Revised version

21 05 October 2022

22

23 **Abstract**

24         This study uses Cu and Zn isotope compositions as proxies of sources and metal  
25 transfers in the planktonic food web from the Mediterranean Sea. Plankton was collected in  
26 spring 2019 in the deep chlorophyll maximum (DCM) along a North-South transect including  
27 coastal and offshore zones (MERITE-HIPPOCAMPE campaign).  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$   
28 compositions were determined on four planktonic size fractions from 60 to 2000  $\mu\text{m}$ .  
29 Combined  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  with geochemical tracers (Ti, POP) showed that geogenic  
30 particles were ubiquitous with plankton assemblages. The  $\delta^{15}\text{N}$  ecological tracer showed that  
31 planktonic food web was enriched in heavy isotopes in the higher trophic levels.  $\delta^{65}\text{Cu}$   
32 compositions were correlated with picoplankton in the offshore zone, and with zooplankton  
33 in the southern coastal zone. *Firmicutes* bacteria were found correlated with  $\delta^{66}\text{Zn}$  zones  
34 suggesting decomposition of particulate matter at the DCM. These findings suggest that  
35 biogeochemical process may impact Cu and Zn isotopy in the planktonic community.

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38 **Keywords:** Mediterranean Sea, planktonic food web, Cu and Zn isotopes, geogenic inputs

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## 48 **1. Introduction**

49 Plankton is an important gateway for the accumulation and transfer of trace metal into  
50 the marine food web (Rossi and Jamet, 2008; Chauvelon et al., 2019). According to their role  
51 in biogeochemical cycles, copper (Cu) and zinc (Zn) can be categorized as essential for  
52 marine food web. Indeed, both elements serve as co-factors in enzymes of key metabolic  
53 pathways. Cu is a key micronutrient that contributes to photosynthesis, denitrification and  
54 iron uptake by primary producers in the food web (Peers et al., 2005; Peers and Price, 2006;  
55 Zumft and Kroneck, 2007). However, above 10 pM, free dissolved copper ( $\text{Cu}^{2+}$ ) exerts acute  
56 toxic effects on phytoplankton (Moffett et al., 1997) which in turn may release organic  
57 ligands that strongly complex Cu as an effective detoxification mechanism (Moffett and  
58 Brand, 1996; Croot et al., 2000; Rue and Bruland, 2001). Zn containing enzymes are carbonic  
59 anhydrases, essential during biomass buildup from reduced carbon and light (Domsic et al.,  
60 2008), or alkaline phosphatases for the acquisition of organic phosphorus when phosphate is  
61 scarce (Morel and Price, 2003; Shaked et al., 2006; Cox and Saito, 2013). Because the  
62 interactions between metal and organisms are numerous and complex, it is still difficult to  
63 understand transfers of Cu and Zn in the planktonic food web.

64 The new generation of high-resolution mass spectrometers, such as the multi-collector  
65 inductively coupled plasma mass spectrometers (MC-ICP-MS), have allowed measuring  
66 accurately isotope compositions of trace metal in natural marine samples (Araújo et al.,  
67 2022a). Cu has two stable isotopes,  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$ , with natural average abundances of 69.17  
68 and 30.83%, respectively, while Zn has five stable isotopes,  $^{64}\text{Zn}$ ,  $^{66}\text{Zn}$ ,  $^{67}\text{Zn}$ ,  $^{68}\text{Zn}$ , and  $^{70}\text{Zn}$ ,  
69 with natural average abundances of 48.63, 27.90, 4.10, 18.75, and 0.62%, respectively  
70 (Maréchal et al., 1999). In natural ecosystems, the relative abundance of stable isotopes varies  
71 according to isotopic fractionation phenomena occurring during biogeochemical processes,  
72 between reactants and products, phases or molecules (Wiederhold, 2015). These changes in

73 isotope ratios have proven useful in tracing sources and biogeochemical processes in  
74 hydrothermal systems (Larson et al., 2003; Rouxel et al., 2004; Maher and Larson, 2007), ore  
75 deposits (Graham et al., 2004; Mason et al., 2005; Lemaitre et al., 2020a) and open ocean  
76 (Conway and John, 2014; Conway et al., 2021). In coastal marine ecosystems, these isotopes  
77 have been relevant to track metal dispersion and to differentiate the type and nature of  
78 particles (natural *versus* anthropogenic, mineral *versus* organic) by river inputs or atmospheric  
79 deposition (Araújo et al., 2022a; b).

80         However, the Cu and Zn isotopic fractionation related to planktonic organisms,  
81 colloids and the dissolved matrix in seawater is not yet clearly elucidated. Cu speciation is  
82 dominated by Cu<sup>2+</sup> complexation with organic ligands (Coale and Bruland, 1988; Moffett et  
83 al., 1990) and induces a significant isotopic fractionation ( $\Delta^{65}\text{Cu}_{\text{complex-free}}$  ranging from +0.1  
84 to +0.8‰) during equilibrium exchanges between organically complexed and free inorganic  
85 dissolved Cu (Bigalke et al., 2010; Fujii et al., 2013; Ryan et al., 2014). Zn bound to organic  
86 ligands exhibits an isotopic fractionation ( $\Delta^{66}\text{Zn}_{\text{cell-sw}}$ ) ranging from -0.8 to -0.2‰ depending  
87 on homeostasis pathways during phytoplankton photosynthesis (John et al., 2007a; Peel et al.,  
88 2009; John and Conway, 2014). While many studies have investigated the magnitude of Cu  
89 and Zn isotopic fractionations under controlled laboratory conditions, few field studies  
90 applied these insights to constrain the transfer mechanisms of the elements in real  
91 environmental conditions.

92         The Mediterranean Sea is a semi-enclosed sea that covers ~ 0.7% of the total ocean  
93 surface area (~ 0.25% by volume), contains 4 to 18% of the world's marine diversity (Bianchi  
94 and Morri, 2000; UNEP/MAP-RAC-SPA, 2008) and is characterized by oligotrophic  
95 conditions due to its low content in nutrient (The Mermex group, 2011; Marañón et al., 2021).  
96 The Mediterranean Sea is subject to natural and anthropogenic exogenous chemical  
97 substances coming from rivers, effluents and atmospheric deposition (Guieu et al., 2002;

98 [Heimbürger et al., 2010, 2011](#); [Pey et al., 2010](#); [Llamas-Dios et al., 2021](#); [Desboeufs et al.,](#)  
99 [2022](#)). Important changes have been observed in recent decades in small pelagic fish  
100 populations of the northwestern Mediterranean Sea related to various environmental factors  
101 ([Coll et al., 2019](#)). Multiple drivers including climatic (temperature, UV irradiance),  
102 acidification, demersal fishing, ship traffic and coastal pollution would affect 20% of the  
103 entire basin and 60–99% of the territorial waters of EU member states of the Mediterranean  
104 and Black Seas ([Micheli et al., 2013](#)). Among the pollutants, trace metals are a recurrent  
105 concern ([Luy et al., 2012](#)).

106 In order to further understand the biogeochemical cycles of Cu and Zn in the  
107 Mediterranean Sea, isotopic compositions of these elements were determined within the  
108 planktonic food web (from bacteria to zooplankton) according to three Mediterranean  
109 geographical areas: the northern and southern coastal zones, and the offshore zone. Based on  
110 these geographical areas, we aim thus to identify geogenic (natural and anthropogenic mineral  
111 sources) and biological (planktonic sources) fingerprints and to evidence their underlying  
112 biogeochemical processes. The abundance, diversity and taxonomy of plankton assemblages  
113 (bacterio-, phyto-, and zoo-plankton) are presented as companion papers ([Quéméneur et al.,](#)  
114 [2022](#); [Bellaaj Zouari et al., 2022](#); [Fierro-González et al., 2022](#)) and are used in this study to  
115 support the discussion.

116

117

## 118 **2. Materials and methods**

### 119 *2.1. Study zone and sampling procedure*

120 The MERITE-HIPPOCAMPE campaign was conducted on the R/V *Antea* ([Tedetti and](#)  
121 [Tronczynski, 2019](#)) in spring 2019 along a north-south Mediterranean transect, between the  
122 French (Toulon and Marseille, northwestern Mediterranean Sea) and Tunisian coastal zones

123 (Gulf of Gabès, southeastern Mediterranean Sea) according to two legs and ten sampling  
124 stations (Table 1). Leg 1 was carried out between April 13<sup>th</sup> and 28<sup>th</sup> with sampling of 5  
125 stations (St2, St4, St3, St10 and St11; in this chronological order) during the transect from La  
126 Seyne-sur-mer to Tunis. Leg 2 was carried out between April 30<sup>th</sup> and May 14<sup>th</sup> with  
127 sampling of 5 stations (St19, St17, St15, St9, St1; in this chronological order) during the  
128 return transect from the Gulf of Gabès to La Seyne-sur-mer (Fig. 1; Table 1). Sampling  
129 strategy implied a wide spatio-temporal variability between stations. They were chosen  
130 according to different criteria of physical, biogeochemical and biological conditions (Tedetti  
131 et al., 2022). We divided the transect in three geographical areas: i) the northern coastal zone  
132 (St1 to St4), located in the bays of Toulon and Marseille, ii) the southern coastal zone (St15,  
133 St17 and St19), located along the Tunisian coasts and iii) the offshore zone (St9 to St11),  
134 located in near the northern zone of the Balearic Thermal Front (St9 and St10), and in the  
135 Algerian ecoregion (St11).

