Application of Zn Isotope Compositions in Oysters to Monitor and Quantify Anthropogenic Zn Bioaccumulation in Marine Environments over Four Decades: A “Mussel Watch Program” Upgrade

Ferreira Araujo Daniel ^{1,*}, Ponzevera Emmanuel ¹, Weiss Dominik Jakob ^{2,3}, Knoery Joel ¹, Briant Nicolas ¹, Yepez Santiago ⁴, Bruzac Sandrine ¹, Sireau Teddy ¹, Brach-Papa Christophe ⁵

¹ Laboratoire de Biogéochimie des Contaminants Métalliques, IFREMER, Centre Atlantique, F44311 Nantes, Cedex 3, France

² Department of Earth Science and Engineering, Imperial College London, London SW7 2BP, United Kingdom

³ Department of Civil and Environmental Engineering, Princeton University, Princeton, New Jersey 08540, United States

⁴ Department of Forest Management and Environment, Faculty of Forestry, University of Concepcion, Calle Victoria, 500 Concepción, Bio-Bio, Chile

⁵ Laboratoire Environnement Ressources Provence-Azur-Corse, Ifremer, Centre Méditerranée, Zone Portuaire de Brégaillon, CS20 330, 83507 La Seyne-sur-Mer Cedex, France

* Corresponding author : Daniel F. Araujo, email address : daniel.ferreira.araujo@ifremer.fr

Abstract :

The application of zinc (Zn) isotope compositions in bivalve organisms to quantify anthropogenic Zn bioaccumulation in marine biota is of great interest to environmental marine management programs such as the “Mussel Watch Program”. Field studies, however, are urgently needed to test its practical value. To this end, we investigated Zn isotope variations in the oyster *Crassostrea gigas* collected over four decades near the Loire estuary (France), where previous geochemical studies provided evidence for a regionally uniform but temporally variable metal contamination. We show that the Zn temporal isotope profile of oysters matches that of the sedimentary records with an isotope offset of approximately +0.5–0.7‰, tentatively attributed to compromised estuarine processes and trophic transfer. A Zn isotope model for quantifying anthropogenic Zn bioaccumulation suggests an overall decrease in anthropogenic estuarine Zn levels over the past 40 years. This first successful application of Zn isotope ratios in a bivalve species to quantify anthropogenic Zn bioaccumulation confirms their utility for supporting environmental management strategies in marine biomonitoring programs.

Graphical abstract

Keywords : Loire estuary, Bay of Biscay, nontraditional isotopes, metal contamination, biomonitoring, marine pollution, bivalve mollusk, *Crassostrea gigas*

41 Introduction

42 Marine ecosystems are under intense pressure from human activities and monitoring and
43 assessing the pollution status is critical when developing emission control, remediation, and
44 policy strategies.¹ Bivalve mollusks are widely used as low-cost biomonitors of metal pollution
45 of coastal and marine environments in ‘Mussel Watch Programs’ implemented worldwide.^{2–5}
46 The use of these biomonitors is advantageous as they provide information about changing
47 trends in metal bioavailability of marine and coastal environments,⁶ allowing for a better
48 temporal resolution than sediment core profiles^{7,8} and requiring less laborious and time-
49 consuming sample preparation than water samples.

50 Over the last two decades, stable isotopes of metals, such as, Zn, Cu, Cd, Cr, Hg, and
51 others have been studied for possible applications as tracers for pollutant sources in sediment,^{9–}
52 ¹¹ water^{12–14} and aerosols.^{15–17} and their ability to identify contaminant pathways using abiotic
53 samples (sediments, aerosols, soils) has been demonstrated^{11,18}. However, their use in
54 biomonitor organisms remain scarce. First successful applications were related to atmospheric
55 pollution, with the use of Zn isotope signatures in leaves and lichens to discriminate
56 anthropogenic emissions from road traffic, mining and combustion sources.^{19,20} In the marine
57 environment, a first laboratory experiment has shown that oyster tissues record conservatively
58 the isotope compositions of sources, being unaffected by salinity effects and Zn internal
59 distribution.²¹ This important finding make it worthwhile to verify in the field the possibilities
60 of extracting Zn source information from “Mussel Watch Programs” or environmental
61 specimens banks. Pioneering surveys on Zn isotope compositions in bivalves from Canadian,
62 American, Brazilian, and French coasts have reported a broad geographical and inter-species
63 isotope variability.^{10,22–24} Despite promising results, all previous field studies could not perform
64 an accurate identification and apportionment of the anthropogenic Zn bioaccumulation. In most
65 part, either due to the lack of an initial isotope characterization of local sources, a lack of isotope
66 variability in samples, or use of different bivalve species at large spatial scales.

