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# Cu isotope records of Cu-based antifouling paints in sediment core profiles from the largest European Marina, The Port Camargue

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**Abstract :**

The intensive use of copper (Cu) compounds as an alternative biocide in antifouling paints (APs) has resulted in wide Cu contamination into the marine environment, especially near marina harbor activities. In this work, the applicability of Cu isotopes to discriminate Cu origins related to the use of Cu-based APs in marine environments was tested. To this, Cu isotopes in APs, shipyard sludges, and sediment cores sampled in the Cu-contaminated Mediterranean marina of Port Camargue were determined. APs represent an important dominant anthropogenic source for metals in this site, making it ideal to test Cu isotopes as tracers. The overall isotope composition of four sediment cores and a surface sample varied between −0.13 and 0.44 ‰ ($\delta^{65}Cu$ relative to NIST-976). Selected APs brands show a similar Cu concentration ~0.15 % and $\delta^{65}Cu$ average of 0.54 ± 0.05 ‰. The plot of $\delta^{65}Cu$ vs concentration for all datasets allowed dissociating natural and APs end-members. However, sample isotope systematics were not consistent with a conservative mixing binary source process. Heavily Cu-contaminated sediments show isotope signatures lighter than APs brands. However, the most Cu-contaminated sample, located directly above the careening area, shows a $\delta^{65}Cu$ slightly lighter than APs (0.44 ‰ vs 0.54 ‰, respectively). Results suggest the preferential releasing of a heavy isotope pool by APs when these compounds are solubilized in seawater. The isotope fractionation was attributed to potential chemical Cu coordination changes during its elemental partition between paint and marina seawater and the fractionation induced by the organic ligands in the water column, before deposition. Further laboratory experiments are recommended to model the isotope fractionation mechanisms related to Cu release by APs. Because the APs' isotope signature is modified in marine environments, the use of Cu isotopes as tracers of AP in marine environments is challenging and needs more investigation.

**Graphical abstract**
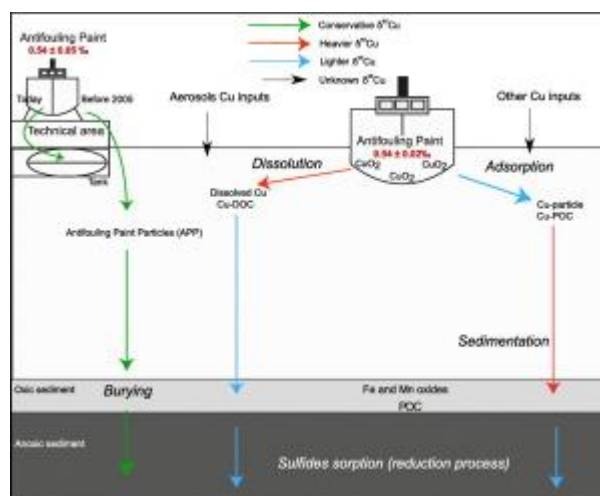


**Highlights**

► Antifouling paint (APs) brands show a similar Cu concentration and $\delta^{65}Cu$ ratios. ► Highly Cu-contaminated sediment shows $\delta^{65}Cu$ slightly lighter than APs brands' $\delta^{65}Cu$. ► Investigations are needed to use Cu isotopes as an effective tracer of APs in marine environments.

**Keywords** : Antifouling paints, Isotopes, Copper, Harbor

## 1. Introduction

Antifouling paints (APs) are biocidal products usually used as coats in ship hulls and aquatic man-made structures to avoid the proliferation of fouling organisms, including bacteria, macroalgae, mussels, barnacles, and invertebrates (Almeida et al., 2007; Turner, 2010). In the recent past, tributyltin (TBT) was used as the main biocide compound in APs, but its deleterious effects on non-target marine ecosystems lead it to be banned from the antifouling paint market in 2003 and posteriorly, phase out from all vessels by 2008 (Antizar-Ladislao, 2008) (IMO, 2001). Such environmental regulations boosted the use of copper (Cu) compounds as an alternative biocide (Amara et al., 2018), resulting in often emissions at high concentrations of this metal into marine environments, notably those under influence of marina and harbor activities (Alzieu, 2000; An & Kampbell, 2003; Briant et al., 2013; Claisse & Alzieu, 1993; Eklund et al., 2009; Guerra-García et al., 2021; Kylin & Haglund, 2010; Lagerström et al., 2016; Schiff et al., 2004). The Cu contamination induced in marine environments by APs is also problematic because, despite constituting an essential micronutrient for organisms, it can be toxic when bioaccumulated in excess (Amara et al., 2018; Strivens et al., 2020; Sussarellu et al., 2018).

Cu-based APs have high Cu contents, normally varying between 5 to 50 wt% (dry weight), and may release it in dissolved, nano, and particulate forms, at variable rates (expressed as µg/cm$^2$/day) depending on several water parameters, such as temperature, pH, Cu concentration and speciation, paint chemical composition, salinity and exposition time of the paint (Adeleye et al., 2016; Holmes & Turner, 2009; Lagerström et al., 2018, 2020; Lindgren et al., 2018; Miller et al., 2020; Ytreberg et al., 2021). Once released in the water column, dissolved Cu ions may participate in many biogeochemical processes (adsorption on solid surfaces, biological uptake, solid precipitation, complexation with dissolved ligands, etc.) that will control its speciation, mobility, and toxicity (de Souza Machado et al., 2016; Rainbow, 2007). In marinas, seawaters are commonly semi-enclosed by hinged walls, with low hydrodynamic energy and limited renewal by open seawater exchange (Guerra-García et al., 2021). Such features lead to anoxic conditions in the water column favoring the trace metals trapping, like Cu, in sediments by forming insoluble sulfide salts (Araújo, Peres, et al., 2017; Guerra-García et al., 2021; Huerta-Diaz & Morse, 1992). Deposition of antifouling solid wastes on boatyards represents also a significant secondary hazardous exposure source of Cu for local biota and humans via runoff and wind transport into nearby marina sediments and soils (Turner, 2010; Turner et al., 2009). Marine benthonic organisms exposed to paint-contaminated sediment particles can incorporate Cu through acid-particle leaching in the

digestive tract and then act as a vector for the Cu transfer up the marine food web (Turner, 2010).

