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Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1. Study zone and sampling procedure

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

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

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

2.2. Analytical procedures

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_3, 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_3 (10%). The digested samples
152 were then diluted using HNO_3 (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_2O 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

assure no bias effect on isotope measurements (Maréchal and Albarède, 2002; Petit et al., 2008; Schleicher et al., 2020). Procedural blanks were below 1% of Zn and Cu mass contents in fractions. After purification, Cu and Zn fractions were near-dried and re-dissolved in HNO_3 for isotope ratio measurements.

2.2.3. Isotopic analysis

Cu and Zn isotopic compositions were measured by MC-ICP-MS (Neptune Plus™; ThermoScientific) in low mass resolution mode using a SIS (Stable Introduction System) nebulization chamber and a low flow PFA self-aspiration nebulizer. Cu isotopes (^{63}Cu, ^{65}Cu) were measured at the PSO platform (Ifremer, Brest). Cu fractions were dissolved at 250 µg/L in HNO_3 (2%, v/v) and introduced at 50 µL/min. Zinc isotopes (^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn) were measured at the AETE-ISO platform (OSU-OREME university, Montpellier). Zn fractions were dissolved at 250 µg/L in HNO_3 (1%, v/v) and introduced at 70 µL/min. Zinc isotopes (^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn), Cu isotopes (^{63}Cu, ^{65}Cu) and Ni isotope (^{62}Ni) were monitored simultaneously and ^{62}Ni was used to correct possible isobaric ^{64}Ni interferences on ^{64}Zn.

Instrumental mass fractionation was corrected as described by Bermin et al. (2006) and Zhao et al. (2014). For the Cu isotope analyses, a Zn internal standard (Zn_{ETH}) was added to the purified Cu fractions and a mixture of external standards (Cu_{SRM976} + Zn_{ETH}) was run with a standard bracketing sequence. The mass bias correction on the $^{65}Cu/^{63}Cu$ ratio was performed combing the internal and external normalization using Zn isotopes ratios ($^{66}Zn/^{64}Zn$, $^{67}Zn/^{64}Zn$, $^{68}Zn/^{64}Zn$, and $^{68}Zn/^{66}Zn$) and an exponential law correction. The final Cu isotopic compositions ($\delta^{65}Cu$) were reported relative to NIST SRM976 as follow (Eq.1):

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

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

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

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

2.2.4. Isotopic analysis

Freeze-dried samples were ground to fine powder with an agate mortar and pestle. Approximately 0.5 mg of powder was weighed into a tin cup (8 × 5 mm) using a precision balance (d = 0.00001 g). N isotopic compositions were measured using elemental analyzer mass spectrometer Integra CN Sercon (Raimbault et al., 2008; Lacoste et al., 2016). N isotopes (^{14}N, ^{15}N) were carried out at the PACEM platform (Mediterranean Institute of Oceanography, Marseilles). The $\delta^{15}N$ composition was reported relative to conventional standard (atmospheric N_2) as follow (Eq. 3):

$$\delta^{15}N_{N2}(‰) = \left(\frac{\left(\frac{^{15}N}{^{14}N}\right)_{sample}}{\left(\frac{^{15}N}{^{14}N}\right)_{N2}} - 1 \right) \times 1000 \qquad \text{(Eq. 3)}$$

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

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).

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

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

where a is Ti-normalized elemental ratio in the geogenic component, b is P-normalized elemental ratio in the biotic component, $[M]_{sample}$ is elemental concentration in the sample, $[Ti]_a$ is Ti concentration in the geogenic component, and $[POP]_b$ 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 ($[POP]_{sample} \equiv [POP]_b$;

