Regional Studies in Marine Science 41 (2021) 101618

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Trace elements and δ^{13}C and δ^{15}N isotopes in sediments, phytoplankton and oysters as indicators of anthropogenic activities in estuaries in the Brazilian Amazon

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ARTICLE INFO

Article history:
Received 18 June 2020
Received in revised form 30 December 2020
Accepted 9 January 2021
Available online 11 January 2021

Keywords:
Bioaccumulation
Food chain
Trophic transfer
Bioindicators
Chemical composition

ABSTRACT

This study investigated two estuaries on the northern coast of Brazil submitted to different anthropic activities. The main objective was to assess the use of oysters (*Crassostrea gasar* and *Paxyodon ponderosus*) and phytoplankton as bioindicators of environmental contamination. Water, sediments, phytoplankton and oysters were chemically analyzed by ICP-OES and ICP-MS. The source for metal bioaccumulation in oysters and phytoplankton was assessed using BSAF (Biota Sediment Accumulation Factor) and the isotropic relations of stable carbon (δ^{13}C and δ^{15}N). The oyster-sediment BSAF showed bioaccumulation of Mn, Cu, Zn, Se, Sr, As, Cd and Ba, which was supported by the correlations of Fe, Mn, Cu, Zn, Se, Sr, Al, Ba and Pb with the isotopes (δ^{15}N and δ^{13}C), which confirmed bioaccumulation as well as biodilution and biomagnification in oysters and phytoplankton. That reflects the efficiency of organisms in bioconcentrating metals and their capacity as bioindicators of contamination.

1. Introduction

Oysters and phytoplankton are known for accumulating high concentrations of chemical elements in their tissues (Torres et al., 2008; Amado-Filho et al., 2008), and are thus used in several countries as bioindicators of contamination by metals (Marin-Guirao et al., 2008; Shiel et al., 2012).

A large number of physiological reactions and environmental factors control the bioaccumulation of metals by oysters and involve trophic transference and the potential for biomagnification (Abbe and Sanders, 1990; Abbe et al., 2000). Metals are transferred to oyster tissues through plankton (phytoplankton), which is at the base of the marine and estuarine food chain (Barwick and Maher, 2003; Wallace and Luoma, 2003; Bragigand et al., 2004).

The main source of metals for organisms are sediments (at the bottom or suspended) that accumulate levels of metals that are higher than those in the water, and can cause serious problems due to their toxicity and propensity for bioaccumulation (Cheng et al., 2013). One way of assessing the transference of metals to sediments-organisms is to determine the factors for bioaccumulating metals (BSAF), calculated in order to determine the capacity for bioaccumulating metals from their environments (Qiu et al., 2011; Cheng et al., 2013). BSAF is the factor for assessing the risk and the transport of sediments to organisms and may reflect the degree of environmental contamination (Qiu et al., 2011; Monferrána et al., 2016).

More efficient than the bioaccumulation factors, stable carbon (δ^{13}C)/nitrogen (δ^{15}N) isotope ratios have proven a valuable tool for ecotoxicological research, and are used to trace the movement and assimilation of nutrient and organic matter sources (Forsberg et al., 1993; Piola et al., 2006; Asante et al., 2008). Because δ^{13}C is little fractionated (0.2 to 1‰ per trophic level), it is used to evaluate carbon sources available for different organisms whenever the isotopic signature of these sources is different (Qiu et al., 2011; Cheng et al., 2013; Liu et al., 2018).

The δ^{15}N isotopes are frequently used to explain the potential for biomagnification or biodilution of organic and inorganic contaminants in aquatic food chains and may undergo constant enrichment (3 to 4‰) with each trophic transfer (Bucci et al.,

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https://doi.org/10.1016/j.rsma.2021.101618
2352-4855/

2007; Bond, 2010; Sang et al., 2019). In addition, the isotope δ^{15}N can be correlated with contaminant concentrations to help estimate metal concentrations and biomagnification rates (McIntyre and Beauchamp, 2007; Ikemoto et al., 2008). The proportions of stable isotopes in organisms depend not only on the food consumed by the organism at the time, but also during all phases of development (Ofukany et al., 2014; Xia et al., 2019).

