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Metal stable isotopes in transplanted oysters as a new tool for monitoring anthropogenic metal bioaccumulation in marine environments: The case for copper

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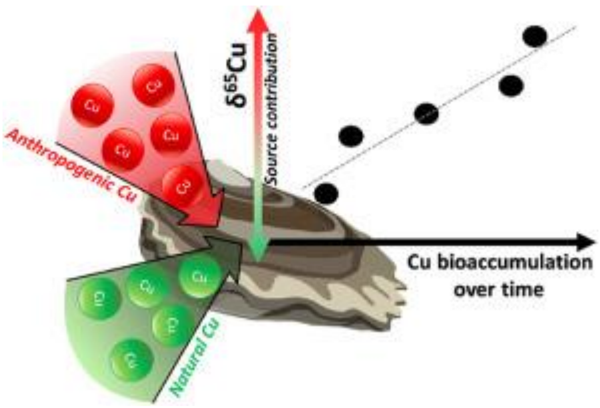
Abstract :

Metal release into the environment from anthropogenic activities may endanger ecosystems and human health. However, identifying and quantifying anthropogenic metal bioaccumulation in organisms remain a challenging task. In this work, we assess Cu isotopes in Pacific oysters (C. gigas) as a new tool for monitoring anthropogenic Cu bioaccumulation into marine environments. Arcachon Bay was taken as a natural laboratory due to its increasing contamination by Cu, and its relevance as a prominent shellfish production area. Here, we transplanted 18-month old oysters reared in an oceanic neighbor area into two Arcachon Bay mariculture sites under different exposure levels to continental Cu inputs. At the end of their 12-month long transplantation period, the oysters' Cu body burdens had increased, and was shifted toward more positive δ65Cu values. The gradient of Cu isotope compositions observed for oysters sampling stations was consistent with relative geographic distance and exposure intensities to unknown continental Cu sources. A binary isotope mixing model based on experimental data allowed to estimate the Cu continental fraction bioaccumulated in the transplanted oysters. The positive δ65Cu values and high bioaccumulated levels of Cu in transplanted oysters support that continental emissions are dominantly anthropogenic. However, identifying specific pollutant coastal source remained unelucidated mostly due to their broader and overlapping isotope signatures and potential post-depositional Cu isotope fractionation processes. Further investigations on isotope fractionation of Cu-based compounds in an

aqueous medium may improve Cu source discrimination. Thus, using Cu as an example, this work combines for the first time a well-known caged bivalve approach with metal stable isotope techniques for monitoring and quantifying the bioaccumulation of anthropogenic metal into marine environments. Also, it states the main challenges to pinpoint specific coastal anthropogenic sources utilizing this approach and provides the perspectives for further studies to overcome them.

Graphical abstract



Highlights

► Cu isotopes in transplanted oysters were used for monitoring Cu bioaccumulation in a marine environment. ► 18-month old oysters were transplanted into Cu-contaminated French mariculture sites. ► Oysters have their Cu isotope compositions shifted toward more positive values after a one-year exposure period. ► A binary isotope model allowed quantifying the bioaccumulation of anthropogenic Cu. ► This approach is an alternative to marine sites with unavailable sample banks of costly environmental monitoring networks.

Keywords : Metal bioaccumulation, Non-traditional isotopes, Bivalve mollusk :Arcachon bay, MC-ICP-MS

Introduction

Metal pollution of aquatic environments inherently alters their chemical composition and poses health risks to ecosystems and humans (Fu et al., 2016; Gaetke et al., 2014; Reilly, 2004). Identifying the origins of metals that accumulate at any given site is a key step in developing successful emission control strategies and targeting contaminated sites for remediation (Barletta et al., 2019; de Souza Machado et al., 2016; Lu et al., 2018; Weiss et al., 2008). To this end, the study of the composition of metal stable isotopes in low-cost biomonitoring organisms may be a promising approach for identifying and quantifying anthropogenic inputs (Martín et al., 2018; Shiel et al., 2012; Smith et al., 2021, 2020). However, a prerequisite step is to examine the ability of this approach in discriminating between natural Cu (the geochemical background) and anthropized Cu, the latter being Cu released to the environment after its transformation within the anthroposphere.

In coastal and marine ecosystems, bivalve mollusks have been widely used in “Mussel Watch programs” for monitoring among other pollutants, marine trace metal (Krishnakumar et al., 2018; Lu et al., 2017; Zhou et al., 2008). Indeed, bivalves, such as oysters and mussels, combine several features that are advantageous for biomonitoring purposes: they are suspended matter filter-feeders, abundant, sessile, and relatively easy to collect and handle in the laboratory (Araújo et al., 2021a). Datasets of elemental levels in these organisms help obtain qualitative information about spatial and temporal trends on metal bioaccumulation, but physiological (e.g., body size, homeostasis) and environmental (e.g., salinity, primary production, phytoplankton assemblages) factors may be confounding, and prevent an accurate assessment of the influence of anthropogenic metal inputs in the environment (Briant et al., 2017; Cossa and Tabard, 2020; Lu et al., 2019, 2017; Pourmozaffar et al., 2019). In turn, metal stable isotopes and mixing models can potentially help track metals from their respective natural and/or anthropogenic origins, thus providing a more direct appreciation of anthropic influence (Araújo et al., 2021b). Indeed, isotope signatures of metals within the anthroposphere are associated with manufactured materials or their by-products. They result from their original sources (e.g., coal and ore deposits), modulated by their transformation processes, such as electroplating and smelting (Borrok et al., 2010; Brocza et al., 2019; Gonzalez and Weiss, 2015; Shiel et al., 2010; Sun, 2019; Tonhá et al., 2020; Zeng and Han, 2020; Zhong et al., 2021). Isotope signatures of metals circulating in the anthroposphere tend to differ from isotope compositions of metals occurring naturally in waters and sediments, which are modulated by weathering and biological activity (Araújo et al., 2019a; Babcsányi et al., 2014; Guinoiseau et al., 2017; Mulholland et al., 2015; Vance et al., 2016). In previous studies, Zn and Cd isotope records in wild bivalve’s soft tissues allowed to gauge and/or quantify anthropogenic inputs in marine environments (Araújo et al., 2021b; Shiel et al., 2013, 2012). These previous works used bivalve samples provided by samples banks feed by Mussel Watch programs from France and United States, c

cover all marine sites, and they are inexistent in most countries. Transplanting bivalves from one ground to another is an alternative to circumvent the unavailability of bank samples. It has been commonly practiced to observed metal bioaccumulation trends in these organisms over determined periods (Geffard et al., 2002; Regoli and Orlando, 1994; Riget et al., 1997; Roméo et al., 2003; Séguin et al., 2016; Senez-Mello et al., 2020, 2020; Wallner-Kersanach et al., 2000). We are unaware of studies using “non-traditional” isotopes in transplanted organisms.