136 At each station, a Multiple Horizontal Plankton Sampler (Midi type, Hydro-Bios<sup>®</sup>),  
137 referred to hereafter as “MultiNet”, was deployed to collect horizontally plankton in the deep  
138 chlorophyll maximum (DCM; Table 1). In a clean lab-container, recovered plankton was  
139 fractionated into four size fractions (F3: 60–200 µm, F4: 200–500 µm, F5: 500–1000 µm and  
140 F6: 1000–2000 µm) using nylon sieves by wet-sieving with seawater previously filtered  
141 through a glass fibre filter (GF/F, Whatman<sup>®</sup>). The four plankton size fractions were  
142 partitioned for various analyses (Tedetti et al., 2022) and aliquots for trace metal and isotopic  
143 analysis were transferred into pre-cleaned (HCl, 10%) polycarbonate jars and stored at -20 °C.

144

## 145 2.2. Analytical procedures

### 146 2.2.1. Elemental concentrations

147 The sample processing was carried out in a trace metal clean laboratory (ISO 5), using  
148 high purity acids (Optima grade, Fisher), and ultrapure water (Milli-Q® EQ 7000, Millipore).  
149 Planktonic samples (~200 mg dw) were digested in PFA vials (Savillex) with 5 mL of a  
150 mixture of pure acids (HF, 0.5 mL; HNO<sub>3</sub>, 4.5 mL), heated on a hot-block (120 °C, 24 h),  
151 evaporated to near dryness, and re-dissolved in 3 mL of HNO<sub>3</sub> (10%). The digested samples  
152 were then diluted using HNO<sub>3</sub> (2%) before running the trace metal analyses. Elemental  
153 analyses were performed by an inductively-coupled plasma mass spectrometer (ICP-MS;  
154 ICAP-Q™; ThermoScientific®) at the Laboratoire de Biogéochimie des Contaminants  
155 Métalliques (LBCM, Ifremer, Nantes). Details of laboratory and analytical procedures  
156 concerning trace metal analyses were described in a companion paper ([Chifflet et al., 2022](#)).

157

### 158 *2.2.2. Chromatographic separation*

159 Prior to isotopic analyses, Cu and Zn were separated from the digested samples by ion  
160 exchange chromatography according to the following modified protocol ([Borrok et al., 2007](#)).  
161 All critical laboratory equipment (PFA vials, micropipette tips, columns and PFA storage  
162 bottles) were acid cleaned before use. Separations were performed under a free-metal laminar  
163 flow hood installed in the clean laboratory (ISO 5). Aliquots of digested samples containing ~  
164 1000 ng of Cu were evaporated in PFA vials and re-dissolved in 1 mL HCl (10 M). Columns  
165 were loaded with 2 mL of pre-cleaned AG-MP1 anion exchange resin (100–200 mesh, Bio-  
166 Rad®), rinsed with 10 mL H<sub>2</sub>O and conditioned with 10 mL HCl (10 M). Samples were  
167 loaded onto the columns and rinsed with 5 mL HCl (10 M) to elute the matrix. Then, Cu  
168 fractions were eluted with 20 mL HCl (5 M) followed by Zn fractions with 15 mL HCl (0.01  
169 M). All steps were repeated 3 times to complete purification of Cu and Zn. The analyte  
170 content recoveries (100 ± 10%) and presence of interfering elements (Ba, Ca, Cr, Mg, Na, Ni,  
171 Ti, V) in Cu and Zn fractions were checked by ICP-MS (ICAP-Q™; ThermoScientific) to

172 assure no bias effect on isotope measurements (Maréchal and Albarède, 2002; Petit et al.,  
173 2008; Schleicher et al., 2020). Procedural blanks were below 1% of Zn and Cu mass contents  
174 in fractions. After purification, Cu and Zn fractions were near-dried and re-dissolved in HNO<sub>3</sub>  
175 for isotope ratio measurements.

176

### 177 2.2.3. Isotopic analysis

178 Cu and Zn isotopic compositions were measured by MC-ICP-MS (Neptune Plus™;  
179 ThermoScientific) in low mass resolution mode using a SIS (Stable Introduction System)  
180 nebulization chamber and a low flow PFA self-aspiration nebulizer. Cu isotopes (<sup>63</sup>Cu, <sup>65</sup>Cu)  
181 were measured at the PSO platform (Ifremer, Brest). Cu fractions were dissolved at 250 µg/L  
182 in HNO<sub>3</sub> (2%, v/v) and introduced at 50 µL/min. Zinc isotopes (<sup>64</sup>Zn, <sup>66</sup>Zn, <sup>67</sup>Zn, <sup>68</sup>Zn) were  
183 measured at the AETE-ISO platform (OSU-OREME university, Montpellier). Zn fractions  
184 were dissolved at 250 µg/L in HNO<sub>3</sub> (1%, v/v) and introduced at 70 µL/min. Zinc isotopes  
185 (<sup>64</sup>Zn, <sup>66</sup>Zn, <sup>67</sup>Zn, <sup>68</sup>Zn), Cu isotopes (<sup>63</sup>Cu, <sup>65</sup>Cu) and Ni isotope (<sup>62</sup>Ni) were monitored  
186 simultaneously and <sup>62</sup>Ni was used to correct possible isobaric <sup>64</sup>Ni interferences on <sup>64</sup>Zn.

187 Instrumental mass fractionation was corrected as described by Bermin et al. (2006)  
188 and Zhao et al. (2014). For the Cu isotope analyses, a Zn internal standard (Zn<sub>ETH</sub>) was added  
189 to the purified Cu fractions and a mixture of external standards (Cu<sub>SRM976</sub> + Zn<sub>ETH</sub>) was run  
190 with a standard bracketing sequence. The mass bias correction on the <sup>65</sup>Cu/<sup>63</sup>Cu ratio was  
191 performed combing the internal and external normalization using Zn isotopes ratios  
192 (<sup>66</sup>Zn/<sup>64</sup>Zn, <sup>67</sup>Zn/<sup>64</sup>Zn, <sup>68</sup>Zn/<sup>64</sup>Zn, and <sup>68</sup>Zn/<sup>66</sup>Zn) and an exponential law correction. The final  
193 Cu isotopic compositions (δ<sup>65</sup>Cu) were reported relative to NIST SRM976 as follow (Eq.1):

$$194 \quad \delta^{65} Cu_{SRM976}(\text{‰}) = \left( \frac{\left( \frac{^{65}Cu}{^{63}Cu} \right)_{sample}}{\left( \frac{^{65}Cu}{^{63}Cu} \right)_{SRM\ 976}} - 1 \right) \times 1000 \quad (\text{Eq. 1})$$

195 For the Zn isotope analyses, a Cu internal standard (Cu<sub>SRM976</sub>) was added to the purified Zn  
 196 fractions and the mixture of external standards (Cu<sub>SRM976</sub> + Zn<sub>ETH</sub>) was unchanged. Due to the  
 197 depletion of the Zn standard (Zn<sub>JMC-Lyon</sub>), we replaced this latter by a new reference standard  
 198 (Zn<sub>ETH</sub>). However, we chose to express our  $\delta^{66}\text{Zn}$  values relative to JMC-Lyon in order to  
 199 compare our data with the literature (Araújo et al., 2017). Therefore, the  $\delta^{66}\text{Zn}_{\text{ETH}}$  values were  
 200 converted to  $\delta^{66}\text{Zn}_{\text{JMC-Lyon}}$  values by adding a factor of 0.28‰ as recommended by Archer et  
 201 al. (2017). The final Zn isotopic compositions ( $\delta^{66}\text{Zn}$ ) relative to JMC-Lyon were expressed  
 202 as follow (Eq. 2):

$$203 \quad \delta^{66}\text{Zn}_{\text{JMC-Lyon}}(\text{‰}) = \left( \frac{\left( \frac{{}^{66}\text{Zn}}{{}^{64}\text{Zn}} \right)_{\text{sample}}}{\left( \frac{{}^{66}\text{Zn}}{{}^{64}\text{Zn}} \right)_{\text{ETH}}} - 1 \right) \times 1000 + 0.28 \quad (\text{Eq. 2})$$

204 External precision ( $2\sigma$ ) was obtained from repeated (2 to 3) measurements of samples.  
 205 Long-term precision was assessed from plankton certified materials (BCR 414). The  
 206  $\delta^{65}\text{Cu}_{\text{BCr414}}$  ( $-0.13 \pm 0.12\text{‰}$ ; n=2) agreed with reported values in literature ( $-0.29 \pm 0.10\text{‰}$ ;  
 207 Yang et al., 2020;  $-0.27 \pm 0.05\text{‰}$ ; Takano et al., 2020). The  $\delta^{66}\text{Zn}_{\text{BCr414}}$  ( $+0.42 \pm 0.04\text{‰}$ ; n=2)  
 208 fell in the same range of published recommended value ( $+0.36 \pm 0.10\text{‰}$ ; Druce et al., 2020)  
 209 and agreed with reported values in literature ( $+0.42 \pm 0.04\text{‰}$ ; Maréchal et al., 2002).