The use of stable isotope (δ^{13}C and δ^{15}N) bioindicators to detect anthropogenic metal contamination processes in the Amazon basin may be more promising than traditional environmental assessment methods that compare concentrations from contaminated areas with control samples (Ikemoto et al., 2008; Piraino and Taylor, 2009). Interpretation of results with these traditional methods may be hindered by aspects such as large river water-volume dilution, high rainfall index and high suspended particulate matter concentrations.

The main objective of this study is to assess the use of oysters (*Paxyodon ponderosus* and *Crassostrea gasar*) and phytoplankton as bioindicators of environmental contamination in two Amazon estuaries with different degrees of anthropogenic impacts. We also seek to describe bioaccumulation and sediment–phytoplankton–oyster transfer of metals, based on use of the bioaccumulation factor (BSAF) and isotopes $\delta^1$3C and δ^{15}N.

2. Materials and methods

2.1. Characteristics of the study sites

This study was conducted in estuaries of the Mocajuba, Tijoca and Pará Rivers in Northern Brazil (Eastern Amazon; Fig. 1), close to Curuça and Barcarena municipalities. Curuça Municipality is located at the confluence of the Mocajuba and Tijoca Rivers, whose estuary is essentially saline. Barcarena Municipality is located in front of Southern Marajó Island, close to the confluence of the Pará and Tocantins Rivers, at its estuary is predominantly formed by freshwater (Fig. 1). The climate in the region is hot and humid, with frequent and abundant rainfall throughout the year. Water in these estuaries is slightly saline from August to November.

The geology of the study site is characterized by clastic, silty-clayey, sandy-clayey and intensely-lateritized sediments of the Barreiras Formation (Tertiary) with yellow latosols and Quaternary sediments (Costa et al., 2004; Vilhena et al., 2010). The silty-clayey sediments in the Mocajuba River estuary are covered with mangrove forests (*Rhizophora mangle* and *Avicennia germinans*). The banks along the Pará River estuary are dominated by swampy vegetation, which is composed of species such as *Euterpe oleracea* Mort., *Montrilia fluxuosa* Mort., and *Montrichardia arborescens* Shott (Macedo et al., 2005); these species grow on silty-sandy sediments. Since the 1970s, industrial installations focused on processing bauxite and kaolin, as well as on producing NPK fertilizers, have been built in this region. A major shipping terminal was built in Vila do Conde.

2.2. Sampling

Water, sediment, phytoplankton and oyster samples were collected (January, April, August and November), at three sampling points (P1C, P2C and P3C; Fig. 1) in the Mocajuba and Tijoca River estuaries. Samples were collected at four different points in the Pará River estuary: in harbors and industrial sites, at Itupanema beach and in Vila do Conde (P1B, P2B, P3B, and P4B; Fig. 1). Collection point P5B (control sample) was located 37 km away from Barcarena Municipality. This point was included in the study because it represents the background values of the site (Vilhena et al., 2016).

Surface water samples were collected during both flood and ebb tides. They were placed in 1 L polyethylene bottles and vacuum-filtered with a 0.45 μm acetate filter. These samples were preserved in nitric acid at pH lower than 2 for additional major and trace element analysis. Physical–chemical parameters (pH, salinity and temperature) were measured *in situ* with a Horiba probe.

Bottom sediments were collected with the aid of a Petersen-type stainless steel dredger. A total of 16 samples were collected. Samples were transferred to plastic bags and stored under refrigeration (4 °C). They were dried at 50 °C and crushed with agate mortar in a laboratory environment.

Samples to determine phytoplankton were collected with “Standard” plankton nets (64 μm), which were horizontally dragged 50 cm down in the water during flood tides. This is the most widely used mesh in the Amazon estuary due to the large quantity of suspended solids in its waters (Paiva et al., 2006). Subsequently, samples were subjected to wet sieving (20 μm mesh) for phytoplankton removal. Samples containing phytoplankton (only) were washed in distilled water and visualized with a Zeiss SV binocular stereo microscope to remove residues such as leaves, stems and shells. These samples were lyophilized (Labconco, Freeze Dry Systems, 7752000), macerated in agate mortar and subjected to total and isotopic chemical analysis. A 250 mL aliquot of plankton material was fixed with formaldehyde solution (4%) for phytoplankton identification. This process was based on the morphological and morphometric features of the colonies. Relative phytoplankton abundance was measured based on Lobo and Leighton (1986).