A first examination on the variations of Cu isotope abundances (^{65}Cu and ^{63}Cu) in soft tissues of wild bivalves (mussels and oysters) revealed the potential to obtain information related to Cu sources, bioaccumulation mechanisms, and physiological status (Araújo et al., 2021a). The latter study was conducted in a low-contaminated Atlantic site (Vilaine Bay) and integrated a 10-year biomonitoring period. It attributed the observed temporal isotope fractionation patterns of mussels to homeostatic regulation processes, involving changes in uptake and excretion rates with increasing Cu bioavailability. For oysters, Cu isotope compositions evolved linearly with Cu body burden, indicating a conservative isotope fractionation with Cu bioaccumulation over time. However, the use of this particular isotope bioaccumulation pattern observed in oysters to identify and quantify the biological incorporation of anthropogenic Cu remained untested. To continue this work, we conducted an *in-situ* experiment with transplanted and caged oysters in Atlantic oyster-rearing site recognized by its Cu-contamination history.

With up to 12000 tons/year, France is at present Europe’s top producer and consumer of Pacific oysters (*Crassostrea gigas*, Buestel et al., 2009), and environmental concerns with high Cu anthropogenic bioaccumulation in France’s farmed oysters dates back to the 1930s (Hinard, 1932). Arcachon Bay (AB) is the oldest French oyster-producing basin, where a continuous increase in Cu concentrations since 1982 is observed (Claisse and Alzieu, 1993; Fig. 1). Although Cu is an essential micronutrient for oysters, excessive environmental concentrations of this metal can damage endocrine systems and affect larval life stages, thus impacting ecological services provided by these organisms, and up to compromising mariculture production and food safety (Gamain et al., 2017; Mai et al., 2012; Sussarellu et al., 2018; Wang et al., 2011; Wijsman et al., 2019). The origin of the increased Cu observed in AB oysters was putatively attributed to the growing use by nautical activities of Cu-based antifouling paints after the ban of tributyltin (TBT), a biocide that had long entered in the composition of such paints (Claisse and Alzieu, 1993). Nevertheless, previous studies using Cu concentration data in sediment, water, and bivalves did not allow to pinpoint the origin of this increased Cu, and/or ascertain its bioaccumulation.

As a new approach for our study, bivalves originating from a coastal neighboring site were transplanted into AB and monitored for one year. Parameters included their elemental Cu levels, Cu isotope compositions, and other biometric data, including shell length and weight of soft tissue parts. Transplanted, caged bivalves share their previous and identical environmental chemical exposure a to variations in environmental and biological factors (Benedicto et al., 2011; Caro et al., 2015; Ostrander, 1996; Senez-Mello et al., 2020). Since oysters were reared together and at the same site since their larval stage, we hypothesize that the differences in isotope fingerprints observed in transplanted oysters after a one-year exposure period reflect the different local Cu isotope signatures at the new site. In our study of the AB, observed isotope shifts in bivalves' soft tissues are attributable mainly to the bioaccumulation of Cu coming from coastal anthropogenic sources, rather than isotope changes affecting marine Cu. Thus, using Cu as an example, this work combines for the first time a well-known caged bivalve approach with metal stable isotope techniques for monitoring and quantifying the bioaccumulation of anthropogenic metals by these organisms into marine environments. Also, it states the main challenges to pinpoint specific coastal anthropogenic sources utilizing this approach and provides the perspectives for further studies to overcome them.

Methods

Study area

The Arcachon Bay (AB, 44°40'N, 01°10'W, Fig. 1) is a French macrotidal coastal lagoon (1 - 5 m tidal range, 156 km^2) connected to the Atlantic Ocean by a 5 km long channel (Deborde et al., 2008). Surface seawater temperature ranges between 1 and 30 °C, and salinity is between 22 and 32 psu, with a significant difference between western and eastern basins that are under the influence of oceanic and continental waters, respectively (Deborde et al., 2008). Shellfish farming activities are characteristic of the local culture and economy, which started at the end of the 19th century. The perimeter of AB is entirely lined by suburban centers and associated marinas (Fig. 1). The port of Arcachon is one main leisure port of of the French Atlantic coast, accounting about 12,000 registered boats, which 95% of pleasure crafts, and the rest are used for fishing, oyster farming and maritime transport (Le Berre et al., 2010). A census performed in the early 2000's showed that most of these embarkations now use annually about 4,3m^3 of copper-based antifouling paints since TBT paints were banned (Auby and Maurer, 2004).. Thus, the sheer intensity of leisure boating activities interacts strongly with AB's natural environment and its professional users (Le Berre et al., 2010). The inner basin is affected by a major source of fresh water by the Leyre River, and other small rivers that flow into the lagoon (Rimmelin et al., 1998). The farm activities in the Leyre river watershed (Fig. 1) are a source of pesticides in the AB (Fauvelle et al., 2018).

Oyster transplantation experiment and wild oyster collection

One thousand 18-month-old *C. gigas* oysters were used for the transplantation experiment. They originated from the "Arguin Banc" site in the open Bay of Biscay, and presumably fully under oceanic influence (Fig. 1), and were transplanted in AB where they were held in polyethylene mesh bags used as cages. This pool of oysters was sub-divided into two 500-organism batches that were t and "Grand Banc" (outer AB, Fig. 1). At each time point, ca. 80 individual oysters were collected from each site after 3-, 6-, and 12-month exposure periods.