210

#### 211 2.2.4. Isotopic analysis

212 Freeze-dried samples were ground to fine powder with an agate mortar and pestle.  
 213 Approximately 0.5 mg of powder was weighed into a tin cup ( $8 \times 5$  mm) using a precision  
 214 balance ( $d = 0.00001$  g). N isotopic compositions were measured using elemental analyzer  
 215 mass spectrometer Integra CN Sercon (Raimbault et al., 2008; Lacoste et al., 2016). N  
 216 isotopes ( $^{14}\text{N}$ ,  $^{15}\text{N}$ ) were carried out at the PACEM platform (Mediterranean Institute of  
 217 Oceanography, Marseilles). The  $\delta^{15}\text{N}$  composition was reported relative to conventional  
 218 standard (atmospheric  $\text{N}_2$ ) as follow (Eq. 3):

$$\delta^{15}\text{N}_{\text{N}_2}(\text{‰}) = \left( \frac{\left( \frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{sample}}}{\left( \frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{N}_2}} - 1 \right) \times 1000 \quad (\text{Eq. 3})$$

External precision (0.5 ‰) was obtained from repeated (2 to 3) measurements of samples.

221

## 2.3. Data processing

### 2.3.1. Biotic trace metal concentration

Marine particles are composed of complex assemblages of biotic (e.g., organic matter from living and dead organisms) and geogenic (e.g., minerals from natural and anthropogenic sources) components (Jeandel et al., 2015; Lam et al., 2018). The relative contribution of the latter component depends on the proximity and intensity of sources. In the field, the separation between biotic and geogenic particles remains an operationally challenging and requires an ultra-clean environment and specific equipment to avoid contaminations (Cullen and Sherrell, 1999; Plaquette and Sherrell, 2012).

However, using normalized ratios of particulate organic phosphorus (P-normalization), a biotic component equated to the fraction of organic matter can be estimated in marine particles (Ho et al., 2007; Liao et al., 2017).

$$[M]_{\text{sample}} = b \cdot [\text{POP}]_{\text{b}} + a \cdot [\text{Ti}]_{\text{a}} \quad (\text{Eq. 4})$$

$$\frac{[M]_{\text{sample}}}{[\text{Ti}]_{\text{sample}}} \equiv b \cdot \frac{[\text{POP}]_{\text{sample}}}{[\text{Ti}]_{\text{sample}}} + a \quad (\text{Eq. 5})$$

where  $a$  is Ti-normalized elemental ratio in the geogenic component,  $b$  is P-normalized elemental ratio in the biotic component,  $[M]_{\text{sample}}$  is elemental concentration in the sample,  $[\text{Ti}]_{\text{a}}$  is Ti concentration in the geogenic component, and  $[\text{POP}]_{\text{b}}$  is concentration of particulate organic phosphorus in the biotic component. Assuming that POP and Ti in samples are mainly due to the biotic and geogenic components, respectively ( $[\text{POP}]_{\text{sample}} \equiv [\text{POP}]_{\text{b}}$ ;

241  $[\text{Ti}]_{\text{sample}} \equiv [\text{Ti}]_{\text{a}}$ ), we can turn the mass balance formula into a first-order equation (Eq. 5) and  
242 resolved the model.

243 This technique has been extensively detailed by Chifflet et al. (2022) as companion  
244 paper and results (biotic, %) are summarized in Table 2.

245

### 246 2.3.2. Statistical analyses

247 Spearman correlation analyses were performed to identify relations between  
248 environmental variables using Xlstat software package version 2019.1.1 (Addinsoft 2020,  
249 Boston, USA, <https://www.xlstat.com>). A principal component analysis (PCA) was carried out  
250 to extract the key biological factors driving Cu and Zn isotopic composition based on the  
251 Spearman correlation test, with a statistical significance level higher than 95% ( $p < 0.05$ ). It  
252 was performed using the abundance of bacterial, phytoplanktonic and zooplanktonic  
253 communities identified in samples and associated Cu, Zn isotope compositions.

254

255

## 256 3. Results and discussion

### 257 3.1. Cu and Zn isotopic composition in plankton

258 In the present study, isotopic compositions ( $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$ ) in samples are explored  
259 in relation to their respective concentrations (Cu and Zn) in four particles size fractions (F3:  
260 60–200  $\mu\text{m}$ , F4: 200–500  $\mu\text{m}$ , F5: 500–1000  $\mu\text{m}$  and F6: 1000–2000  $\mu\text{m}$ ) collected into the  
261 DCM in order to better understand the biogeochemical cycles of Cu and Zn within the  
262 Mediterranean planktonic food web (Fig. 2; Table 3). Note that some samples (*i.e.*, St1-F3  
263 and St1-F6) had too little amount of Cu and Zn ( $< 1000$  ng) to perform isotopic analysis,  
264 which explains some missing data in Table 3. All other data of the MERITE-HIPPOCAMPE  
265 campaign for trace metal (As, Cd, Cr, Cu, Fe, Mn, Ni, Sb, Ti, V, Zn) and associated

266 particulate organic phosphorus (POP) in the four size fractions are detailed in a companion  
267 paper (Chifflet et al., 2022).

268 The Cu isotopic composition ( $\delta^{65}\text{Cu}$ ) in the different stations and planktonic size  
269 fractions (60–2000  $\mu\text{m}$ ) ranged from +0.05 to +1.23‰ and was isotopically heavier than the  
270 particulate fraction (-0.03 to +0.54‰; 0.8-51  $\mu\text{m}$ ; Little et al., 2018) and marine aerosols  
271 (+0.00 to +0.18‰; Little et al., 2014) measured in the South Atlantic. The Zn isotopic  
272 composition ( $\delta^{66}\text{Zn}$ ) in the different stations and planktonic size fractions (60–2000  $\mu\text{m}$ ) was  
273 relatively narrower, varying from +0.33 to +0.59‰. It was also isotopically heavier than  
274  $\delta^{66}\text{Zn}$  measured in marine particles of the central Atlantic Ocean (+0.15 to +0.43‰; Maréchal  
275 et al., 2000). It should be noticed that the MERITE-HIPPOCAMPE campaign was conducted  
276 in spring and, an increase of  $\delta^{66}\text{Zn}$  values was already observed during bloom events in  
277 oceanic waters (Maréchal et al., 1998) as well as in lacustrine systems (Peel et al., 2009). A  
278 general examination of data did not allow to identify any clear trend between  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$   
279 and their respective concentrations (Cu and Zn), neither as a function of spatial distribution,  
280 nor as a function of size fractions (Fig. S1 as supplementary information).

281 The distribution of trace metal in planktonic food web is driven by complex  
282 biogeochemical cycles (Collier and Edmond, 1984; Bruland and Lohan, 2003; Sunda, 2012).  
283 As micronutrients, Cu and Zn are essential to phytoplankton activities through photosynthesis  
284 processes and construction of macromolecules (Morel et al., 2008). Then, trace metal are  
285 transferred into the trophic food web through dietary pathway (Sun et al., 2020). Besides  
286 biological processes, geogenic particles issued from rivers, effluents and atmospheric  
287 deposition (Radakovich et al., 2008; Oursel et al., 2013) may contribute to the  $\delta^{65}\text{Cu}$  and  
288  $\delta^{66}\text{Zn}$  signatures found in plankton. Hence, the  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  fingerprint observed here in  
289 the different planktonic size fractions may result from both biological/trophic transfer  
290 processes and geogenic inputs, the discrimination between the two remaining technically

291 challenging (Cullen and Sherrell, 1999; Cloquet et al., 2008; Plaquette and Sherrell, 2012;  
292 [Bacconnais et al., 2019](#)).