Oysters (5–12 cm) from the estuaries of Mocajuba (*Crassostrea gasar*) and Pará Rivers (*Paxyodon ponderosus*) were collected at five sampling points (Fig. 1). In the Pará River estuary four samplings were performed and fifteen *P. ponderosus* oysters were collected in each sampling, totaling sixty samples. In the Mocajuba River estuary four samplings were also performed, totaling sixty samples of oysters of type *C. gasar*.

Tissues were removed with stainless steel scissors and tweezers. Next, the tissue samples were washed in deionized water. The soft tissues of each oyster (120 individuals) were chemically analyzed. Subsequently, samples were lyophilized (Labconco, Freeze Dry Systems, 7752000), crushed, ground in agate mortar, and chemically analyzed.

2.3. Granulometric fractioning

Samples were treated with hydrogen peroxide (30%) for organic matter removal, before the sediments were subjected to granulometric analysis. Grain-size fractioning was conducted in a laser particle size analyzer (Analysette Microtec Plus sedigraph) in order to assess particles of approximately 0.04 to 2500 μm. Data were analyzed using Mscontrol VO. 6.0.76 software at the Geosciences Institute, Federal University of Pará, Brazil.

2.4. Chemical analysis of water and sediments

The chemical composition of bottom sediments (major and trace elements) was determined through alkaline fusion with lithium metaborate/tetraborate and acid solubilization. This process was followed by inductively-coupled plasma mass spectrometry (ICP-MS) analysis. Analyses were conducted in compliance with the Litho-Geochemical Standard Package Codes method and with STD OREAS45CA reference materials (Group Website), AcmeLabs, Acme Analytical Laboratories (Vancouver), Ltd. Filtered and acidified water samples were analyzed in the same laboratory along with the STD TMDA-70 reference material.