After collection, bivalves were depurated in laboratory tanks during 24 h, using filtered local seawater. Then, 30 specimens were taken for biometric measurements, including shell length and weight, total body weight, and the weight of their lyophilized soft tissues (namely dry tissue), to quantify their growth using a body condition index: (weights of dry tissue / (total body tissue – shell) * 1000; (Lawrence and Scott, 1982). Depending on the weight of the organisms, soft parts of between 20 and 50 individuals were pooled, homogenized, and dried for the metal analyses presented here. The average bioaccumulated Cu body burden (µg Cu per individual bivalve) in the soft tissues of each pool was estimated by multiplying Cu concentrations of the pool by the corresponding mean dry weight of the soft tissues. This is meant to accommodate differences is growth rates and neutralize biodilution.

In AB, there are three oyster sampling stations from the French marine chemical contamination biomonitoring network ROCCH. These stations, which include the Comprian site, have been using indigenous oysters to monitor marine contaminants, including TBT and Cu, since 1980 (https://wwz.ifremer.fr/surval/). For an example, Fig. 1 shows a time series of Cu and TBT levels in oysters from Comprian. For our study, an additional sample made of lyophilized soft tissues of ca. ten indigenous oysters from Comprian and collected in the winter of 2019 and prepared identically to the transplanted oysters within the biomonitoring framework of the ROCCH.

Sample preparation and analyses

Bivalve sample digestion and chemical analysis have already been detailed in previous publications (Araújo et al., 2019,a,b; 2021a,b). Briefly, aliquots of freeze-dried bivalve tissues (~200 mg) were digested in closed vessels by a concentrated nitric acid solution and using microwave energy. Copper elemental analyses were performed by quadrupole inductively coupled plasma mass spectrometry (ICP-MS). For isotope analyses, aliquots of digested samples containing 500 ng of Cu were purified using an AG-MP1 resin, and Cu isotope abundances determined by multicollector ICP-MS (Neptune, Thermo Scientific) at the Pôle Spectrométrie Océan (PSO) laboratory (Ifremer, France). Reference materials (RMs) of other animal tissues (oyster SRM 1566b-NIST®; protein fish DORM-4, NRC-CNRC®) and procedural blanks were included in each sample batch for analytical control. All sample preparation procedures were carried out with ultrapure water and high-purity acid blends.

Isotope analyses samples were dissolved in diluted acid nitric (2% v/v) and analyzed at concentrations around 250 ng g^{-1}. A Stable Introduction System (SIS: cyclonic spray chamber and PFA nebulizer at 50 µL min^{-1}, ESI) was used to introduce samples into spectrometer. The raw Cu isotope ratios were corrected for mass bias using the standard bracketing technique and the final Cu i

reference material NIST SRM-976 (Eq. 1):

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

For unknown samples and RMs, $\delta^{65}Cu_{SRM-976}$ values represent the average and the two-standard deviation (2s) of two or three individual measurements performed during a single analytical session. The precision average obtained for individual samples and RMs was better than ±0.05‰. The obtained $\delta^{65}Cu_{SRM-976}$ value for the RM fish protein DORM-4 (+0.55 ± 0.02‰, Table 1) fell in the same range of values published for this material (+0.52 ± 0.08‰, Sullivan et al., 2020; +0.48 ± 0.06‰, Sauzéat et al., 2021). The $\delta^{65}Cu_{SRM-976}$ value obtained for three full replicates of the oyster tissue SRM 1566b of +0.25 ± 0.03‰ is in line with the long-term reproducibility for this RM (+0.22 ± 0.03‰, 2s, n = 8, Araújo et al., 2021) at PSO laboratory.

Results and discussion

Evolution of bioaccumulated Cu and its isotope composition in transplanted oysters

The dataset is presented in Table 1, and all biometric data are included in the Supplementary Material (Table 1S). The biometric data indicate a faster and higher body growth of oysters in Grand Banc (outer AB) compared to those in Comprian (inner AB, Table 1S). Hence, to cancel out effects of biodilution in Cu concentrations and to be able to compare Cu bioaccumulation despite growth rate differences between these sites, we computed the oysters' Cu body burden (µg Cu/per individual, Table 1) rather than Cu concentrations.

Oysters transplanted in Comprian and Grand Banc sites shared temporal patterns of Cu concentration, body burden, and isotope compositions, i.e., a significant increase in the concentration of bioaccumulated Cu accompanied by a shift toward more positive $\delta^{65}Cu$ values (Fig. 2, Table 1). In Comprian, over the one-year exposure period, mean oyster Cu body burdens increased continuously from 44 to 189 µg (four-fold increase), while $\delta^{65}Cu$ values shifted from +0.35 to +0.60‰. The latter is remarkably close to indigenous oysters from Comprian harvested in winter 2019 (+0.59‰, Fig. 2). In Grand Banc, the Cu body burden levels are lower than in Comprian at the 3-month and 6-month time steps, but reach a very similar 188 µg/per individual (Fig. 2a) at the end of the exposure period. In turn, the Cu isotope composition does not evolve monotonously, with an initial shift to lower $\delta^{65}Cu$ values at the first-time step followed by an increase and stabilization near 0.4‰ (Fig. 2b). Despite comparable Cu body burdens at the 2 AB sites reached at the end of the transplantation experiment, the oysters' final isotope compositions differ by 0.17‰ (Fig. 3a). It is also notable that the largest isotope shift occurs at Comprian, about 0.25‰.

