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A critical examination of the possible application of zinc stable isotope ratios in bivalve mollusks and suspended particulate matter to trace zinc pollution in a tropical estuary☆

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ABSTRACT

The application of zinc (Zn) isotopes in bivalve tissues to identify zinc sources in estuaries was critically assessed. We determined the zinc isotope composition of mollusks (*Crassostrea brasiliana* and *Perna perna*) and suspended particulate matter (SPM) in a tropical estuary (Sepetiba Bay, Brazil) historically impacted by metallurgical activities. The zinc isotope systematics of the SPM was in line with mixing of zinc derived from fluvial material and from metallurgical activities. In contrast, source mixing alone cannot account for the isotope ratios observed in the bivalves, which are significantly lighter in the contaminated metallurgical zone ($\delta^{66}Zn_{JMC} = +0.49 \pm 0.06‰$, 2σ, $n = 3$) compared to sampling locations outside ($\delta^{66}Zn_{JMC} = +0.83 \pm 0.10‰$, 2σ, $n = 22$). This observation suggests that additional factors such as speciation, bioavailability and bioaccumulation pathways (via solution or particulate matter) influence the zinc isotope composition of bivalves.

1. Introduction

Zinc isotopes are established as versatile tracers of sources in various environmental reservoirs and of different biogeochemical processes (Wiederhold, 2015). They undergo significant isotopic fractionations during industrial processes such as ore refining and coal combustion, resulting in byproducts and man-made materials that are isotopically distinct from those found naturally, and this has the promise to discriminate zinc from natural or anthropogenic origin (Ochoa and Weiss, 2015; Thapalia et al., 2015; Yin et al., 2016). Furthermore, stable metal isotopes are fractionated during low temperature processes, and hence can be used to determine reactions pathways in the environment such as adsorption on organic and inorganic solid surfaces, biological uptake, redox reactions, complexation and more (Cloquet et al., 2008 and references therein; Veeramani et al., 2015; Yin et al., 2016; Szynkiewicz and Borrok, 2016; Markovic et al., 2017).

The possible application of zinc isotope ratios as a bio-monitoring tool for tracing metal bioavailability and sources in estuaries using tissues of bivalve mollusks has been recently assessed (Shiel et al., 2012, 2013; Petit et al., 2015). The findings, however, did not confirmed the applicability of Zn isotopes for source tracing using biomonitor organisms. One possible explanation for this is that these investigations were carried out in large estuaries, representing open systems mixing multiple point and diffuse anthropogenic sources and natural sources. This is making source identification challenging given the typically small isotope

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variability between zinc sources.

To get a better appreciation of the potential of zinc isotopes as biomonitoring tool in aquatic systems, we conducted a study in a small tropical estuary system, Sepetiba Bay, in southeastern Brazil. This bay is a good estuarine model system as it presents one single dominant anthropogenic zinc source (i.e., associated with old electroplating wastes) and the possible metal contaminants pathways are well-established by previous geochemical and biomonitoring studies (Molisani et al., 2004; Lacerda and Molisani, 2006). A first assessment zinc isotope compositions of sediment cores and suspended particulate matter (SPM) was performed recently in Sepetiba Bay, demonstrating that the stable isotopes signatures of sediments are reliable tracers of anthropogenic sources in the inner bay regions and, therefore, can be useful to reconstruct the temporal and spatial evolution of zinc contamination (Araújo et al., 2017a). In this companion work, we examine the applicability of zinc isotopes to trace anthropogenic sources signatures in oysters (*Crassostrea brasiliana*) and mussels (*Perna perna*) from Sepetiba Bay, using new data on SPM to extend our understanding on zinc source mixing along the fluvial-estuarine continuum.

2. Materials and methods

2.1. Study area

Sepetiba bay is a small semi-enclosed estuary about 519 km^2 located 60 km south of Rio de Janeiro city. It plays an important role as geo-economic center of Brazil, hosting an extensive industrial park with significant harbor activity, besides an increasing human occupation in its drainage basin (Fig. 1). Rivers, estuaries and extensive mangrove ecosystems occur in the northern and eastern portions of the bay. The watershed of approximately 2654 km^2 is drained by rivers crossing agricultural, industrial and urban areas. The São Francisco channel represent the main fluvial system, receiving transposed waters of a neighbor watershed located upstream and attending for over 86% of the total freshwater and most part of fluvial sediment load (Molisani et al., 2004).