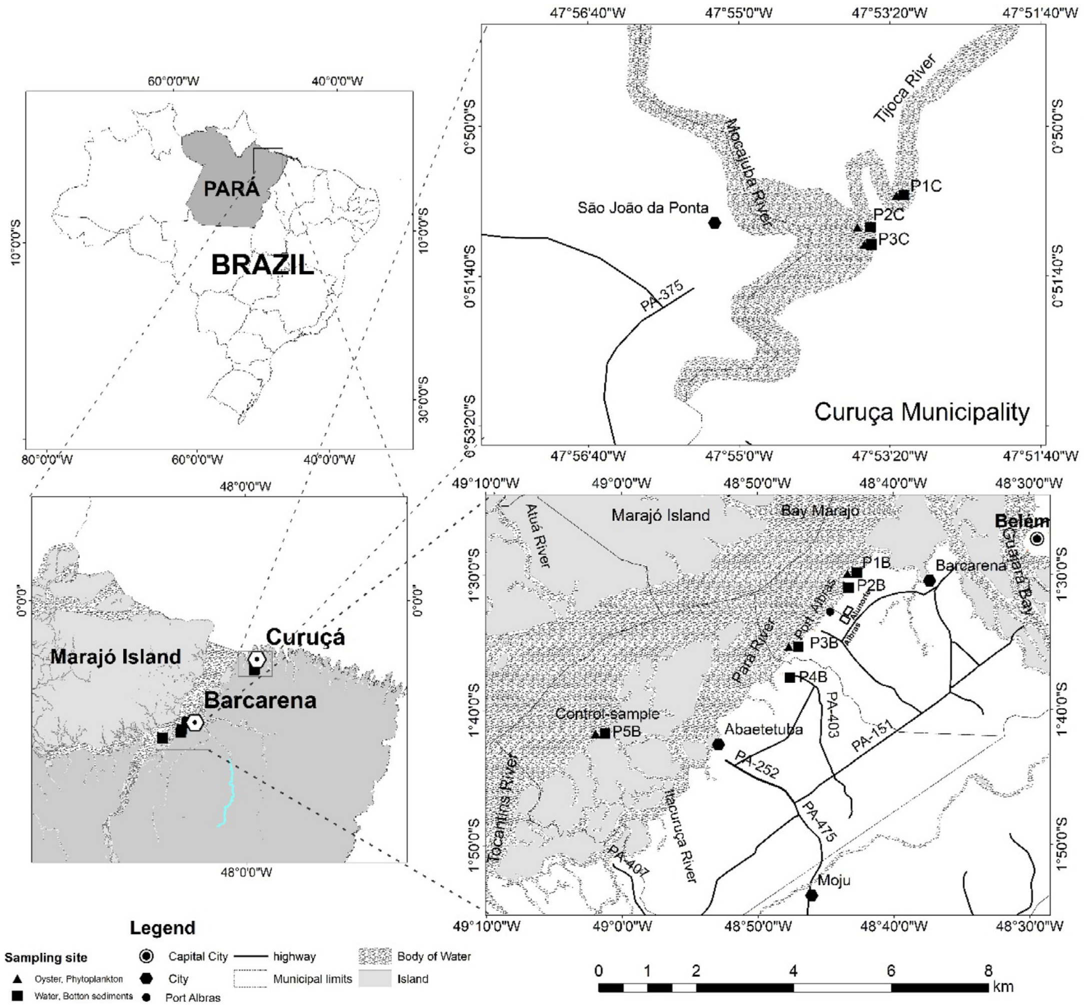

Fig. 1. Location (a) of Mocajuba River in Curuçá region and indicating samples sites P1C, P2C, and P3C; (b) of Pará River in Barcarena region indicating sampling sites P1B, P2B, P3B, P4B, and P5B.

2.5. Chemical analysis of oysters and phytoplankton

Oyster (0.2 g dry weight) and phytoplankton (0.1 g dry weight) samples were digested with HNO_3 and HCl (3:1 v/v), following Lacerda et al. (1987). Each solution was heated at 100 °C for 45 min for complete digestion. Once cooled, the solution was diluted to 25 mL with Milli-Q water. Total Mn, Fe, Cu, Zn, Se, Al, As, Cd, Ba, and Pb concentrations ($\mu g\ g^{-1}$) were determined through inductively-coupled plasma optical emission spectrometry (ICP- MS; HORIBA Jobin Yvan Ultima 2) using argon-induced plasma. Procedures were performed at the Environmental Analysis Laboratory of the GIS Center for Nuclear Energy in Agriculture CENA/USP, Brazil.

The images for micromorphological observations of phytoplankton samples, and for textural and semi-quantitative chemical analyses, were taken in a Scanning Electron Microscope, SEM (LEO 1450, London, England) coupled to an Energy Dispersive X-ray Spectroscope (EDS) 500 DP, Gresham Scientific Instruments (Sirus 10/7.5, London, England) controlled by specific software. Phytoplankton samples were placed in an aluminum holder, under controlled temperature conditions, and metalized with gold. The analyses were conducted at the Goeldi Museum laboratory, Belém Municipality, Brazil.

2.6. Oyster-sediment bioaccumulation factor (BSAF)

The metal bioaccumulation factor (BSAF) in the oysters (*P. ponderosus* and *Crassostrea gasar*) was determined by the ration between the chemical concentration of the metals (in $\mu g\ g^{-1}$) in the oysters and the chemical concentration of the bioavailable metals in the bottom sediments (Arnot and Gobas, 2006; Cheng et al., 2013).

2.7. Analysis of stable $\delta^{13}C$ and $\delta^{15}N$ isotopes

Sediment samples were acidified for carbonate removal and dried at 50 °C to enable the stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ (Lin et al., 1992), which was applied to dry sediment, phytoplankton, and oyster samples. The analysis was conducted in a continuous-flow isotopic ratio mass spectrometer (CF-IRMS). 15 mg of each sample was weighed and used to measure carbon ($\delta^{13}C$ and total carbon) and nitrogen ($\delta^{15}N$ and total nitrogen) contents. Stable isotope abundance was measured by comparing the ratio of the two isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) to the international reference standard (Vienna Pee Dee Belemnite - VPDB), for carbon; and to atmospheric nitrogen (N_2) for nitrogen. The isotopic fraction was expressed as conventional notation (δ) in parts per mill (‰), according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X represents $\delta^{13}C$ or $\delta^{15}N$, and R is the isotopic $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio (Peterson and Fry, 1987). The precision of these analyses (for both C and N) is close to 0.2‰.