body burden and concentration suggest that bioaccumulated Cu in AB has a distinct origin from that offshore, at the Arguin Banc site (Fig. 3a). In line with our initial hypothesis, the simultaneous and linear increases of elemental body burden and isotope compositions shows that there exists a pool of bioavailable Cu inside the AB which is distinct from the offshore marine environment. The faster Cu bioaccumulation and larger magnitudes of isotope variation in oysters from Comprian (inner AB) suggest that this area is under greater exposure to continental Cu emissions. (Fig. 3a). This is possibly due to its geographic proximity to agriculture and urban sources from the Leyre watershed and/or the influence from nautical releases (antifouling paints) from the inner bay. In contrast, the more restricted isotope variations and lower Cu bioaccumulation rates in Grand Banc (outer AB) are consistent with the more oceanic character of this site, which also captures an attenuated and temporally-delayed continental Cu signal. The observed shift to lower δ^{65}Cu values in the first three months of the transplantation experiment (GB-T1 sample in outer AB) is attributed to off-shore seawater during the summer (low river flow). Indeed, estuarine waters from the neighboring Gironde estuary (Atlantic French coast) with low anthropogenic Cu displayed enrichments in light isotope about +0.12 ± 0.08‰ (1s, n= 8, Petit et al., 2013), which supports this proposition. Indeed, as will see in the further discussion, the estimated end-member of marine bioaccumulated Cu pool for oysters matches well with a low δ^{65}Cu value for oceanic waters.

Inferring a Cu binary source model to apportion the continental and natural Cu fractions bioaccumulated in oysters

The gradient of Cu isotope compositions observed for oyster soft parts at Comprian and Grand Banc sampling stations was consistent with exposure intensities to a continental Cu source. Plotting δ^{65}Cu values against its reciprocal concentration (1/[Cu]) is a useful approach to identify source mixing processes (El Azzi et al., 2013; Křίbek et al., 2018; Mihaljevič et al., 2019). The good fit of all oyster samples (indigenous and transplanted) on a straight line (R^2 = 0.93, p <0.05) indicate that bioaccumulated Cu and associated δ^{65}Cu values can be described in terms of a simple binary mixing source model involving two end-members, defined here as representing the continental and marine bioavailable Cu pools in oysters, respectively (Fig. 3b). The “mixture line” obtained by regression analysis allow to estimate the values of these two end-members, which in turn can then be used to quantify the continental Cu fraction bioaccumulated in oysters. As noted, these end-members represent bioaccumulated pools of Cu, rather than actual Cu sources to this environment. Since they are based on the oyster dataset, they already include any potential biological isotope fractionation induced by oyster Cu bioaccumulation. Therefore, they can be used to quantify the continental Cu fraction bioaccumulated in oysters’ soft tissues irrespectively of any biological fractionation. The implications in their use for source identification are discussed in next section.

al., 2019) in the linear regression equation, we obtain +0.02‰ for an isotopic end-member representing the natural marine Cu bioaccumulated pool at our study site. Interestingly, this value is close to some relatively unpolluted estuarine water samples from the neighboring Gironde estuary (Atlantic French coast, +0.12 ± 0.08‰, 1s, *n* = 8, Petit et al., 2013). For the other end-member, we use the highest Cu concentration reported in oysters from the French Mussel Watch program (of approximately 2,500 $mg.kg^{-1}$) to obtain a value about +0.65‰ for the continental bioaccumulated Cu end-member (Fig. 3b). This extrapolation towards low and elevated Cu concentrations is consistent with the exceptional capacity of oysters to bioaccumulate high loads of Cu, as high as 4 % d.w. of whole- body tissue (Wang et al., 2011).The standard error of the regression analysis (S), which represents the average distance that the observed values fall from the regression curve, is about ±0.05 ‰ and is considered the uncertainty associated with the estimation of the two end-member values.

The calculated $\delta^{65}Cu$ end-member values of +0.65 ‰ and +0.02 ‰ enable the use of a simple binary mixing model to quantify respectively the relative fractions of continental and marine Cu fractions bioaccumulated in their soft tissues during the time-course of the transplantation:

$$Cu_{continental(\%)} = \left(\frac{\delta^{65}Cu_{sample} - \delta^{65}Cu_{marine}}{\delta^{65}Cu_{continental} - \delta^{65}Cu_{marine}}\right)*100 \text{ (Eq. 2)}$$

where $\delta^{65}Cu_{sample}$, $\delta^{65}Cu_{natural}$, and $\delta^{65}Cu_{anthropogenic}$ stand for the $\delta^{65}Cu$ values obtained for the sample of interest, and the estimated values for natural and anthropogenic Cu end-members, respectively. The computed values are included in Table 1. Uncertainty values were computed by error propagation in Equation 2 using analytical uncertainties of oyster samples (Table 1) and estimate uncertainties of end-members (±0.05‰) were below 1% for transplanted oysters.

The Cu of continental origin bioaccumulated in oysters before transplantation amounts to 52 %, revealing the transport of continental emissions to the marine environments beyond AB. In the transplanted oysters from Comprian, this percentage climbs to 92%, matching closely this of indigenous oysters (90%). Even if oysters from Grand Banc display similar Cu bioaccumulation loads (Table 1), the continental Cu fraction is significant, with a contribution of 65 % to the Cu body burden. As a consistency check, we use the concentration presented in Fig. 1. It shows that the Cu concentrations in contemporary oysters have nearly quadrupled over the last 40 years, indicating the “new” Cu incoming AB unlikely to derive from natural sources. It is reassuring that the ¾ of continental Cu in present-day oysters estimated from the time series is close to the isotopically-calculated fraction. Most anthropic sources reported in the literature, including antifouling and urban sources, display more positive Cu isotope composition averages than the Upper Continental Crust, which isotope range is about 0‰ (Fig. 4). Therefore, it is plausible that the bioaccumulation c

values, such as observed in our study. Thus, we consider continental Cu emissions in AB dominantly anthropogenic. The following section discusses the potential use of oyster's isotope signatures to pinpoint anthropogenic Cu sources.

A critical assessment of using recorded Cu isotope signals in oysters for source identification

Using oyster isotope signatures for source identification requires verifying if biological uptake and biogeochemical processes in sediment-water interface can overprint original isotope signatures of sources. Here, we assess the potential effect of these factors on Cu isotope signals recorded in oysters.