In the last years, many endeavors have been addressed in implementing environmental regulatory policies for reducing marine environment impacts by Cu-based antifouling paints and more realistic ecological risk assessment associated with these chemicals (Ytreberg et al., 2021). In European Union, the MAMPEC software has been used as a tool in the Biocidal Product Regulation (BPR, Regulation (EU) 528/2012) to predict environmental concentrations and risk levels by the simple input of biocide releasing rates in spreadsheets. However, in many circumstances, multiple anthropogenic Cu sources contributing to its accumulations at any given site prevent an accurate Cu source and appropriate environmental management actions for emission control, remediation, and policy strategies (Weiss & Rehkamper, 2008). Besides, traditional tools using Cu concentrations only are limited to discriminating Cu origins related to APs from other multiple anthropogenic sources, such as agriculture, industry, mining and smelting, and urban wastes in marine environments (Weiss & Rehkamper, 2008).

Today, the high-precision isotope ratio determinations provided by the multi-collector mass spectrometry (MC-ICP-MS) allow the use of metal stable isotope compositions in natural archives (sediment, water, bivalve mollusk, etc.) for mapping spatial and temporal source emissions patterns in the environment (Araújo, Peres, et al., 2017; Kříbek et al., 2018; Martín et al., 2018; Mihaljevič et al., 2019; Petit et al., 2013; Shiel et al., 2012; Wiederhold, 2015). Anthropogenic Cu source signatures derive from original Cu ore deposits and may integrate additional isotope changes artificially induced during ore refining and industrial manufacturing of Cu-based products (Klein & Rose, 2020). Differently, natural Cu isotope compositions are mostly controlled by weathering processes of crustal rocks and primary activity (Guinoiseau et al., 2018; Jouvin et al., 2012; Takano et al., 2014; Vance et al., 2016). These different Cu isotope systematics between natural and anthropogenic Cu sources enable Cu isotopes as a contaminant tracer. In the context of Cu pollution in marine environments, they were useful to identify temporal and spatial contamination evolution of Cu in sites under the influence of urban sources and warfare wastes legacy (Araújo et al., 2019a; Araújo et al., 2019b).

In this work, for the first time, the applicability of Cu isotopes for discriminating Cu origins related to the use of Cu-based antifouling paints in marine environments was verified. For this purpose, Cu isotopes in four sediments cores sampled were determined in the historically Cu-contaminated Mediterranean marina of Port Camargue to assess if temporal

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isotope records on these natural archives allow identifying antifouling paint's isotope fingerprints. The Cu isotope characterization of three commercial Cu-based antifouling paints and a representative sludge sample from holding tank areas fairing ships are compared to sediment data. In Port Camargue, APs represent a single-dominant anthropogenic source well-characterized in previous studies (Briant et al., 2013, 2016), which makes it an interesting environmental setting to test the feasibility of using Cu isotopes as a contaminant tracer in marina Cu pollution contexts. These new insights into a broader perspective of using Cu isotopes in coastal anthropized systems were also discussed.

## 2. Material and methods

### 2.1. Study site and sampling

A full description of the marina of Port-Camargue can be found in Briant et al (2013). Briefly, Port Camargue was built in the Camargue sand area and is one of the largest European marinas with a 60-ha basin and more than 5000 moorings. The boat maintenance area covers an area of 4.5 ha and groups 20 companies specializing in the maintenance of leisure vessels and about 2000 hull cleaning operations are carried out each year. Sedimentation tanks were installed in 2004 in this area for hull cleaning water wash down and other wastewaters. Port Camargue also receives inputs from runoff from a very small (200 ha), densely urbanized drainage basin and from the sea. The sedimentation rate in the marina is heterogeneous and low (around 1 cm yr$^{-1}$) according to bathymetric data provided by the port authorities.

In September 2011, a core sampling campaign was conducted using a UWITEC$^{®}$ corer equipped with 40 cm long polycarbonate tubes with a diameter of 8 cm to assess the vertical distribution of trace elements at 4 sites (Fig. 1). These sites were chosen as representative of the diversity of contamination in the marina. Site C8 is at the entry of the harbor, where water movements and the water column depth are the most important. It represents the less contaminated site. Site C18 is near the harbor's gas station. Site C19 is directly connected to the boat maintenance area runoff and site C25 is located in the northern channel of Port Camargue, where few water movements are present.

Cores were maintained vertically and stored at 4°C until analysis. Sub-samples of each core represented two-centimeter sections were sieved with seawater through a nylon sieve <63 µm fraction. Macroscopic or microscopic observations using scanning and transmission electron microscopy did not detect paint chips in either the sieved or unsieved samples. A sediment sample of the Vidourle River located north of Port-Camargue representing a local non-Cu contaminated site and a sludge sample from the settling tank of the boat maintenance

area of the marina were taken. Samples were not sieved. Samples of antifouling paints from three different brands (Veneziani®, International®, and Hempel®) available commercially were also characterized for elemental concentrations and isotope analysis (Table 1). These were new paintings and had not undergone any alteration. All three paints had the same copper compounds (copper oxides and thiocyanate). All samples were freeze-dried. The sediments were then ground in an agate mortar and stored in the dark at -20 °C until analysis.

### 2.2. Sample preparation

Prior to analysis, aliquots of about 100 mg of sediments, paints, or sludges were digested using a microwave-assisted acid digestion (Discover SP-D Plus, CEM®) using a mixture of 2 mL HF (Suprapur 40%, Merck Millipore®) and 4 mL HNO$_3$ (Suprapur 65%, Merck Millipore®) second the U.S. EPA method SW 846-3052. From the final digested solution, aliquots were taken for elemental and isotope Cu analysis. Cu concentrations were determined with the Thermo Scientific X Series II Q-ICP-MS (Plateforme AETE-ISO – HydroSciences/OSU OREME, Montpellier – France), equipped with a collision cell technology (CCT) chamber. An internal solution, containing In and Bi was added online to the samples to correct signal drifts. Measurements of certified reference marine sediment PACS-2 (the Canadian National Research Council- CNRC) agreed with recommended values ranging within ±5% for all analytes. The blank procedure was negligible (<0.01%) compared to the measured concentrations.

For isotope analysis, Cu was isolated from matrix elements by ion-exchange chromatography following the protocol from Marechal et al. (1999). The whole chromatography procedure was performed in an over-pressured air-filtered cleanroom (Class 10000) at the HydroSciences Montpellier Laboratory (HSM, UMR5151, Montpellier). The recovery yields (around 100 ± 10%) were systematically checked by Q-ICP-MS at the same laboratory and procedural blanks were below 1% of the Cu mass content of the samples.