The study area has been impacted for almost five decades by a stack of wastes (estimated at 600,000 tons) from a zinc electroplating plant. These wastes exposed to the open air and lixiviated during rainfalls reached surrounding mangroves and the bay through a small tidal creek located in the Saco do Engenho mangrove, considered as the *hot spot* area (Fig. 1) (Molisani et al., 2004). Large loads of zinc and cadmium remobilized from the wastes were estimated about 24 t y^{-1} of Cd and 3660 t y^{-1} for Zn (Molisani et al., 2004). High zinc content in oysters have been reported along the last four decades, often exceeding 80,000 μg g^{-1} (Lacerda and Molisani, 2006). Despite the end of zinc refining activities in 1997, the continuous wastes lixiviation by pluvial waters kept the high inputs of metal loads for more than a decade later as confirmed in other studies (Marques et al., 2006; Gomes et al., 2009). The stack of wastes was definitively removed in 2012. However, Zn-enriched sediment particles from the *hot spot* continue to be remobilized throughout the bay during tidal cycles.

2.2. Sampling and sample preparation

In 2014, oysters samples (*Crassostrea brasiliana*) were collected at five different locations within the Sepetiba Bay to obtain a transect between the channel located in the Saco do Engenho mangrove (the *hot spot* area of electroplating impacts, P1; Fig. 1) and the open sea at the southwestern area of the bay (P5; Fig. 1). About 17 to 21 individual oyster samples were collected in each one of the location P1 to P5, accounting to a total of 91 oysters sampled in the bay. Mussels (*Perna perna*) were found only at P5, where 10 individuals were collected to evaluate possible inter-specific differences on Zn isotope compositions, as well as possible differences of gender. For mussels, male and female individuals were identified according to the tissue color, i.e. white for male and orange for female.

Oysters and mussels were depurated under 48 h in local water, and their tissues were extracted with plastic spatula, rinsed and stored frozen individually in polyethylene little flasks for 48 h, prior

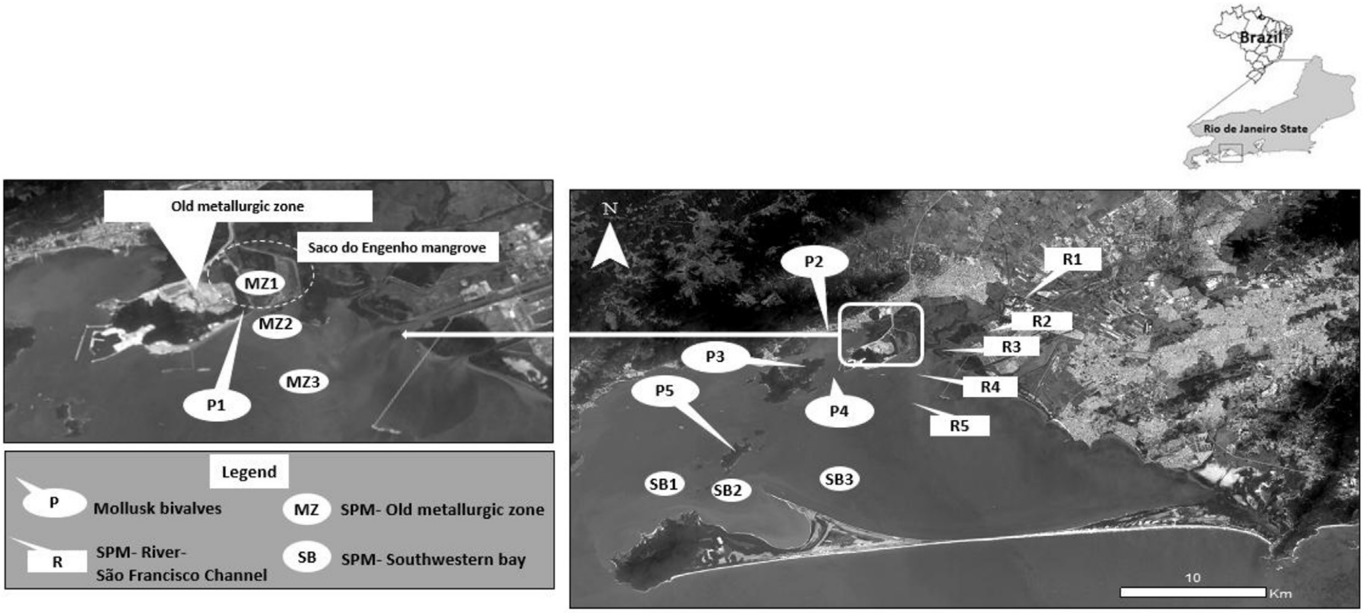

Fig. 1. Map showing the sampling locations at the Sepetiba bay. At left, it is shown a zoom in the old metallurgic zone, where the electroplating activity operated from 1960's to end of 1990's. The dashed circle line highlight the Saco do Engenho mangrove, one site heavily impacted by the wastes lixiviated from the old wastes produced by the electroplating processes. Bivalve sampling stations: P1-Saco do Engenho; P2- Gato; P3-Martins; P4-Itacurussá; P5- Jaguanum. The SPM samples were collected at diferente zones: R (rivers); MZ (old metallurgic zone); and SB (southwest of the bay).

to freeze drying. For each sampling site, oyster tissues were pooled in three different representative aliquots, each one representing a sample. Mussels were identified by sex and analyzed individually.