Oysters can accumulate Cu (and Zn) at high concentrations without serious toxic effects, due to great capacity for detoxifying excess Cu and Zn by making these metals under inert and non-toxic forms. Presumably, this mechanism compensates for their lack of significant cellular Zn and Cu excretion (Kunene et al., 2021; Rainbow, 2018; Wang et al., 2018, 2011). Indeed, the "half-lives" calculated for Cu and Zn excretion from the same *C. gigas* species from the neighboring Gironde estuary Atlantic Coast, are about 1,500 and 3,000 days, respectively (Geffard et al., 2002). These durations are very long compared to the life-spans of our oysters, and are consistent with the limited elimination of these elements into inert, intracellular, metal-rich granules (Geffard et al., 2002; Wang et al., 2018). Thus, these very low excretion rates of Cu and Zn in oysters likely to not affect isotope budget of whole soft tissues, which makes these organisms "integrative isotope recorders" of the source contributions in the bioaccumulated Cu and Zn from their surrounding environment. While this has already been shown for Zn isotopes in oysters in aquarium-based experiments (Ma et al., 2019), it is still to be rigorously confirmed for Cu. However, based on the similarity of bioaccumulation mechanisms for these both micronutrient elements (Kunene et al., 2021; Tan et al., 2015, Weng et al. 2018), and the data presented above, we can speculate that it is also true for Cu.

Thus, we attribute the greatest difficulty to pinpoint anthropogenic Cu not to biological fractionation processes, but rather to the gaps in ours constrain about Cu isotope fractionation in anthroposphere (Tonhá et al., 2020; Viers et al., 2018; Yin et al., 2018). Anthropogenic isotope signatures latter derive mainly from mineral deposits of this element, which present the most extensive range among the natural compartments (−16.5 to +10 ‰, Klein and Rose, 2020; Mathur and Wang, 2019; Moynier et al., 2017; Wang et al., 2017). Consequently, anthropogenic sources also display a wide isotope variability that can overlap each other, hampering the discrimination of individual Cu anthropogenic sources (Fig. 4). This drawback becomes more critical in complex coastal environments where several anthropogenic metal sources normally coexist.

It is worthy also to argue that the direct comparison of Cu isotope compositions between distinct, but connected marine biogeochemical reservoirs (e.g., sediment, water, biota) and a may occur during release and transfer between these environmental compartments before bioaccumulation. As an example, in Cu-polluted soils by Cu-based fungicide, soil particle leaching and surface runoff exhibit a shift up to 0.40 ‰ in comparison to the particulate phase source (Babcsányi et al., 2016; Blotevogel et al., 2018; El Azzi et al., 2013). In marinas and harbors, sediments that have been Cu-contaminated by antifouling paints show isotope signatures slightly lighter than the source anti-fouling paints themselves, but significantly different from the natural background (Briant, 2014). This suggests a preferential release of heavy isotopes from these compounds when solubilized in seawater, or the occurrence of an unaccounted fractionation process between the paint chip and its host sediment. Similar modifications on source isotope signatures induced by changes in the metal speciation have also been observed for Zn, Hg, and Cd isotope systems in sites with industrial and metallurgical contamination legacies or in laboratory involving photochemical reactions of Ag-nanoparticles (Tonhá et al., 2020; Brocza et al., 2019; Li et al., 2021; Shiel et al., 2010; Zhong et al., 2020).

It is noted that anthropogenic fingerprints in natural sample archives like sediments, exceed in amplitude the isotope range from the natural Cu baseline. The latter normally centered around 0‰ (Fig. 4). This isotope pattern is illustrated when comparing sediment from almost pristine to highly contaminated sites, like those of the Loire estuary, Port Camargue, and Toulon Bay (Fig. 4). The latter is characterized by lighter isotope signatures related to warfare and shipbuilding contamination legacies that ranges from -0.79 to +0.34‰ (Araújo et al., 2019a). In contrast, Port Camargue sediments range from -0.13 to + 0.44‰ (Briant, 2014), tending in overall, to more positive values related to Cu-based antifouling paints. In turn, the relatively uncontaminated Loire estuary sediments have a narrower isotope range, with $\delta^{65}Cu$ values between -0.24 and +0.09‰, with an average of −0.04 ± 0.18‰ (2s, n = 31). This average value is close to that of UCC (~0 ‰) and likely reflect the variability of its natural sources, such as weathered particles derived from soils and rocks of the Loire river watershed (Araújo et al., 2019b).

These studies and ours demonstrate that Cu isotopes can be useful to discriminate anthropogenic and natural sources in despite of possible isotope modifications of anthropogenic metals after their release into the environment. While identifying specific coastal sources remain a challenge task because these post-depositional isotope changes, they still carry isotope signals that can be traced and apportioned in particulate and dissolved phases, and ultimately, into the organisms.

Conclusions

This study confirmed, for the first time, the applicability of a “non-traditional” metal stable isotope system in transplanted oysters to monitor metal bioaccumulation. Our findings demonstrate that Cu isotopes can constrain the continental Cu fraction bioaccumulated in oysters and infer its natural or a contamination gradient can be extended to other metal isotope systems and then yield an apportionment of anthropogenic contributions to the metal body burden of these organisms. Furthermore, it does not hinge on the availability of sample banks of costly environmental monitoring networks operating for long time series.

Unfortunately, source pinpointing remains elusive and further studies are sorely needed. Indeed, the biogeochemical reactions of anthropogenic metal-based substances released into aquatic environments may potentially induce fractionation of metal isotopes between particulate and dissolved phases. This results mainly from changes in the metal atom’s coordination, strength of bonds, ligand complexation (inner-sphere vs. outer-sphere formations), and adsorbent features on the water column (Balistrieri et al., 2008; Dong and Wasylenki, 2016; Ducher et al., 2016; Guinoiseau et al., 2016; Li et al., 2015; Lu et al., 2016; Moynier et al., 2017). Thus, further laboratory and field experiments are required to observe and model how anthropogenic metals from a range of compounds known to contaminate marine environment yield different isotope signals in particulate and dissolved phases. For Cu isotopes, in the context of marine pollution, Cu-based anti-fouling paints are an obvious first experimental target.