241 $[Ti]_{sample} \equiv [Ti]_a$), 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 (δ^{65}Cu and δ^{66}Zn) in samples are explored
259 in relation to their respective concentrations (Cu and Zn) in four particles size fractions (F3:
260 60–200 µm, F4: 200–500 µm, F5: 500–1000 µm and F6: 1000–2000 µm) 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 (δ^{65}Cu) in the different stations and planktonic size
269 fractions (60–2000 µm) ranged from +0.05 to +1.23‰ and was isotopically heavier than the
270 particulate fraction (-0.03 to +0.54‰; 0.8-51 µm; 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 (δ^{66}Zn) in the different stations and planktonic size fractions (60–2000 µm) was
273 relatively narrower, varying from +0.33 to +0.59‰. It was also isotopically heavier than
274 δ^{66}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 δ^{66}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 δ^{65}Cu and δ^{66}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 δ^{65}Cu and
288 δ^{66}Zn signatures found in plankton. Hence, the δ^{65}Cu and δ^{66}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

challenging (Cullen and Sherrell, 1999; Cloquet et al., 2008; Plaquette and Sherrell, 2012; Baconnais et al., 2019).

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

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

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

316 composed of lighter isotopes (Ehrlich et al., 2004). As a result, major δ^{65}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, δ^{66}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, δ^{65}Cu and δ^{66}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 δ^{65}Cu and δ^{66}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 µg/mg
335 and from 20.9 to 44.9 µg/mg, respectively). The second pool (Pool 2) was composed of
336 samples with lower δ^{65}Cu and δ^{66}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 µg/mg
338 and from 63.6 to 100.7 µg/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

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

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

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

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

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

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

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

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

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

4. Conclusions

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

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

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

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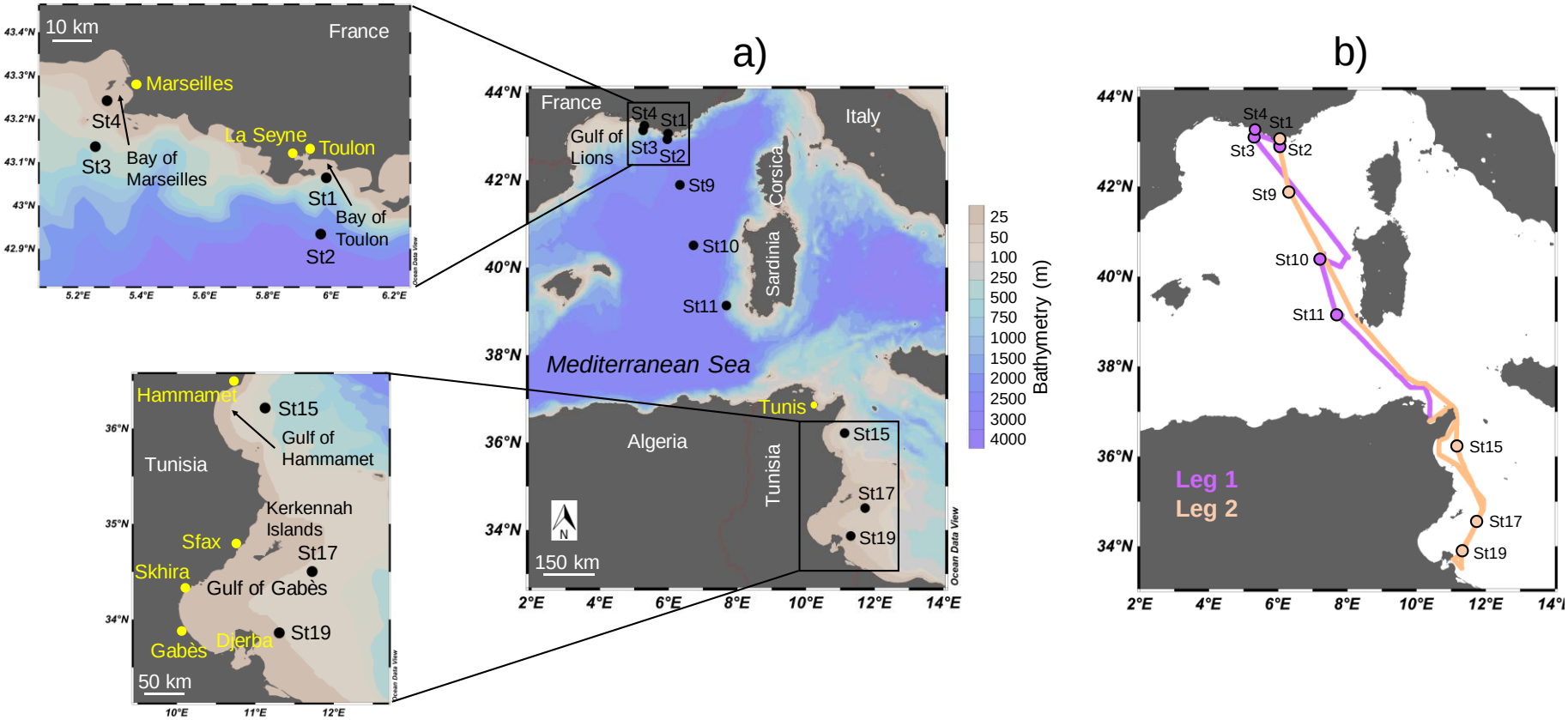