293

### 294 *3.2. Geogenic contribution on Cu and Zn isotopic compositions in plankton*

295 Geogenic particles (*i.e.* minerals from natural and anthropogenic sources) are  
296 ubiquitous and enter in marine ecosystems through various processes such as weathering,  
297 atmospheric deposition, sediment resuspension ([Bianchi, 2007](#)). The variations of  $\delta^{65}\text{Cu}$  and  
298  $\delta^{66}\text{Zn}$  in geogenic particles are greater than 1‰ and encompass multiple natural and  
299 anthropogenic sources ([Fig. 3](#)). For example, in the natural soil, due to the large variability of  
300 geological zones,  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  vary widely from -0.15 to +0.44‰ and from -0.03 to  
301 +0.43‰, respectively ([Fekiacova et al., 2015](#)). Similar variations are also found in Saharan  
302 dusts ( $\delta^{65}\text{Cu} = -0.01 \pm 0.14\text{‰}$  and  $\delta^{66}\text{Zn} = +0.19 \pm 0.15\text{‰}$ ; [Schleicher et al., 2020](#)) or marine  
303 particles ( $\delta^{65}\text{Cu} = +0.23 \pm 0.20\text{‰}$ ; [Little et al., 2018](#) and  $\delta^{66}\text{Zn} = +0.35 \pm 0.08\text{‰}$ ; [Maréchal et](#)  
304 [al., 2002](#)). Based on a compilation of results from basaltic and ultramafic samples from  
305 different geological zones, the isotopic fingerprint of Cu and Zn in a bulk silicate earth (BSE)  
306 can be estimated as  $\delta^{65}\text{Cu} = +0.06 \pm 0.20\text{‰}$  and  $\delta^{66}\text{Zn} = +0.28 \pm 0.05\text{‰}$  ([Chen et al., 2013](#);  
307 [Liu et al., 2015](#)). However, establishing a natural baseline seems difficult to achieve because  
308 similar  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  can also be found in other natural particles such as carbonates ( $\delta^{66}\text{Zn}$   
309 =  $+0.73 \pm 0.41\text{‰}$ ; [Fekiacova et al., 2015](#)), Fe-Mn nodules ( $\delta^{65}\text{Cu} = +0.31 \pm 0.23\text{‰}$  and  $\delta^{66}\text{Zn}$   
310 =  $+0.90 \pm 0.28\text{‰}$ ; [Maréchal et al., 2000](#)), chalcopyrite ( $\delta^{65}\text{Cu} = -1.91 \pm 2.50\text{‰}$ ; [Mathur et al.,](#)  
311 [2005; 2014](#)) and sphalerite ( $\delta^{66}\text{Zn} = +0.17 \pm 0.20\text{‰}$ ; [Sonke et al., 2008](#)).

312 The artificial anthropogenic processes, such as ore refining and electrolysis, can also  
313 fractionate isotopes leading to fingerprints in byproducts distinguishable from natural  
314 background. Redox processes can induce Cu isotopic fractionation, whereby  $\text{Cu}^{\text{II}}$ -bound  
315 products are enriched in heavier isotopes while  $\text{Cu}^{\text{I}}$ -bound products are preferentially

316 composed of lighter isotopes (Ehrlich et al., 2004). As a result, major  $\delta^{65}\text{Cu}$  variations in  
317 anthropogenic reservoirs can be found in pesticides (from -0.49 to +0.89‰; Blotevogel et al.,  
318 2018), antifouling paints (Briant et al., 2022), urban aerosol (Souto-Oliviera et al., 2018;  
319 2019) and in traffic-related inputs (from -0.18 to +0.71‰ with a median value of +0.34‰;  
320 Dong et al., 2017). Due to its low boiling point temperature and high fractionation in  
321 evaporation–condensation processes, Zn isotopic fractionation varies widely in coal activities  
322 (-0.10 to +1.35‰; Desaulty et al., 2020). In addition to contamination from fossil combustion,  
323 industrial activities also release Zn particles. For example,  $\delta^{66}\text{Zn}$  varies from +0.5 to +1.3‰  
324 in metallurgic and electroplating waste (Sirvy et al., 2008; Araújo et al., 2017), from -0.1 to  
325 +0.6‰ in non-exhaust traffic emission (Dong et al., 2017), from +0.11 to +0.71‰ in  
326 galvanized hardware (John et al., 2007b), from +0.38 to +0.76‰ near Pb-Zn smelter (Juillot  
327 et al., 2011), from +0.40 to +0.46‰ in ZnO fumes (Shield et al., 2010), and from +0.18 to  
328 +0.20‰ in N-fertilizer or +0.40 to +0.44‰ in P-fertilizer (Chen et al., 2008).

329 To assess the potential influence of geogenic particles on Cu and Zn isotopic  
330 compositions of Mediterranean plankton,  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations were examined in  
331 relation to Cu/POP and Zn/POP in the four size fractions, according to the three geographical  
332 zones (Fig. 4). Two pools of samples stand out. The first pool (Pool 1) was composed of  
333 samples with great  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations (from +0.05 to +1.23‰ and from +0.33 to  
334 +0.59‰, respectively) in relation to low Cu/POP and Zn/POP ratios (from 1.1 to 3.4  $\mu\text{g}/\text{mg}$   
335 and from 20.9 to 44.9  $\mu\text{g}/\text{mg}$ , respectively). The second pool (Pool 2) was composed of  
336 samples with lower  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations (from +0.29 to +0.59‰ and from +0.35 to  
337 +0.48‰, respectively) in relation to higher Cu/POP and Zn/POP ratios (from 5.9 to 8.7  $\mu\text{g}/\text{mg}$   
338 and from 63.6 to 100.7  $\mu\text{g}/\text{mg}$ , respectively).

339 According to our companion paper (Chifflet et al., 2022), samples from Pool 2 seemed  
340 dominated by a geogenic component since they samples were poor in organic matter with a

341 biotic component < 40%. Otherwise, the samples from Pool 1 were organic rich with a biotic  
342 component ranging from 60 to 100% (Table 2). The Ti is known to be a geogenic element  
343 (Ohnemus and Lam, 2015) unaffected by biological uptake or particulate recycling  
344 (Dammshäuser et al., 2013). Therefore, Ti/POP ratio can be used to represent geogenic vs  
345 biotic particles. While the Cu/POP vs Ti/POP diagram showed clear relationships with  
346 samples from the same geographic area, these trends appeared more nuanced with the Zn  
347 diagram (Fig. 5). Due to geographic characteristics (distance from shore, bathymetry), waters  
348 from the northern and southern coastal zones were very likely more influenced by weathering  
349 processes and sediment resuspension than were waters from the offshore zone. The increase  
350 of Cu/POP and Ti/POP ratios were concomitant to the relative decrease of the biotic  
351 compartment against the geogenic counterpart. The two linear trends observed in the Cu  
352 diagram probably represented a ‘geogenic-dilution’ line of the northern and southern coastal  
353 zones. Slopes suggest different geogenic sources and outpoints (e.g. St1-F6, St2-F3, St4-F5,  
354 St17-F4) could be due to poor separation of plankton size fractions in the field. The offshore  
355 values overlap the lower end of the ‘geogenic-dilution’ lines, showing a mixture of geogenic  
356 particles in both the northern and southern coastal zones. Different anthropogenic source  
357 contributions could explain the different slopes and the isotopic fingerprint overlapping.  
358 Therefore, Cu and Zn geogenic sources could not be identified precisely in our samples. On-  
359 board particle sieving could not be as selective as expected and geogenic particles may have  
360 remained “stuck” or accumulated with some fractions when washing was not performed  
361 optimally.

362

### 363 *3.3. Effects of the trophic transfer and homeostasis on Cu and Zn isotopic compositions*

364 Cu and Zn isotopic compositions increased with  $\delta^{15}\text{N}$ , indicating an enrichment in  
365 heavy isotopes in the higher trophic levels (Fig. 6). Only samples from the southern coastal

366 zone did not follow this pattern. The trophic Cu and Zn isotope fractionation has been already  
367 studied in mammals from terrestrial and marine environments (Jaouen et al., 2013; Araújo et  
368 al., 2021), but isotope patterns in marine food webs remain unknown so far. Despite this gap,  
369 it is recognized that speciation, uptake mechanisms and metabolism are key parameters  
370 controlling trophic isotope fractionation in plankton-zooplankton realm.

371 Cu and Zn are essential elements involved in many metabolic mechanisms making it  
372 difficult to identify the main biological mechanisms driving their isotopic fractionation in the  
373 frame of field studies. Indeed, each metabolic pathway (adsorption, precipitation, redox) can  
374 induce isotopic fractionation in favor of one isotope or another. For example, Cu uptake into a  
375 biofilm can cause a relative enrichment of  $^{65}\text{Cu}$  and Cu uptake across the cell membrane can  
376 favor  $^{63}\text{Cu}$  (Bigalke et al., 2010; Courtaud et al., 2014). Furthermore, the enzymatic activity  
377 involved in  $\text{Cu}^{\text{I}}\text{-Cu}^{\text{II}}$  redox processes modifies the Cu fractionation with  $\text{Cu}^{\text{I}}$  species in favor  
378 of light isotope (Zhu et al., 2002; Ehrlich et al., 2004; Navarrete et al., 2011; Fujii et al., 2013;  
379 Moynier et al., 2017). Similar to Cu, biological processes can also be invoked to explain Zn  
380 isotopic composition in the planktonic food web. Previously,  $^{66}\text{Zn}$  enrichment in planktonic  
381 biomass was found to vary from +0.19 and +0.43‰ depending on species and environmental  
382 conditions (Maréchal et al., 2000; Gélabert et al. 2006; John et al. 2007a). In natural  
383 environment, the main processes inducing Zn fractionation are adsorption (with organic or  
384 inorganic ligands) and diffusion (Cloquet et la., 2008; Coutaud et al., 2014). While Zn  
385 adsorption on organic matter and carrier proteins are mechanisms of heavy isotope  
386 enrichment in marine organisms (Weiss et al., 2005; Gélabert et al., 2006; John and Conway,  
387 2014), diffusion of Zn through the phytoplanktonic cell is preferentially into lighter Zn (John  
388 et al., 2007a; Peel et al., 2009; John and Conway, 2014).