Nevertheless, the combined use of several isotope systems, like Zn, Cd, Ag, and Pb in the so-called “multi-isotope approaches", which have been successfully applied to individual pollutant tracking (Araújo et al., 2021c; Li et al., 2019; Shiel et al., 2012), could enhance their power of discrimination for different metal pollutant sources. This multi-isotope approach is timely for environmental forensic applications addressing pollution source identification in marine environments, since concentrations of trace metals, notably Cu, Zn, and Ag, are on the rise in urbanized marine coasts (Barletta et al., 2019; Zalasiewicz, 2018), or still present in mobile and bioavailable forms in legacy inventories (Araújo et al., 2019a; Briant et al., 2013; Caplat et al., 2005; Dang et al., 2015b, 2015a; Resongles et al., 2014; Tonhá et al., 2020).

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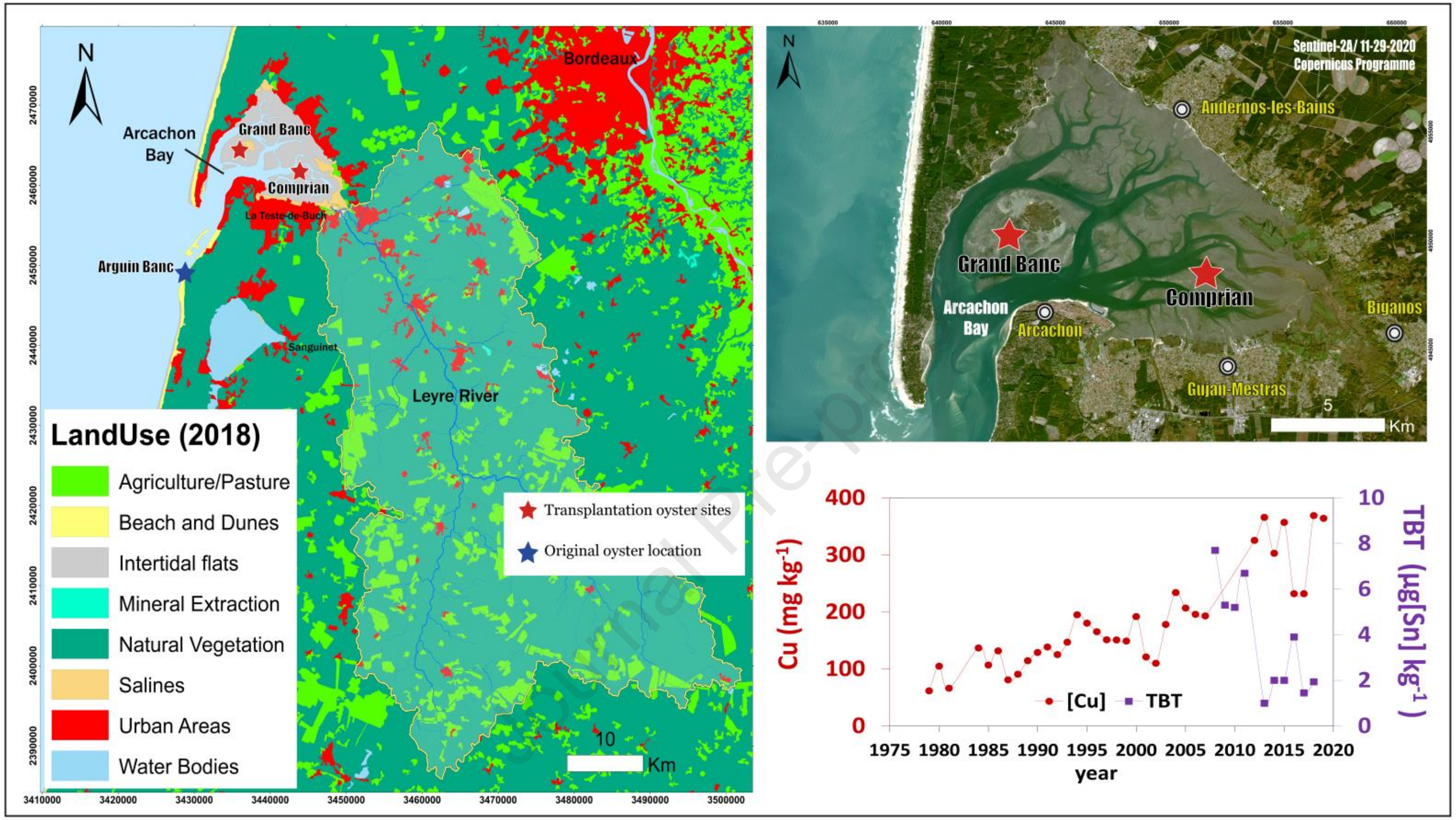

Fig. 1. Study site map and sampling stations. 18-month old oysters harvested from Arguin Banc were transplanted to Comprian and Grand Banc. The historical trends on Cu and TBT concentrations in indigenous oysters from the Comprian site are from ROCCH monitoring network (available at Surval: https://wwz.ifremer.fr/surval/).