Figure 1. a) Location of the sampling stations in the Mediterranean Sea. b) Details of the MERITE-HIPPOCAMPE campaign tracks: Leg 1 (from La Seyne-sur-Mer to Tunis) with five sampling stations (St2, St4, St3, S10 and St11, in this chronological order); Leg 2 (from Tunis to Gulf of Gabès to La Seyne-sur-Mer) with five sampling stations (St15, St17, St19, St9 and St1, in this chronological order).

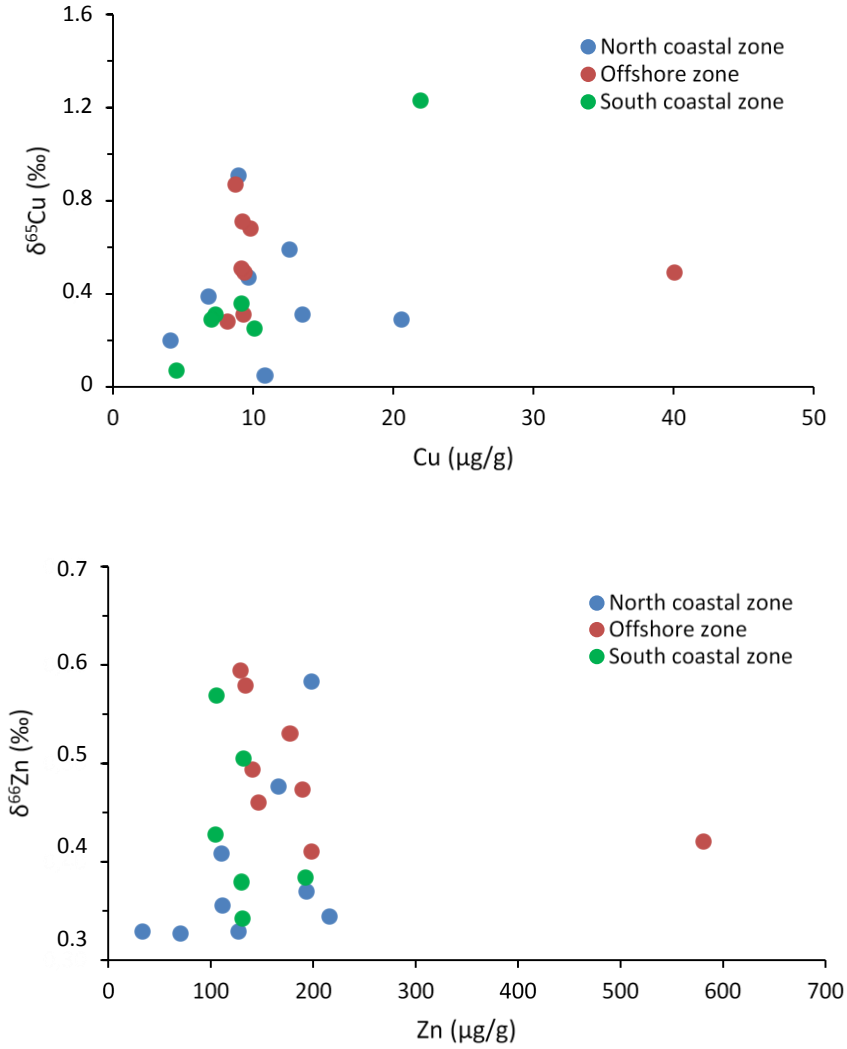