389 During the MERITE-HIPPOCAMPE campaign, samples from four size fractions were  
390 subsampled for bacterial (Quéméneur et al., 2022), phytoplanktonic (Bellaaj Zouari et al.,

391 2022) and zooplanktonic (Fierro-González et al., 2022) diversity, taxonomy and abundance.  
392 Hence, Spearman correlations between  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  values and abundances of various  
393 bacteria, phytoplankton and zooplankton species were investigated (Table S1 as  
394 supplementary information). When considering all stations, significant ( $p < 0.05$ ) but weak  
395 correlations ( $r < 0.5$ ) were found for all parameters. However, some discrepancies were  
396 observed at regional scale. In the northern coastal zone,  $\delta^{66}\text{Zn}$  was strongly correlated with the  
397 relative abundance of the phylum *Firmicutes* ( $r = 0.81$ ,  $p < 0.05$ ) (Table S2 as supplementary  
398 information), which includes heterotrophic bacteria usually present during decomposition  
399 processes in water and sediments (Zhao et al., 2017). In the offshore zone,  $\delta^{65}\text{Cu}$  was strongly  
400 correlated with *Prochlorococcus sp.*, the phylum *Cyanobacteria* and picoeukaryotes ( $r = 0.78$ ,  
401  $p < 0.05$ ) (Table S3 as supplementary information). In the southern coastal zone, while  $\delta^{65}\text{Cu}$   
402 and  $\delta^{66}\text{Zn}$  were moderately correlated with *Firmicutes* ( $r = -0.66$  and  $0.57$ ,  $p < 0.05$ ,  
403 respectively),  $\delta^{65}\text{Cu}$  was strongly correlated with zooplanktonic organisms, i.e., copepods ( $r =$   
404  $-0.94$ ,  $p < 0.05$ ) (Table S4 as supplementary information).

405 Under strong anthropogenic pressure, coastal waters receive both large inputs of  
406 nutrients that can stimulate phytoplanktonic activities (Cruzado and Velasquez, 1990; Macias  
407 et al., 2019) and a cocktail of contaminants that can induce changes in the biodiversity of  
408 primary producers (Hulot et al., 2000; Johnston and Roberts, 2009; Goni-Urriza et al., 2018).  
409 In such conditions, Cu can reduce copepods eggs production, thus negatively impact primary  
410 predator communities (Lauer and Bianchini, 2010). Furthermore, bacteria are known to  
411 strongly fractionate Cu ( $\Delta^{65}\text{Cu}_{\text{media-cell}}$  from +1.0 to +4.4‰; Navarrete et al., 2010), and lower  
412 interactions between bacteria and phytoplankton were already observed by Pringault et al.  
413 (2020; 2021) in contaminated Bizerte Lagoon (southeastern Mediterranean Sea, Tunisia). The  
414 lower  $\delta^{65}\text{Cu}$  values in the southern coastal zones and the strong anti-correlation with copepods  
415 could suggest a discrepancy in the southern coastal zone. The impact of Cu in the Gulf of

416 Gabès (southern coastal zone) should be further investigated as a significant transfer of Cu  
417 into the planktonic food web was also observed in a companion study (Chifflet et al., 2022).

418

419

#### 420 4. Conclusions

421 In this study, we propose an isotopic approach to examine the Cu and Zn transfers in  
422 the planktonic food web in three geographical areas of the Mediterranean Sea: the northern  
423 coastal zone (bays of Toulon and Marseille, France), the offshore zone (near the Balearic  
424 Thermal Front) and the southern coastal zone (Gulf of Gabès, Tunisia). Overall, geogenic  
425 particles were ubiquitous in the different sizes of plankton (60–200, 200–500, 500–1000,  
426 1000–2000  $\mu\text{m}$ ) and the Cu and Zn isotope compositions seems to reflect a complex mixing  
427 of signatures of geogenic (from natural and anthropic sources) and planktonic assemblages.  
428 Nevertheless, elemental and isotope Cu-Zn data combined with proxies for geogenic (Ti) and  
429 organic matter (POP) indicated that geogenic particles in the northern and southern coastal  
430 zone were of different origins, and that in the offshore, geogenic particles were from a  
431 mixture of northern and southern sources. The  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations were examined in  
432 relation to  $\delta^{15}\text{N}$  and showed trends of heavy isotope enrichment in the planktonic food web  
433 from the northern and offshore zones. Discrepancies were observed between the southern  
434 coastal zone and the latter two zones. While  $\delta^{65}\text{Cu}$  was strongly correlated with  
435 *Prochlorococcus sp.* and picoeukaryotes in the offshore zone,  $\delta^{65}\text{Cu}$  was strongly correlated  
436 with copepods in the southern coastal zone. We suggest that the southern coastal zone  
437 exhibits a shift in the community structure due to Cu biogeochemical interactions.  
438 Furthermore, the bacterial phylum *Firmicutes* was found correlated with  $\delta^{66}\text{Zn}$ , suggesting  
439 decomposition of particulate matter at the DCM in Mediterranean coastal ecosystems.

440 In the framework of the MERITE-HIPPOCAMPE campaign, a companion study  
441 showed amplification of essential and non-essential elements (As, Cu, Cr, Sb) in biotic  
442 compartment of southern coastal zones (Chifflet et al., 2022). The present study confirms the  
443 impact of trace metal on planktonic food web of the Mediterranean Sea. During the campaign,  
444 we encountered challenging weather conditions with winds up to 40 knots and 3 m waves. As  
445 a result, collecting and sieving particles on-board was operationally difficult without time for  
446 replicates. For future research, we recommend limiting studies to homogeneous geographic  
447 areas (same physico-chemical characteristics of waters, same anthropogenic influences) and  
448 collecting plankton in size fractions ranging from 0.2 to 2000  $\mu\text{m}$ .

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452 ***Authors contribution.*** All the authors participated in the MERITE-HIPPOCAMPE project  
453 and contributed to the sampling strategy, preparation of the material, field work, laboratory  
454 analyses and/or data processing and interpretation. SC and NB wrote the original manuscript,  
455 and all the authors participated to its reviewing and/or editing.

456

457

458 ***Acknowledgments.*** The MERITE-HIPPOCAMPE project was initiated and funded by the  
459 “Pollution & Contaminants” transversal axis of the CNRS-INSU MISTRALS program (joint  
460 action of the MERMEX-MERITE and CHARMEX subprograms). It has also received the  
461 financial support of the Franco-Tunisian International Joint Laboratory (LMI) COSYS-Med.  
462 The MERITE-HIPPOCAMPE campaign was organized and supported by the French  
463 (Oceanographic Fleet, CNRS/INSU, IFREMER, IRD) and Tunisian institutions (Ministry of  
464 Agriculture, Hydraulic Resources and Fisheries of Tunisia, Ministry of Higher Education and

465 Scientific Research of Tunisia). The project has also received additional funding from the  
466 MIO's incentive "Action Sud" and "Transverse" programs (CONTAM project) and by the  
467 ANR CONTAMPUMP (ANR JCJC #19-CE34-0001-01). We acknowledge the MIO's  
468 platforms: "Service Atmosphère-Mer" (SAM) for the preparation and management of the  
469 embarked material and, "Plateforme Analytique de Chimie des Environnements Marins"  
470 (PACEM), the staff from LBCM-Ifremer laboratory for various chemical analyses and the  
471 staff from AETE-ISO platform from the OSU-OREME university for the MC-ICP-MS  
472 analyses. The authors are deeply grateful for the thorough and constructive corrections and  
473 insightful comments of the anonymous reviewers, who have significantly improved the  
474 original manuscript.