Copper isotopes were measured at the Géosciences Environnement Toulouse (GET, UMR 5569, Toulouse) using MC-ICP-MS (Neptune, Thermo Scientific). Samples were dissolved in 1% (v/v) HNO$_3$ and introduced at concentrations of 300 µg L$^{-1}$ with a quartz SSI Cyclonic/Scott Dual Spray Chamber coupled with a low flow pFA nebulizer (50 to 70 µL/min). A Zn standard (JMC 3-0749 L) was added to the purified Cu fractions and a Cu (NIST 976) + Zn (JMC 3-0749 L) standard mixture was run as a bracketing standard. The mass bias correction on the $^{65}$Cu/$^{63}$Cu ratio was performed by combing the bracketing method 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 isotope compositions are expressed as $\delta^{65}$Cu values in relation to NIST SRM 976 as follows:

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

Where $R\left(\frac{^{65}Cu}{^{63}Cu}\right)_{sample}$ and $R\left(\frac{^{65}Cu}{^{63}Cu}\right)_{NIST}$ represent respectively the copper isotope ratio for the sample and for the mean of the two bracketing standards.

Sediment reference materials measured three times along each analytical session were used for analytical quality control. The $\delta^{65}$Cu$_{NIST}$ average value obtained for three full replicates of the reference marine sediment PACS-2 was 0.11±0.03‰ (2s, $n$= 6 measurements), and therefore, in line with that reported in other studies (0.05 ±0.06, 2s, $n$= 3, Araújo et al., 2019a). The external precision (2s) average obtained from two to three measurements of unknown samples and certified materials was ±0.05‰.

### 3. Results

*3.1. Concentrations and isotope composition of Cu in Port Camargue sediments*

All sediment dataset of the study is summarized in Table 1. All cores show higher Cu concentrations in the upper layers (between 0 and 10 cm) than below, with maximum values reaching 130 µg g$^{-1}$ (core C8), 203 µg g$^{-1}$ (core C25), 1090 µg g$^{-1}$ (core C18), and 1961 µg g$^{-1}$ (core C19). In core C19, after a peak concentration (1961 µg g$^{-1}$) at a depth of 7 cm, the concentrations decrease regularly to reach 76 µg g$^{-1}$ at a depth of 33 cm. On the contrary, the concentrations stabilize in inferior layers, around 100 µg g$^{-1}$ from 9 cm downward for core C8 and about 15 µg g$^{-1}$ from 15 cm to the base for core C25. For this later, these low Cu concentrations are close to the average of the Earth's Crust (25 µg g$^{-1}$, Taylor & McLennan, 1985) and to the Vidourle surface sediment (31 µg g$^{-1}$). Together, these samples can be considered representative of the local geochemical background.

The overall isotope composition for core sediment samples shows limited variability from -0.13 to 0.44‰ in comparison to studied Earth surface materials (-1.5 to +2‰, e.g. Moynier et al., 2017). In core C8, $\delta^{65}$Cu values are almost constant (-0.06 ± 0.05‰, 1s, $n = 4$), while in core C18, it is noticed a continuous decrease with depth, from 0.23 ± 0.04‰ at the surface to -0.13 ± 0.01‰ at a depth of 17 cm (Fig. 2). Core C19 shows the largest isotope

variability (0.15 ± 0.15‰, 1s, $n$ = 8), and an isotope profile accompanying Cu concentrations (linear regression, $R^2$ =0.85), except for the deeper layer (0.25 ±0.01‰ at 31 cm). $\delta^{65}$Cu values increase continuously from 0.09‰ at 30 cm depth to reach a maximum value of 0.44 ± 0.02‰ at a depth of 7 cm, followed by a decrease to around 0.25‰. Core C25 shows relatively a narrow range of $\delta^{65}$Cu values (0.06 ± 0.09‰, 1s, $n$ = 8), with an almost constant profile, except for a positive peak about 0.25‰ occurring at 5 cm depth.

### *3.2. Elemental and isotope copper characterization of antifouling paint brands*

The three selected APs show similar Cu concentration range (around 0.1-0.2% Cu) and a remarkable heavy isotope signature encompassing $\delta^{65}$Cu values between 0.49 and 0.59‰ (Table 1), and an average of 0.54 ± 0.10‰ (2s, $n$ = 3). With a $\delta^{65}$Cu of 0.56 ± 0.04‰, the isotopic composition of Cu in the sludge from heavily polluted retention tanks (Cu concentration around 30,000 μg g$^{-1}$) falls within the isotope range of Cu-based AF paints. Moreover, AP particles collected in the careen site of Banyuls harbor (France), show a similar Cu concentration with a $\delta^{65}$Cu of 0.50 ± 0.02‰ (Freydier, personal comm.).

## 4. Discussion

### *4.1. Examining Cu isotope source mixing processes in sediments from Port Camargue*

This section examines if Cu isotope source mixing processes are conservative at spatial and temporal scales, allowing quantifying Cu source contributions locally. Plotting $\delta^{65}$Cu values against its reciprocal concentration (1/[Cu]) is a useful approach to identifying source mixing processes, as well, to investigate potential biogeochemical processes' effects noising the conservative mixing behavior (El Azzi et al., 2013; Kříbek et al., 2018; Mihaljevič et al., 2019). In Fig. 3, we distinguish two main clusters: the first one, identified as sediment samples with Cu concentrations below 130 μg g$^{-1}$ and $\delta^{65}$Cu values average centered around -0.01 ± 0.13‰ (2s, $n$= 11), overlapping with Upper Continental Crust average (~ 0‰, UCC; Moynier et al., 2017). This group including the Vidourle River sample, all core C8 sub-samples, deeper layers of core C25 (below 6 cm), and the deepest layer of core C18 (16-18 cm), represent the local geochemical background, i.e., the natural Cu isotope compositions. In turn, the second cluster refers to samples with heavier and more variable $\delta^{65}$Cu values, averaging around 0.14 ± 0.24‰ (2s, $n$ = 15) with enriched Cu concentrations (above 130 μg g$^{-1}$). This group linked to various anthropogenic Cu inputs shows a great diversity of signatures and seems to tend toward the APs isotope signature for the most contaminated sample. Considering sediment cores individually, core C19 presents a clear linear gradient of

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isotope compositions accompanying concentrations (expressed as 1/Cu) (Fig. 3; $R^2 = 0.85$; excluding the layer 30-32). Because of its vicinity to the technical area, sediment from the core C19 zone has received direct APs particles (APPs) before the settling of the sludge tank in 2005. Even if the concentrations are lower, core C18 also has a linear gradient of isotope compositions accompanying concentrations ($R^2 = 0.83$). This core is away from the direct deposit of APPs linked to boat maintenance activities. However, the presence of a gas station a few meters away can induce contamination with metallic trace elements, in particular Zn, Pb, and Cu (Dong et al., 2017). Unfortunately, no information on the isotopic signature of diesel has been reported in the literature.