Figure 2. δ^{65}Cu and δ^{66}Zn variations relative to Cu and Zn concentrations in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000 µm), respectively. Isotopic compositions are in ‰ and concentrations in µg/g of dry sample. Values are grouped according to three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

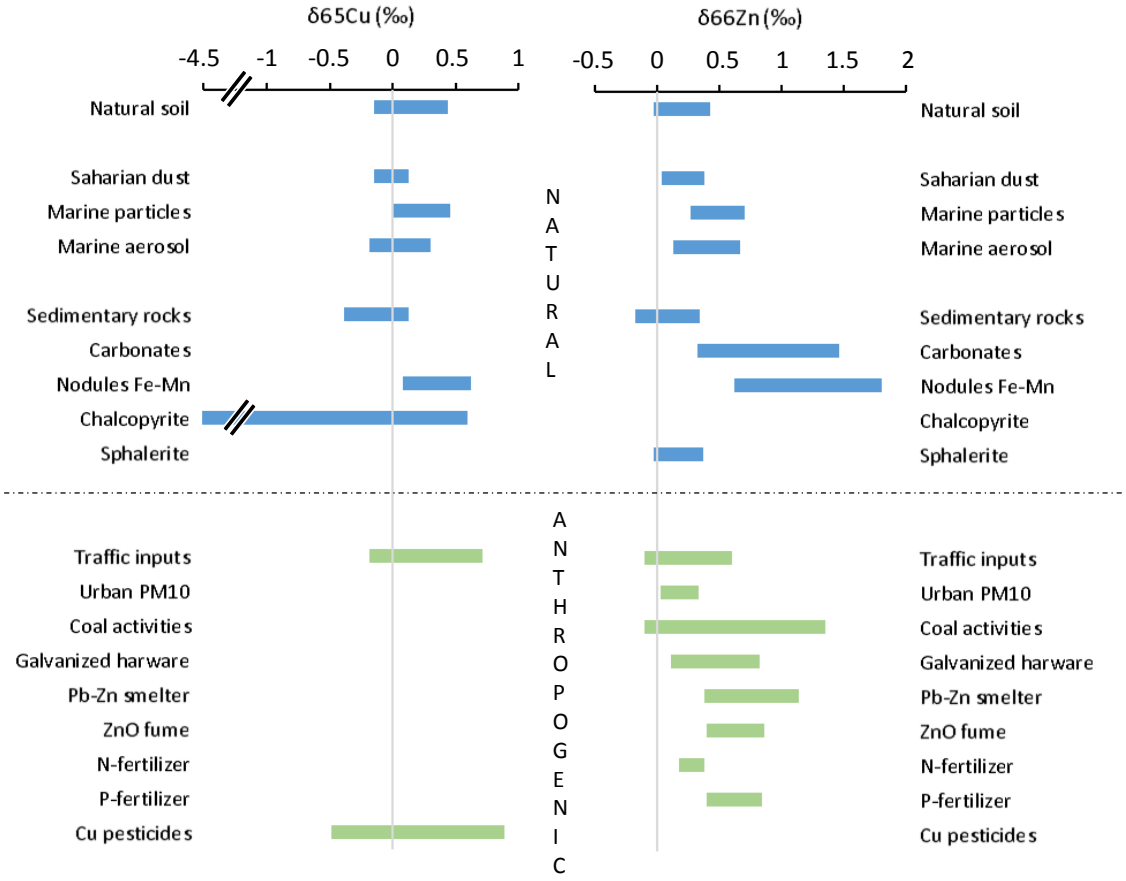

Figure 3. Summary of various $\delta^{65}Cu$ and $\delta^{66}Zn$ compositions in geogenic (natural and anthropogenic) sources.