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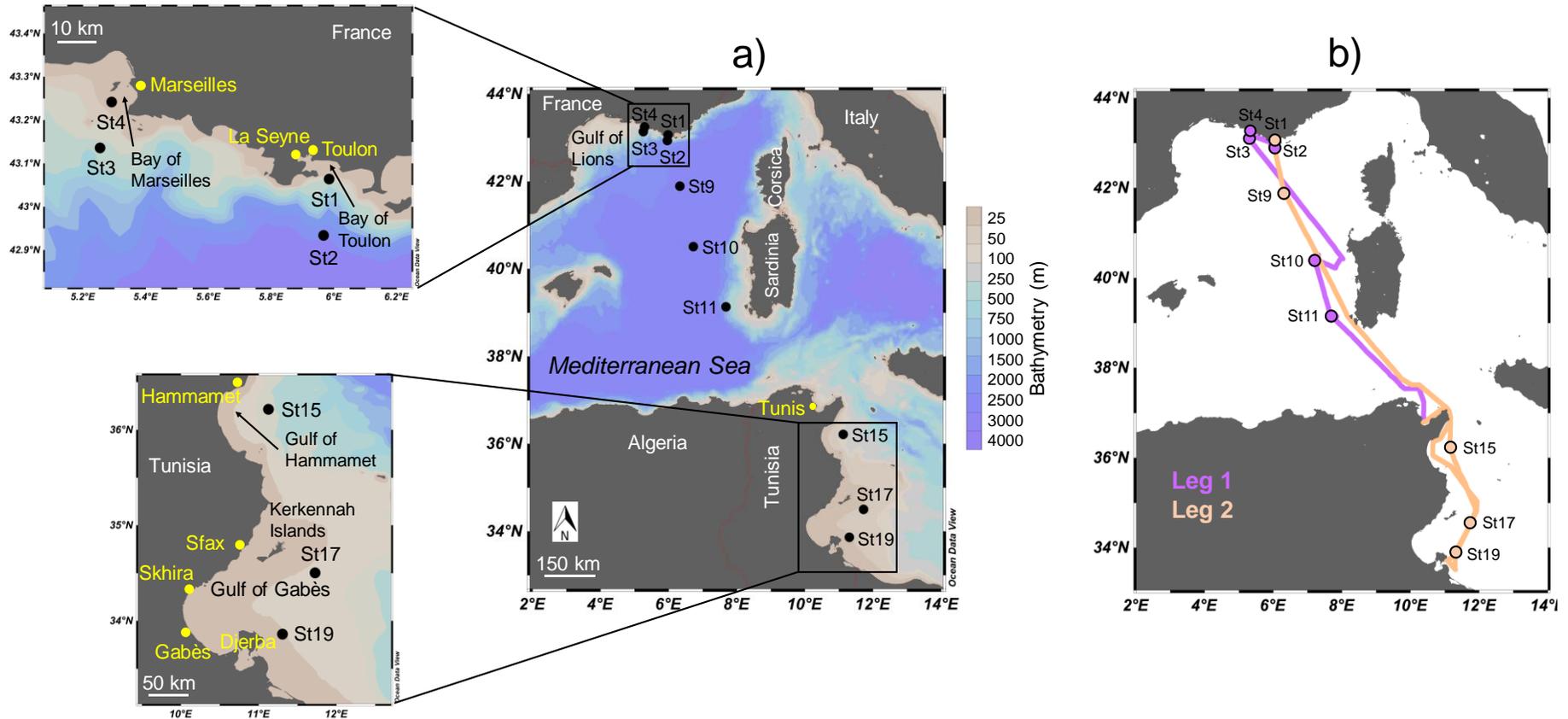
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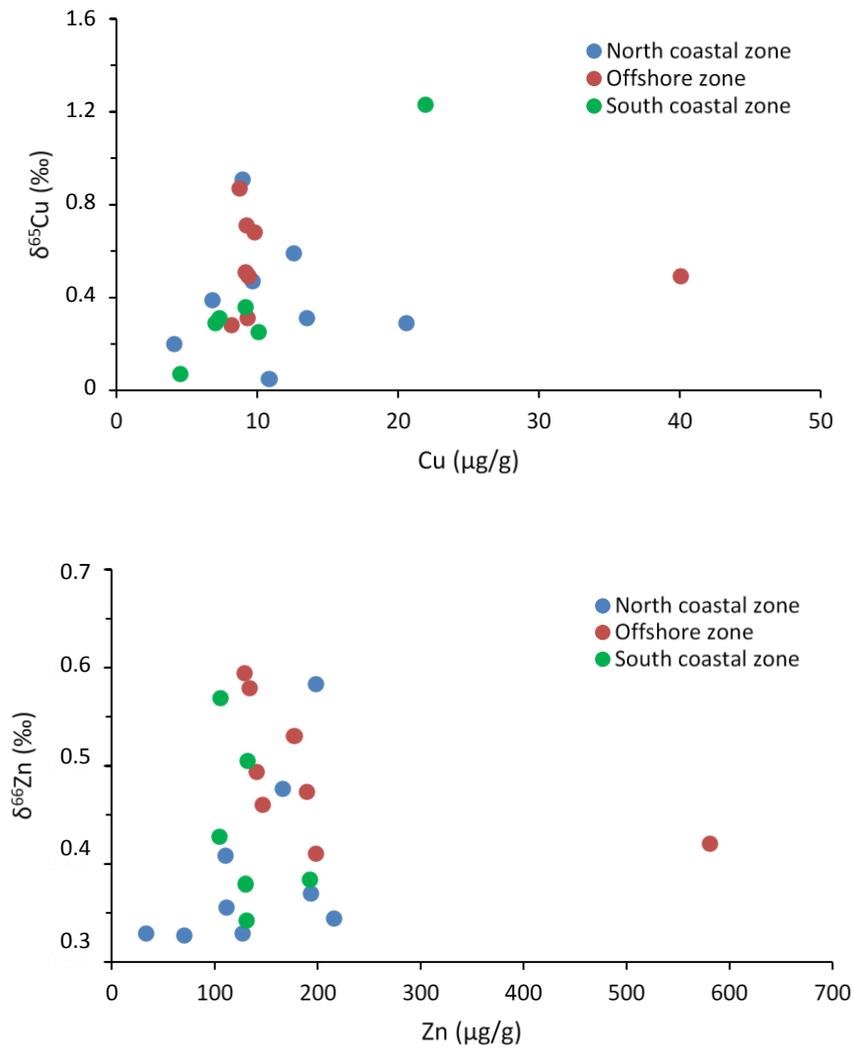
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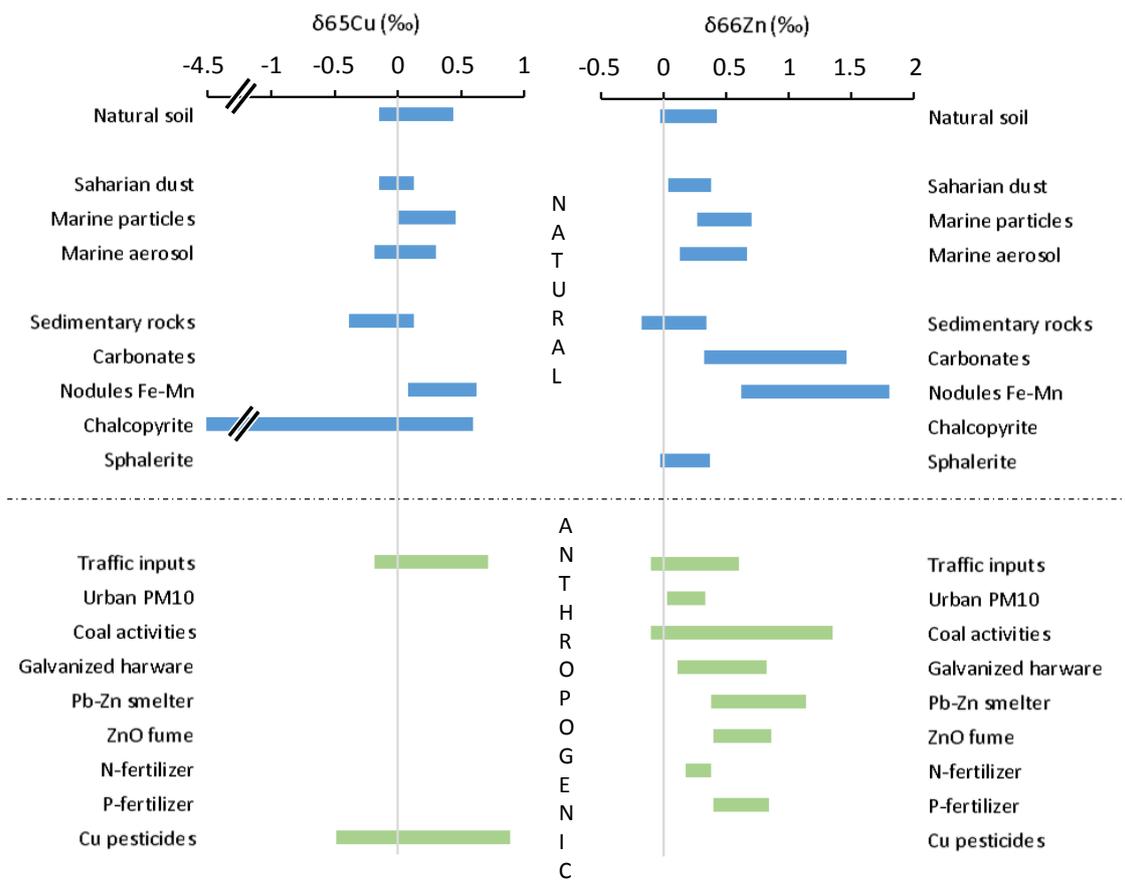
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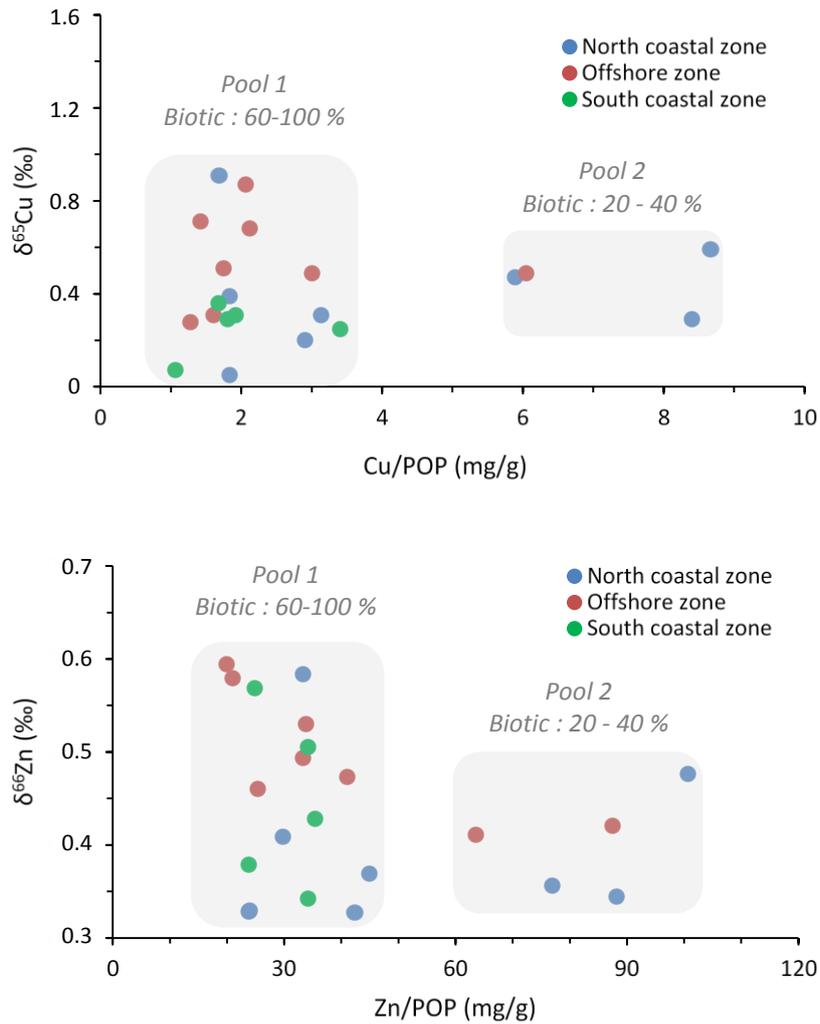
**Figure 1.** a) Location of the sampling stations in the Mediterranean Sea. b) Details of the MERITE-HIPPOCAMPE campaign tracks: Leg 1 (from La Seyne-sur-Mer to Tunis) with five sampling stations (St2, St4, St3, St10 and St11, in this chronological order); Leg 2 (from Tunis to Gulf of Gabès to La Seyne-sur-Mer) with five sampling stations (St15, St17, St19, St9 and St1, in this chronological order).