In 2015, water samples and suspended particulate material (SPM) were collected: i) along a transect from the São Francisco Channel to the inner bay, to represent the main input of freshwater and continental materials into the bay; ii) at the southwestern mouth of the bay, to represent the marine input; and iii) in the Saco do Engenho Channel, close the old metallurgic plant, to characterize the major anthropogenic source in the bay (Fig. 1). A precise volume of water samples (between 150 ml and 500 ml) were filtered through a 0.45 μm acetate cellulose membrane previously acid cleaned, dried and weighted. The dissolved phase was acidified to pH = 2 and stored at 4 °C until concentration was determined. Colloidal-sized particles smaller than 0.45 μm are operationally defined as solutes in this study. The filters containing the SPM samples were dried and weighed to calculate the mass of sediment.

All reagents, acid cleaning of bottles and of labware and sample preparation were performed using <18.2 MΩ H_2O (Nanop System®) and ultra-pure acids (Merck®) distillated by sub-boiling in teflon stills. The chemical procedures were performed at the clean laboratory suites at USP and UNB. Suspended Particlate Matter samples and certified reference materials obtained from the United States Geological Survey (USGS), i.e. BHVO-2, BCR-2, and AGV-2, were digested in Savillex® Teflon on a hot plate using a multiple-step acid procedure with HF, HNO_3, and HCl. The oyster samples (about 100 mg) were dissolved using an acid mixing (HF, HCl and HNO_3) using a closed vessel microwave system (Speedwave 4, Berghof). After total sample digestion, aliquots were taken for the determination of Zn concentrations and isotopic compositions. Zinc was separated from other elements by ion-exchange chromatography technique employing a Bio-Rad PolyPrep columns filled with 2.0 ml of the anion exchange resin AG-MP1, 100–200 mesh, (Araújo et al., 2017b). For subsequent mass bias corrections, the copper standard NIST 976 was added to the purified fractions after the ion exchange procedure and concentration matched with the ratio 1:1 (300 μg/ml in 0.05 M HNO_3).

2.3. Zinc concentrations and isotope ratio analysis

Zinc concentrations were determined by flame absorption atomic spectrometry (F-AAS), model Varian AA240FS, at the GEOPS laboratory at the Université Paris-Sud. External calibration curves were prepared with elemental Merck® standards and the accuracy and precision of analysis were verified with certified reference materials from United States Geological Survey-USGS (BHVO-2 and BCR-2 basalt), National Research Council Canada (Dorm 3 fish protein) and of SCP SCIENCE (EP-L-3 water). The experimentally determined values agreed within of 10% of certified values. Detection limits for zinc were around 20 μg L^{-1}.

Zinc isotope ratios were measured using the ThermoFinnigan Neptune *Plus* MC-ICP-MS at the Centro de Pesquisas Geocronológicas (CPGeo) at the University of São Paulo (USP). The samples, dissolved in 0.05 M HNO_3, were introduce with a stable introduction system (SIS: double Scott/cyclonic spray chamber) coupled with a low flow PFA nebulizer (50 μL min^{-1}). Time integrations for blanks were 80 s, while samples and standards took 320 s. The zinc isotope ratios were measured relative to an in-house Zn single element standard from MERCK (Lot #9953) (denoted as Zn_{UNB}) and expressed in δ-notation as below:

$$\delta^{66}Zn(‰) = \left(\frac{^{66}Zn/^{64}Zn_{sample}}{^{66}Zn/^{64}Zn_{standard}} - 1\right)$$

The $\delta^{68}Zn$ values were calculated using the ratio $R(^{68}Zn/^{64}Zn)$ to test for mass dependency and absence of interferences (Fig. 2). The data in this paper are reported relative to the Johnson Matthey 3–0749-L (JMC) reference material, which was previously calibrated against our Zn UnB reference material ($\Delta Zn_{JMC-UnB} = +0.17‰$, $n = 30$, $2\sigma \pm 0.05$ Araújo et al., 2017b). Error propagation was used to establish an estimate of the analytical uncertainty (expressed as 2σ) of the $\delta^{66}Zn_{JMC}$ values. Mass bias corrections were done using the exponential law combined with sample-standard bracketing (Araújo et al., 2017b).

For quality control, the certified isotope reference material Zn IRMM 3702 was measured two or three times along each session analysis yielding a $\delta^{66}Zn_{JMC}$ value of −0.27 ± 0.06‰ ($n = 30$, 2σ), in line to values published previously (Sossi et al., 2015). In addition, replicate samples of the USGS and NIST reference materials were subjected to the full analytical protocol including digestion, ion-exchange chromatography and isotopic measurements. The $\delta^{66}Zn_{JMC}$ values obtained for BHVO-2 basalt (+0.25 ± 0.10‰, 2σ, $n = 5$), BCR-2 basalt (+0.25 ± 0.08‰, 2σ, $n = 1$), and AGV-2 andesite (+0.29 ± 007, 2σ, $n = 2$) agreed all well with those reported in the literature for silicate rocks (Chen et al., 2013; Sossi et al., 2015). The average external reproducibility of unknown samples (SPM, oysters and mussels) was ±0.07‰ (2σ, $n = 36$) and was chosen to represent external reproducibility of the entire analytical procedure.