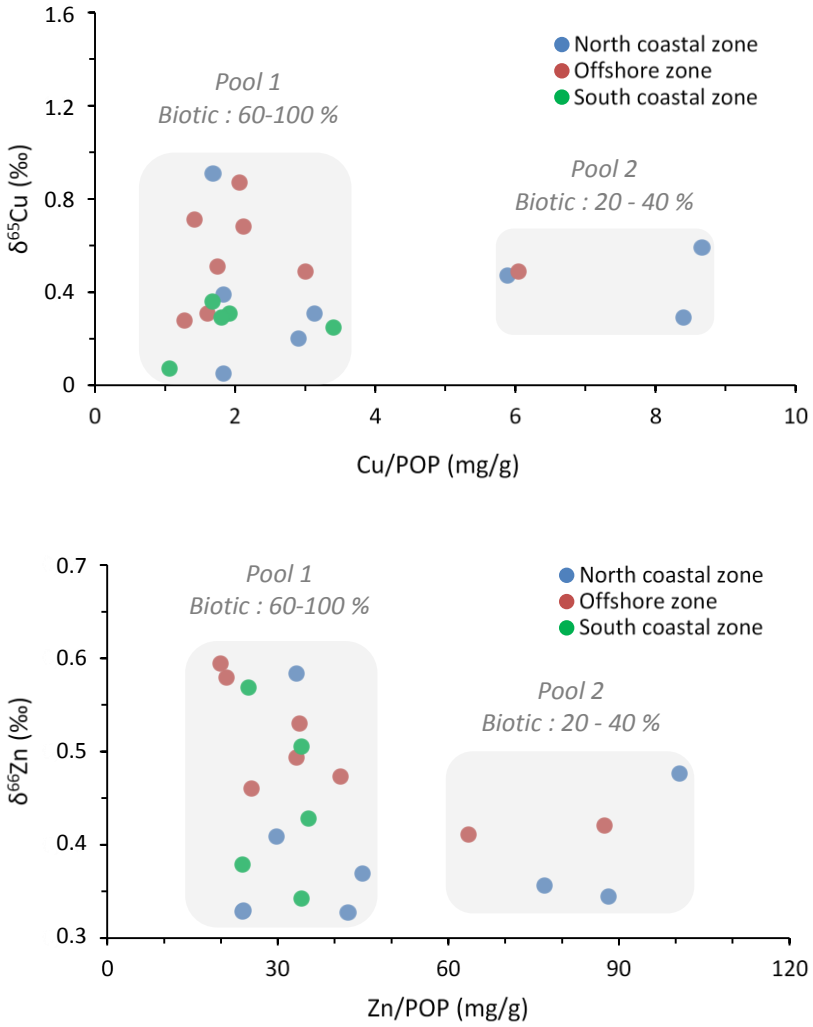

Figure 4. $\delta^{65}Cu$ and $\delta^{66}Zn$ variations relative to their corresponding Cu/POP and Zn/POP ratios in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000 µm). Values are grouped according to three geographical zones: the northern coastal zone (blue),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