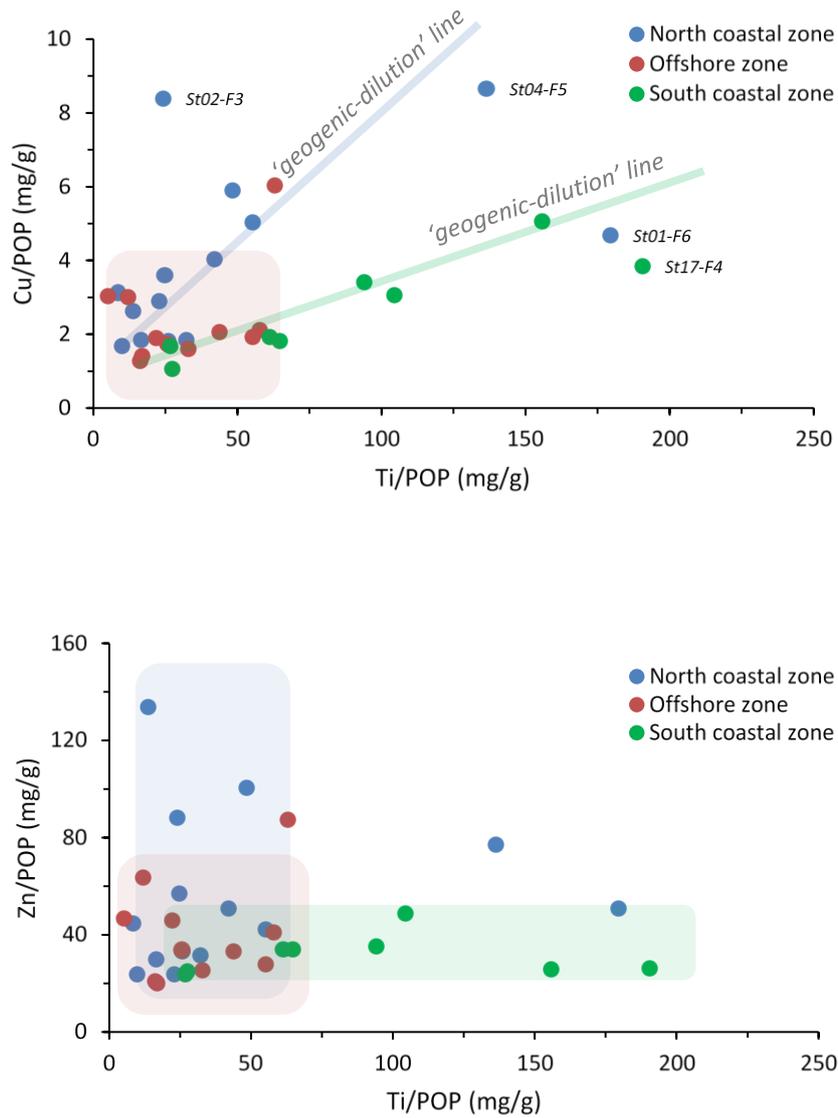
**Figure 2.**  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations relative to Cu and Zn concentrations in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000  $\mu\text{m}$ ), respectively. Isotopic compositions are in ‰ and concentrations in  $\mu\text{g/g}$  of dry sample. Values are grouped according to three geographical zones: the northern coastal zone (blue), the offshore zone (red) and the southern coastal zone (green).



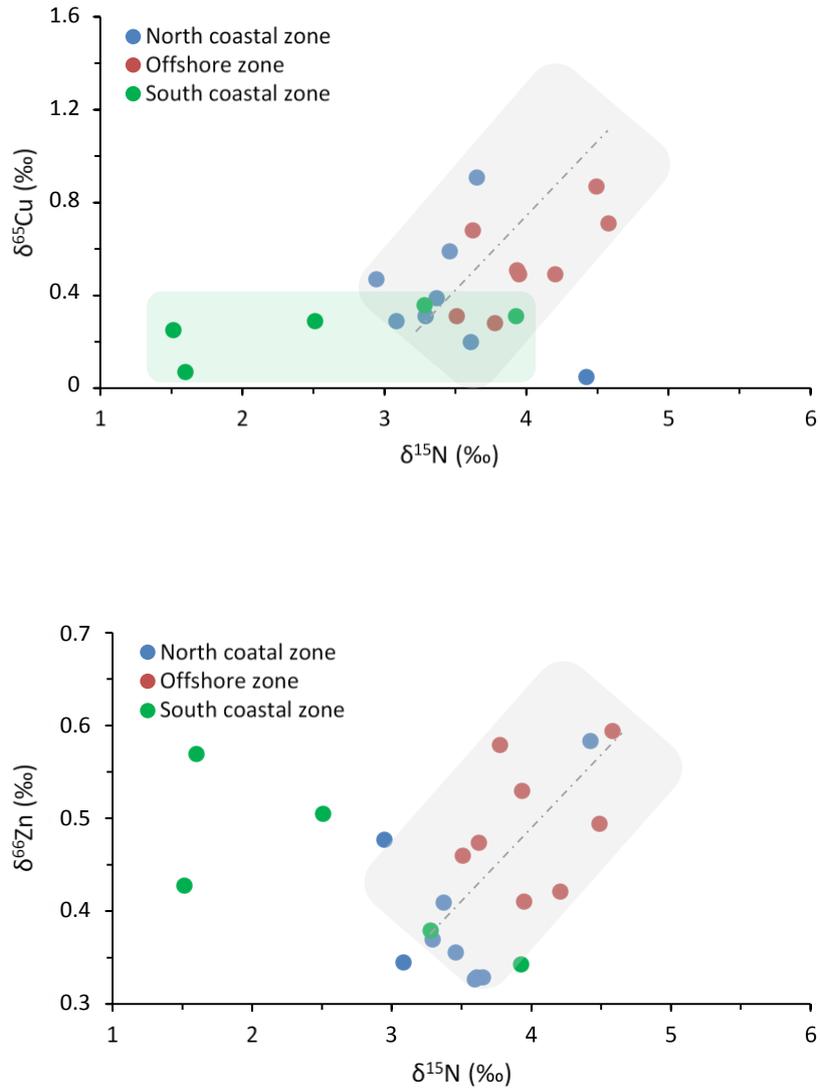
**Figure 3.** Summary of various  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  compositions in geogenic (natural and anthropogenic) sources.



**Figure 4.**  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations relative to their corresponding Cu/POP and Zn/POP ratios in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000  $\mu\text{m}$ ). Values are grouped according to three geographical zones: the northern coastal zone (blue), the offshore zone (red) and the southern coastal zone (green).