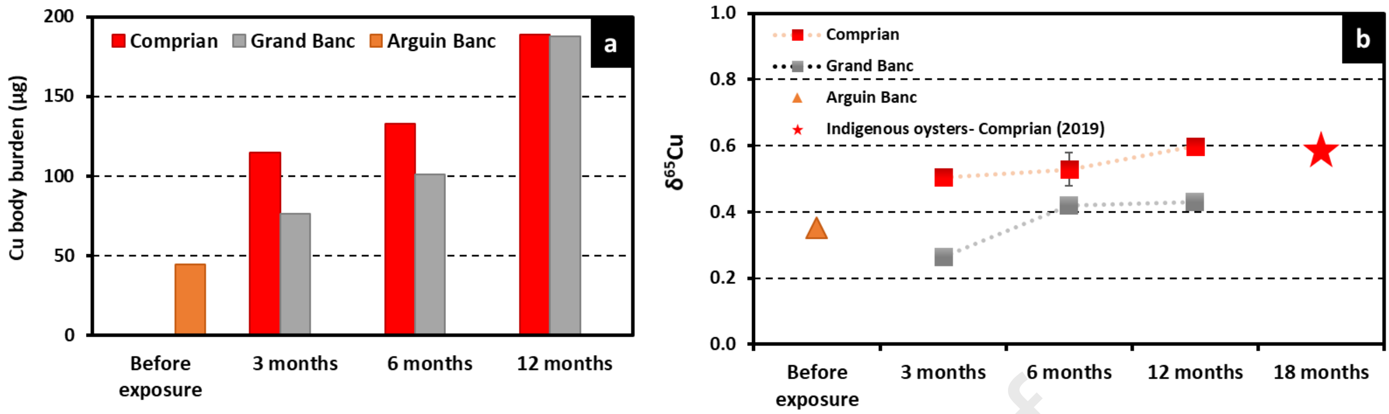

Fig. 2. (a) Temporal evolution of Cu body burden levels (Cu µg/per individual) and (b) δ^{65}Cu values (‰) for oysters before (Arguin Banc) and after their transplantation in Arcachon Bay (AB), at Comprian and Grand Banc. The data refer to pools of oysters collected after three-, six-, and twelve-month long transplantation periods. An additional 2019 sample composed of 18-month old indigenous oysters was acquired from the French Mussel Watch program (ROCCH) environmental sample bank.

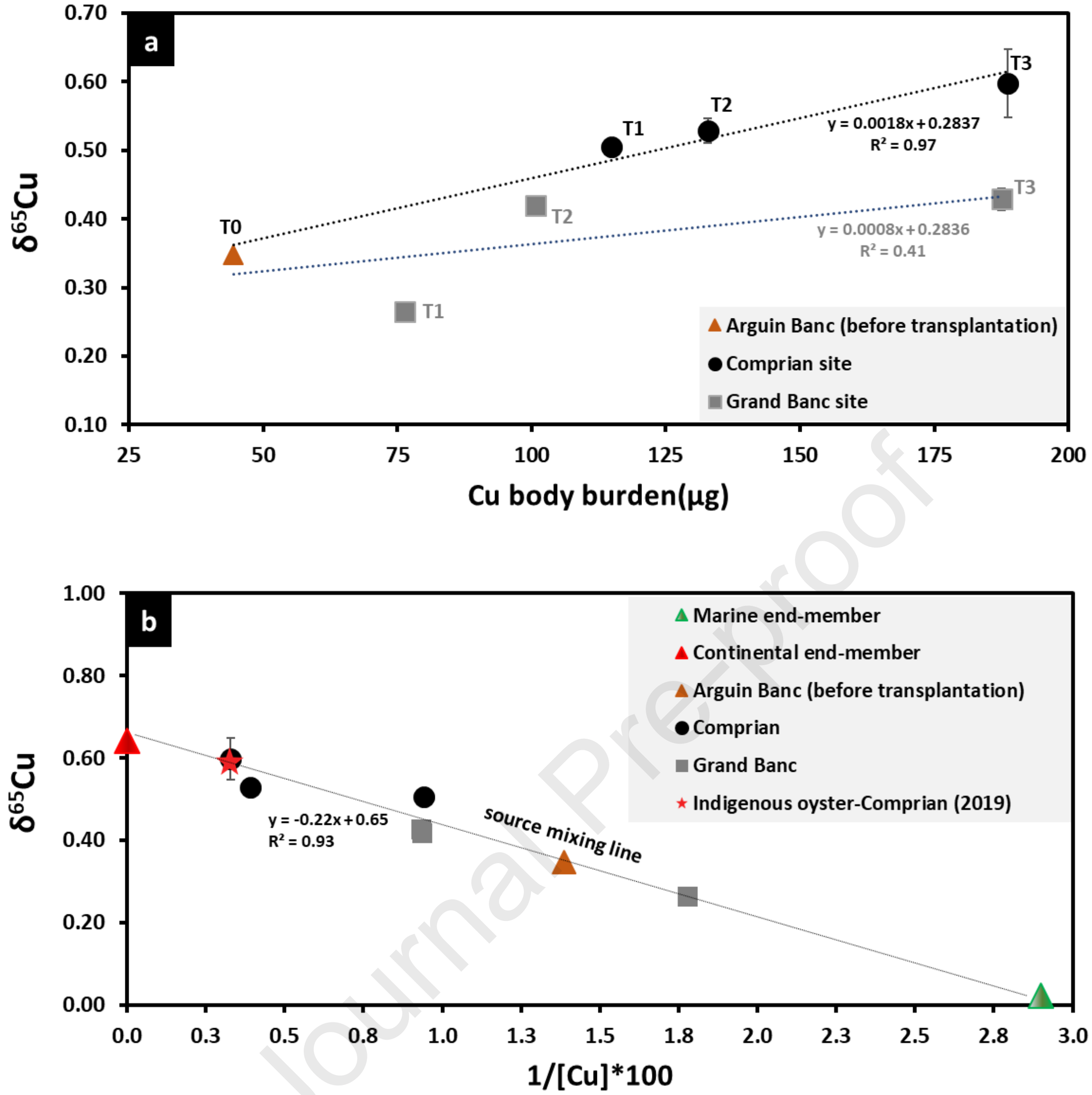

Fig. 3. (a) Plot of $\delta^{65}Cu$ values (‰) against Cu body burden levels (Cu µg/individual bivalve) for oysters transplanted from Arguin Banc to the Comprian and Grand Banc sites; T1, T2, T3 labels refers to three-, six-, and twelve-month long transplantation periods. (b) Binary isotope source mixing model based on the regression analysis of oyster samples. The 2019 sample composed of indigenous oysters was acquired from the ROCCH sample bank. The natural bioaccumulated end-member is estimated at +0.02‰ using worldwide concentration baseline for oysters (Lu et al., 2019). The continental Cu end-member (+0.65‰) is estimated using the highest Cu concentration in the ROCCH database for Pacific oysters (2,5000 mg.kg^{-1}). Error bars represent the analytical precision (2s) obtained for two measurements performed for each sample.