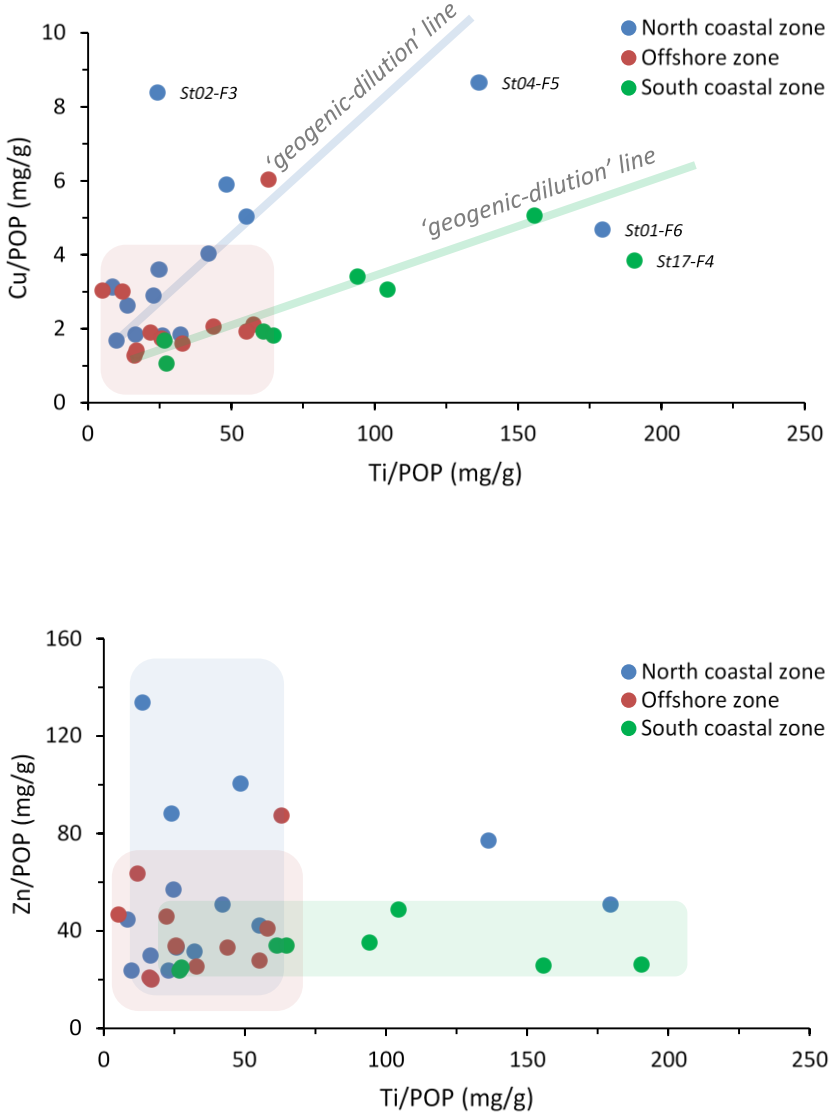

Figure 5. Cu/POP vs Ti/POP and Zn/POP vs Ti/POP diagrams in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000 µm). Values are grouped according to three geographical zones: the northern coastal zone (blue), the offshore zone (red) and the southern coastal zone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