2.4. Statistical analysis

Comparison of zinc concentrations and isotope compositions among the different sampling sites were verified by statistical tests using the SPSS® software v.18.0 (IBM®, Armonk, NY, USA). The normality distribution of data was verified using Kolmogorov–Smirnov and Shapiro-Wilk tests, while Levene's test was applied to check the homogeneity of variances (confidence level of 95%). One-way ANOVA and Student test *t* were applied to data normally distributed. If data deviate from the normal distribution or fail in Levene's test, the Welch ANOVA was used instead of a one-way ANOVA.

3. Results and discussions

3.1. Zinc isotope signatures of SPM samples in the fluvial-estuarine system

The dataset for the zinc isotope compositions and concentrations of filtered water and SPM is shown in Table 1. The $\delta^{66}Zn_{JMC}$ values of the SPM samples varied between +0.15 and + 0.95‰ (Table 1, Figs. 2 and 3).

The SPM collected around the old metallurgic zone (Saco do Engenho, Fig. 1) displays the highest zinc concentrations (325–2339 μg g^{-1}) and the heaviest isotope compositions (+0.87 to +0.95‰) in the Sepetiba bay. These $\delta^{66}Zn_{JMC}$ values fall within the range of the isotopic signatures associated to metallurgic wastes and effluents reported in previous studies which vary mostly between +0.5 and + 1.49‰ (Sivry et al., 2008; Juillot et al., 2011; Yin et al., 2016). These isotopic signatures reflect an isotopic record of the contamination by the old electroplating wastes that took place along the last five decades. The higher zinc concentrations in dissolved and particulate phases in samples collected in the old metallurgic zone indicate a high remobilization of Zn-enriched sediments during low tides and point to the fact that this source is still a significant zinc supplier to the Sepetiba bay system, despite the waste removal taking place in 2012. We note that the filtered water of the sampling site MZ1 exceeds the Brazilian legislation threshold value for marine water of 170 μg L^{-1} (CONAMA, 2005).

Along the transect from the upper São Francisco channel to the

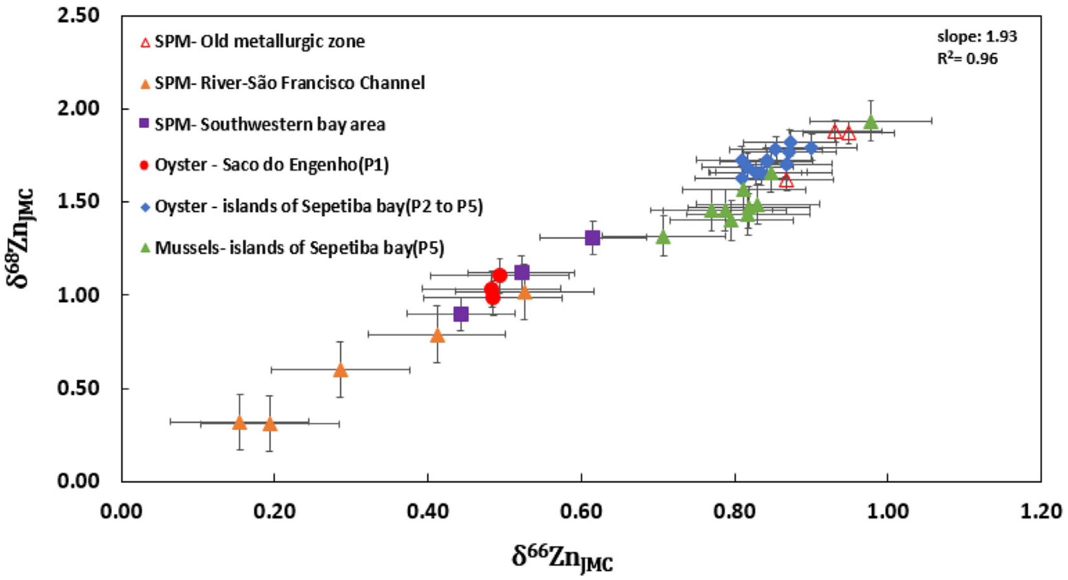

Fig. 2. Three-isotope plot of Zn measured in SPM, oyster and mussel samples follow the mass-dependent fractionation law with the slope 1.93.

Table 1
Zinc concentration and $\delta^{66}Zn_{JMC}$ values in suspended particulate matter (SPM) and water, SPM concentration, conductivity and pH of water samples.