In this work, we studied Zn isotope variations in oyster soft tissues collected over the course of four decades in the vicinities of the Loire estuary (North Bay of Biscay, France). Being the outlet of one of the major European rivers, this estuary integrates Zn contaminants delivered by diverse sources upstream, including urban, industrial, agricultural, maritime and fossil fuel burning activities.²⁵ Geochemical records from sediment cores collected along the riverine and estuarine Loire watersheds revealed an accentuated polymetallic contamination of Zn, Pb, Hg, Cd, and Cu, during the period from 1950 to 1980,^{25–27} the so called “Great Acceleration”.²⁸ In our previous study in Loire estuary and neighboring coasts, including Bourgneuf bay, we used Zn isotopes to establish a mixing model for natural and anthropogenic Zn sources.²⁵ However, a quantitative information on the transfer of this toxic metal from abiotic (water and sediment) compartments to the biosphere was still missing. This information is of utmost importance for regional aquaculture management and shellfish-farming. For example, oyster-farming areas of Bourgneuf Bay (south Loire estuary), ranking sixth in France with a production up 8,600 tons,²⁹ are reached by the estuarine plume of the Loire river with its low-salinity and high turbidity and associated contaminants discharged by the Loire estuary.^{30–}
³³ Given the sampling stations of the French “Mussel Watch program” ongoing since early 1980’s, and our previous Zn source isotope characterization,²⁵ the Loire estuary and Bourgneuf Bay constitute an ideal site to assess the potential of Zn isotope signatures of harvested oysters to reconstruct temporal anthropogenic Zn bioaccumulation. It will yield information on the anthropogenic Zn uptake by filter-feeders and validate the use of Zn isotopes in oysters employed in biomonitoring networks (in the so-called “Mussel Watch” programs) to support environmental management strategies.

Material and methods

Study area

The macrotidal Loire estuary is ca. 100-km long and ends the longest French river (1012 km, mean annual water discharge of 846 m³/s, Fig. 1a-c).² The maximum turbidity zone (MTZ),

where suspended particulate matter (SPM) in the column water reach $> 1 \text{ g L}^{-1}$, is 20- to 50-km long^{34,35}. The Loire river watershed has hosted important economic activities along its recent history including textile industry, leather and fur-tanning in the XVIth century, and then based on exploitation of geological resources (mining and metallurgy), raw materials and energy production from the Industrial Revolution in the XIXth century until present.^{25,26,36,37} The Loire Estuary basin *stricto sensu* is highly industrialized and urbanized comprising the metropole region of Nantes and Saint-Nazaire and its $> 800,000$ inhabitants (Fig. 1c).^{25,26,36,37} Spatial and temporal correlations of trace metal concentrations in surface and sediment core in the Loire estuary and coastal vicinities suggest common sources and mechanism of dispersion.^{25,36,38} At a smaller scale, hydrodynamic models demonstrate a strong accumulation of fine sediments in Bourgneuf bay derived from the Loire estuary as result of the sediment plume dispersion induced by wind and tidal regimes, and density flows (Fig. 1b). Map elaboration details are included in Supporting Information (SI, Appendix 1).³⁹ These findings are consistent with the Loire estuary macro-tidal character associated with high hydrodynamic energy that promotes the transport of fine-sediment particles enriched in metal contaminants at long-distances along the western Atlantic continental margin of the Bay of Biscay.^{31,40} Inputs of fine material from estuarine plumes from the Loire, Gironde, and Vilaine rivers settle in muddy areas along the Bay of Biscay continental shelf. One hundred km from the Loire estuary mouth, the “large mud patch” (i.e., “La Grande Vasière”) is the main zone of fine material deposition, with 250 km long and 30 km wide (Fig. 1a).^{41–43} The use of elements of anthropogenic origin, such Zn and Pb enabled to distinguish sources of sedimentary material between the estuaries of Loire and Gironde.^{38,40}

South of the Loire estuary, the Bourgneuf Bay is a 340 km² macrotidal embayment, of which ca. 30% are intertidal zones.^{29,44} It is connected to the Atlantic Ocean only by its NW quadrant, and bounded by the mainland and the Noirmoutier island.⁴⁵ No major river drains into the bay, being it is surrounded by extensive marshes, drained by dozens of creeks.^{46,47} The Bay of Bourgneuf is the main locale for oyster-farming in the Pays-de-la-Loire administrative

region, representing 8% of national French production.²⁹ There are currently 283 mainly small oyster farms settled over the intertidal zone, producing Pacific oysters (*Crassostrea gigas*) in approximately three-year growth cycles for sale in the local market.^{45,48}

North of the Loire estuary, the Vilaine Bay encompasses an area of 200 km², its main tributary, is the 200km-long Vilaine river.⁴⁹ Its 10-m water column is subjected to intense vertical mixing, which can lead to sediment resuspension and high turbidity.⁵⁰ The Pénérf river discharges into the northeastern part of Vilaine Bay. Its upper course drains rural areas, and small agri-food industries. The lower part drains more densely populated areas including two resort towns, Tour du Parc and Damgan.⁵¹ Local mariculture has been an important activity since the end of the XIXth century.

Bivalve sampling and sample preparation

The bivalve mollusks' tissue samples were acquired from sample collection of the French National Monitoring Network RNO-ROCCH (Observation Network of chemical contamination of the marine environment) managed by the French Institute for the Exploitation of the Sea (IFREMER). Each monitoring site along the French shoreline is sampled at least twice yearly. In this study, eleven oyster samples (*Crassostrea gigas*) harvested at Bourgneuf bay ("La Coupelasse" site, 47° 0' 44" N; 2° 1' 23" W, Fig. 1c) were taken to cover a decadal period between 1985 and 2016. Samples were from the same season (autumn) to reduce possible influences of seasonal parameters like spawning, diet, and fluvial water discharge fluxes. For the year 1997, we used a complete seasonal sampling (winter-spring-summer-autumn) to investigate possible seasonal effects on Zn isotope records in oysters from Bourgneuf bay. In Vilaine bay, ten oyster samples from "La Pointe Er Fosse" sampling station (47° 30' 31.788" N; 2° 38' 46.8168" W, Fig. 1a), close to Pénérf river outlet, were harvested annually in the winter season comprised the period between 2009 and 2018.

Bivalve sampling and preparation were performed as described by Claisse et al.⁵² After collection from their natural bed, ca. dozens of oysters were cleaned from epibiota and kept for

24 h in approximately 20 L of filtered seawater taken from the collection site. This depuration step eliminates feces and pseudo-feces from the digestive tracts of the animals. Then, 2 -to-3-year old individual oysters were selected on the basis of their shell length to decrease biases related to body size or age. At least ten oysters were pooled into a single sample. Bivalves were then shucked, dripped dry in a Buchner funnel, and frozen whole. Subsequently, frozen samples were thawed, homogenized, and freeze-dried. They were stored until analysis in acid-cleaned glass containers at dark, ambient conditions, in a hygrometry-controlled room.

Sediment sampling

In this discussion, we include Zn isotope results of a sediment core and surface sediments previously published by Araújo et al.²⁵ (Table S1). This sediment core, labeled “PV1” (~300 cm length), was sampled on an inner intertidal mudflat of the Loire estuary (47° 17' 14" N; 2° 3' 48" W, Fig. 1c), during the Paleovase mission in Mars 2015. Coring was carried out using aluminum tubes (6m long, 74mm inside diameter and 1mm thick), and subsampled into 2 cm-thick sediment slices, every 5 cm in the upper meter and every 10 cm for the rest of the sediment core. After collection, the sub-samples of core PV1 were frozen, lyophilized, homogenized, and sieved at 2 mm. A sedimentation rate of approximately 0.7 cm/year was estimated using the excess ²¹⁰Pb dating technique, demonstrating that each core slice represents at least three years of sedimentation. This chronometer combined with geochemical metal concentration profiles allowed to mark the beginning of post-industrialization period at 96 cm deep in the PV1 core, corresponding approximately to the end of XIXth century.²⁵ For the lateral mapping of isotopic compositions of sediment, 17 surface sediments (1-2 cm thick) were collected using Ekman grab in 2014 through the Loire estuary and Bourgneuf Bay (Fig. 1c). After collection, surface sediment samples were stored in plastic bags, deep-frozen, lyophilized, homogenized and sieved at 2 mm. All surface sediments were acquired from the ROCCH sediment sample bank.

Sample preparation and elemental analysis

About 200 mg aliquots of dry bivalve tissue samples were digested with nitric acid solutions using a microwave system (Ethos Up, ThermoFisher). Dry aliquots of sediments were digested in Teflon® bombs on a coated graphite block using a multiple-step acid procedure with concentrated HF, HCl, and HNO₃ acids. After sample digestion, aliquots from the final extract solution were split for subsequent elemental and isotope analyses. Procedural blanks and international certified reference materials (CRM) were processed with each batch of samples (wet digestion, elemental analysis, ion chromatography and isotope determination). CRM included oyster and mussel tissues (SRM 1566b, NIST® and ERM-CE278k, ERM®) and sediments (MESS-3 and PACS-2 from NRC – CNR®). All reagents, labware acid cleaning and dilution solutions for elemental and isotope analyses were performed using 18.2 MΩ.cm⁻¹ H₂O (Nanop System®) and ultra-pure acids (PlasmaPure Plus grade, SCP Science®). Zinc concentrations were determined by inductively coupled plasma mass spectrometry (Q-ICP-MS, iCAP Qc, Thermo Fisher Scientific) at the Laboratoire de Biogéochimie des Contaminants Métalliques (LBCM, IFREMER).

Zinc isotope ratio determinations

An ion-exchange chromatographic column was employed for the separation of Zn analytes from matrix elements before isotope analysis. Aliquots of the final extract of digested samples containing around 1-4 µg of Zn were processed through a column bed packed with AG-MP1 resin (Biorad®) following an adapted elution protocol of Maréchal et al.⁵³ Chromatographic recovery yields were 100 ±10% for all samples. Procedural blanks corresponded to less than 1% of the total Zn aliquot mass loaded in the chromatographic column.

Zinc isotopes were measured by MC-ICP-MS (Neptune, Thermo Scientific) at the Pôle Spectrométrie Océan laboratory - PSO (IFREMER, Brest, France). Samples were dissolved in 2% (v/v) HNO₃ to match concentrations at 300 ng g⁻¹ and introduced into the spectrometer using a SIS system. Sample standard bracketing combined with external normalization technique using Cu NIST standard doping was employed to correct the instrumental

fractionation. The final Zn isotope compositions are expressed in this paper as $\delta^{66}\text{Zn}$ values in relation to the “JMC” (Johnson Matthey Company, lot 3-0749-L), as follow:

$$\delta^{66}\text{Zn}_{\text{JMC}} (\text{‰}) = \left(\frac{R\left(\frac{^{66}\text{Zn}}{^{64}\text{Zn}}\right)_{\text{sample}}}{R\left(\frac{^{66}\text{Zn}}{^{64}\text{Zn}}\right)_{\text{JMC 3-0749-L}}} - 1 \right) \times 1000 \quad (\text{Eq.1})$$

The average precision (2s) of $\delta^{66}\text{Zn}$ determinations was $\pm 0.04\text{‰}$, obtained from repeated MC-ICP-MS determinations of unknown samples and reference materials (RMs). The uncertainty bars in the figures corresponds to this value. The RMs MESS-3 marine sediment ($n = 9$) and SRM 1566b oyster tissue ($n = 4$) were prepared in replicates which followed the complete analytical protocol, i.e., sample digestion, ion-exchange chromatography, and replicated isotopic analyses. The $\delta^{66}\text{Zn}_{\text{JMC}}$ value for MESS-3 was $+0.26 \pm 0.06\text{‰}$ (2s, $n = 9$), in line with the previously published value, $+0.28 \pm 0.02\text{‰}$ (2s).⁵⁴ Certified reference materials for bivalve tissues matrixes for Zn isotope analysis are inexistent. As an alternative, research groups have reported $\delta^{66}\text{Zn}_{\text{JMC}}$ values for reference materials that are commercially available. A first reported $\delta^{66}\text{Zn}_{\text{JMC}}$ value to NIST 1566a oyster reference material was about of $-0.18 \pm 0.07\text{‰}$ (1s, $n = 4$). This value differs significantly from our obtained value of $+0.70 \pm 0.05\text{‰}$ (2s, $n = 5$) for the SRM 1566b. These new reported values extend the RM repertory for organic matrixes,^{55–57} and can allow future inter-laboratory comparisons and quality assurances.

Results and discussions

Temporal and spatial evolution of Zn isotope composition in sediments

The sub-samples of sediment core PV1 core show a narrow range of $\delta^{66}\text{Zn}_{\text{JMC}}$ values centering around $+0.26 \pm 0.07\text{‰}$ (2s, $n = 11$) and Zn concentrations vary between 159 and 237 $\mu\text{g g}^{-1}$. The chronology of Zn concentrations and isotope profiles in the PV1 core suggest three main stages of Zn pollution (Fig. 2): (i) a pre-industrial period, with low Zn concentrations and Zn isotope

227 compositions close to an Upper Continental Crust (UCC) average;⁵⁸ (ii) an industrial period,
 228 with a noticeable increase of Zn concentrations at the end of XIXth century, supplemented by
 229 lower $\delta^{66}\text{Zn}_{\text{NMC}}$ values; (iii) a period of weakening Zn contamination from the 1980s onwards,
 230 associated with significant diminution on Zn concentrations, and a return of $\delta^{66}\text{Zn}_{\text{NMC}}$ values to
 231 the UCC isotope range. These distinct different stages point to progressive changes of Zn
 232 sources through the time. Therefore, Zn isotope geochemical profiles of sedimentary archives
 233 are useful to establish temporal trends of anthropogenic inputs (i.e., pollution) on the decadal
 234 and longer time-scale. The $\delta^{66}\text{Zn}_{\text{NMC}}$ values in surface sediments (from +0.13 to +0.33, Fig. 3,
 235 Table S1) and water samples from upstream Loire river (from +0.21 to +0.38) fell in the same
 236 range values observed for the PV1 core. This is expected once Zn anthropogenic inputs from
 237 the Loire estuary are dispersed in the Bay of Biscay, associated with estuarine particles and
 238 waters. Zn isotope variability in Loire fluvial water samples was associated with mixing
 239 processes between sources like WWTP effluents and/or non-exhaust road-traffic emissions.¹¹
 240 Conversely, a mixing between natural sources ($\sim +0.3\text{‰}$), represented by terrigenous material
 241 and the anthropogenic Zn sources, ($\sim +0.1\text{‰}$, Fig. 3) was attributed for the underlying estuarine
 242 sediments. The single identified anthropogenic end-member in the Loire-Bourgneuf system
 243 was related to the mixing of multiple anthropogenic sources in urban sewage networks and/or
 244 tributaries. Overall, Zn anthropogenic sources tend to have lighter $\delta^{66}\text{Zn}_{\text{NMC}}$ values than
 245 geological background, such as tire wear (from +0.00 to +0.20‰),^{16,59} gasoline (between -0.50
 246 and +0.07‰),^{11,60} urban runoff (from +0.13 to +0.15‰),⁶¹ wastewaters and sludge
 247 ($\sim 0.05\text{‰}$),^{11,61} industrial effluents (chemical and agro-food industry, between +0.10 and +
 248 0.15‰)¹¹ and atmospheric industrial emissions (from -0.6‰ to +0.15).^{62–64} As noted, most
 249 industrial and urban sources have isotope averages centered around 0.1‰, which make their
 250 discrimination an analytical challenge. Despite this drawback, $\delta^{66}\text{Zn}$ signatures constitute a
 251 “global” tool for tracking urban and industrial anthropogenic fingerprints in aquatic systems
 252 (Fig. 3), including the case of Loire estuary.¹¹ A complete review about Zn isotopes
 253 contaminant systematic in aquatic systems can be found in Desaulty and Giraud.¹¹

254 *Oyster temporal isotope profiles*

255 Ten years of biomonitoring (2009-2018) of oysters from Vilaine bay shows uniform $\delta^{66}\text{Zn}_{\text{NMC}}$
256 values around $+0.71 \pm 0.06\text{‰}$ (2s, $n=10$). In contrast, Zn concentrations vary largely from 1.5
257 and 3.5 mg g^{-1} (average of $2.3 \pm 1.2 \text{ mg g}^{-1}$, 2s, $n=10$), with temporal Zn concentration profile
258 characterized by an increase followed by a decrease during the sampling period (Table S2, Fig.
259 S1). Vilaine bay has no documented Zn pollution history, and the constant temporal Zn isotope
260 profile confirms the lack of substantial change on Zn source apportionment in this system for the
261 studied period. Indeed, we assume that our sampling scheme (same-season samplings) is
262 appropriate to alleviate potential seasonal Zn isotope changes, unrelated to changes on Zn source
263 contributions.

264 In turn, Zn concentrations vary by a factor of nearly 2. This suggests a dynamic cycling
265 of Zn in this shallow estuarine environment, where Zn bioavailability may vary. Likely drivers
266 for such changes include the tidal effects on geochemical conditions like salinity, pH and Eh
267 mediated by a variable primary productivity, frequency/intensity of particle resuspension
268 episodes (e.g., storms), changes in riverine fluxes of metal or watershed run-off.^{1,65,66} This
269 finding evidence that, for a same season, biogeochemical processes does not induce significantly
270 change on Zn isotope records of oysters.

271 In the second sampling site, Bourgneuf Bay, oyster tissue samples exhibit $\delta^{66}\text{Zn}_{\text{NMC}}$
272 values varying between $+0.67$ and $+0.94\text{‰}$, while Zn concentrations range from 1.4 to 3.7 mg
273 g^{-1} (Table S2). Seasonally, in spite of co-occurring Zn concentrations changes, $\delta^{66}\text{Zn}_{\text{NMC}}$ values
274 fell in a narrow range comprising $+0.78$ and $+0.86\text{‰}$ (Table S2, Fig. S2). Like for the Vilaine
275 bay, this confirms that Zn source apportionment is constant through the different seasons, even
276 though estuarine Zn bioavailability may change drastically. The decadal temporal evolution of
277 the isotope signatures and elemental concentrations of Zn in oyster samples from Bourgneuf
278 Bay are presented in the Fig. 4a: $\delta^{66}\text{Zn}_{\text{NMC}}$ increases progressively from 1985 to 2013 before
279 slightly decreasing, while Zn concentrations overall decrease as well ($r = -0.72$, $p < 0.05$).
280 Interestingly, the decadal trend of oyster data since the 1980s match with the one observed in

the PV1 core (Fig. 4b). The data of the contamination trend from the two independent environmental records materialized by a sediment core and by biomonitoring can be used to infer that Zn isotope systematics in oysters is controlled by a mixing source processes analogous to sediments. Indeed, lighter isotope compositions show more pronounced Zn anthropogenic source contribution, and heavier $\delta^{66}\text{Zn}$ a more natural Zn origin. Based on that premise, we propose a similar mixing model between natural and urban anthropogenic sources to describe Zn isotope variability in these organisms. Such a simple model allows to quantify the anthropogenic Zn bioaccumulation in bivalve populations over time in the Loire estuary. While similar approaches with Pb isotope ratios have been useful to reconstruct the temporal evolution of Pb pollution with its compared isotopic signature in soil vs. peat bogs, and in sediment vs. lichens,^{67–69} we tested here for the first time the use of Zn isotope signatures in a biomonitoring organism to quantify Zn bioaccumulation and to reconstruct anthropogenic Zn loads in the marine environment.

Quantifying Zn anthropogenic bioaccumulation over time

The $\delta^{66}\text{Zn}$ data from oyster samples enable us to use a simple binary mixing model to quantify the Zn anthropogenic bioaccumulation ($\text{Zn}_{\text{anthropogenic}}(\%)$) over the time:¹⁸

$$\text{Zn}_{\text{anthropogenic}}(\%) = \left(\frac{\delta^{66}\text{Zn}_{\text{sample}} - \delta^{66}\text{Zn}_{\text{natural}}}{\delta^{66}\text{Zn}_{\text{anthropogenic}} - \delta^{66}\text{Zn}_{\text{natural}}} \right) \text{ (eq. 2)}$$

where $\delta^{66}\text{Zn}_{\text{sample}}$, $\delta^{66}\text{Zn}_{\text{natural}}$, and $\delta^{66}\text{Zn}_{\text{anthropogenic}}$ stand for the $\delta^{66}\text{Zn}_{\text{NMC}}$ values obtained for sample of interest, and the values for natural and anthropogenic Zn end-members, respectively. These end-members correspond to hypothetical Zn pools able to oyster's uptake constituted by 100% natural and 100% anthropogenic Zn.

Binary isotope models are based on a conservative and linear relationship between the relative contributions of the two-isotope end-members, and can be mathematically modeled by a straight line, known as “mixing line”.^{70,71} To predict the unknown $\delta^{66}\text{Zn}_{\text{NMC}}$ values of end-

308 members of the theoretical mixing line for bivalve samples, we use a linear regression between
 309 $\delta^{66}\text{Zn}$ values and Zn concentrations ($\delta^{66}\text{Zn}$ v.s. $[\text{Zn}]$, and from the correspondent reciprocals
 310 ($\delta^{66}\text{Zn}$ v.s. $1/[\text{Zn}]$, Fig. 5a). The natural end-member value of +1.02‰ is then computed using a
 311 Zn concentration of $340 \mu\text{g.g}^{-1}$, which is taken from the global baseline for oysters.⁷² This Zn
 312 value is based on a meta-analysis of 775 oyster samples distributed in more than 71 sites around
 313 the world, and accounting with five *Crassostrea* species, including the *C. gigas*.⁷² Conversely,
 314 the anthropogenic end-member value is used as the intercept (+0.65‰) of the regression line
 315 using the reciprocal concentration (Fig. 5a). The standard error of the regression analysis (S),
 316 which represents the average distance that the observed values fall from the regression curve,⁷³
 317 is about $\pm 0.07\text{‰}$ and is considered the uncertainty associated with the estimation of end-
 318 member values. Source apportionment estimated using isotope end-members in equation 2
 319 suggest a maximum contribution of 95% of anthropogenic Zn in 1985, suggesting elevated Zn
 320 contamination in bioavailable forms in the Loire estuary system for this period. From 1985 to
 321 2016, the anthropogenic Zn bioaccumulation decreases to about 59 % (Fig. 5b). This 1/3
 322 abatement in anthropogenic Zn being bioaccumulated is coherent with an overall decrease in
 323 levels of total Hg, Cd and Pb observed for the same time span (ROCCH database, available at
 324 <https://wwz.ifremer.fr/surval/>). The abatement likely due to several converging factors. They
 325 include the de-industrialization in the Loire watershed,²⁶ the implementation of environmental
 326 regulations like the EU Water Framework Directive²¹ and/or increased recycling of metals
 327 within the anthroposphere.⁷⁴ Pb isotopes in mussels from Loire estuary attested a substantial
 328 attenuation of Pb contamination related to the end Pb metallurgical activities in the inner
 329 estuary.⁷ On a regional scale, this punctual source closure may also have impacted Zn
 330 contamination in the Loire estuary, since Zn often tracks Pb in metallurgical emissions.^{16,75} In
 331 a broader national perspective, part of reductions on Zn, Pb, Cu, Hg, and Cd emissions have
 332 been explained by the ban of leaded gasoline (year 2000), fume trapping systems in industry
 333 plants, and gradual shift from coal-burning to nuclear power in the energy sector in the 1970's.⁷⁶
 334 Similar decreasing tendencies for these trace metals were observed in sedimentary archives

from the four major French watersheds (Loire²⁶, Seine^{74,76}, Garonne,⁷⁷ and Rhône^{78,79}) and attributed to the same economic and regulatory reasons.⁷ All these results appoint for an overall diminution of anthropogenic Zn circulation in the French watersheds in the last two decades, and consequently, of its inputs into marine and coastal systems.

Zinc transfer across trophic levels and Zn isotope record in oysters

Zn isotope records in oyster soft tissues depend on relative contributions on the Zn body burden of bioaccumulated Zn via dietary inputs and dissolved phases.²¹ Phytoplankton is the main dietary input to oysters, and once they taken Zn from the dissolved phase, this last is ultimately the main source of Zn to oysters. Biogeochemical processes and sources control Zn isotope ratios in the dissolved phase and biotic systems, and then, both factors should be considered to explain the final $\delta^{66}\text{Zn}$ values found in oysters.

In the previous section, we estimated the isotope values for oysters from Bourgneuf bay hypothetically bioaccumulating 100% natural Zn and 100% anthropic Zn (+1.02, and +0.65‰, respectively). They are higher than the estimated $\delta^{66}\text{Zn}$ values ranges for natural rivers (+0.4) and anthropogenic urban and industrial sources (+0.1‰) in sediment and dissolved phases of the Loire estuary. Interestingly, these isotope differences are the same (0.3‰). Considering the three oyster (*C. gigas*) datasets (Loire estuary, Vilaine bay, Gironde estuary) from Atlantic French turbid estuaries, we see an overall offset up +0.4‰ from the $\delta^{66}\text{Zn}$ values of their natural and anthropogenic sources. Our dataset shows higher concentrations and remarkably more positive $\delta^{66}\text{Zn}_{\text{NMC}}$ values than same species oysters harvested in other localities (Fig. 6), such as the English Channel ($\delta^{66}\text{Zn}_{\text{NMC}} = +0.53 \pm 0.04$, 2s, $n = 2$)²⁴, and West Canadian Coast ($\delta^{66}\text{Zn}_{\text{NMC}} = +0.31 \pm 0.08$, $n = 3$).²³ These results imply that the sole $\delta^{66}\text{Zn}$ values of anthropogenic and natural Zn inputs cannot explain the heavy isotope compositions in French Atlantic oysters, and an additional biogeochemical process should be considered. A first hypothesis is the shift in the $\delta^{66}\text{Zn}$ values of planktonic in relation the surrounding seawater is the influence of Zn adsorption on cell walls and/or metabolic uptake processes. The latter tends to enrich

362 phytoplankton cells in the light isotope due kinetically-driven biological Zn isotope
363 fractionation.^{80,81} This processes unlikely to be the a dominant processes, since Zn isotope of
364 oysters are heavy compared to waters and not light. On the other hand, free Zn (II) ions
365 adsorption on the binding sites on external walls of planktons cells retain preferentially the
366 heavy isotope,⁸⁰ and could explain the final heavy Zn isotope in oysters. Based on controlled
367 adsorption batch experiment in diatom cells, this process can lead to isotope fractionation
368 factors ($\Delta^{66}\text{Zn} = \delta^{66}\text{Zn}_{\text{diatom}} - \delta^{66}\text{Zn}_{\text{solution}}$) between 0.0 and 0.3‰. Applying this factor average
369 (ca. +0.15‰) on the $\delta^{66}\text{Zn}$ value average of rivers worldwide ($\sim +0.3\text{‰}$),⁸² we obtain a final
370 budget about +0.45‰ for the phytoplanktonic pool, which remains lower than those observed
371 for oysters.

372 To close this isotope budget, an additional shift to positive δ -values to sorption processes
373 within the “estuarine geochemical filter” should be considered. In turbid estuaries, dissolved
374 Zn concentrations tend to be low due to the water column scavenging by particle flocculation
375 processes at Maximum Turbid Zone (MTZ).⁸³ In contrast, primary productivity increases
376 toward the Chlorophyll Maximum Zone (CMZ), typically located further downstream than the
377 MTZ, since the sediment plume does not obstruct light penetration.⁸³ Studies with paired
378 particulate and dissolved water samples in Gironde¹⁰ and at Pearl Harbor¹⁴ demonstrated that
379 estuaries can act as “geochemical isotope filters”, removing preferentially the light Zn isotope
380 from dissolved fraction by sorption processes on surface particles through Maximum Turbid
381 Zone (MTZ), and resulting in a strong heavy isotope enrichment in this fraction. In the Gironde
382 estuary, this process leads to significant shifts in $\delta^{66}\text{Zn}$ values in dissolved phase up 0.8‰. Petit
383 et al.¹⁰ suggest that the heavy $\delta^{66}\text{Zn}$ values of estuarine waters can then be transposed to their
384 indigenous phytoplankton and recorded in filter-feeders preying on this biomass. Our full oyster
385 dataset covering the entire Atlantic French Coast support Petit et al.’s findings. Indeed, the data
386 presented here suggest that $\delta^{66}\text{Zn}$ isotope values of natural and anthropogenic sources observed
387 in rivers are in turn imprinted onto oysters, albeit with an isotope off-set related to Zn light
388 isotope removal of the dissolved phase in the estuary. Since Zn isotope fractionation through

MTZ affects both natural and anthropogenic signals equally, it is likely that isotope systematic and amplitude (i.e., $\delta_{\max} - \delta_{\min} \approx 0.3\text{‰}$) between end-members is preserved from abiotic compartments to oysters (Fig. 7).

Implications

We demonstrated for the first time that mixing source isotope systematics in sediment and water are transposed to oysters through Zn bioaccumulation. This finding enables the quantification of Zn origins in these organisms, and the reconstruction of Zn pollution history in environmental sample banks. Since the Zn isotope record provided by these organisms detects variations in anthropogenic inputs at decadal time scales, Zn isotope information can be incorporated in “Mussel Watch” programs to support or quantify environmental management strategies and policies.

Over the last decade, Zn anthropogenic fractionation in coal, metallurgic and industrial processes has been well established, being these processes systematized in terms of enrichment or depletion in the heavy isotope.^{9,15,24,64,84–87} Identifying these isotope patterns in biomonitoring network sampling stations allows to use analogous mixing source models to monitor and quantify Zn anthropogenic bioaccumulation in oysters related to different sources at the regional and global scales.

Acknowledgements

The authors would like to thank all IFREMER personnel contributing to the ROCCH program. This work was conducted within the POLLUSOLS project framework and was financed by the regional government of the Pays de la Loire (France). Sentinel-2 products were download from the Copernicus Open Access Hub (<https://scihub.copernicus.eu/>).

Associated contents

Supporting information (SI) contains the complete datasets for sediments and bivalve mollusks dataset, and additional plots for bivalve temporal series mentioned in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Fig. 1. Study site and sampling locations: (a) strong accumulation of fine sediments in Bourgneuf bay derived from the Loire estuary as result of the sediment plume dispersion induced by wind and tidal regimes, and density flows (adapted with permission from Penaud et al.⁴³); (b) trend of the surface velocity of the current, along the coastal margin from the Vilaine Bay to the Bourneuf Bay; (c) Land cover of the Loire River estuary watershed.

Fig. 2. Temporal profile of Zn concentrations ($\mu\text{g g}^{-1}$) and Zn isotope compositions (‰) in the PV1 sediment core. The green stripe represent the $\delta^{66}\text{Zn}_{\text{MMC}}$ value average of the Upper Continental Crust ($+0.28 \pm 0.05\text{‰}$ ⁵⁸). Data published previously in Araújo et al.²⁵

Fig. 3. Zinc isotope compositions for sediments influenced by urban and industrial Zn sources. Zn isotope variability in these sediments can be described using mixing processes models between natural ($\sim 0.3\%$) and anthropogenic ($\sim 0.1\%$) Zn sources. Concentrations are reported in $\mu\text{g g}^{-1}$ for Zn, and % for Al.

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781 **Fig. 4.** Temporal profile of Zn concentrations (mg g^{-1} dry weight) and Zn isotope compositions
782 (‰) in oyster series from Bourgneuf Bay for the period between 80s and 2016 (a); compared
783 Zn isotope profiles between oyster series and the PV1 core for the same period.

Fig. 5. (a) The equations obtained from the linear regression analysis of $\delta^{66}\text{Zn}_{\text{JMC}}$ vs. $[\text{Zn}]$ vs. $1/[\text{Zn}]$ predicted $\delta^{66}\text{Zn}_{\text{JMC}}$ values of the natural and anthropogenic end-member about +1.02‰ and +0.65‰, respectively; (b) Temporal evolution on contributions of Zn anthropic sources in the Loire estuary using a binary mixing source model. The green dashed line marks the most recent anthropogenic Zn level (36%, 2016 year), which corresponds an attenuation of 59% compared to 1985. Concentrations are expressed in mg g^{-1} (dry weight).

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798 **Fig. 6.** Zn isotope compositions (‰) and Zn concentrations (mg g^{-1} dry weight) in oysters worldwide. Except for Sepetiba bay (*Crassostrea Brasiliana*)²²
799 and Pearl River Esutary (*Crassostrea hongkongensis*)²¹, all data is reported for *Crassostrea gigas*.^{10,23,24} Dashed lines represent isotope average for
800 uncontaminated river waters⁸² and urban and industrial Zn sources reported elsewhere.¹¹ The compiled data is included in the Table S2 (SI).

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804 **Fig. 7.** Zinc isotope systematic in rivers are transposed to oysters through Zn bioaccumulation via water and diet. In estuaries, Zn isotope fractionation in
 805 the water dissolved phase at Maximum Turbid Zone (MTZ) induce an enrichment in the heavy isotope, transposed to biomass at Chlorophyll Maximum
 806 Zone (CMZ), and posteriorly to oysters by feeding. A Zn isotope model in oysters from Bourgneuf Bay estimated end-member values that conserve the
 807 same isotope amplitude and systematic observed for the correspondent end-members in abiotic compartments (Fig. 7).