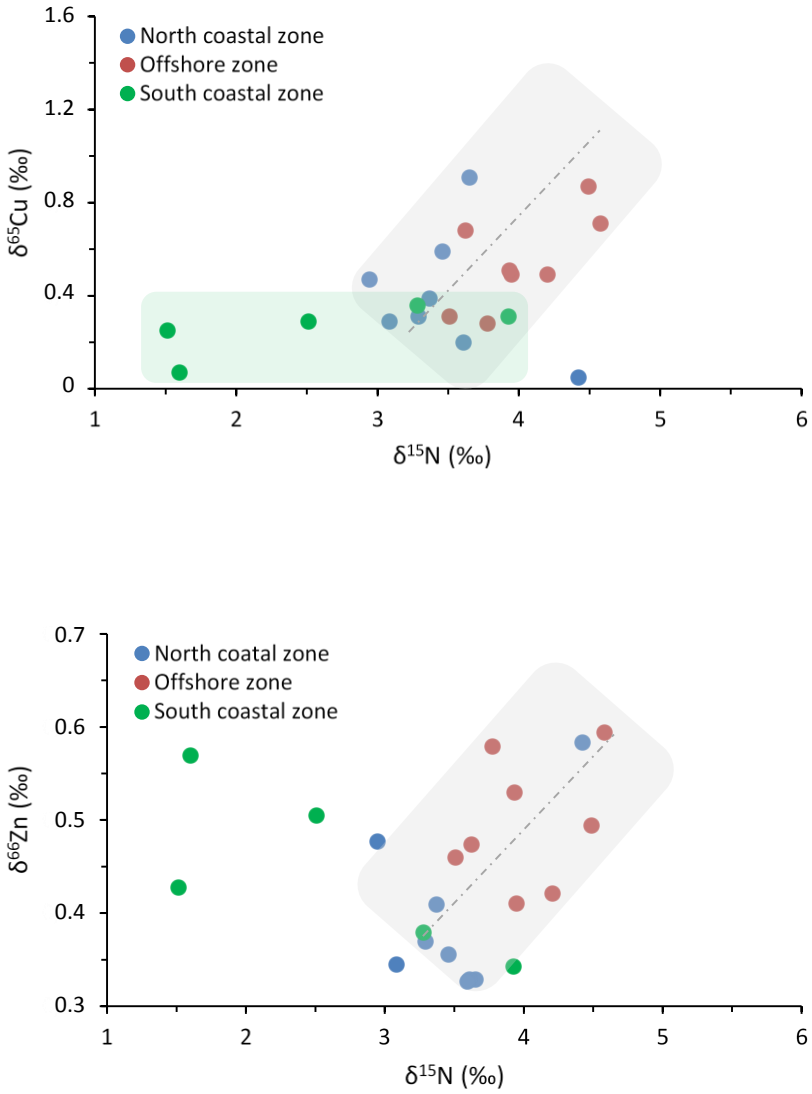

Figure 6. δ^{65}Cu and δ^{66}Zn variations relative to their corresponding δ^{15}N in the four size fractions plankton (F3: 60–200, F4: 200–500, F5: 500–1000 and F6: 1000–2000 µm). Isotopic compositions are in ‰. Values are grouped according to three geographical zones: the northern coastal zone (blue),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

Station	Latitude (N)	Longitude (E)	Localisation	Depth (m)	DCM (m)	Characteristics
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 µm; F4: 200-500 µm; F5: 500-1000 µm; F6: 1000-2000 µm) and the 10 stations (St1-4, St9-11, St15, St17, St19). Details of trace metal (Ti, µg/g), particulate organic phosphorus (POP, mg/g) and biotic component (Biotic, %) were presented in Chifflet et al (2022) and, details of nitrogen isotopic compositions ($\delta^{15}N$, ‰) were presented in Tésan-Onrubia et al. (2022) as companion papers.

Station	Fraction	Biotic (%)	Ti (µg/g)	POP (mg/g)	$\delta^{15}N$ (‰)
Northern coastal zone (Marseilles-Toulon bays)					
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	-	-
Offshore zone (near the Balearic Thermal Front and the Algerian ecoregion)					
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
Southern coastal zone (Gulf of Gabès)					
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	-	-

Table 3. Concentrations (µg/g) and isotopic compositions (‰) of Cu and Zn in four planktonic size fractions (F3: 60-200 µm; F4: 200-500 µm; F5: 500-1000 µm; F6: 1000-2000 µm) 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 (µg/g)	Zn (µg/g)	δ^{65}Cu (‰)	$2\sigma_{Cu}$ (‰)	δ^{66}Zn (‰)	$2\sigma_{Zn}$ (‰)
Northern coastal zone (Marseilles-Toulon bays)							
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	-	-	-	-
Offshore zone (near the Balearic Thermal Front and the Algerian ecoregion)							
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
Southern coastal zone (Gulf of Gabès)							
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	-	-	-	-