**Figure 5.** Cu/POP vs Ti/POP and Zn/POP vs Ti/POP diagrams in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000  $\mu\text{m}$ ). Values are grouped according to three geographical zones: the northern coastal zone (blue), the offshore zone (red) and the southern coastal zone (green).



**Figure 6.**  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  variations relative to their corresponding  $\delta^{15}\text{N}$  in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000  $\mu\text{m}$ ). Isotopic compositions are in ‰. Values are grouped according to three geographical zones: the northern coastal zone (blue), the offshore zone (red) and the southern coastal zone (green).

**Table 1.** General information about the 10 stations sampled during the MERITE-HIPPOCAMPE campaign (April 13-May 14, 2019) aboard the *R/V Antea*.

<b>Station</b>	<b>Latitude (N)</b>	<b>Longitude (E)</b>	<b>Localisation</b>	<b>Depth (m)</b>	<b>DCM (m)</b>	<b>Characteristics</b>
St1	43°03.819'	5°59.080'	Toulon	91	20	Urbanized bay; intermittently bloom area
St2	42°56.020'	5°58.041'	Toulon	1770	25	Limit of continental shelf; intermittently bloom area
St3	43°08.150'	5°15.280'	Marseilles	95	55	Southeast entrance to the Gulf of Lions; intermittently bloom area
St4	43°14.500'	5°17.500'	Marseilles	58	31	Urbanized bay; intermittently bloom area
St9	41°53.508'	6°19.998'	Offshore	2575	20	North of the Balearic Thermal Front; Bloom area
St10	40°18.632'	7°14.753'	Offshore	2791	50	South of the Balearic Thermal Front; intermittently bloom area
St11	39°07.998'	7°41.010'	Offshore	1378	40	Algerian ecoregion; no bloom area
St15	36°12.883'	11°07.641'	Gulf of Hammamet	100	66	Near Sicily channel; No bloom area and high density of small pelagic fishes
St17	34°30.113'	11°43.573'	Gulf of Gabes	48	40	Gabès ecoregion; coastal bloom area, high density of small pelagic fishes
St19	33°51.659'	11°18.509'	Gulf of Gabes	50	40	Gabès ecoregion; coastal bloom area, high density of small pelagic fishes

**Table 2.** Multiparametric values obtained during the MERITE HIPPOCAMPE campaign (spring 2019) in the four planktonic size fractions (F3: 60-200  $\mu\text{m}$ ; F4: 200-500  $\mu\text{m}$ ; F5: 500-1000  $\mu\text{m}$ ; F6: 1000-2000  $\mu\text{m}$ ) and the 10 stations (St1-4, St9-11, St15, St17, St19). Details of trace metal (Ti,  $\mu\text{g/g}$ ), particulate organic phosphorus (POP,  $\text{mg/g}$ ) and biotic component (Biotic, %) were presented in [Chifflet et al. \(2022\)](#) and, details of nitrogen isotopic compositions ( $\delta^{15}\text{N}$ , ‰) were presented in [Tésan-Onrubia et al. \(2022\)](#) as companion papers.

<b>Station</b>	<b>Fraction</b>	<b>Biotic</b> (%)	<b>Ti</b> ( $\mu\text{g/g}$ )	<b>POP</b> ( $\text{mg/g}$ )	<b><math>\delta^{15}\text{N}</math></b> (‰)
<i>Northern coastal zone (Marseilles-Toulon bays)</i>					
St01	F3	68	150.3	4.67	3.20
St01	F4	83	52.7	5.32	3.65
St01	F5	67	153.9	5.94	4.42
St01	F6	35	445.1	2.48	4.26
St02	F3	43	59.1	2.45	3.09
St02	F4	98	36.7	4.31	3.29
St02	F5	81	93.6	3.78	3.48
St02	F6	81	132.6	3.16	3.52
St03	F3	30	79.5	1.64	2.94
St03	F4	42	54.8	3.97	3.39
St03	F5	93	61.6	3.71	3.37
St03	F6	-	-	3.17	3.34
St04	F3	70	32.3	1.40	3.61
St04	F4	40	91.9	1.67	3.60
St04	F5	22	197.6	1.45	3.46
St04	F6	-	243.1	-	-
<i>Offshore zone (near the Balearic Thermal Front and the Algerian ecoregion)</i>					
St09	F3	100	59.4	2.70	1.99
St09	F4	98	16.5	3.19	2.73
St09	F5	90	37.6	3.11	3.95
St09	F6	-	37.8	-	-
St10	F3	74	267.0	4.61	3.62
St10	F4	90	133.9	5.24	3.94
St10	F5	84	186.2	4.24	4.49
St10	F6	89	274.7	4.98	5.67
St11	F3	80	191.1	5.80	3.51
St11	F4	89	103.7	6.39	3.78
St11	F5	23	417.4	6.64	4.20
St11	F6	85	110.4	6.48	4.58
<i>Southern coastal zone (Gulf of Gabès)</i>					
St15	F3	66	316.0	3.03	3.56

St15 F4	83	234.9	3.83	3.93
St15 F5	88	146.1	5.47	3.28
St15 F6	-	264.5	-	-
St17 F3	61	178.1	1.14	1.86
St17 F4	64	290.3	1.52	2.41
St17 F5	-	590.4	-	-
St17 F6	-	-	-	-
St19 F3	60	278.6	2.96	1.51
St19 F4	97	116.5	4.26	1.60
St19 F5	-	251.6	3.88	2.51
St19 F6	-	842.9	-	-

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**Table 3.** Concentrations ( $\mu\text{g/g}$ ) and isotopic compositions ( $\text{‰}$ ) of Cu and Zn in four planktonic size fractions (F3: 60-200  $\mu\text{m}$ ; F4: 200-500  $\mu\text{m}$ ; F5: 500-1000  $\mu\text{m}$ ; F6: 1000-2000  $\mu\text{m}$ ) collected at the deep chlorophyll maximum (DCM) according to three geographical areas (the northern coastal zone, the offshore zone, and the southern coastal zone).

Station	Fraction	Cu ( $\mu\text{g/g}$ )	Zn ( $\mu\text{g/g}$ )	$\delta^{65}\text{Cu}$ ( $\text{‰}$ )	$2\sigma_{\text{Cu}}$ ( $\text{‰}$ )	$\delta^{66}\text{Zn}$ ( $\text{‰}$ )	$2\sigma_{\text{Zn}}$ ( $\text{‰}$ )
<i>Northern coastal zone (Marseilles-Toulon bays)</i>							
St1	F3	8.63	148.10	-	-	-	-
St1	F4	8.95	126.70	0.91	0.02	0.33	0.03
St1	F5	10.85	197.97	0.05	0.01	0.58	0.03
St1	F6	11.60	125.54	-	-	-	-
St2	F3	20.57	216.00	0.29	0.01	0.34	0.03
St2	F4	13.49	193.30	0.31	0.09	0.37	0.03
St2	F5	13.58	214.78	-	-	-	-
St2	F6	12.81	160.32	-	-	-	-
St3	F3	9.69	165.67	0.47	0.01	0.48	0.04
St3	F4	10.42	530.69	-	-	-	-
St3	F5	6.82	110.16	0.39	0.06	0.41	0.03
St3	F6	-	-	-	-	-	-
St4	F3	4.07	33.63	0.2	0.06	0.33	0.03
St4	F4	8.37	70.51	-	-	0.33	0.02
St4	F5	12.55	111.47	0.59	0.01	0.36	0.03
St4	F6	16.99	149.42	-	-	-	-
<i>Offshore zone (near the Balearic Thermal Front and the Algerian ecoregion)</i>							
St9	F3	5.14	124.11	-	-	-	-
St9	F4	9.65	149.54	-	-	-	-
St9	F5	9.36	197.92	0.49	0.03	0.41	0.03
St9	F6	7.50	132.38	-	-	-	-
St10	F3	9.81	189.76	0.68	0.00	0.47	0.04
St10	F4	9.14	177.28	0.51	0.07	0.53	0.04
St10	F5	8.76	141.08	0.87	0.06	0.49	0.04
St10	F6	9.59	139.39	-	-	-	-
St11	F3	9.32	147.16	0.31	0.02	0.46	0.04
St11	F4	8.17	133.56	0.28	0.02	0.58	0.04
St11	F5	40.09	580.68	0.49	0.02	0.42	0.03
St11	F6	9.21	129.32	0.71	0.02	0.59	0.03
<i>Southern coastal zone (Gulf of Gabès)</i>							
St15	F3	9.28	148.03	-	-	-	-
St15	F4	7.33	130.82	0.31	0.02	0.34	0.02
St15	F5	9.14	130.38	0.36	0.01	0.38	0.03

St15	F6	8.18	108.30	-	-	-	-
St17	F3	5.81	29.63	-	-	-	-
St17	F4	5.85	39.70	-	-	-	-
St17	F5	21.92	192.53	1.23	0.01	0.38	0.04
St17	F6	-	-	-	-	-	-
St19	F3	10.08	104.96	0.25	0.05	0.43	0.04
St19	F4	4.52	105.83	0.07	0.01	0.57	0.04
St19	F5	7.02	132.35	0.29	0.01	0.51	0.04
St19	F6	5.62	67.74	-	-	-	-

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