Even if we identified natural and APs end-members, the feasibility of using Cu isotopes to discriminate sources in the marine environment is still a challenge. As a whole, the sample isotope dataset did not fall in a typical linear array as expected for binary mixing source processes (Araújo et al., 2019a). Indeed, even in the highly Cu-enriched sediment layers associated with a high input of this element derived from APs, $\delta^{65}Cu$ values were overall lower than those obtained for commercial APs (average $\delta^{65}Cu = 0.54 \pm 0.05‰$, 1s). These outcomes suggest that Cu isotopes from APs are not transposed to sedimentary stratum in a straightway, and therefore, biogeochemical processes during releasing, deposition, and post-deposition should be considered. In aquatic environments, dissolved Cu will complex with a range of organic and to a lesser extent inorganic ligands (e.g., Coale & Bruland, 1988; Moffett & Dupont, 2007). Each of these processes (biological uptake, sorption, organic complexation) is associated with Cu isotope fractionation (e.g., Bigalke et al., 2010; Navarrete et al., 2011; Pokrovsky et al., 2008; Ryan et al., 2014; Sherman, 2013).

A first hypothesis could be related to an isotope fractionation in the Cu partition between paints and liquid phases during releasing processes by paints in water column associated with underlying speciation changes involving oxidation state, metal coordination, and complex geometry (Moynier et al., 2017; Wiederhold, 2015). As a rule of thumb, metal complexes with lower coordination states and shorter bond lengths should prefer the heavy isotope (Moynier et al., 2017). Cu normally occurs bound to oxygen as oxides in APs, while released Cu ions may bound to chlorine, sulfides, and/or organic ligands present in the seawater. *Ab initio* calculations show isotope fractionation as high as 2‰ in the isotope partitioning between aqueous free ion and Cu complexes involving common ligands present in seawater, such as sulfate, sulfides, chloride, and carbonate species (Fujii et al., 2013, 2014). Batch experiments determining release rates of APs have demonstrated the increase of solubilization of $Cu_2O$ particles with the salinity increase, suggesting an important role of $Cl^-$

ligands in this process (Adeleye et al., 2016; Lagerström et al., 2020). We hypothesize that Cu releasing by APs leads to an isotope fractionation with the preferential retention of the light isotope in non-soluble oxide Cu forms, and the preferential releasing of the heavy isotope in the form of dissolved chloro-complex. Knowing the importance of organic ligands as the main Cu dissolved complex, it is important to mention the potential fractionation induced. Organic Cu-binding ligands preferentially complex heavy isotopes (Bigalke et al., 2010; Ryan et al., 2014; Sherman, 2013). Experiments with hydroxymate, malonate, and histadine as organic ligands, show that these ligands favor the heavy isotope over inorganic free $Cu^{2+}$ ($\Delta^{65}Cu_{\text{complex-free}}$) by +0.75 to 1.5‰ (Sherman, 2013). The experiments of Ryan et al. (2014) on correlations between the stability constants (log K) of organic Cu complexes and the extent of isotopic fractionation, showed that, within the typical range of stability constants for Cu ligands in seawater ( ~11.5 to 14, Coale & Bruland, 1988; Heller & Croot, 2014; Moffett & Dupont, 2007), $\Delta^{65}Cu_{\text{complex-free}}$ fractionation in seawater fluctuates from about +0.2 to +0.5‰. The complexes formed with these ligands prevent a direct attribution of high Cu isotopic signatures from APs.

In the sediment and the associated pore water, the cycle of Cu in the core C19 of Port Camargue has been fully described in a previous study (Briant et al., 2016). Briefly, the study has shown that in summer, pore water profiles showed a steep redox gradient in the top 24 cm with the reduction of Fe and Mn oxy-hydroxides at the sediment-water interface (SWI) and of sulfate immediately below. Because Port Camargue sediments know anoxic episodes during summertime (Briant et al., 2016), an additional isotope effect could occur in the water-sediment interface and in sediments. Sulfides are common ligands in euxinic seawater systems together with chlorides, and therefore, may have a key role in the isotope fractionation of Cu. Both field observations and theoretical calculations appoint a preferential light isotope enrichment in sulfides resect to dissolved aqueous Cu (Fujii et al., 2013; Mathur et al., 2009; Savage et al., 2015). Transposing these findings to marina contexts, it is plausible that the trapping of Cu in sediments by forming insoluble Cu(I)S salts could induce an additional isotope fractionation, possibly shifting $\delta^{65}Cu$ values of sediments to negative values. On the other hand, a quantitative Cu precipitation as sulfides would transpose the isotope compositions of dissolved Cu to sulfide salts, i.e., no fractionation would occur between these two Cu-bearing phases. For last, fractionation induced by Cu sorption on solid surface processes cannot be disregarded, once it is recognized their potential fractionation is associated (Balistrieri et al., 2008; Komárek et al., 2021; Pokrovsky et al., 2008; Sherman and

Little, 2020). However, predicting the magnitude and direction is not evident due to the complex nature of particles in coastal systems and the difficulty in determining if the processes are kinetically or thermodynamically controlled.

Unlike organic and inorganic ligands, biological uptake did not show strong evidence for isotope fractionation. Limited variability of the $\delta^{65}Cu$ of dissolved Cu in various studies (e.g. Baconnais et al., 2019; Little et al., 2018; Yang et al., 2020) indicates that net associated isotopic fractionation with biological uptake, remineralization, and scavenging is probably insignificant. Yang et al. (2020) conclude that it may reflect the important role of organic ligands in the marine Cu cycle, which probably have masses of several hundred Daltons (Boiteau et al., 2016; Dupont et al., 2004), and the fact that phytoplankton may take up ligand-bound Cu directly (Semeniuk et al., 2015). Complexation with ligands makes the relative mass difference between $^{63}Cu$ and $^{65}Cu$ extremely small, potentially making mass-dependent fractionation insignificant for Cu-ligand complexes. In sediment, bacteria-metal interactions occur and could imply a fractionation. Navarrete et al. (2011) have investigated Cu-bacteria interactions with experiments conducted with common bacteria: *Bacillus subtilis* and *Escherichia coli*. Results ($\Delta^{65}Cu_{solution-solid}$ ranging from ~+1.0‰ to +4.4‰) indicate that live bacterial cells preferentially sequester the lighter Cu isotope regardless of the experimental conditions. The fractionation mechanisms involved are likely related to active cellular transport and regulation, including the reduction of Cu(II) to Cu(I). This process could modify the sediment Cu isotopic signature at the microscopic scale. However, as far as we look at bulk sediment, bacteria can only substantially change the overall Cu isotopic signature of the sediment.

All the processes evoked here explain the isotope discrepancy observed between the contaminated sediments and APs. However, post-depositional processes occurring in sedimentary archives early diagenesis probably unaffected isotope records. Obviously, being based on theoretical and field shreds of evidence, further laboratory experiments under controlled conditions are necessary to investigate the potential isotope fractionation related to Cu releasing by APs.

### 4.2. *Copper isotope composition in anthropized coastal systems worldwide: a comparative assessment*

The plot of Cu isotope compositions against Cu concentration data in sediments from Port Camargue and other anthropized coastal systems indicate distinct isotope patterns for each site enabling their discrimination (Fig.4 and Supplementary File Table 1). In this plot, Port

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Camargue stands out as the site with the highest Cu concentrations in sediments and with a general tendency for heavy $\delta^{65}$Cu values in the most Cu-enriched samples (above 130 µg g$^{-1}$), while samples with lower Cu concentrations have $\delta^{65}$Cu centered around 0‰, close to the average of the UCC. Same $\delta^{65}$Cu values, close to the UCC, were observed for the low Cu-contaminated sediments (core and surface sediments) and overall homogenous from the Loire estuary (Araújo et al., 2019b). They fell mostly in the UCC isotope range ($-0.04 \pm 0.18$‰, 2s, $n = 31$), being associated with weathering processes and soil particles export.

Another isotope trend was observed in anthropized Toulon Bay. The bay is divided into two sub-systems by a sea wall, namely Small bay and Large bay, with high and low levels of Cu pollution, respectively (Araújo et al., 2019a). In the Small bay link to Toulon city and the military harbor, surface sediments display lighter isotope compositions around -0.17 ±0.19‰ ($n$ =24, 1s). Authors have suggested that it reflects a possible mixing between new diffuse anthropogenic sources and old warfare material legacy from World War II (Araújo et al., 2019a). Contrary to Port Camargue and the identified APs source, the main anthropogenic sources seem to be light. In the Large bay, which is open to the Mediterranean Sea, the more positive values in sediments (0.08 ±0.23‰, $n$ =21, 1s) are attributed to the dominance of local natural sources.

The evolution of $\delta^{65}$Cu values in the marine environment is subject to physicochemical processes as well as to inputs related to different sources. In an estuarine environment, anthropogenic sources are varied. Thus, in the Scheldt estuary (Belgium), a preliminary study sampling seven surface sediment along a transect in the estuarine-marine continuum reported a progressive increment on $\delta^{65}$Cu values from -0.38 to 0.23‰, which was interpreted as a potential conservative mixing process between a continental-polluted source and a marine source component (Petit et al., 2008). In the Gironde fluvial-estuarine system (France), Cu isotope compositions of suspended particulate matter were consistent with a binary mixing process involving geogenic and anthropogenic inputs, this last one possibly related to the use of Cu sulfate fungicides in the vineyards surrounding the fluvial–estuarine system (Petit et al., 2013). In the Itchen estuary (England), $\delta^{65}$Cu values were not correlated to Cu concentrations and displayed remarkable negative values as low as -1.02‰ (Vance et al., 2008). If the range of $\delta^{65}$Cu values, in such environments, can be wider than in the Port Camargue environment, the $\delta^{65}$Cu values do not reach the heavier isotope ratio, nor the APs isotope ratio.

As noted, the different source apportionment involving detrital-continental, marine-autochthonous, and anthropogenic materials control the Cu isotope variability in sediment reservoirs. Natural and continental Cu is mostly derived from rock weathering processes.

While the Upper Continental Crust (UCC) presents an overall restricted isotope variation around 0.0 ±0.1‰ (Savage et al., 2015), sediments may present relatively larger isotope variability due to chemical weathering reactions (oxidation, hydrolysis, carbonation, etc.) mediated (or not) by biological activity. The combination of biotic and abiotic processes results in the Cu isotope fractionation between the primary minerals of rocks and secondary phases newly formed in the processes (Mathur et al., 2012). In the aquatic environment (freshwater, coastal water, open ocean) Cu isotopes tend to be partitioned between aqueous organic complexes enriched in the heavy isotope and residual particulate phases (Fe-Mn oxyhydroxides, clay minerals) enriched in the light isotopes (Little et al., 2014; Vance et al., 2016, Sherman and Little 2020). Experimental Cu adsorption on the surface of kaolinite under controlled conditions (T, pH, ionic, strength initial Cu concentration) also corroborates preferential adsorption of the light Cu isotope (Li et al., 2015). In macrotidal estuaries, as in the Loire estuary, large sediment loads seem to inherit the slightly lighter isotope signatures from weathered rock materials. In smaller coastal areas, under favorable hydrodynamic and geochemical conditions, marine-autochthonous sources linked to algal biomass can contribute significantly to the Cu sediment budget. In the Large bay of Toulon, the dominant natural Cu characterized by a positive $\delta^{65}Cu$ value average was linked to Cu-organic matter compounds. This hypothesis is based on the experimental Cu adsorption on insolubilized humic acid, which indicated the heavy isotope preferential retention in the organo-Cu complexes (Bigalke et al., 2010).

Main anthropogenic Cu sources in coastal systems include industrial effluents, agriculture soil runoff (El Azzi et al., 2013), urban waste sludge, and copper smelting waste (Araújo, Boaventura, et al., 2017). The origins of anthropogenic Cu signatures are intrinsically attached to feeding ores used in the metallurgical processes, to identify well-marked and "universal" isotope patterns for the distinct anthropogenic Cu sources is still challenging. Cu ore deposits have large isotope ranges spanning all soil, sediment, and water compartments, varying second the mineral type (oxide vs. sulfides), and passive to changes along the metallurgical ore refining. Indeed, as observed in the literature, anthropogenic Cu delivered in the environment can undergo isotope fractionation during post-depositional biogeochemical processes. Despite these drawbacks, Cu anthropogenic signatures of sources tend to exhibit large ranges, or more extreme values toward positive or negative directions, than the natural range of rocks and weathered materials. For example, the $\delta^{65}Cu$ value ranged from −0.95‰ to 0.44‰ in the agricultural slurry contaminated by pigs; 0.30 to 0.61‰ in cement, (Souto-Oliveira et al., 2018); from 0.46 to 0.59‰ in vehicular aerosol (Souto-

Oliveira et al., 2019); and around 0.54 ±0.05‰ in Cu-based antifouling paints (this study). Consistent with the Cu isotope systematic of anthropogenic sources, we note that the Cu-contaminated coastal sites of the Gironde estuary, Toulon bay (Small bay), and Port Camargue, tend to have sediments isotope values felling out far from the UCC range around 0‰. The respective heavy or light isotope enrichments are likely to reflect the Cu isotope from anthropogenic sources and/or environmental processes. Despite the potential APs related isotopic fractionation upon Cu release, the final anomalous isotopic signatures recorded in the sediments appear to be differentiated from the natural geochemical baseline. However, further studies are needed to directly link an anthropogenic source to a signature recorded in the sediment.

## 5. Conclusions

In this work, we tested the applicability of Cu isotopes for discriminating Cu origins related to the use of Cu-based antifouling paints in the largest European Marina, the Port Camargue. The overall isotope composition of four sediment cores and a surface sample ranges from -0.13 to 0.44‰. The three selected brands of APs showed similar Cu concentrations in the range 0.1-0.2% and $\delta^{65}Cu$ values average of 0.54 ± 0.05‰. The plot of $\delta^{65}Cu$ values against reciprocal concentration (1/Cu) for all dataset allowed to identify natural (-0.01 ± 0.13‰) and anthropogenic (0.14 ± 0.24‰) end-members. However, sample isotope systematics were not consistent with a conservative mixing binary source process. Even if heavier Cu-contaminated sediment shows isotope signatures slightly lighter than those obtained for the APs brands, results indicate that biogeochemical processes on water column are modifying the isotope records of sources. As a first hypothesis, we attribute to a potential isotope fractionation during the Cu release by APs, with the preferential retention of the light isotope in non-soluble Cu oxides and the heavy isotope in the form of dissolved chloro-complexes or organic ligand complexes. Thus, the isotope signature of APs is not directly transposed to sedimentary archives linking to many fractionation processes in the water column. Post-depositional processes occurring sedimentary archives early diagenesis probably unaffected isotope records. In terms of mass balance, the exchange of Cu with porewater is negligible to the bulk solid phase. However, in extremely particular conditions they could be discriminable from the natural range (e.g. core C19). Cu isotopes could be a promising tool for investigating Cu pollution in marinas systems. Nevertheless, further laboratory experiments under controlled conditions are recommended to model the potential isotope fractionation related to Cu release by APs in aquatic systems.

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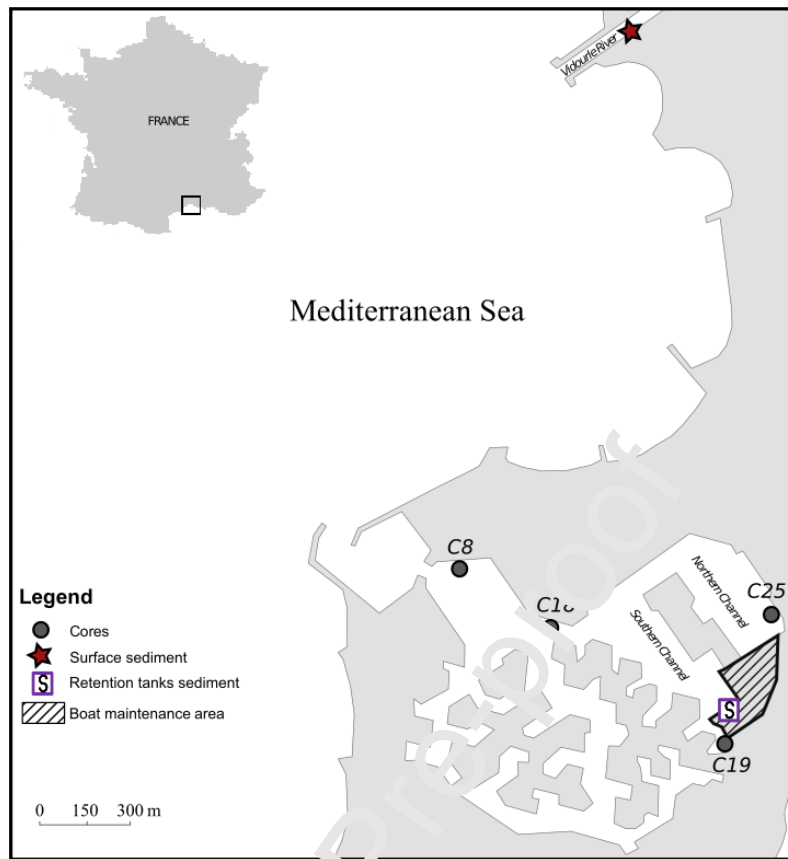
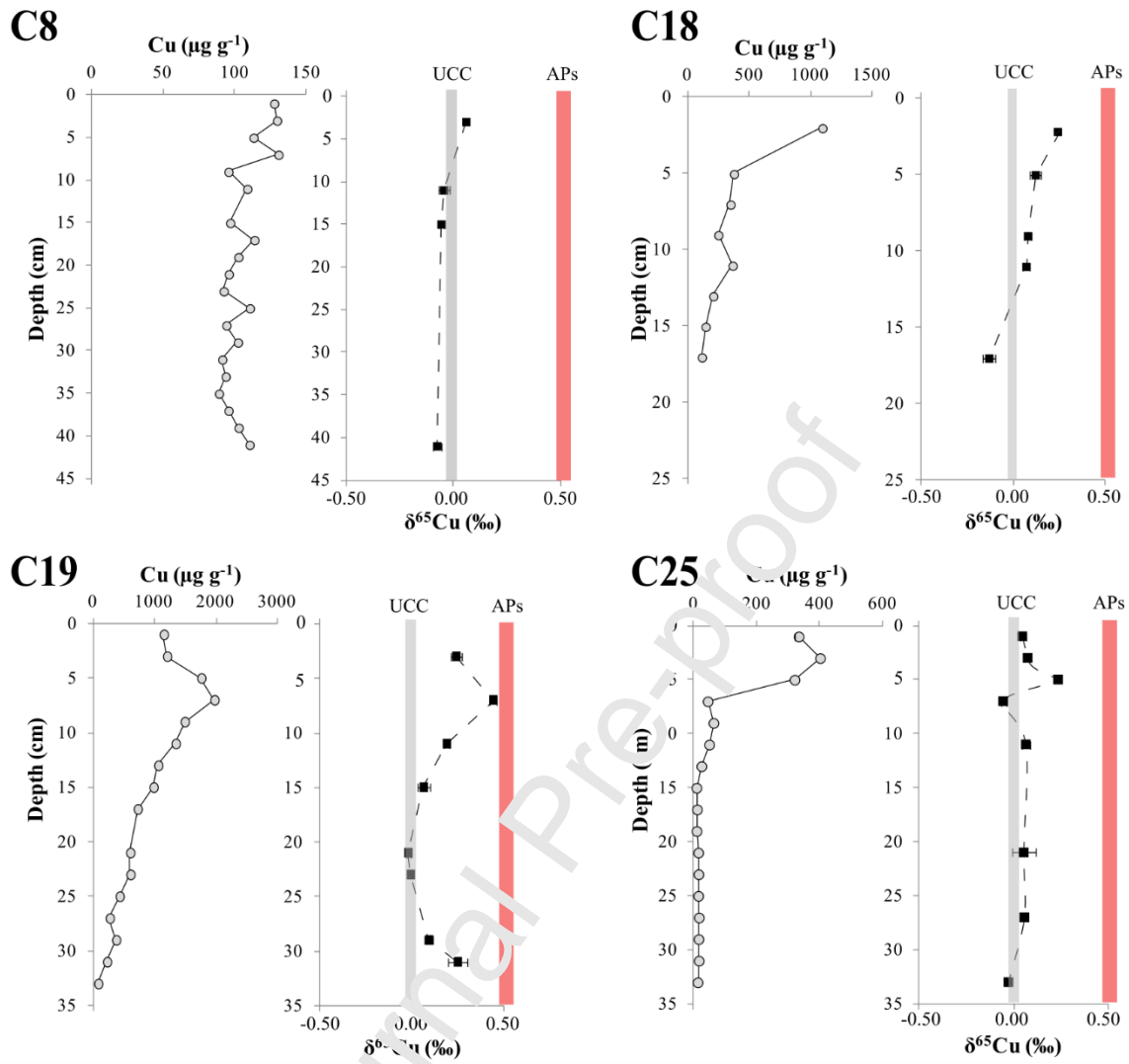**Figure 1:** Map of Port Camargue with sediment sampling stations.

**Figure 2**: Isotope and elemental Cu profiles of sediment cores. The vertical grey strips represent the isotope range of the Upper Continental Crust (UCC) and the red stripes represent the isotope range of antifouling paints (APs).
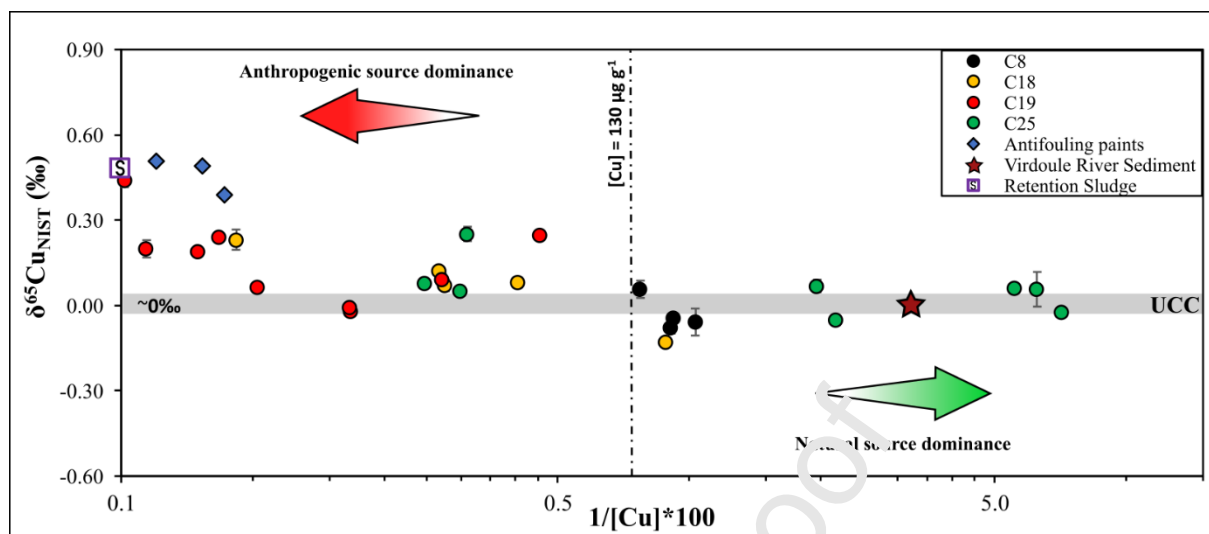
**Figure 3**: Cu isotope compositions ($\delta^{65}$Cu in ‰) vs the inverse of Cu concentration (1/µg g$^{-1}$) for all study samples. The horizontal gray strip corresponds to the isotope average of the Upper Continental Crust (UCC). The vertical dashed line represents the Cu concentration boundary between samples with a high influence of anthropogenic and natural sources, respectively. The regression analysis of core C19 sub-samples (excluding the base layer 30-32 cm) shows an isotope gradient accompanying the elemental Cu enrichment with R² = 0.85. The regression analysis of core C18 shows an isotope gradient accompanying the elemental Cu enrichment with R² = 0.83 while R²= 0.54 for core C25.

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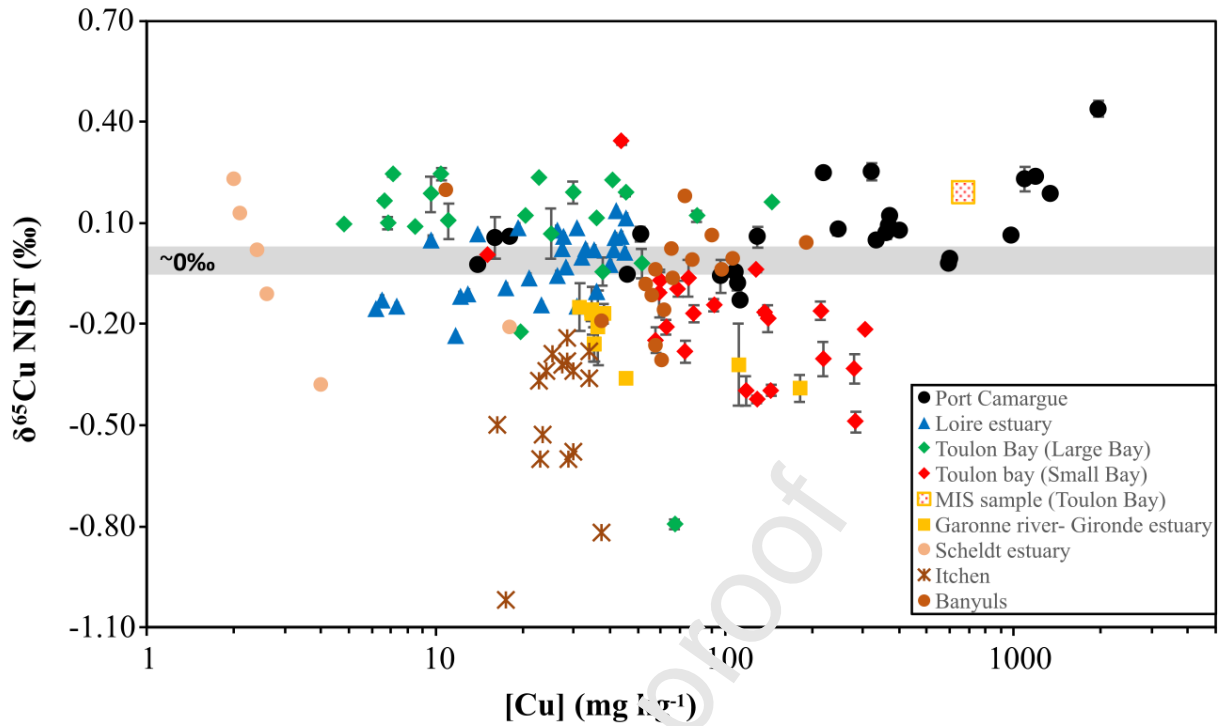**Figure 4:** Cu isotope compositions (‰) vs Cu concentrations (µg g$^{-1}$) for anthropized coastal systems: Port Camargue (this study); Loire estuary (Araújo et al., 2019b); Toulon Bay (Araújo et al., 2019a); Garonne river- Gironde estuary (Petit et al., 2013); Scheldt estuary (Petit et al., 2008); Itchen estuary (Vance et al., 2008), Banyuls (Freydier et al., personal comm).

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**Table 1**: Cu concentrations and $\delta^{65}$Cu ratios of studying samples. n.a = non-analysed.

| Samples | | $\delta^{65}$Cu (‰) | $\sigma$ (‰) | Depth (cm) | [Cu] ($\mu g\ g^{-1}$) | 1/Cu ($g\ \mu g^{-1}$) |
|---|---|---|---|---|---|---|
| *C8* | 2-4 | 0.06 | 0.03 | 3 | 129.4 | 0.008 |
| | 10-12 | -0.05 | 0.01 | 11 | 108.6 | 0.009 |
| | 14-16 | -0.06 | 0.05 | 15 | 96.7 | 0.010 |
| | 40-42 | -0.08 | 0.02 | 41 | 110.2 | 0.009 |
| *C18* | 0-4 | 0.23 | 0.04 | 2 | 1090.0 | 0.001 |
| | 4-6 | 0.12 | 0.00 | 5 | 373.2 | 0.003 |
| | 8-10 | 0.08 | 0.02 | 9 | 246.2 | 0.004 |
| | 10-12 | 0.07 | 0.02 | 11 | 362.3 | 0.003 |
| | 16-18 | -0.13 | 0.01 | 17 | 113.0 | 0.009 |
| *C19* | 2-4 | 0.24 | 0.00 | 3 | 1193.0 | 0.001 |
| | 6-8 | 0.44 | 0.02 | 7 | 1961.0 | 0.001 |
| | 10-12 | 0.19 | 0.00 | 11 | 1333.0 | 0.001 |
| | 14-16 | 0.06 | 0.01 | 15 | 973.7 | 0.001 |
| | 20-22 | -0.02 | 0.01 | 21 | 595.7 | 0.002 |
| | 22-24 | -0.01 | 0.00 | 23 | 599.6 | 0.002 |
| | 28-30 | 0.09 | 0.02 | 29 | 367.4 | 0.003 |
| | 30-32 | 0.25 | 0.01 | 31 | 219.4 | 0.005 |
| | 32-34 | n.a | n.a | 33 | 76.1 | 0.013 |
| *C25* | 0-2 | 0.05 | 0.02 | 1 | 335.0 | 0.003 |
| | 2-4 | 0.08 | 0.01 | 3 | 402.7 | 0.002 |
| | 4-6 | 0.25 | 0.03 | 5 | 321.9 | 0.003 |
| | 6-8 | -0.05 | 0.02 | 7 | 46.0 | 0.022 |
| | 10-12 | 0.07 | 0.02 | 11 | 51.0 | 0.020 |
| | 20-22 | 0.06 | 0.06 | 21 | 16.0 | 0.063 |
| | 26-28 | 0.06 | 0.00 | 27 | 18.0 | 0.056 |
| | 32-34 | -0.02 | 0.00 | 33 | 14.0 | 0.071 |
| *AF paint* | *Veneziani®* | 0.59 | 0.05 | | 1655.8 | 0.001 |
| | *International®* | 0.53 | 0.03 | | 1296.0 | 0.001 |
| | *Hempel®* | 0.49 | 0.01 | | 1153.5 | 0.001 |
| *CRM* | PACS (n=6) | 0.11 | 0.03 | | 299.0 | 0.003 |
| *Surface sediment* | Retention tanks | 0.56 | 0.04 | | 30260.0 | 0.000 |
| | Vidourle river | 0.00 | 0.01 | | 31.0 | 0.032 |

**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:

## Authors contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Rémi Freydier, Nicolas Briant, and Sophie Delpoux. The first draft of the manuscript was written by Nicolas Briant. Rémi Freydier, Daniel F. Araujo and Françoise Elbaz-Poulichet commented on previous versions of the manuscript. All authors read and approved the final manuscript.