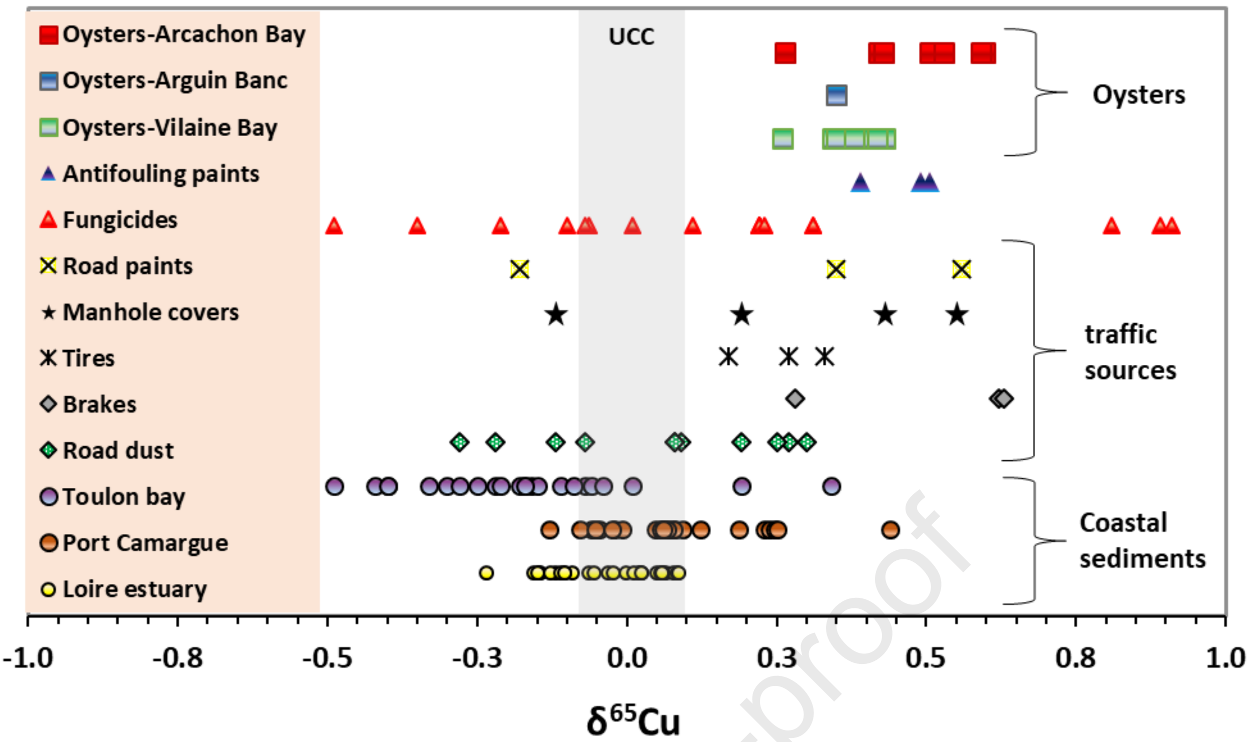

Fig. 4. Copper isotope compositions (δ^{65}Cu, values in ‰) of oysters (this study) and anthropogenic materials reported in the literature: antifouling paints (Briant, 2014), fungicides (Babcsányi et al., 2016; Blotevogel et al., 2018; El Azzi et al., 2013), vehicule traffic-related sources (Dong et al., 2017; Schleicher et al., 2020; Souto-Oliveira et al., 2019). Oyster isotope data from Vilaine bay (Biscay Bay, Atlantic French shore) were published previously (Araújo et al., 2021a) and are included here for comparison. Coastal sediments include samples from Toulon bay (Araújo et al., 2019a), Port Camargue (French Mediterranean shore, Briant, 2014) and Loire estuary (Araújo et al., 2019c) (Biscay Bay, Atlantic French shore). They represent sediment isotope signatures related to harbor activities mixed to warfare contamination legacy, Cu-based antifouling paints and a low-Cu contaminated system, respectively. Grey band centered around 0 ‰ represents Cu isotope range average reported for Upper Continental Crust (UCC) (Liu et al., 2015).

Table 1. Copper concentrations, Cu body burden, and isotope compositions for dry-pooled oyster samples. The quantification of the continental Cu fraction (%) bioaccumulated in oysters using a binary isotope model is detailed in the text.

Oyster sample ID	Exposure period	[Cu] (mg kg-^{1})	Cu body burden (µg)	δ^{65}Cu (‰)	2s	Bioaccumulated continental Cu fraction (%)
Oysters before exposure experiment (T0)	before exposure	72.1	44	0.35	0.06	52
Indigenous oyster (2019) in Comprian	18 months	305	ND	0.59	0.01	90
Transplanted oysters in Comprian						
Com-T1	3 months	106	115	0.50	0.01	77
Com-T2	6 months	257	133	0.53	0.02	81
Com-T3	12 months	304	189	0.60	0.05	92
Transplanted oysters in Grand Banc						
GB-T1	3 months	56	76	0.26	0.01	39
GB-T2	6 months	107	101	0.42	0.00	63
GB-T3	12 months	107	188	0.43	0.02	65

Highlights

- Cu isotopes in transplanted oysters were used for monitoring Cu bioaccumulation in a marine environment.
- 18-month old oysters were transplanted into Cu-contaminated French mariculture sites.
- Oysters have their Cu isotope compositions shifted toward more positive values after a one-year exposure period.
- A binary isotope model allowed quantifying the bioaccumulation of anthropogenic Cu.
- This approach is an alternative to marine sites with unavailable sample banks of costly environmental monitoring networks

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: