The estuarine geochemical reactivity of Zn isotopes and its relevance for the biomonitoring of anthropogenic Zn and Cd contaminations from metallurgical activities: Example of the Gironde fluvial-estuarine system, France

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Abstract :

Zinc stable isotopes measurements by MC-ICP-MS, validated by laboratory intercalibrations, were performed on wild oysters, suspended particles and filtered river/estuarine water samples to provide new constraints for the use of Zn isotopes as environmental tracers. The samples selected were representative of the long range (400 km) transport of metal (Zn, Cd, etc.) contamination from former Zn-refining activities at Decazeville (i.e. $\delta^{66}Zn > 1$ ‰) and its phasing out, recorded during 30 years in wild oysters from the Gironde Estuary mouth (RNO/ROCCH sample bank). The study also addresses additional anthropogenic sources (urban and viticulture) and focuses on geochemical reactivity of Zn in the turbidity gradient and the maximum turbidity zone (MTZ) of the fluvial Gironde Estuary. In this area, dissolved Zn showed a strong removal onto suspended particulate matter (SPM) and progressive enrichment in heavy isotopes with increasing SPM concentrations varying from $\delta^{66}Zn = -0.02$ ‰ at 2 mg/L to +0.90 ‰ at 1310 mg/L. These signatures were attributed to kinetically driven adsorption due to strongly increasing sorption sites in the turbidity gradient and MTZ of the estuary. Oysters from the estuary mouth, contaminated sediments from the Lot River and SPM entering the estuary showed parallel historical evolutions (1979-2010) for Zn/Cd ratios but not for $\delta^{66}Zn$ values. Oysters had signatures varying from $\delta^{66}Zn = 1.43$ ‰ in 1983 to 1.18 ‰ in 2010 and were offset by $\delta^{66}Zn = 0.6 - 0.7$ ‰ compared to past (1988) and present SPM from the salinity gradient. Isotopic signatures in river-borne particles entering the Gironde Estuary under contrasting freshwater discharge regimes during 2003-2011 showed similar values ($\delta^{66}Zn \approx 0.35 \pm 0.03$ ‰; 1SD, n=15), i.e. they were neither related to former metal refining activities at least for the past decade nor clearly affected by other anthropogenic sources. Therefore, the Zn isotopic signatures in Gironde oysters reflect the geochemical reactivity of Zn in the estuary rather than signatures of past metallurgical contaminations in the watershed as recorded in contaminated river sediments. The study also shows that the isotopic composition of Zn is

strongly fractionated by its geochemical reactivity in the Gironde Estuary, representative of meso-macrotidal estuarine systems.

1. Introduction

Zinc is a ubiquitous biolimiting micronutrient, involved as a cofactor in enzymes and regulatory proteins in all living beings (Cloquet et al., 2008) but may become toxic in contaminated environments, where concentrations can exceed background levels by orders of magnitude. This duality observed for many metallic trace elements (MTE), explains why

environmental studies using Zn isotopes have focused on both past/present pristine environments (Maréchal et al., 2000; Pichat et al., 2003; Viers et al., 2007; Zhao et al., 2014) and metal contaminated systems (Weiss et al., 2007; Borrok et al., 2008; Chen et al., 2008; Sivry et al., 2008; Sonke et al., 2008; Chen et al., 2009; Mattielli et al., 2009; Bigalke et al., 2010). Both kinds of applications deal with a variation range of δ^{66}Zn values greater than 1 ‰, which is about more than 20 times the analytical precision achieved with modern MC-ICP-MS measurements.

The similarity of Zn isotope signatures in continental siliciclastic rocks and marine detrital sediments (Maréchal et al., 1999; Albarède, 2004; Cloquet et al., 2008) and the small isotopic difference between natural and common anthropogenic Zn (John et al., 2007; Cloquet et al., 2008; Sivry et al., 2008; Sonke et al., 2008) may explain why Zn isotope studies on estuarine systems are extremely rare (Petit et al., 2006; Petit et al., 2008a). In contrast to the small isotopic variations expected for sedimentary samples in estuaries, a range of δ^{66}Zn values over 1 ‰ has been observed for dissolved Zn in the Seine (Chen et al., 2008; Chen et al., 2009) and various World Rivers (Little et al., 2014), as well as in coastal and open ocean surface waters (Bermin et al., 2006; Boyle et al., 2012; Zhao et al., 2014). This clearly highlights the importance of studying dissolved Zn isotopes at the ocean/continent interface in order to understand potential fractionation related to biogeochemical processes in these highly dynamic environments. In fact, the partitioning between solids and solution plays a critical role in the distribution, transport and behavior of metals in aquatic environments, with adsorption/desorption processes being a major control on dissolved metal loads in fluvial-estuarine systems (Turekian, 1977; Benoit and Rozan, 1999), potentially modifying Zn isotopic signatures.

Several studies have established how anthropochemical processes related to the production of metallic Zn account for the diversification of Zn isotopic signatures in commercial products and by-products (John et al., 2007; Sivry et al., 2008; Sonke et al., 2008; Mattielli et al.,

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2009). The use of Zn isotopes as a tracer of anthropogenic contamination often addresses natural systems affected by Cd contamination (associated to other metals) from pyrometallurgical Zn production. Due to environmental regulation and remediation strategies implemented since the mid-eighties, globally decreasing Cd concentrations and consistent changes of δ^{66}Zn signals in contaminated systems have been observed (Sivry et al., 2008; Sonke et al. 2008; Petit et al., 2008a; Mattielli et al. 2009). However, Zn isotope studies have mainly focused on tracing point-source pollutions in the nearby environment rather than following their long-range transport at the watershed scale. At this scale, the relative proportions of natural and anthropogenic sources vary in time and space due to a complex interplay of transient processes controlled by a combination of natural (floods vs. low discharge periods, mixing between tributaries) and anthropogenic forcing (urban and industrial activity, remediation, reservoir management, dredging, etc.). Studying the evolution of metal-contaminated large scale systems requires long-term and high-resolution monitoring of suspended and dissolved metal loads allowing establishing balanced fluxes at different physical, hydrodynamic and geochemical boundaries of the river-estuary continuum.

Since 1979, the French "Mussel Watch" program (RNO/ROCCH) has been monitoring MTE exposure in French coastal systems using bivalves sampled along the coastline and in estuaries. These organisms are useful sentinels for information on time-integrated bioavailable metal exposure in coastal sites and the related sample banks allow for the monitoring of spatial and temporal trends over decades.

Bivalves from the RNO/ROCCH sample bank have been analysed for Cd (Claisse, 1989; Chiffoleau et al., 2001), Ag (Chiffoleau et al., 2005; Lanceleur et al., 2011), Ni and V (Chiffoleau et al., 2004), Cr (Chiffoleau et al., 1994), Pb and Hg (Claisse, 1989), and more recently, Zn and Cd stable isotopes (Shiel et al., 2013). The latter study has shown Cd and Zn isotopic signatures (δ^{66}Zn > 1 ‰; δ^{114}Cd < -1 ‰) in two RNO oysters samples from the La Fosse site (Gironde Estuary mouth) clearly different than in bivalves from other French

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coastal sites under stronger marine influence (δ^{66}Zn $\approx$ 0.5 ‰; δ^{114}Cd $\approx$ -0.5 ‰). Shiel et al. (2013) have attributed the heavy δ^{66}Zn in Gironde oysters to the resilience and the river-borne resuspension/transport of Cd-contaminated sediments from the Lot River (1 ‰ < δ^{66}Zn < 1.6 ‰, Sivry et al., 2008), downstream the former Decazeville metal processing plant (Fig. 1). In the same study, the light δ^{114}Cd values in oysters were explained by airborne emissions of metallic Cd from the former Decazeville industry, presumably characterized by very light Cd isotope signatures, as observed in a Zn smelter in Northern France (Cloquet et al., 2006). However, atmospheric dust from the same smelter had negative Zn isotope signatures (δ^{66}Zn $\approx$ -0.7 ‰; Mattielli et al., 2009) and Cd contaminated sediments from the Lot River show δ^{114}Cd values close to 0 ‰ (Sivry et al., 2006). Given that the main transport of Cd from the Decazeville area to the Gironde Estuary and adjacent coastal area occurs via the Lot-Garonne-Gironde fluvial-estuarine system (Schäfer et al., 2002; Audry et al., 2004b; Dabrin et al., 2009) rather than by atmospheric deposition, a dual transport pathway for Cd and Zn accumulated in Gironde oysters appears unlikely. We hypothesize that other processes must account for the particular Zn and Cd isotopic compositions measured in oysters from the Gironde Estuary.

The potential of metal stable isotopes for fingerprinting and discriminating anthropogenic sources, and the scientific relevance of using bivalves to monitor contamination levels in coastal sites are well established. However, the use of stable metal isotope signatures in coastal biomonitoring of remote metal contaminations must account for constraints and complexities inherent to transport, mixing and reactivity in rivers and estuaries.

The objective of this study is to provide new constraints on the use of Zn stable isotopes for biomonitoring studies using bivalves, applied to the phasing out of the Cd-rich metal contamination affecting the Gironde fluvial-estuarine system and characterized by uncommon Zn isotopic signatures, i.e. strongly enriched in heavy isotopes. This work also

aims at studying possible isotopic fractionation related to geochemical reactivity of Zn in estuaries and its relevance for signals recorded in marine bivalves. In addition, we also refer to Cd isotope data for the Gironde Watershed from two previous publications (Shiel et al., 2013 and Sivry et al., 2006), in order to compare Zn isotope behaviour and potential Cd isotope fractionation related to their contrasting geochemical reactivities in turbid estuaries, as a perspective for future research.

2. Study area and scientific context

There are numerous studies on physical, hydrological and geochemical characteristics of the Gironde Estuary and its tributaries (Isle, Dordogne and Garonne Rivers) making it a globally known model system, especially for the understanding of trace element transport and reactivity. The Gironde fluvial-estuarine system (Fig. 1) represents one of the largest (80000 km²; i.e. 18% of French metropolitan land surface area) and least industrialized watersheds in Europe. In addition to urban runoff and wastewater inputs (Deycard et al., 2014), as well as Zn-rich Cu sulfate fungicides used for intensive wine production in the area (Masson et al., 2006; Petit et al., 2013b), the Gironde Estuary and adjacent coastal zones (i.e. Marennes-Oléron Bay, see Fig. 1) are affected by historical multiple metal contamination. This multiple metal contamination, originating from former Zn refining activities near Decazeville (1842-1987, Fig. 1) implies very strong Cd pollution (Jouanneau et al., 1990; Audry et al., 2004a) and is associated with Zn isotopic signatures enriched in heavy isotopes, typical of residual by-products related to Zn processing (Sivry et al., 2008), as observed in the 50-years (1959-2001) sediment record of the Cajarc reservoir (Fig.1A; Sivry et al., 2008) downstream the Decazeville site. Although located more than 400 km upstream from the Gironde Estuary Mouth (i.e. the oyster sampling site), former Zn refining activities at Decazeville are responsible for very high Cd bioconcentrations in wild oysters (>100 mg/kg dry weight (d.w.)), evidenced at the end of the seventies by the French Mussel Watch program (Claisse, 1989). These Cd bioconcentrations were up to 50 times higher than those

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in oysters from other sites along the French coast and resulted in the ongoing prohibition of shellfish consumption, production and purification in this area ("Zone D"; French prefectural decree 18/11/1996). The metallurgical activity ended after a major pollution accident in 1986 followed by remediation efforts such as the treatment of drainage water and the confinement of a part of the waste deposits in storage basins. This has efficiently reduced total Cd fluxes in the Garonne River at the La Réole site (i.e. the main fluvial entry to the Gironde Estuary), with a remarkable decrease of dissolved Cd fluxes (Fig. 1B) over the period 1990-2007 (Blanc et al., 1999; Schäfer et al., 2002; Dabrin et al., 2009). However, metal contamination along the fluvial-estuarine continuum persists due to erosion/transport of contaminated sediments from banks and hydroelectric reservoirs (i.e. Cajarc site) during flood events (Audry et al., 2004b, 2007; Coynel et al., 2007).

Following the remediation at the Decazeville site, dissolved and particulate Cd concentrations in the Gironde Estuary decreased from the early eighties (Jouanneau et al., 1990) to 2007 (Dabrin et al., 2009), resulting in decreasing Cd bioconcentrations in oysters over this period (e.g. from >100 mg/kg to 26 mg/kg d.w. at the La Fosse site; Fig. 1C; Lanceleur et al., 2011). Compared to Cd, little is known on the "historical" evolution of particulate Zn levels in the brackish estuary, but early studies (Etcheber 1986; Jouanneau et al., 1986) report higher Zn concentrations (average of 335 $\pm$ 113 mg/kg (1SD, n=13)) for the period 1978-1981, compared to the present average values of 221 $\pm$ 54 mg/kg (1SD, n=140, data for the period 2001-2007 from TGM monitoring surveys). This is in agreement with higher inputs of Zn during the period of industrial activity at Decazeville, as recorded by SPM samples from the La Réole site, showing higher average Zn concentrations of 1410 $\pm$ 700 mg/kg (n=12, Etcheber, 1986) in 1980-1981, against 703 $\pm$ 373 mg/kg (n=208, data from TGM monitoring surveys) in 1999-2011.

Along the salinity gradient of the Gironde Estuary, average dissolved Zn concentration of 1.8 $\pm$ 1.7 µg/L (1SD, n=114, period 2001-2007, for salinities between 0 and 20), are clearly lower

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than the average dissolved Zn concentration at the La Réole site of 5.4 $\pm$ 22 µg/L (1SD, n=192, period 1999-2011). This difference strongly suggests that removal of dissolved Zn may occur and could be a permanent feature in this hyperturbid estuary.

In contrast to Zn, the geochemical reactivity of Cd and the role of the MTZ are well understood through extensive monitoring campaigns in the salinity and turbidity gradients. Dissolved Cd typically shows bell-shaped concentration profiles with a mid-salinity maximum reflecting the balance between chloride-induced desorption/complexation and dilution with seawater (Strady et al., 2008; Dabrin et al., 2009). During this process, up to 95% of the fluvial particulate Cd gross flux is transferred towards the dissolved phase in the brackish estuary (Dabrin et al., 2009). This addition increases tremendously the Cd exposure of the oysters at the Gironde mouth. Subsequent dilution of dissolved Cd by seawater results in systematically decreasing Cd concentrations in Gironde oysters from the most inner (La Fosse) to the most outer sampling sites (Bonne Anse and Pontaillac), which reflects the distribution of dissolved Cd in the estuary mouth (Lanceleur et al., 2011). At present, Gironde oysters remain the most contaminated ones for Cd (>6 times the national median), but are similar to oysters from other French coastal sites for Zn bioconcentrations (RNO data). This clearly suggests that exposure levels of Cd and Zn are different in the estuary and could be related to their contrasted geochemical reactivity,described above.

3. Material and methods

3.1 Sampling and sample preparation

Two years-old wild Japanese oysters *Crassostrea Gigas* were sampled by IFREMER at the La Fosse site in the Gironde Estuary, showing a typical salinity range of 15-20 (Fig.1). The sampling frequency varied from 4 times (from 1979 to 2002) to twice a year (from 2003 to

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2007) and annually (from 2007 to 2010). A detailed description of the oyster sampling and conditioning is provided in Claisse (1989). Briefly, the oysters were cleaned with seawater for 24h on site and frozen for transport. After freeze-drying, oyster's soft tissues were separated from shells and pooled by >10 individuals, thawed, ground, homogenized and stored in the RNO/ROCCH specimen bank.

River and estuarine water was sampled along a 12 points longitudinal profile of ~70 km in the Garonne Branch of the Gironde Estuary (Fig. 1D) from La Réole (upper limit of the tidal influence, KP (kilometric point) -70) to Bordeaux (upper limit of the salinity gradient, KP -6), after a long period of low freshwater discharge (113 m³/s on the day of the sampling). Sampling was performed in the main channel at ~30 cm depth, during a single 4 hours cruise following the flow of water masses. Volumes of 3 to 4 L were sampled in acid-cleaned PET bottles after rinsing with ambient water. Approximately ~15 ml were immediately filtered on site through an acid-cleaned syringe and disposable 0.2 μm polycarbonate filters and acidified with 100 μl of conc. HNO_3 (ultrex) for the determination of dissolved Zn concentrations.

In addition, soils, sediments and SPM from the EPOC-TGM sample bank were selected according to their geochemical and environmental characteristics and spatial distribution in the Lot-Garonne-Gironde Continuum (Fig. 1): (1) SPM from two major flood events sampled at the La Réole site (one flood originating from the Lot River in December 2003 (Coynel et al., 2007) and one from the Upper Garonne River in February 2003); (2) surface and deep sediments from the Marcenac and Cajarc reservoirs, respectively upstream and downstream from the confluence of the Lot River with the Riou-Mort River draining the Decazeville area (Audry et al., 2004a; Sivry et al., 2008); (3) a recent SPM sample from the Gironde Estuary sampled in 2007 (KP 52, salinity = 12); (4) a "historical" SPM sample from the Gironde Estuary (KP 30, salinity ≅ 15) sampled in 1988; (5) two soil samples from vineyard areas adjacent to the Gironde Estuary and contaminated by the use of Cu sulfate fungicides

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(Larrose et al., 2011); (6) a Zn contaminated river bank sediment from a small urban river of the Bordeaux urban agglomeration.

3.2 Cleaning of labware and reagents

The physical and chemical treatments of samples and cleaning of all types of material and consumables in contact with samples were performed under an extraction hood and/or a laminar flow hood in a class 1000 laboratory. All dilutions were performed with 18.2 MΩ milli-Q® (MQ) water. Acid cleaning of labware was performed with 10 % HNO_3 (made from 14 mol/l HNO_3 Baker Analyzed), for at least 72 h and rinsed thoroughly three times with MQ. High density polyethylene beakers and screw top Teflon beakers intended for the recovery of Zn after purification by anion exchange chromatography were cleaned in three sequential steps of 24 h at 125 °C (1) with HCl ~6 mol/l (made from 12 mol/l Baker Analyzed); (2) and (3) with conc. HNO_3 and rinsed with abundant MQ between each step (Petit et al., 2008b). Polycarbonate filters were soaked in HNO_3 1 mol/l in two successive bathes for 48 h then rinsed several times and stored in MQ (de Jong et al., 2007). Acids used for the digestion of solid samples, the acidification of filtered river water and anion-exchange chromatography were of high purity (22 mol/l suprapur HF, 14 mol/l Ultrex HNO_3 and 12 mol/l instrA Baker Analyzed HCl), while isotopic measurements were performed using subboiled conc. HNO_3 (Petit et al., 2008b).

3.4 Quantification of SPM, centrifugation and filtration

The determination of SPM concentrations was performed by filtration of homogenous and precise volumes of water (between 1000 ml and 40 ml) through calcined (550 °C; 4 hours) and pre-weighed GFF filters (45 mm in diameter and 0.7 µm in porosity) and dried to constant weight at 50°C. The ~3-4 L samples underwent repeated centrifugations (Jouan CR4-12, 10 min at 4000 rpm) carried in four acid-cleaned conic vials of 250 ml. The centrifuged SPM were recombined, dried to constant weight at 50 °C and further crushed

and homogenized in an agate mortar. The centrifuged supernatant was filtered through 0.2 µm polycarbonate acid cleaned filters (140 mm in diameter). The 3-4 L samples of filtered water were acidified at pH ~2 with conc. HNO_3 (ultrex) and stored in the dark. Aliquots of these filtered water samples were compared with samples filtered on board to control potential contaminations by the filtration system.

3.5 Pre-concentration of dissolved Zn

The pre-concentration of filtered river water samples intended for Zn isotope measurements was performed by evaporation as described in Vance et al. (2008) and used in Little et al. (2014) for the preparation of samples devoted to isotopic measurements of dissolved Zn. Each sample was repeatedly evaporated to dryness in 4 pre-weighed acid-cleaned 50 ml Polypropylene (PP) tubes (DigiTUBE®,SCP SCIENCE) at 100 °C in a temperature-controlled digestion system (DigiPREP MS®, SCP SCIENCE) under an extraction hood in a class 1000 clean lab facility. Finally, the 4 aliquots were recombined in 3 ml HCl 7 mol/L (instrA) at 125 °C. These straightforward approaches provided low blanks of ~10 ng Zn obtained from the evaporation of 1300 ml of MQ, i.e. 2-3 orders of magnitude less than the amount of Zn recovered from the pre-concentration of the samples.

3.6 Digestion

Precise amounts of dry, ground and homogenized samples, corresponding to at least 2500 ng of Zn were digested in acid cleaned 50 ml PP tubes (DigiTUBE®, SCP SCIENCE). Freezed-dried oysters were digested using 2 ml aqua regia mixture (2/3 conc. HCl instrA and 1/3 conc. HNO_3 ultrex, in v/v) following existing protocols (Chiffoleau et al., 2005; Shiel et al., 2009). Additional 4 ml HF (Suprapur) were added to the aqua regia mixture for the total digestions of sediments, soils and SPM samples. All solid samples were heated at 110 °C for 2 h in a temperature-controlled digestion system (DigiPREP MS®, SCP SCIENCE) and evaporated to dryness.

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The residue was re-dissolved in 10 ml HCl 7 mol/L (instrA), repeatedly heated at 110 °C and ultra-sonicated (Bransonic 2510E-NT) for 30 min until a clear solution was obtained. For the determination of Zn concentrations, a known aliquot of the solution was evaporated to dryness, re-dissolved in 100 µl of conc. HNO_3 (Baker ultrex) and evaporated again to eliminate chlorine ions. This residue was finally re-dissolved in 1 ml HNO_3 1 % (Baker ultrex). The remaining solution was evaporated and stored for anion-exchange chromatography. The digestion protocol provided low blanks of 5 ng Zn, which represented a contribution less than 0.2 % of Zn loaded on the column.

3.7 Anion-exchange chromatography

The purification of Zn was performed by loading precise amounts equivalent to at least 2500 ng of Zn in 2 ml of HCl 7 mol/L on Biorad polyprep columns filled with 2 ml AG-MP1-M anion-exchange resin. Washing and conditioning of the resin batch was performed as described in de Jong et al. (2007). A single pass purification following the protocol of Mason et al. (2003) was applied, allowing a better purification of Zn from Cd and Sn compared to the protocol of Maréchal et al. (1999). The sample was loaded in 2 ml HCl 7 mol/L. The matrix, the Cu, the Fe, the Zn and the Cd fractions were recovered in 10 ml HCl 7 mol/L, 20 ml HCl 7 mol/L, 10 ml HCl 2 mol/L+HF 8 mol/L, 10 ml HNO_3 0.5 mol/L+ 0.1 mol/L HBr, and 10 ml HNO_3 0.5 mol/L, respectively. Full recovery of Zn was quantitatively checked in 100 µl aliquots, analyzed by Quadrupole ICP-MS (Thermo X7 series II) and was >95% in any case. We also performed column calibrations on sediment and oyster samples to fine-tune the elution protocol and control potential Zn breakthrough related to column saturation (Chapman et al. 2006). The column blanks were ~10 ng Zn, while total (procedural) blanks were always better than 20 ng Zn. The remaining dried residue was dissolved in 100 µl conc. HNO_3 (ultrex) and evaporated to dryness prior to isotopic measurements.

3.8 Quadrupole ICP-MS measurements.

Dissolved Zinc (Zn_D), particulate Zn (Zn_P) and particulate Cd (Cd_P) concentrations in water samples and digestates were measured by Q-ICPMS on a Thermo X7 series II (ThermoScientific) at EPOC, Université de Bordeaux. Appropriate dilutions were performed to achieve typical working concentrations always 100 times higher than the detection limits of 0.01; 0.02 and 0.1 µg/l respectively for Zn_D, Cd_P and Zn_P. Duplicate (digestion + analysis) measurements of SPM and oyster samples always agreed within less than 4 % RSD for Zn_P and Cd_P and 10 % RSD for Zn_D. The accuracy of the analytical methodology (digestion + analysis) was verified with several certified reference materials (MESS-2, NCSDC-311, NCSDC-317; TORT-2, SLRS-5 and TMRAIN-04) covering a wide range of concentrations and matrix compositions. The measured Zn and Cd concentrations were always within 10% of the certified value, and measurements of Zn and Cd concentrations in oysters always agreed with the RNO/ROCCH data, respectively within 8% and 4% on average.

3.9 Multi-Collector ICP-MS measurements.

Zinc isotopic analyses were performed on a Nu plasma 015 MC-ICP-MS (Nu Instruments) at G-TIME (Université Libre de Bruxelles), following the methodology described in Petit et al. (2008b). Dried purified Zn fractions were redissolved in 0.05 mol/L HNO_3, made from sub-boiled conc. HNO_3. Measurements were acquired with on-mass zero, in wet plasma and low resolution (M/ΔM=300), using a microconcentric nebulizer (100 µml/min), plasmashield and type A nickel sampler and skimmer cones. Instrumental mass bias was corrected by sample standard bracketing and external normalization to a common Cu standard (Mason et al., 2004; Petit et al., 2008b), assuming an exponential law of mass fractionation. The measurement of several Cu-normalized Zn isotope ratios allowed checking for mass-dependent fractionation and potential isobaric interferences on Zn masses (Petit et al., 2013a). Samples were typically run in triplicate and bracketed by two standards. The conventional delta notation was used to report Zn isotopic compositions, as the deviation of a

given Zn isotopic ratio in the sample relative to that of the reference bracketing standards, in per mil (Eq.1).

$$^{6X}Zn = \left(\frac{\left(^{6x}Zn / ^{64}Zn \right)_{Sample}}{\left(^{6x}Zn / ^{64}Zn \right)_{Std}} - 1 \right) * 1000 \quad Eq.1$$

Where $^{6X}Zn/^{64}Zn$ were Cu-normalized Zn isotopic ratios, with $X \in [6, 7, 8]$; and $^{6X}Zn/^{64}Zn_{Std}$, the average isotopic ratio of the bracketing standards.

All Zn isotopic data reported in this study were expressed relative to the "Lyon" JMC3-0749-L reference Zn standard but measured against the in-house Zn standard (JMC 0620611.10) previously calibrated to the Lyon JMC Zn (Petit et al., 2008b). Precision and (analytical and procedural) accuracy were controlled by (1) repeated measurements of the Zn IRMM3702 certified reference material, (2) duplicate or triplicate measurements (mineralisation + purification + analysis) performed on one dissolved and two particulate samples and (3) intercalibrations of two oyster and four sediment samples with other labs (GET-Université Paul Sabatier, Toulouse III (France) and PCIGR- University of British Columbia, Canada).

4. Results

4.1 SPM concentrations

The SPM concentrations (Table 1) in the Garonne Branch of the fluvial Gironde Estuary defined a sharp gradient with turbidities increasing from ~2 mg/L to ~1000 mg/L within a distance of 16 km only (samples T1-T3). In the MTZ, SPM concentrations were above 1600 mg/L on average but varied by a factor 2-3 due to the natural heterogeneous distribution of particles forming local turbid volutes (samples T4-T12). Other samples had SPM concentrations of 474 mg/L for GMC319 (salinity 12); 653 mg/L and 88.7 mg/L at the La

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Réole site for the two floods, on the 06/02/2003 (4740 m³/s) and the 15/12/2003 (4550 m³/s), respectively.

4.2 Elemental concentrations

- **Particles**. Particulate Zn (Zn_P) and Cd (Cd_P) concentrations exhibited similar longitudinal profiles from T1 (near La Réole) to T12 (near Bordeaux) in the fluvial estuary. Concentrations decreased from 2140 mg/kg and 15.2 mg/kg at T1 to 376 mg/kg and 1.42 mg/kg at T3, respectively, for Zn_P and Cd_P. Particles in the MTZ (T4-T12) showed low and constant values of Zn_P = 262 $\pm$ 43 mg/kg and Cd_P = 0.46 $\pm$ 0.08 mg/kg (average $\pm$ 1SD), similar to those typically observed in recent (2001-2008) particles from the brackish estuary (e.g. sample GMC319; Dabrin et al., 2009). Samples from the contaminated sediment core of Cajarc showed high concentrations varying from 9970 to 907 mg/kg and from 247 to 11.8 mg/kg, respectively for Zn_P and Cd_P. Suspended particulate matter samples from the La Réole site showed Zn_P and Cd_P concentrations of 488 mg/kg and 6.44 mg/kg, and 242 mg/kg and 0.89 mg/kg, respectively for the December 2003 flood (from the Lot watershed) and the February 2003 flood (from the Upper Garonne Watershed). Vineyard soils exhibited rather low Zn_P and Cd_P concentrations not higher than 137 and 1.12 mg/kg, respectively. The urban river sediment showed more elevated values with ~370 mg/kg and ~1.50 mg/kg, respectively. All these samples had consistently higher Zn_P and Cd_P concentrations than samples from the Marcenac sediment core, representative of typical regional geochemical background (Audry et al., 2004a; Masson et al., 2006).

- **Dissolved phase.** Dissolved Zn concentrations (Zn_D) in the fluvial estuary (Fig. 1D) varied with distance from 4.47 µg/L at T1, similar to average Zn_D concentrations at the La Réole site (5.4 µg/L $\pm$ 22 (1SD, n=192), TGM monitoring surveys), to 1.53 µg/L at T3. Dissolved Zn concentrations reached low and constant values of 1.03 $\pm$ 0.18 µg/L on average for the MTZ (T4-T12).

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- Oysters. Zinc and Cd bioconcentrations in oyster's soft body tissues, expressed as mg/kg dry weight (d.w.) were mostly dependent on seasonal weight gains and losses during the reproduction cycle (Páez-Osuna et al., 1995), such that maximum values systematically occurred in winter months. In winter oyster samples (Table 1), Zn bioconcentrations varied from 10100 to 3010 mg/kg but showed no remarkable trend with time. In contrast, Cd bioconcentrations, showing a regular decrease over time, varied from 133 mg/kg in the early eighties to 25.9 mg/kg in 2010.

4.3 Zn stable isotopes

4.3.1 Intercalibration and quality controls

The precision of Zn isotopic measurements of a sample is given by the reproducibility (2SD) for n≥2 replicates. Precision was δ^{66}Zn = 0.06 ‰ (n=53) on average for all the samples, and close to 0.04 ‰ (n=55) on average, for all the bracketing standards. The IRMM3072 Zn certified reference solution (Ponzeverra et al., 2006), measured to verify the analytical repeatability and accuracy, gave δ^{66}Zn = 0.36 ± 0.06 ‰ (n=12), in agreement with δ^{66}Zn = 0.32 ± 0.16 ‰ (Cloquet et al., 2006) and 0.30 ± 0.03 ‰ (Petit et al., 2008b), as shown in Fig. 2A. Duplicates and triplicates (full protocol including digestion, ion-exchange chromatography and replicated isotopic analyses) agreed within precision of isotopic measurements (see Table 1) with δ^{66}Zn = 0.44 ± 0.06 ‰ (n=3); 0.46 ± 0.09 ‰ (n=4) and 0.47 ± 0.04 ‰ (n=3) for sample T1-P and δ^{66}Zn=0.20 ± 0.06 ‰ (n=3) and 0.24 ± 0.11 ‰ (n=4) for sample AP22. In addition, duplicates for dissolved Zn (sample T12-D) were indistinguishable, with Zn isotopic compositions of δ^{66}Zn = 0.90 ± 0.01 ‰ (n=2) and 0.90 ± 0.02 ‰ (n=2). These quality controls indicated that the whole protocol was repeatable and suggested that the main source of errors was related to measurement rather than chemistry. This was confirmed by the intercalibration of two oysters (H87-26 and H2005-14, reported in Shiel et al. (2013); PCIGR University of British Columbia, Vancouver, Canada) and four sediments (M31, Ci8, D27 and

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C18 reported in Sivry et al. (2008); LMTG Université Paul Sabatier, Toulouse, France). Zinc isotopic compositions of these six samples always agreed within the precision of isotopic measurements (Fig. 2A), despite the processing of these samples by independent users using different (1) chromatographic protocols (Maréchal et al. (1999) at the LMTG and Masson et al. (2003) at the PCIGR); (2) instruments (Neptune at the LMTG and Nu Plasma 021 at the PCIGR; with dry and wet plasma); (3) analysis routines (e.g. off mass zero at the PCIGR); and (4) in-house reference standards (δ^{66}Zn =-0.11 $\pm$ 0.03 ‰; Sivry et al., 2008) and 0.12 $\pm$ 0.05 ‰ (Shiel et al., 2013), respectively for the Zn$_{LMTG}$ and Zn$_{PCIGR-1}$).

4.3.2 Zn isotope variations in the Gironde system

The isotopic composition of all samples showed mass dependent fractionation (Fig. 2B), suggesting that no significant isobaric interferences affected Zn isotopes. They varied from δ^{66}Zn = -0.02 to 0.90 ‰ (average of 0.54 $\pm$ 0.31 (1SD, n=13)) for dissolved Zn; from δ^{66}Zn = 1.18 to 1.43 ‰ (average of 1.28 $\pm$ 0.08 (n=11)) for oysters; and from δ^{66}Zn = 0.34 to 0.44 ‰ (average of 0.38 $\pm$ 0.03 (1SD, n=12)) for SPM from the Garonne Branch (sample T1-T12). Metal contaminated samples from the Cajarc sediment core had heavy isotopic signatures consistent with measurements by Sivry et al. (2008). Suspended particulate matter from the brackish MTZ sampled in 1988 showed moderate enrichment in heavy Zn isotopes (δ^{66}Zn = 0.67 ‰). Other potential Zn-rich sources, i.e. one vineyard soil and one urban sediment, showed enrichments in light Zn isotopes (δ^{66}Zn = 0.13 ‰ and 0.20 ‰, respectively) compared to SPM sampled at the La Réole site during floods (δ^{66}Zn = 0.32 and 0.38 ‰), in the brackish MTZ of the Gironde Estuary (δ^{66}Zn = 0.43 ‰), or in uncontaminated sediments from the Marcenac reservoir (δ^{66}Zn = 0.31 ‰).

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5. Discussion

5.1 Elemental and isotopic signatures in oysters (1979-2010)

The regular decrease of Cd bioconcentrations in oysters from ∼120 mg/kg in the eighties to ∼26 mg/kg in 2010 (Fig. 3A) is attributed to the phasing out of the Cd contamination from Decazeville (Chiffoleau et al., 2001; Lanceleur et al. 2011) and contrasts with Zn bioconcentrations (Fig. 3B) showing no remarkable evolution over the same period. This striking difference between Cd and Zn record in RNO oysters could be related to contrasted regulation mechanisms of Cd (toxic metal) and Zn (micronutrient), potentially causing different behaviors with respect to uptake and detoxification (Mouneyrac et al., 1998), but may also reflect different environmental exposure related to contrasting geochemical reactivities of Cd and Zn in the estuary (see section 2).

Reconstructing temporal and spatial trends for metal contamination in the Gironde watershed requires comparing different types of samples (sediments, SPM, oysters). Absolute trace metal concentrations in these samples are not readily comparable since they are either affected by differences in grain size (for particles) or intra-annual changes in biomass (for oysters, see section 4.1). The use of Zn/Cd concentration ratios for these different samples contributes to overcome such uncertainty, because element ratios (not affected by grain size variations or dilution etc.) are much more robust than concentrations. In fact, Previous work has shown that Zn/Cd ratios in sediments of the Lot-Garonne river system provide reliable signatures of particle provenance (Coynel et al., 2007), with typical Zn/Cd concentration ratios in Decazeville smelter waste as low as 40, whereas uncontaminated sediments and SPM from the Garonne/Gironde watershed show Zn/Cd ratios of 150-300 (Audry et al., 2004a).

Temporal changes of the Zn/Cd ratio in the Lot-Garonne-Gironde continuum display a 4 stages (i-iv) historical evolution (Fig. 4A). During (i) the period 1979-1992, when the influence of the Decazeville contamination in the Lot-Garonne-Gironde continuum was the strongest, low Zn/Cd ratios of 67±17 (mean ± 1SD, n=51) in oysters were close to Zn/Cd ratios from the Cajarc core data for the same period (40± 4, n=14). This first period was followed by (ii) a decade-long (1992-2003) regular increase of Zn/Cd ratios in oysters, reaching a value of 220 for the oyster sample H2003-154. This trend is consistent with globally increasing Zn/Cd ratios measured in SPM samples at the La Réole site (ongoing high resolution surveys with18 samples per year, started in 1999) and is related to the deployment of efficient remediation strategies designed to hold back the metal pollution at the Decazeville site. These high Zn/Cd ratios were followed by (iii) a sudden drop in December 2003 recorded in SPM samples and contemporaneous oyster samples, related to erosion/resuspension and downstream transport of Cd contaminated sediments during a major flood in the Lot River (Coynel et al., 2007). Finally, (iv) regular increases in Zn/Cd ratios for oysters and SPM are related to the continued transport of less contaminated particles into the estuary since 2004. Therefore, the Zn/Cd ratio is a robust tracer of the Decazeville signal because the "historical" evolution of this ratio in oysters strongly reflects contemporaneous changes in the geochemical signatures of SPM entering the estuary and is consistent with the evolution of contaminated sediments at Cajarc in the Lot River. In fact, particulate fluxes dominate inputs of Cd and Zn from the Garonne and Dordogne Watersheds into the Gironde Estuary (>95%; Audry et al., 2004b; Audry et al., 2007), which will then undergo geochemical transformations in the estuarine salinity and turbidity gradients.

Matching historical evolutions of Zn/Cd ratios in river sediments and oysters suggest that oysters may also record uncommon anthropogenic Zn isotopic signatures originating from the Decazeville site (Fig. 1A; Sivry et al., 2008). Our measurements of Zn isotopic compositions in oysters (Table 1) confirm values of δ^{66}Zn >1 ‰ previously reported for two

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oysters sampled in 1987 and 2005, presumably attributed by Shiel et al. (2013) to the resilience of the contamination by the former Zn-processing activities at Decazeville. The historical evolution of Zn isotopic signatures in oysters shows an overall decreasing trend in heavy isotopes from δ^{66}Zn = 1.43 ‰ in 1983 to δ^{66}Zn = 1.18 ‰ in 2005-2010 (Fig. 4B) that could apparently reflect a mixing between the Decazeville smelter waste signal and increasing proportions of natural Zn (i.e. δ^{66}Zn around 0.35 ‰). However, the temporary increase in δ^{66}Zn values in oysters (between 1997-2001) is not consistent with contemporaneous changes in Zn/Cd ratios (Fig. 4A), nor with continued depletion in heavy Zn isotopes in the corresponding sections of the Cajarc sediment core (δ^{66}Zn $\approx$ 0.82 ‰ in 1999 and 2001; Sivry et al., 2008). Most importantly, recent oysters samples (2003-2010) still show δ^{66}Zn values > 1 ‰ but have Zn/Cd ratios of ~200, approaching geochemical background values, i.e. not geochemically consistent with pre-1986 oysters samples affected by the Decazeville contamination and showing Zn/Cd ratios of ~60. These apparent contradictions between isotopic and geochemical data signify that Zn isotopic signatures in the Gironde oysters are not simple records of the Decazeville contamination, as assumed in Shiel et al. (2013). This statement is further discussed through the following sections.

5.2 Elemental and isotopic composition of Zn in particles of the Gironde Watershed.

Particles entering the Gironde Estuary from the Garonne River at the La Réole site show variable Cd_P and Zn_P concentrations and Zn/Cd ratios (e.g. Fig. 4A), depending on their nature and origin. Major floods from the Lot Watershed and construction work affecting riverbed sediments, causing erosion and downstream transport of old contaminated sediments to the Gironde Estuary (Audry et al., 2004b; Coynel et al., 2007), carry particles with low Zn/Cd ratio (i.e. as low as 76 for sample LR12-2003, or lower). Floods of comparable importance from the Upper Garonne Watershed (upstream of the Lot River

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confluence) typically show elevated Zn/Cd ratios (as high as 272 for sample LR02-2003; or higher), much more typical of geochemical background. In low freshwater discharge periods, particles with high organic carbon concentrations (up to 14 % POC) related to phytoplankton blooms may occur at the fluvial entry of the Gironde Estuary (Petit et al., 2013b). These samples show Cd_P and Zn_P enrichments (e.g. sample T1, near the La Réole site) but intermediate Zn/Cd ratios of 140. In the fluvial estuary profile (Garonne Branch, Fig. 1D), Cd_P and Zn_P concentrations strongly decrease and Zn/Cd ratios continuously increase from 140 at T1 to 650 ± 148 in the MTZ (average $\pm$ 1SD, n=8; samples T4-T12). This evolution is consistent with the mixing of incoming particles from the Garonne River with Cd-depleted particles from the brackish Gironde Estuary, typically showing high Zn/Cd ratios (e.g. sample GMC319, Zn/Cd=524).

Despite strongly varying Zn/Cd ratios, all the above samples have remarkably constant zinc isotopic signatures (Table 1). Transient signals at the La Réole site for floods from (1) the Lot River (δ^{66}Zn = 0.38 ‰), or (2) the Upper Garonne River (δ^{66}Zn = 0.32 ‰) and (3) during low freshwater discharge periods (δ^{66}Zn=0.47 ‰) have δ^{66}Zn values similar to those of the fluvial estuary (0.34 ‰< δ^{66}Zn < 0.40 ‰, average δ^{66}Zn = 0.37 ± 0.02 ‰ (1SD, n=11; samples T2-T12) and the brackish estuary (δ^{66}Zn = 0.43 ‰; Table 1). Consequently, mixing of incoming particles from the River with Cd-depleted particles from the brackish estuary does not result in significant changes in δ^{66}Zn values. All these particulate samples show Zn isotopic signatures close to the regional background value of δ^{66}Zn = 0.29-0.33 ‰, as recorded at the Marcenac site (this study and Sivry et al., 2008) and typical of Natural Zn (Cloquet et al 2008). Potential inputs of other anthropogenic sources into the estuary by erosion of nearby vineyard soils (δ^{66}Zn as low as 0.13 ‰) and runoff from urban areas of Bordeaux (δ^{66}Zn = 0.22 ‰, sample AP22) cannot explain Zn (and Cd) enrichments eventually observed (e.g. sample T1, also slightly enriched in Zn heavy isotopes) because these secondary sources

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show isotopic compositions typical of "common anthropogenic Zn" (John et al., 2007), i.e. slightly enriched in light isotopes compared to the regional geochemical background.

Therefore, particles entering the Gironde Estuary from the Garonne Branch do not show the "uncommon anthropogenic" Zn signatures (John et al., 2007; Sivry et al., 2008) related to smelting residues from the metal refining site of Decazeville, for the past ten years at least. Moreover, their isotopic signatures are not distinguishable from the natural background value. Two factors explain this observation: (i) the remediation at the Decazeville site which has strongly decreased anthropogenic Cd and Zn fluxes in the Lot River and (ii) the dilution of this signal by natural particles from the Upper Lot, Upper Garonne and Gironde Watersheds.

The dilution of geochemical and isotopic signals from the Lot Watershed at the main hydrological boundaries of the continuum, i.e. (1) the confluence of the Lot and Upper Garonne Rivers and (2) the confluence of the Garonne with the Dordogne (and Isle) Rivers, can be roughly estimated from the Cajarc core data (1956-2001), by applying simple mixing equations. Historical changes in Zn/Cd ratios (see Fig. 4A) and δ^{66}Zn values (see Fig. 4B) can be reconstructed at these boundaries by assuming that (i) sediments deposited at Cajarc are representative of contemporaneous SPM exported from the Lot River; (ii) natural particles (Zn_P = 500 mg/kg, Cd_P = 1.8 mg/kg (Coynel et al., 2007) and δ^{66}Zn = 0.30 ‰ (Sivry et al., 2008)) mix with the Lot River signal at the two above boundaries; and (iii) mixing proportions of rivers reflect inter-annual average freshwater discharges. These values consist in 151 m³/s for the Lot River (at the Villeneuve-sur-Lot station), 393 m³/s for the Upper Garonne River (at the Lamagistère station, upstream from Port Ste Marie), and 602 m³/s, 251 m³/s, and 63 m³/s, respectively for the fluvial inputs to the Gironde Estuary from the Garonne (at the Tonneins station), the Dordogne (at the Pessac sur Dordogne station) and the Isle (at the Courtras station) Rivers ("Banque hydro 2014" database, *Ministère de l'Environnement et du Développement Durable)*.

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Calculated Zn/Cd ratios for particles reaching the two above hydrological boundaries show closer values and globally more consistent evolutions in time with those measured in oysters and SPM at the La Réole site (Fig. 4A). This attests for the necessity of considering this dilution effect by natural particles when investigating long range transport and correlations with Zn isotopic signals recorded in the Cajarc core. The above calculations show a strong depletion in heavy isotopes between 1993 and 1998, reflecting remediation at the Decazeville site (Fig. 4B). They predict δ^{66}Zn values as low as ≈ 0.41 ‰, for particles reaching the estuary in 2001, i.e. much closer to values of δ^{66}Zn = 0.32 ‰ and 0.38 ‰ measured in 2003 and δ^{66}Zn = 0.46 ‰ in 2011 (La Réole sampling site, this study). Considering also that over 95 % of Zn entering in the Gironde Estuary is carried by particles (Audry et al., 2004b; Masson et al., 2006; Audry et al., 2007), the above results signify that Zn isotopic signatures measured in oysters after 1998 cannot be explained by the resilience of the Decazeville signal in the continuum.

There is also evidence for heavier Zn isotopic signature of δ^{66}Zn = 0.67 ‰ (sample MES88) in the brackish estuary in 1988 compared to δ^{66}Zn = 0.48 ‰ (sample GMC319) in 2007, consistent with higher Zn_P and Cd_P concentrations and lower Zn/Cd ratios, before the implementation of efficient remediation around the former Decazeville smelter. These isotopic signatures are significantly lighter than contemporaneous oyster samples (H87-26 and H2009-54) showing δ^{66}Zn = 1.27 ‰ in 1987 and δ^{66}Zn = 1.18 ‰ in 2009 (Table 1). In fact, isotope signatures of particles are systematically lower by 0.6-0.7 ‰ compared to those of oysters in the estuary. Again, this supports the statement that the simple transposition of the Decazeville smelter waste signal cannot explain uncommon Zn isotopic signatures in the Gironde oysters.

5.3 Isotopic composition of dissolved Zn in the fluvial estuary.

In contrast to constant $\delta^{66}Zn$ values in particles from the estuarine turbidity gradient (sample T1-T12, see section 4.3 and 5.2), dissolved Zn showed increasingly strong enrichment in heavy isotopes from $\delta^{66}Zn$ = -0.02 ‰ (at T1) to $\delta^{66}Zn$ = +0.90 ‰ (at T12) in the fluvial estuary (Fig. 1D).

As for particulate Zn, variations in dissolved Zn isotopes are not consistent with physical mixing of potential anthropogenic sources. Potential waste water inputs from the Bordeaux city do not measurably increase Zn_D concentrations nor change Zn_D isotopic compositions at the sampling site nearest to Bordeaux (T12), even in low freshwater discharge situations (Deycard et al., 2014).

As described in section 4.2, Zn_D concentrations strongly decreased with distance in the fluvial estuary (Fig.1D), along with strongly increasing SPM concentrations, due to the presence of the MTZ. At the most upstream sampling site T1 (near the La Réole site), The Zn_D concentration of 4.47 µg/L was similar to the long term average of 5.4 $\pm$ 22 µg/L, obtained from high resolution sampling at the La Réole site (n=192, period 1999-2011). In the MTZ (sampling sites T4-T12), Zn_D concentrations were constant and reached low values of 1.03 $\pm$ 0.18 µg/L (average $\pm$ 1SD), close to long term average of 1.8 $\pm$ 1.7 µg/L (1SD, n=114, period 2001-2007) measured in the brackish estuary (i.e. salinity 0-25). These observations strongly suggest that an efficient removal of Zn_D occurs in the MTZ. Because of the permanent presence of the MTZ in the Gironde estuary, such removal is probably a permanent process, as well.

Oxic to hypoxic oxygen conditions (100-60% saturation, data not shown) and elevated SPM concentrations in the water column of the fluvial estuary probably rule out sulfide precipitation and biological uptake of dissolved Zn as potential removal processes of light Zn_D isotopes. In

contrast, the close relation between SPM concentrations and both the partitioning between particulate and dissolved Zn forms (Fig. 5A) and the strong and regular enrichment in heavy Zn isotopes in the dissolved phase, (Fig. 5B, C), suggest that adsorption is intense and implies that light isotopes are preferentially scavenged onto the particulate phase during this removal.

For mass balance reasons, this removal process does not necessarily require a measurable increase in Zn_P concentrations for particles, nor a measureable shift of their $\delta^{66}Zn$ signatures towards lighter values. In fact, the removal of Zn from solution involves amounts of Zn_D smaller by several orders of magnitudes than those of Zn_P in mass per liter units, especially in these highly turbid waters. For the particulate phase, the corresponding amount of authigenic/adsorbed Zn_P is expected to be very small compared to the overwhelming presence of detrital particles in the fluvial estuary, having the isotopic signature of the geochemical background (see Wiederhold (2015) for a similar example). Preferential adsorption of light Zn isotopes is not generally predicted by experimental approaches, showing rather small to moderate effects on Zn isotopes, with fractionation factors typically less than 1.00050 (e.g. Pokrovsky et al., 2005; Balistrieri et al., 2008; Juillot et al., 2008; Jouvin et al., 2009) and enrichment of heavy isotopes in solid phases. Such experiments, designed to reach chemical and isotopic equilibrium, are performed in closed systems, use pure Zn solutions and pure mineral/organic substrates. They do not address the kinetic fractionation factors for the individual sorption reactions, i.e. the dynamic balance which controls the overall adsorption and isotopic fractionation. We cannot assume that conditions for reversible equilibrium reactions are met in open natural systems such as estuarine transition zones, characterized by strong SPM, pH and redox gradients, involving complex organo-mineral particles covering a wide grainsize range, including colloidal forms, probably affected by flocculation. If our results are not consistent with fractionation experiments at isotopic equilibrium, they agree with the few data available on natural water samples showing

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enrichment in heavy Zn isotopes in the dissolved phase and lighter isotopic signatures in the corresponding suspended solids. This was observed in small streams from a mountain watershed (Borrok et al., 2008) and in the Seine River (Chen et al., 2008; Chen et al., 2009). Dissolved Zn isotopic data from the Seine River (Fig. 5C) were interpreted as a two components physical mixing between urban wastewaters and a natural source (i.e. dissolved Zn from carbonate rocks). Although Zn isotopes in the Seine show a similar trend as those in the Gironde Estuary, such a mixing model is not conceptually applicable to the environmental context of the fluvial Gironde Estuary. Given the extremely high SPM concentrations and the rapid degradation of phytoplankton in the MTZ (Masson et al., 2011; Petit et al. 2013b), metabolic uptake of light Zn isotopes by phytoplankton invoked in Borrok et al. (2008) is probably not applicable. However, kinetic isotope fractionation, favoring faster reaction rates for light isotopes and strong deviations from equilibrium fractionation factors (e.g. Borrok et al. 2008; Hoefs, 2009; Wiederhold, 2015), may most probably explain the fractionation trend observed for dissolved Zn in the turbidity gradient of the MTZ. In this system, the increase in total available sorption sites by three orders of magnitude over a short distance could drive intense adsorption of Zn, favoring higher reaction rates for light Zn_D isotopes. Important flocculation, typical in estuarine MTZ, probably enhances the irreversibility of adsorption and the isolation of the reaction products, therefore contributing to a strong depletion in light isotopes of dissolved Zn.

The variation range for dissolved Zn isotopes observed in the turbidity gradient and MTZ of the Gironde Estuary nearly covers the whole range of values reported from very different river systems worldwide (Chen et al., 2008, Little et al., 2014). The removal of dissolved Zn, that we attribute to kinetic adsorption and fractionation in the Gironde Estuary is probably not the unique explanation for the variation in these systems all over the world (Fig. 6, modified from Little et al., 2014), because different processes, including physical mixing, lead to linear trends in this diagram. However, kinetic fractionation is probably a very important mechanism

in turbid estuaries, potentially controlling the isotopic composition of dissolved Zn reaching the ocean, especially in meso- to macrotidal estuarine zones, where SPM concentrations are generally high.

5.4 Implication for the isotopic record of Zn in RNO/ROCCH oysters.

Assuming that dissolved Zn removal in the MTZ occurs all the time, as explained above, the related isotopic fractionation of Zn_D as observed in the fluvial estuary is also expected to be a permanent feature in the Gironde Estuary, producing values as high as $\delta^{66}Zn$ = +0.90 ‰ (at the most downstream sampling site at KP -6) which is close to the isotopic composition of Gironde oysters.

Based on these observations, we propose that the geochemical reactivity of Zn in the MTZ (section 5.3), rather than the resilience of the Decazeville contamination (section 5.2) is the best explanation for uncommon Zn isotopic signatures recorded in the Gironde oysters (section 5.1). This model is also consistent with homogeneous $\delta^{66}Zn$ values of 0.54 ± 0.02 ‰ (1SD, n=6), similar to the isotopic composition of seawater ($\delta^{66}Zn \cong 0.50$ ‰, Boyle et al., 2012; Zhao et al., 2014), for oysters and bivalves from the other RNO/ROCCH sampling sites reported in Shiel et al. (2013), more exposed to marine/oceanic waters than oysters from the La Fosse site (salinity range of 15-20) of the Gironde Estuary. Even though ecotoxicological considerations are beyond the scope of this study, interpretations are consistent with the hypothesis of preferential direct (across gills) pathway for Zn in natural oysters. However, the strong enrichment in heavy isotopes for dissolved Zn could also be transposed to phytoplankton grown in estuarine waters and recorded in oysters feeding on this biomass.

5.5 Implication for future research on Zn and Cd isotopes in estuarine systems.

In addition to extreme enrichment in heavy Zn isotopes, Gironde oysters show extreme enrichment in light Cd isotopes compared to other RNO/ROCCH sites, with similar δ^{114}Cd values of -1.03 ‰ in 1987 and 2005 (Shiel et al., 2013), despite strongly decreasing Cd bioconcentrations (121 mg/kg and 29 mg/kg, respectively). Bivalves from the Marennes-Oléron Bay, adjacent to the Gironde Estuary, and from the Seine Estuary have comparable Cd isotopic signatures (δ^{114}Cd = -1.00 $\pm$ 0.11 ‰, n=2 (average $\pm$ 1SD) and δ^{114}Cd = -0.88 ‰, respectively), but much lower Cd concentrations (Cd = 2 $\pm$ 1.2 mg/kg, n=2 and Cd = 2.2 mg/kg, respectively). bivalves sampled at other coastal sites with more marine influence show heavier δ^{114}Cd values of -0.51 $\pm$ 0.18 ‰, and low Cd bioconcentrations of 3.1 $\pm$ 3.7 mg/kg (n=10).These observations by Shiel et al. (2013) support the idea that the most negative Cd isotopes signatures are observed at or near estuarine zones but are not always correlated with elevated Cd bioconcentrations.

This is supported by the two constant δ^{114}Cd values for Gironde oysters despite strongly decreasing Cd bioconcentrations over this period (Fig 3A) suggesting that Cd isotope signatures of Natural and Anthropogenic sources are not distinguishable in oysters. Moreover, Cd isotopic compositions of Gironde oysters are clearly different than those in the Cajarc sediment core (-0.1 ‰<δ^{114}Cd< 0.14‰; Sivry et al., 2006), which are typical of Zn-ores but also similar to natural sediments and sedimentary rocks (Schmitt et al., 2009; Rehkamper et al., 2011). These data were not considered in Shiel et al. (2013), who propose that airborne inputs of reduced Cd (presumably enriched in light isotopes), emitted from Zn smelting activities at Decazeville are the source of isotopically light anthropogenic Cd in oysters. However, this assumption is inconsistent with both the dominant riverborne particulate Cd transport in the watershed and isotope signals in contaminated Lot River sediments (see references in section 2, Fig.1 and Fig. 4A). In fact, the offset of ~1 ‰ in δ^{114}Cd values between oysters and Cd-contaminated sediments, probably implies a more complex mechanism and cannot be explained by a simple transposition of isotopic

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signatures, as demonstrated for Zn in the previous sections. The confrontation of Shiel et al. (2013) and Sivry et al. (2006) data, both covering several decades, suggests that the isotopic composition of Cd recorded in Gironde oysters results from a long-lived or permanent process.

As explained in section 2, Cd bioconcentrations in oysters exhibit spatial trends from higher values for the most inner sampling site (La Fosse) to lower values for the more marine sites (Pontaillac and Bonne Anse), strongly reflecting the average position of the dissolved Cd mid-estuarine maximum (Chiffoleau et al. 2001; Lanceleur et al. 2011). Therefore, Cd bioconcentrations are strongly related to dissolved Cd exposure in ambient estuarine waters. The geochemical reactivity of Cd is controlled by a strong addition due to desorption of particulate Cd as chlorocomplexes, typically observed in the Gironde (Dabrin et al., 2009) and other estuarine systems. As a consequence, ~90% of Cd at the estuary mouth is present in the dissolved phase (Dabrin et al., 2009), resulting in contamination of oysters either by direct exposure, or indirectly, by trophic exposure via the phytoplancton (e.g. Strady et al., 2011). Given the very negative δ^{114}Cd values in oysters from the Gironde Estuary and adjacent coastal zone, we propose that the isotopic composition of dissolved Cd in the Gironde Estuary is enriched in light isotopes due to desorption and chlorocomplexation compared to other RNO/ROCCH sites with more marine influence. Potential biological fractionation processes of Cd isotopes during its transfer from water to oysters (including plankton or not) are probably not the explanation for these very light Cd isotopic signatures, because their fractionation effects are not specific to the Gironde estuary and adjacent coastal zone. Unfortunately, fractionation of Cd isotopes during estuarine biogeochemical processes is still unknown, because the scientific community currently focuses on open ocean water-column processes, linked with the biological pump, in the context of climate change (e.g. GEOTRACES program). Further research on metal isotope fractionation during estuarine biogeochemical processes will be necessary.

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6. Conclusions

Wild oysters from the Gironde Estuary mouth (La Fosse site, RNO/ROCCH) are enriched in heavy Zn isotopes compared to bivalves from other RNO sites (bays and coastal area), showing a slightly decreasing trend in Zn isotopic compositions from $\delta^{66}Zn$ = 1.43 ‰ in 1983 to 1.18 ‰ in 2010. These $\delta^{66}Zn$ values are not consistent with a simple transposition of uncommon ($\delta^{66}Zn$ >1 ‰) isotopic signatures recorded in contaminated sediments from the Lot River, related to smelting residues of the former Zn refining activities at Decazeville. Due to on-site remediation and dilution by natural Zn in the fluvial-estuarine continuum, particles entering the estuary do no longer exhibit the Zn isotope signature of the Lot River, at least since 2003 and probably as early as 1999. This is supported by geochemical modeling and Zn isotopic measurements on a wide range of SPM samples in the fluvial and brackish Gironde Estuary. These samples show similar Zn isotopic compositions ($\delta^{66}Zn \approx 0.35 \pm 0.03$ ‰ (average $\pm$ 1SD), n=15), typical of regional background values, despite strongly variable absolute Zn and Cd concentrations and ratio. In this respect, the Zn/Cd ratio could be a much more discriminating proxy than $\delta^{66}Zn$ and allows correlating geochemical evolutions recorded in sediments (Lot River), SPM (Lower Garonne River) and wild oysters (Gironde Estuary mouth) from the fluvial-estuarine continuum.

Dissolved Zn undergoes strong removal in the MTZ, associated to a strong and progressive enrichment in heavy isotopes (from $\delta^{66}Zn$ = -0.02 ‰ (upstream) to $\delta^{66}Zn$ = +0.90 ‰ (downstream)), closely related to increasing SPM concentrations. This removal is interpreted as kinetically driven adsorption, favoring the removal of light Zn isotopes. Due to lower Zn_D concentrations in the Gironde Estuary compared to the Garonne River on an interannual basis, and the permanent presence of the MTZ in this hyperturbid system, the heavy isotopic composition of dissolved Zn in the estuary is probably a persistent feature. Finally, we

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propose that the geochemical reactivity and uptake of dissolved Zn by oysters, rather than the transposition the Zn isotopic signature from the Cd contaminated sediments, are probably responsible for the enrichment in heavy Zn isotopes in oysters from the Gironde Estuary. Accordingly, this study highlights the prevalence of estuarine geochemical processes in controlling the isotopic composition of dissolved Zn reaching the ocean from major, meso- to macrotidal turbid estuarine systems. Integration of long-term monitoring in rivers, sediment archives and all available Zn and Cd isotope data from the Gironde fluvial-estuarine system suggests that the very low and apparently invariable δ^{114}Cd values in RNO/ROCCH oysters reflect dynamic fractionation of Cd isotopes in the Gironde Estuary rather than source-derived signatures. We hypothesize that desorption and chlorocomplexation induce enrichment in light Cd isotopes in the dissolved phase. However, direct evidences will be essential to support this. Further investigations should aim at confirming contrasting isotopic fractionation patterns resulting from different geochemical reactivities of Cd and Zn in estuarine systems. This is also necessary to validate the combined use of bivalve and metal stable isotopes for environmental studies.

Acknowledgments

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Table caption

Geochemical and isotopic data for particles and dissolved phase in the fluvial Gironde Estuary, for RNO/ROCCH oysters (Ifremer data, dry-weight (d.w.) values) at the La Fosse site and for various particulate samples from the Gironde Watershed. Kilometric point values (KP) correspond to distance from Bordeaux and are positive in the downstream direction. Zn and Cd concentrations and Zn isotopic compositions of particulate and dissolved samples are given as average $\pm$ 1SD and average $\pm$ 2SD, respectively, based on n replicates. Zn isotopes measurements were intercalibrated with (*) Shiel et al. (2013) for oysters and ($) Sivry et al. (2008) for sediments.

Figure Captions

Figure 1: Map of the Gironde Watershed with geochemical data showing temporal evolutions of (A) Cd concentrations (white dots) and δ^{66}Zn (black dots) in the Cajarc sediment core (Audry et al., 2004a; Sivry et al., 2008); (B) Dissolved Cd fluxes at La Réole (Blanc et al., 1999; Schäfer et al., 2002; Audry et al., 2004b; Audry et al.2007, Dabrin et al.; 2009); (C) Cd dry-weight (d.w.) biocentrations in oysters (Chiffoleau et al., 2001). (D) Map of the Garonne Branch of the Fluvial Gironde Estuary showing sampling stations (T1-T12).

Figure 2: Quality controls for Zn isotopic measurements. (A) Intercalibration of the certified reference material IRMM 3702 Zn (Cloquet et al. 2006 and Petit et al. 2008b) as well as sediments (Sivry et al. 2008, at the LMTG, Toulouse) and oysters (Shiel et al. 2013, at the PCIGR, Vancouver). (B) Mass-dependency of Zn isotopic measurements with X $\in$ [7, 8]. Error bars are 2SD based on n replicates.

Figure 3: Temporal evolution (thin line) and 4-periods sliding average (bold line) of (A) Cd and (B) Zn bioconcentrations (dry weight) in RNO/ROCCH oysters from the La Fosse site.

Figure 4: Four stages (i-iv) temporal evolution of the Decazeville contamination and transfer in the Lot-Garonne-Gironde continuum. (A) Zn/Cd ratios for RNO/ROCCH oyster samples, for the Cajarc sediment core and for high resolution SPM samples at the La Réole site (18 samples/year). (B) δ^{66}Zn values for RNO-ROCCH winter oyster samples, for the Cajarc sediment core and for samples from the La Réole site, the fluvial estuary (SPM and dissolved phase) and brackish estuary (with connecting line). Modeled Zn/Cd ratios and δ^{66}Zn values for SPM at (1) the Lot+Garonne confluence (thin discontinuous line) and at (2) the Garonne+Dordogne confluence (thin dotted line) were estimated from the Cajarc sediment core assuming dilution by natural particles (see text for details). Error bars are 2SD based on n replicates (not shown for 2011 data).

Figure 5: Behavior of Zn in the fluvial Gironde Estuary (Garonne Branch, this study) and in the Seine River (Chen et al. 2008, 2009). (A) Relative proportion of dissolved Zn as a function of SPM concentrations, for Log K_d values varying from 2 to 6 (Garonne: black circles; Seine: grey diamonds). (B) Evolution of dissolved δ^{66}Zn and SPM concentrations (grey stars) in the fluvial estuary. (C) Dissolved δ^{66}Zn as a function of SPM concentrations for the Seine (Chen et al., 2008; Chen et al., 2009) and the Gironde samples. The arrow indicates the downstream direction in the fluvial Gironde Estuary. Error bars are 2SD based on n replicates (see Table 1).

Figure 6: Dissolved δ^{66}Zn as a function of reciprocal Zn concentrations (ng/L) for World Rivers (Little et al., 2014), Seine River (Chen et al., 2008) and fluvial Gironde Estuary (this study). Modified from Little et al. (2014). Error bars are 2SD based on n replicates (see Table 1).
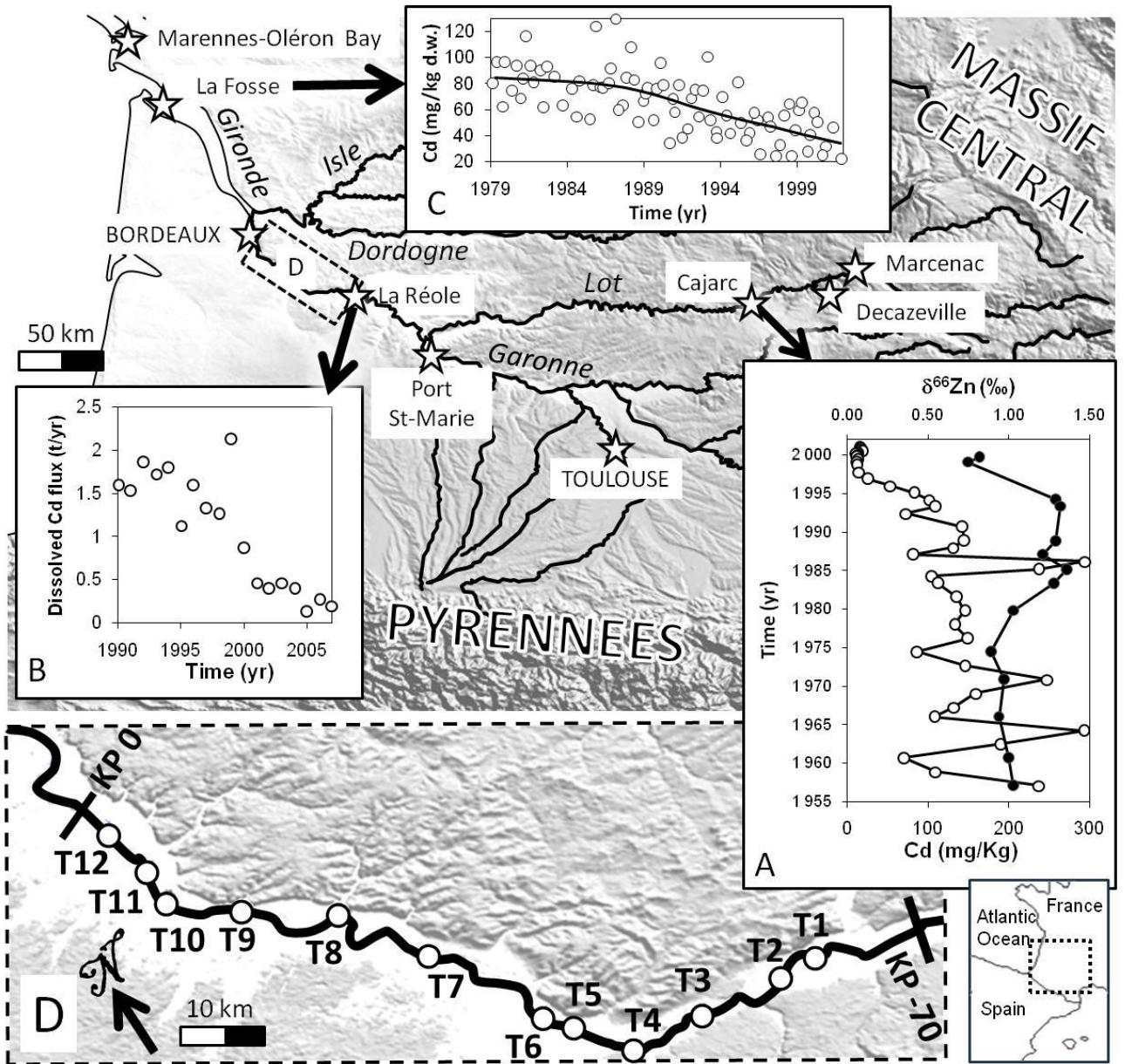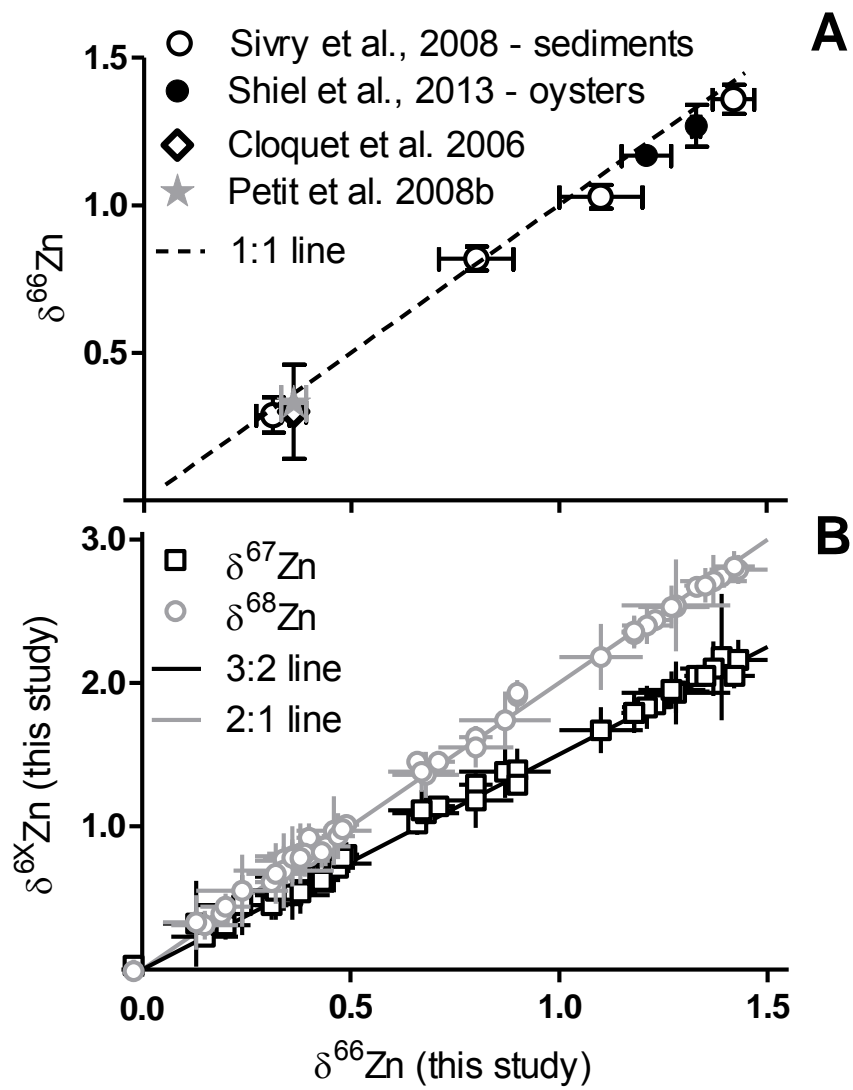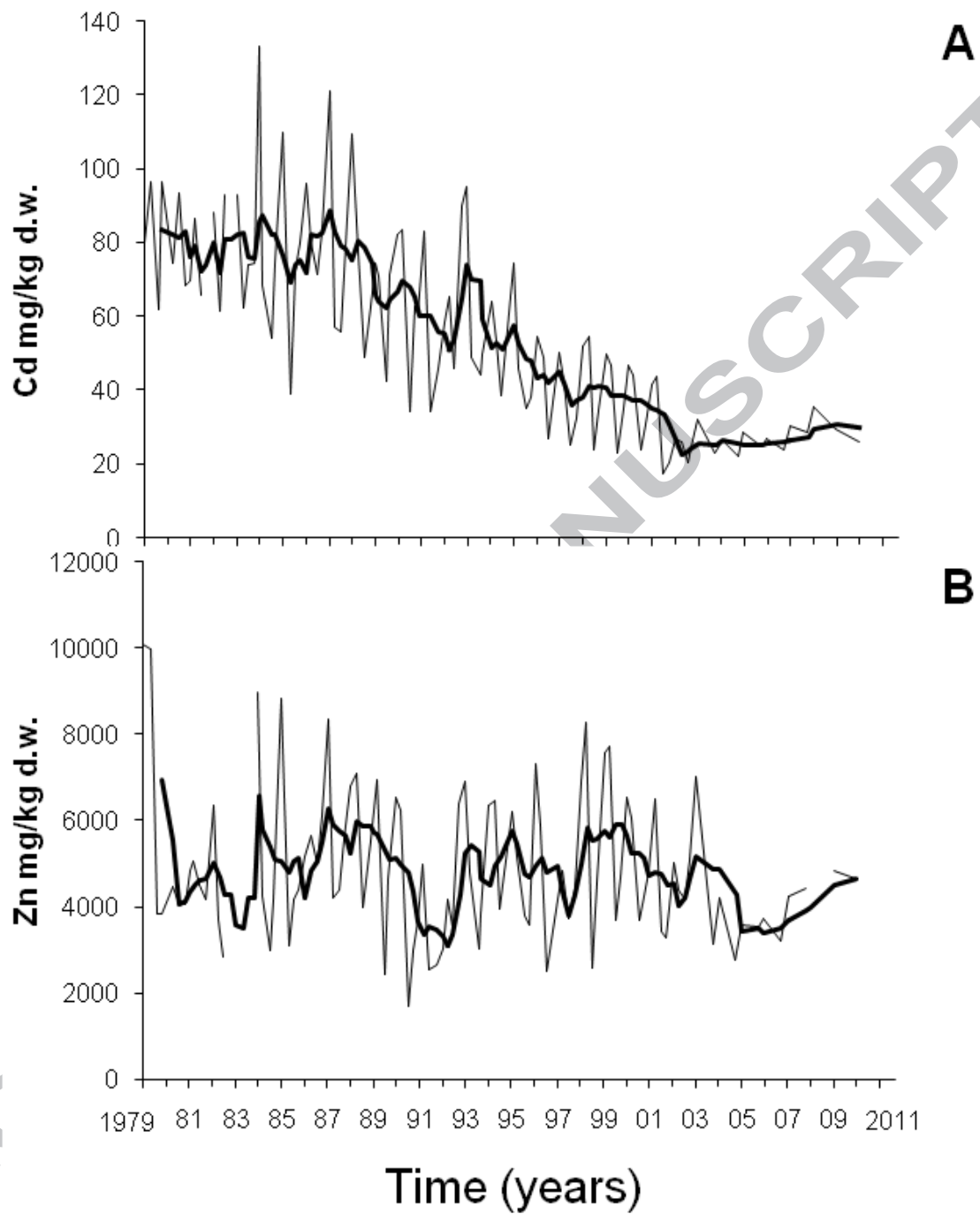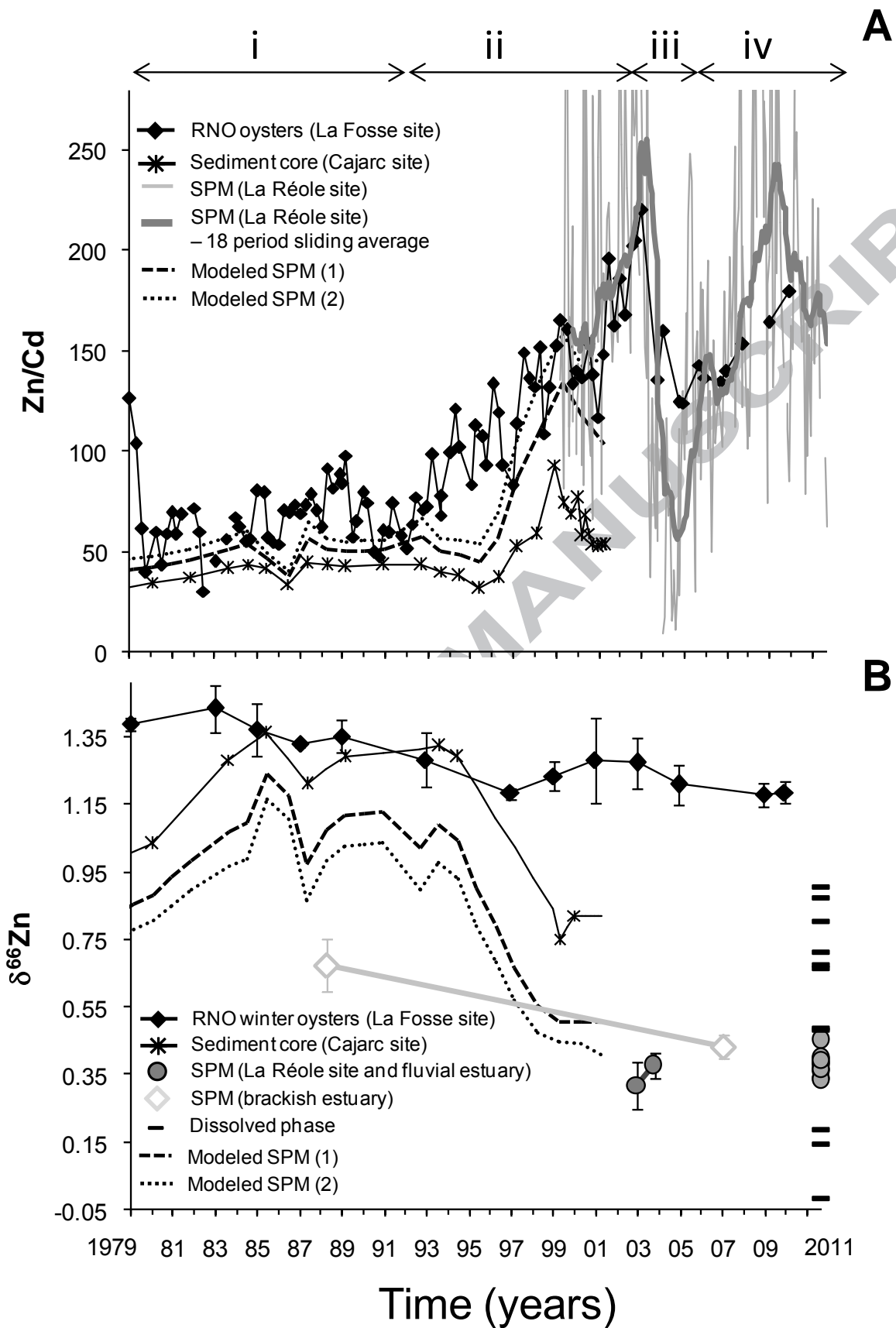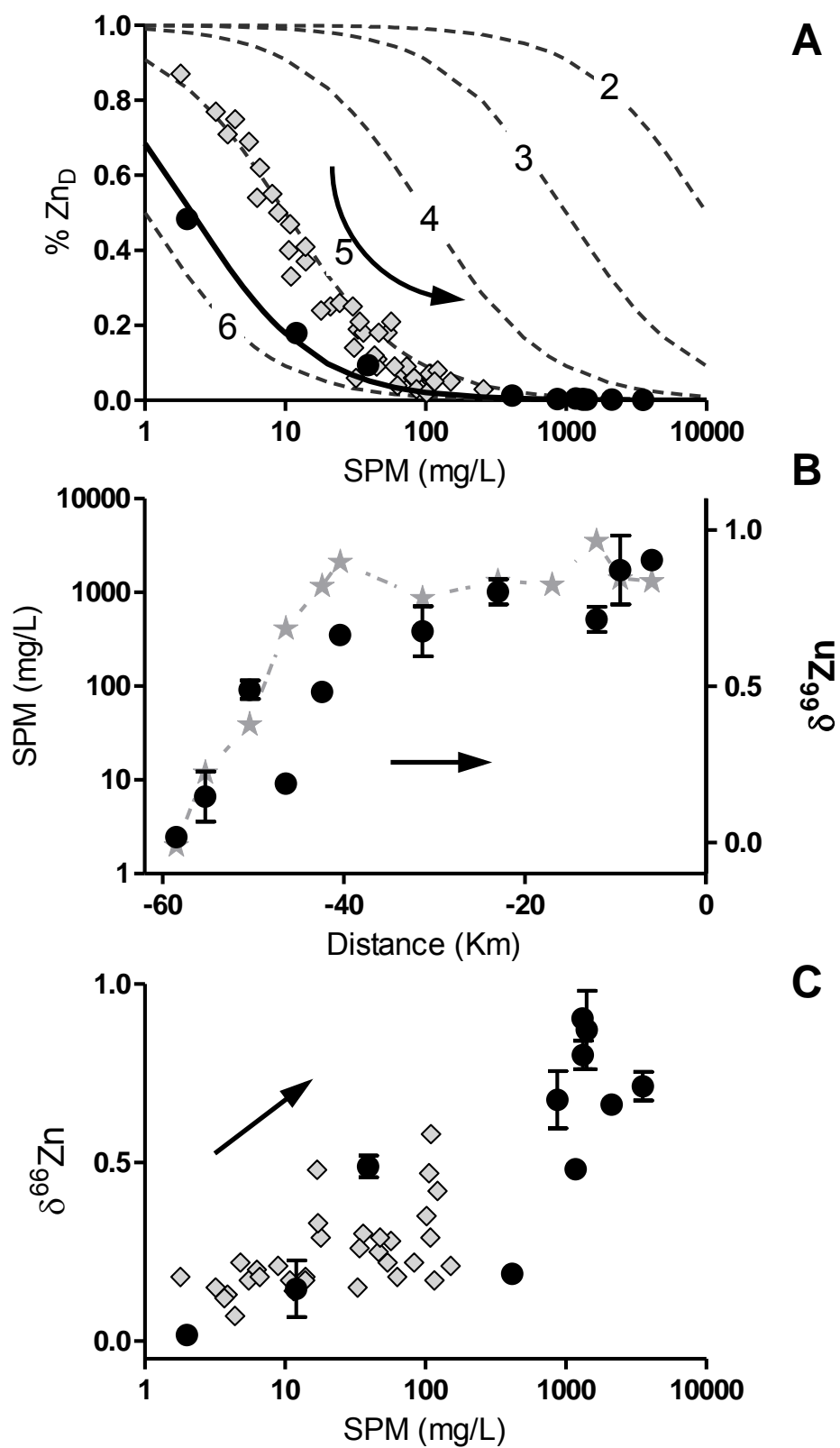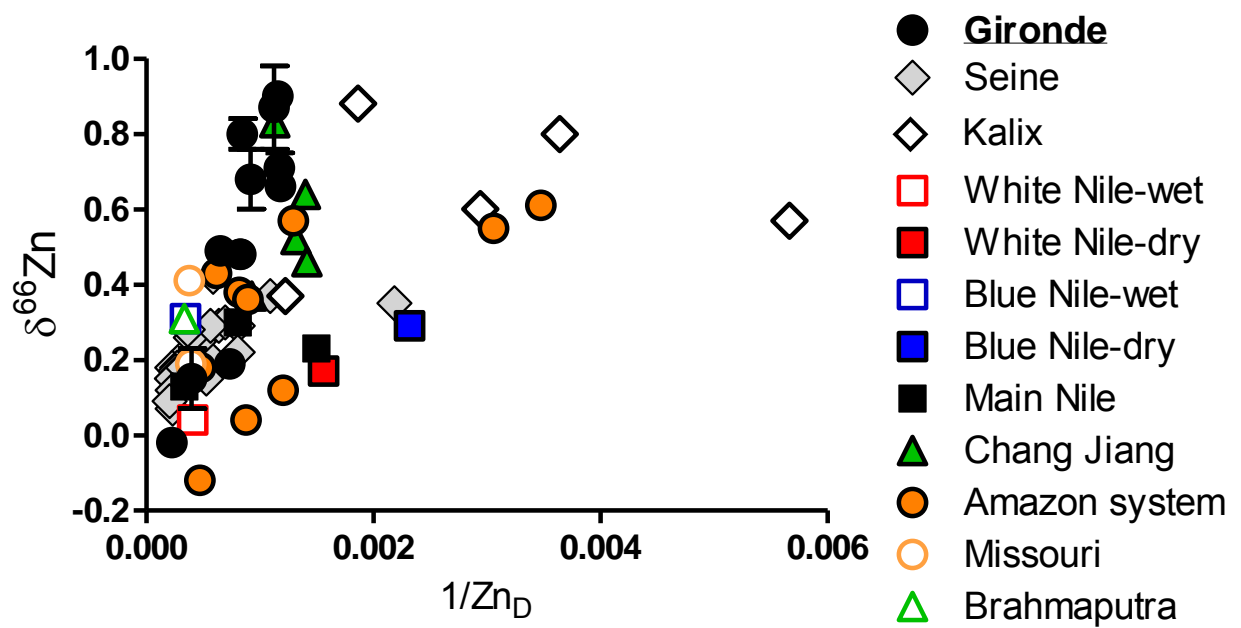
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FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

Table 1

ACCEPTED MANUSCRIPT

	Sampling date	KP (km)	SPM (mg/L)	Concentrations Zn	Cd mg/kg	n	Zn stable isotopes δ66/64Zn ‰	δ67/64Zn ‰	δ68/64Zn ‰	n
Fluvial estuary - Particulate phase				mg/kg						
							0.44 ± 0.06	0.69 ± 0.14	0.86 ± 0.14	3
T1-P	23/09/2011	-58.5	2	2140 ± 27	15.2 ± 0.4	2	0.46 ± 0.09	0.74 ± 0.20	0.97 ± 0.24	4
							0.47 ± 0.04	0.71 ± 0.10	0.93 ± 0.16	3
T2-P	23/09/2011	-55.3	12	957	4.44	1	0.38 ± 0.06	0.65 ± 0.16	0.81 ± 0.09	3
T3-P	23/09/2011	-50.4	39	376 ± 6	1.42 ± 0.10	2	0.38 ± 0.08	0.59 ± 0.20	0.81 ± 0.21	4
T4-P	23/09/2011	-46.4	413	248 ± 1	0.68 ± 0.03	2	0.36 ± 0.09	0.65 ± 0.11	0.79 ± 0.21	4
T5-P	23/09/2011	-42.4	1170	199 ± 1	0.44 ± 0.01	2	0.40 ± 0.03	0.67 ± 0.16	0.92 ± 0.10	4
T6-P	23/09/2011	-40.4	2120	235	0.46	1	0.40 ± 0.04	0.62 ± 0.06	0.79 ± 0.07	4
T7-P	23/09/2011	-31.3	868	349	0.61	1	0.36 ± 0.09	0.52 ± 0.17	0.76 ± 0.15	4
T8-P	23/09/2011	-23	1320	268	0.41	1	0.34 ± 0.05	0.57 ± 0.13	0.77 ± 0.09	3
T9-P	23/09/2011	-17	1210	282	0.38	1	0.34 ± 0.12	0.55 ± 0.14	0.69 ± 0.26	4
T10-P	23/09/2011	-12.1	3530	243	0.42	1	0.38 ± 0.06	0.57 ± 0.13	0.78 ± 0.09	4
T11-P	23/09/2011	-9.5	1410	263	0.35	1	0.36 ± 0.02	0.65 ± 0.21	0.78 ± 0.11	3
T12-P	23/09/2011	-6	1315	272	0.34	1	0.39 ± 0.04	0.66 ± 0.17	0.81 ± 0.16	3
Fluvial estuary - Dissolved phase				µg/L						
T1-D	23/09/2011	-58.5		4.47 ± 0.31		3	-0.02 ± 0.02	0.03 ± 0.04	-0.01 ± 0.08	3
T2-D	23/09/2011	-55.3		2.51 ± 0.23		3	0.15 ± 0.08	0.23 ± 0.01	0.31 ± 0.1	2
T3-D	23/09/2011	-50.4		1.53 ± 0.15		3	0.49 ± 0.03	0.80 ± 0.09	1.01 ± 0.05	3
T4-D	23/09/2011	-46.4		1.36 ± 0.13		3	0.19 ± 0.01	0.31 ± 0.08	0.39 ± 0.02	3
T5-D	23/09/2011	-42.4		1.21 ± 0.12		3	0.48 ± 0.02	0.78 ± 0.02	0.98 ± 0.01	3
T6-D	23/09/2011	-40.4		0.85 ± 0.09		3	0.66 ± 0.01	1.22 ± 0.08	1.45 ± 0.05	3
T7-D	23/09/2011	-31.3		1.09 ± 0.11		3	0.68 ± 0.08	1.09 ± 0.13	1.36 ± 0.16	3
T8-D	23/09/2011	-23		1.18 ± 0.11		3	0.80 ± 0.04	1.29 ± 0.07	1.62 ± 0.08	3
T9-D	24/09/2011	-17		1.01 ± 0.09		3				
T10-D	23/09/2011	-12.1		0.85 ± 0.07		3	0.71 ± 0.04	1.14 ± 0.06	1.45 ± 0.05	3
T11-D	23/09/2011	-9.5		0.89 ± 0.07		3	0.87 ± 0.11	1.38 ± 0.18	1.74 ± 0.2	3
T12-D	23/09/2011	-6		0.86 ± 0.08		3	0.90 ± 0.01	1.39 ± 0.15	1.91 ± 0.11	2
							0.90 ± 0.02	1.29 ± 0.02	1.93 ± 0.01	2
RNO Oysters at La Fosse				mg/Kg d.w.						
H81-811	23/02/1981	85		4830	70		1.39 ± 0.02	2.18 ± 0.44	2.74 ± 0.05	4
H83-91	28/02/1983	85		4200	93		1.43 ± 0.07	2.16 ± 0.14	2.79 ± 0.10	3
H85-79	22/02/1985	85		8840	110		1.37 ± 0.08	2.10 ± 0.19	2.71 ± 0.18	3
H87-26	02/03/1987	85		8350	121		1.33 ± 0.01	2.05 ± 0.04	2.67 ± 0.03	3
H87-26*							**1.27 ± 0.07**			
H89-16	21/02/1989	85		6260	74		1.35 ± 0.05	2.05 ± 0.06	2.68 ± 0.12	3
H93-67	04/02/1993	85		6900	95		1.28 ± 0.08	1.94 ± 0.15	2.53 ± 0.12	3
H97-62	10/02/1997	85		4170	50		1.18 ± 0.02	1.79 ± 0.03	2.34 ± 0.05	3
H99-53	03/03/1999	85		7590	50		1.23 ± 0.04	1.85 ± 0.09	2.44 ± 0.07	3
H2001-17	09/02/2001	85		4850	41		1.28 ± 0.13	1.93 ± 0.22	2.54 ± 0.32	3
H2003-154	18/02/2003	85		7030	32		1.27 ± 0.08	1.95 ± 0.13	2.53 ± 0.15	3
H2005-154	09/02/2005	85		3570	29		1.21 ± 0.06	1.83 ± 0.15	2.40 ± 0.13	3
H2005-154*							**1.17 ± 0.03**			
H2009-54	12/02/2009	85		4850	30		1.18 ± 0.03	1.79 ± 0.15	2.35 ± 0.11	3
H2010-54	03/02/2010	85		4670	26		1.18 ± 0.03	1.79 ± 0.11	2.36 ± 0.11	3
Watershed samples				mg/Kg						
Lot River (Cajarc)										
Ci8	2001	~-400		907	11.8		0.80 ± 0.09	1.18 ± 0.19	1.55 ± 0.14	4
Ci8$							**0.82 ± 0.04**			
C 18/CT18	1986	~-400		9970	237		1.42 ± 0.05	2.05 ± 0.09	2.81 ± 0.11	3
C 18/CT18$							**1.36 ± 0.05**			
CT2(27)/D27	1967	~-400		6530	247		1.10 ± 0.1	1.67 ± 0.16	2.18 ± 0.23	4
CT2(27)/D27$							**1.03 ± 0.04**			
Lot River (Marcenac)										
M 31	1977	~-450		82	0.41		0.31 ± 0.04	0.45 ± 0.1	0.61 ± 0.05	3
M 31$							**0.29 ± 0.06**			
Garonne River at La Réole										
LR02-2003	06/02/2003	-70	653	242	0.89		0.32 ± 0.07	0.55 ± 0.20	0.67 ± 0.21	3
LR12-2003	15/12/2003	-70	89	488	6.44		0.38 ± 0.04	0.54 ± 0.09	0.77 ± 0.02	3
Gironde Estuary SPM										
LR1988	?/?/1988	-70	?	302	1.85		0.67 ± 0.08	1.11 ± 0.14	1.38 ± 0.14	4
GMC319	04/03/2007	52	474	236	0.45		0.43 ± 0.03	0.62 ± 0.02	0.82 ± 0.11	3
Vineyard soils										
Côte de Bourg				114	0.57		0.38 ± 0.02	0.68 ± 0.2	0.78 ± 0.03	2
St-Estèphe				137	1.12		0.13 ± 0.08	0.32 ± 0.3	0.33 ± 0.19	4
Urban River sediment										
AP22				369	1.49		0.20 ± 0.06	0.31 ± 0.10	0.44 ± 0.09	3
							0.24 ± 0.11	0.45 ± 0.21	0.55 ± 0.25	4

TABLE 1