Sample location	Zn_{SPM} (μg g^{-1})	Zn_{Diss}[a] (μg L^{-1})	$\delta^{66}Zn_{JMC\text{-}SPM}$	±2σ	$\delta^{68}Zn_{JMC\text{-}SPM}$	±2σ	*n*[b]	SPM (mg L^{-1})	Conductivity (mS cm^{-1})	pH
Saco do Engenho (Old metallurgic zone)										
MZ1	2339	3384	0.87	0.05	1.62	0.10	2	25	57	7.3
MZ2	1169	85	0.93	0.10	1.88	0.09	2	21	55	7.8
MZ3	325	66	0.95	0.05	1.87	0.09	2	59	51	7.8
River – SF Channel										
R1	66	87	0.15	0.09	0.32	0.15	4	22	1.5	6.9
R2	23	<L.D[a]	0.19	0.06	0.31	0.06	2	118	7	7.0
R3	68	<L.D	0.41	0.11	0.79	0.10	4	84	10	7.2
R4	247	<L.D	0.29	0.14	0.60	0.31	4	169	11	7.3
R5	208	<L.D	0.53	0.06	1.02	0.11	2	20	29	7.9
Southwestern bay										
SB1	60	<L.D	0.44	0.07	0.90	0.10	2	29.4	69	7.9
SB2	629	<L.D	0.62	0.06	1.31	0.10	6	16.9	68	8.0
SB3	82	<L.D	0.52	0.08	1.12	0.08	2	24.8	68	8.1

[a] The limit of detection (L.D.) was estimated to 20 μg.L^{-1}.
[b] Number of Zn isotope measurements on MC-ICP-MS.

inner bay, the SPM displays an enrichment of heavy isotopes from the upstream site at the San Francisco channel to the inner bay, from +0.15‰ to +0.53‰ (Table 1), correlating with conductivity along the estuarine mixing zone ($R^2 = 0.80$, $n = 5$, Fig. 3). The zinc concentrations of SPM samples increase toward the bay (Table 1). The results likely reflect mixture between fluvial and metallurgic-anthropogenic zinc in the estuary. The heavier isotope values of the sample from the outermost location of the fluvial transect (R5) point towards the old metallurgic zone as the main source of zinc in the bay. Zinc isotope compositions of upstream samples of the fluvial system, R1 (+0.15‰) and R2 (+0.19‰) are more likely natural terrigenous materials mixed with potential industrial and urban diffuse sources from the watershed's bay. However, the low zinc concentrations of R1 and R2 samples suggest a dominant input of terrigenous materials. In line with this conclusion is the observation that the zinc isotope compositions are close to values reported previously for granites from the Sepetiba Bay watershed (+0.21 ± 0.01‰, 2σ, $n = 2$, Araújo et al., 2017a).

The southwestern area of the bay, SB1 and SB3, displays low zinc concentrations (60 and 82 μg g^{-1}) and isotope compositions (+0.44‰ and +0.52‰, respectively). In contrast, SB2 presents tenfold higher zinc concentrations (629 μg g^{-1}) and heavier $\delta^{66}Zn_{JMC}$ value (+0.62‰). The SB2 sample results is likely reflecting admixing of anthropogenic zinc derived from the old metallurgic zone. Long distance transport of metal contamination within the bay has been demonstrated previously using sediment core analyses (Patchineelam et al., 2011) and the zinc isotope compositions of a sediment core sampled in the southwestern area of the bay was associated to the metallurgic waste contamination (Araújo et al., 2017a). In turn, SB1 and SB3 reflect essentially the marine end-member.

3.2. *Zinc isotope compositions of oysters and mussels*

Zinc isotope compositions and concentrations of oyster and mussels are displayed in Table 2 and Fig. 4. Zinc concentrations of oyster tissues decrease away from the metallurgic contamination *hotspot* at Saco do Engenho along the transect to the south bay area, i.e. from site P1 to site P5 (Table 2). This suggests that zinc concentrations are directly controlled by metallurgic-anthropogenic zinc amount exported to the bay. This finding is in good agreement with previous studies in the study area (Rebelo et al., 2003; Lacerda and Molisani, 2006). The observed zinc concentrations in oysters around the old zone of metallurgy are lower than those

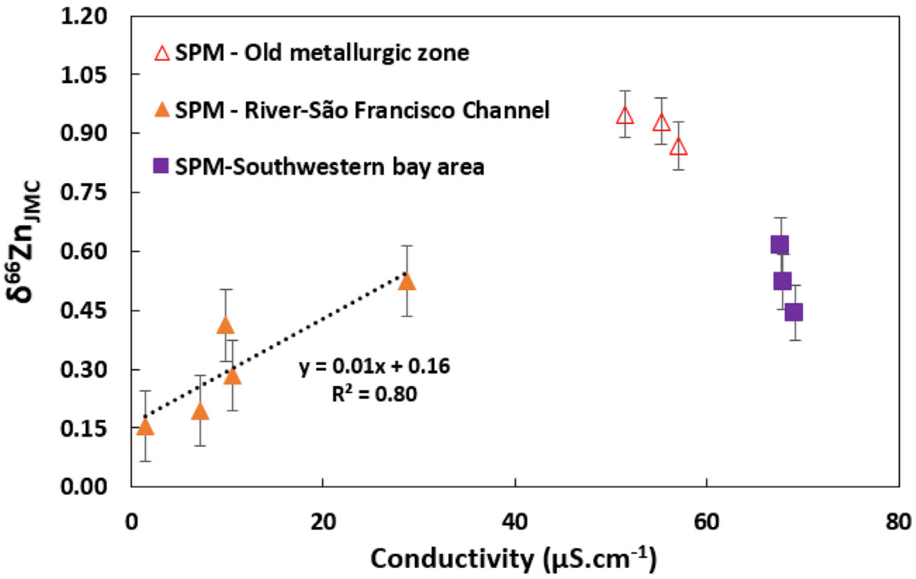

Fig. 3. $\delta^{66}Zn_{JMC}$ of SPM samples against conductivity.

observed in previous studies (Rebelo et al., 2003) but remain at least two fold higher than in pristine areas nearby (Rebelo et al., 2003).

The $\delta^{66}Zn_{JMC}$ values, in contrast, do not display spatial gradients or significant correlations with zinc concentrations (Fig. 4). In contrast, the zinc isotope ratios of oysters are grouped in two distinct regions: one representing the hotspot area of the old metallurgic zone with $\delta^{66}Zn_{JMC}$ values of $+0.49 \pm 0.06$‰ (2σ, $n = 3$) and one comprising ratios displayed by the other sampling stations with $\delta^{66}Zn_{JMC}$ values around $+0.85 \pm 0.06$‰ (2σ, $n = 12$). This pattern suggests that mixing of different sources is not the main controlling factor of the isotopic signatures of the oysters. Even oysters collected in the contaminated old metallurgic zone shows large Zn isotopic fractionation compared to the SPM (Fig. 4, $\Delta^{66}Zn_{SPM\text{-}Oyster} > +0.38$‰). We suggest hence other possible explanations for the observed results, including the variability in zinc bioaccumulation pathways and zinc speciation and bioavailability. This is discussed in more detail below.

In a previous study, Petit et al. (2015) suggested that oysters from the Gironde estuary (France) incorporate dissolved zinc which isotope signatures is controlled by previous adsorption processes occurring during the mixing of fresh and marine waters. In our study, the dissolved phase collected from the small channel inside the mangrove of Saco do Engenho (Fig. 1) have concentrations exceeding 3000 µg L^{-1} (Table 1). This suggests a higher bioavailability of zinc from the dissolved phase, once metals can enter bivalve organisms by facilitated diffusion via grills and actively pumped across the membranes (Carpene and George, 1981; Gosling, 2003; Wang and Rainbow, 2005). Experimental and empirical studies suggest that the dissolved zinc pool is enriched in the light isotope due the preferential adsorption of the heavy isotope on solid surface of particles (Bryan et al., 2015; Szynkiewicz and Borrok, 2016). Thus, the incorporation of a dissolved zinc phase is consistent with the lighter isotope compositions of oysters in comparison to the SPM samples ($\Delta^{66}Zn_{SPM\text{-}Oyster} > +0.38$‰) in the old metallurgic zone. Fig. 5 illustrates the proposed processes involved in determining the zinc isotopes signals observed for the Sepetiba Bay bivalves.

The zinc isotope compositions of oysters collected at the others sampling sites (P2 to P5) are homogeneous ($+0.85 \pm 0.06$‰, 2σ,

Table 2
Zinc concentration and $\delta^{66}Zn_{JMC}$ values for Oysters and mussels.

	Zn ($\mu g.g^{-1}$)	$\delta^{66}Zn_{JMC}$	2σ	$\delta^{68}Zn_{JMC}$	2σ	n[b]
Oyster Crassostrea Brasiliana						
P1- Saco do Engenho						
SI	20,824	0.48	0.06	1.03	0.06	2
SII	21,097	0.49	0.06	0.98	0.07	2
SIII	20,922	0.49	0.05	1.10	0.05	2
Average	**20,947** ± **138**	**0.49** ± **0.01**		**1.04** ± **0.12**		
P2-Gato						
GAI	4506	0.90	0.05	1.79	0.10	2
GAII	5696	0.87	0.05	1.77	0.08	2
GAIII	4953	0.87	0.06	1.82	0.07	2
Average	**5052** ± **601**	**0.88** ± **0.03**		**1.79** ± **0.05**		
P3-Martins						
MAI	6182	0.82	0.05	1.69	0.05	2
MAII	6561	0.81	0.05	1.73	0.07	2
MAIII	7693	0.81	0.05	1.63	0.10	2
Average	**6812** ± **786**	**0.81** ± **0.01**		**1.68** ± **0.07**		
P4- Itacurussá						
ITI	4454	0.83	0.07	1.66	0.08	2
ITII	7625	0.87	0.05	1.70	0.06	2
ITIII	3657	0.84	0.05	1.66	0.06	2
Average	**5245** ± **2099**	**0.84** ± **0.04**		**1.67** ± **0.05**		
P5- Jaguanum						
JI	2836	0.84	0.06	1.72	0.07	2
JII	2941	0.85	0.07	1.78	0.08	2
JIII	3945	0.84	0.07	1.72	0.07	2
Average	**3241** ± **613**	**0.85** ± **0.01**		**1.74** ± **0.07**		
Mussels Perna Perna[a]						
P5- Jaguanum						
Mal1	107	0.81	0.05	1.57	0.07	2
Mal2	105	0.82	0.07	1.47	0.13	4
Mal3	123	0.79	0.11	1.45	0.10	4
Mal4	130	0.83	0.06	1.49	0.11	4
Mal5	127	0.98	0.12	1.94	0.18	2
Mal6	127	0.85	0.10	1.66	0.11	2
Fem1	187	0.82	0.06	1.44	0.10	4
Fem2	128	0.71	0.08	1.32	0.08	2
Fem3	141	0.77	0.07	1.46	0.07	2
Fem4	189	0.80	0.10	1.40	0.21	4
Average	**136** ± **29**	**0.82** ± **0.14**		**1.52** ± **0.35**		

[a] Male and female mussels are designed by the abbreviations “mal” and “fem”, respectively.

[b] Number of Zn isotope measurements on MC-ICP-MS.

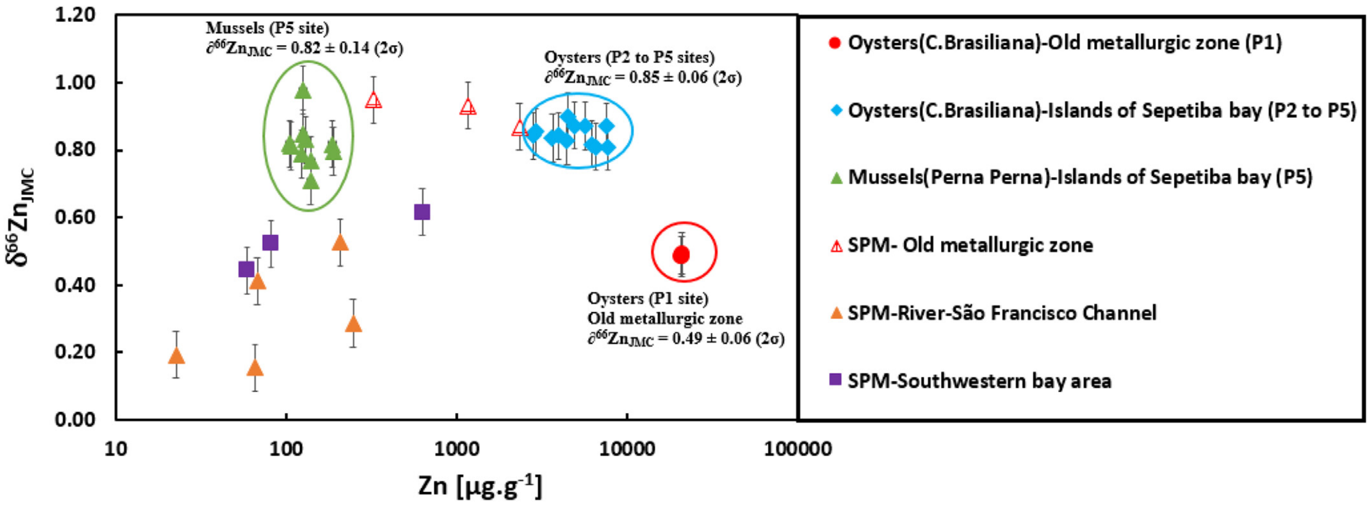

Fig. 4. $\delta^{66}Zn_{JMC}$ (‰) and Zn concentrations ($\mu g\ g^{-1}$) of SPM, oyster and mussel samples of the Sepetiba bay. The error bars (2σ) correspond to 0.07‰ which represents the average reproducibility of unknown samples. The circles highlight the $\delta^{66}Zn_{JMC}$ average of different groups of bivalve samples: oysters sampled in the old metallurgic zone (Saco do Engenho, P1 site); oysters sampled in the islands of Sepetiba bay (P2 to P5 sites); and mussels sampled in one of the islands of Sepetiba bay (P5 site).

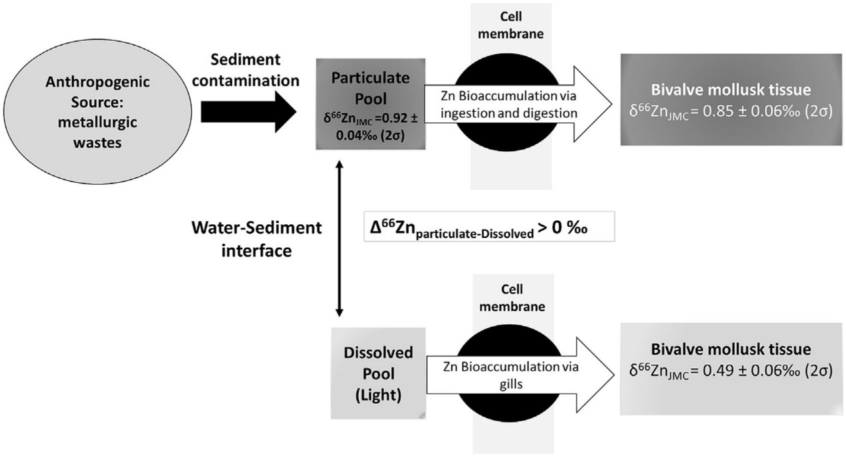

Fig. 5. A simplified scheme of possible processes controlling the Zn isotope systematics for bivalve mollusks from Sepetiba bay. The $\delta^{66}Zn_{JMC}$ values can be explained by two different routes of Zn bioaccumulation: one via dissolved phase and other via particulate phase, with preferential incorporation of the labile Zn bound-particles. Light refers to enrichment of light isotopes in the dissolved phase promoted by sorption processes at interfaces of solid surfaces, as demonstrated and predicted in experimental and theoretical studies (Bryan et al., 2015; Szynkiewicz and Borrok, 2016).

$n = 12$) and may be related to a bioaccumulation mechanisms common to the bivalves in these areas but different from the oysters collected in the old metallurgic zone (P1). In the areas outside the hotspot of contamination (Saco do Engenho), zinc concentrations in the dissolved phase decrease to values below the analytical detection limit (20 $\mu g\ L^{-1}$), suggesting that the particulate phase becomes indeed an important pathway for zinc into the bivalves. Trace metals weakly bound to particles ingested by bivalves can be desorbed by enzymes and non-enzymatic ligands in the gastrointestinal tract and become available for uptake and subsequent incorporation by the gut epithelial cells (Griscom and Fisher, 2004; Birch and Hogg, 2011). Correlation between bioavailable (HCl-extractable) zinc concentrations in fine-grained bottom sediments and oyster tissue concentrations have been reported in Australian estuaries (Birch and Hogg, 2011). Previous studies carried out in Sepetiba Bay observed large percentage of zinc (~80% wt.) in the weakly bound fraction of SPM samples, operationally defined as the zinc extracted with 0.1 mol L^{-1} HCl (Lacerda and Molisani, 2006). Therefore, these two exposure pathways (dissolved and particulate) associated with the speciation and bioavailability could explain the isotopes signals observed Sepetiba Bay bivalves, as illustrated in Fig. 5.

Mussels from site P5 have zinc concentrations at least one order of magnitude below the oyster concentrations (Table 2), with an average of $136 \pm 29\ \mu g\ g^{-1}$ (σ, $n = 10$). Despite this large difference, the isotope compositions of mussels *Perna perna* (+0.82 ± 0.14‰, 2σ, $n = 10$, Fig. 4) are statistically similar (*t-test*, $p > 0.05$) to oysters collected in the islands sites (P2 to P5, +0.85 ± 0.06‰, 2σ, $n = 12$). Average zinc concentrations of male ($120 \pm 11\ \mu g\ g^{-1}$, σ, $n = 6$) and female ($161 \pm 31\ \mu g\ g^{-1}$, σ, $n = 4$) are statistically different (*t-test*, $p < 0.05$), but not the isotope compositions (test t, $p > 0.05$). Thus, no effects on zinc isotope compositions associated to interspecies differences or gender are observed.

4. Conclusions

Zinc isotope composition of SPM enables the identification of the end-members (old electroplating wastes and terrestrial zinc transported by the main river of the bay) and hence to subsequently constrain mixing process in the fluvial-estuarine system.

The isotope signatures of the bivalves, in contrast, cannot be explained by the mixing of identified end-members and group in two distinct groups: One group with a lighter signatures consisting of molluscs collected in the hotspot area of the old metallurgic zone (P1) and one group with the heavier signatures consisting of molluscs collected from the remaining sampling stations (P2 to P5). Our results suggest that the isotope record of zinc in bivalve's tissues may be controlled by additional factors such as speciation and bioavailability and bioaccumulation pathways (via solution or particulate matter). Therefore, their applicability to source tracing in biomonitoring studies is not straightforward. Future work on controlled laboratorial experiments with bivalves are needed to constrain the relationships among speciation, uptake mechanisms and the bioisotopic record in bivalve tissues.

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