2.8. Quality control

The analytical quality of the collected data was assured through the implementation of laboratory quality assurance and of quality control methods such as the use of standard operating procedures, calibration standards, reagent blank analysis, certified material analysis and replicate analysis.

The accuracy of the method and quality control were checked through chemical analyses applied to the samples (in triplicate), as well as through analysis applied to reference materials such as STD TMDA-70 (water samples), STD OREAS45CA (sediments) and DORM-2 (oysters and phytoplankton). There was good matching between analytical results and the certified values of STD TMDA-70 — recovery rates ranged from 90.6% to 105.2%. The accuracy of the analytical procedures – expressed as relative standard deviation (RSD) – ranged from 5% to 10%. The same procedures were performed in sediments with certified values of STD OREAS45CA. Recovery rates ranged from 88.8% to 108.6%. The accuracy of the analytical procedures – expressed as relative standard deviation (RSD) – was lower than 10% in all metals analyzed through ICP-MS. The analytical quality of the oyster (*P. ponderosus and C. gasar*) and phytoplankton samples was assessed based on a certified reference material (DORM-2) of dogfish muscle tissue. The recovery rates of the certified samples ranged from 90% to 102% in Mn, Fe, Cu, Zn, Se, Al, As, and Ba, and recorded 88% for Cd and Pb. Detection limits were 0.01 $\mu g\ g^{-1}$ in Mn, Fe, Cu, Zn, Cd, and Ba, 0.02 $\mu g\ g^{-1}$ in Se and As, and 0.04 $\mu g\ g^{-1}$ in Pb and Al.

2.9. Statistics

The statistical analysis of the collected data was performed using the Statistica 6.0 software. Results of chemical element concentrations in water, sediments, phytoplankton and oyster tissues were tested beforehand for normality and homogeneity through the Kolmogorov–Smirnov and Levene tests, respectively. One-way analysis of variance (ANOVA) was applied to $\delta^{13}C$ and $\delta^{15}N$, whereas Mn, Fe, Cu, Zn, Se, Al, As, Cd, Ba and Pb were used to investigate significant differences between compartments (water $N = 54$, sediments $N = 16$, phytoplankton $N = 20$) and between oyster species *Crassostrea gasar*, $N = 10$ and *Paxyodon ponderosus*, $N = 10$. Variables were subjected to the nonparametric Kruskal–Wallis test whenever they did not show normal distribution, or when data homogeneity did not meet the assumptions of the analysis (even when the logarithm or the square root of the raw data were used).

Simple linear regression analysis was used to assess the relation between trace element concentrations and $\delta^{13}C/\delta^{15}N$ ratio (oysters and phytoplankton). Given the different number of observations between matrices (phytoplankton and oysters), regressions were performed based on the weighted least squares model to assure that all matrices could contribute to the regression analysis. Probability value < 0.05 was taken into consideration to indicate statistical significance in our study.

3. Results

3.1. Characteristics of sediments and surface water

The estuary of the Mocajuba River presents silty-clayey sediments: silt (65%), clay (31%), and sand (4%). The chemical composition of these sediments comprises SiO_2(57.37 ± 11.47%), Al_2O_3 (15.26 ± 2.85%), and Fe_2O_3 (5.0 ± 1.01%), as well as low Na_2O, K_2O, MgO (< 2%) and CaO (< 0.5%) levels, besides trace elements. These values are lower than the concentrations in the Continental Upper Crust (Table 1). The water draining these sediments is slightly acidic (pH 6.2–6.9), mainly during the peak of the rainy season, but becomes more saline (salinity 0–18) during the dry season. The water is bicarbonate-dominated, sodic-magnesian-calcic ($Na^{+} > Mg^{2+} > Ca^{2+} > K^{+}$) and rich in Cl^{-} and Br^{-}. Fe was the trace element presenting the highest concentration, which was followed by Al and Sr (Table 2).

Table 1
Mean values and standard deviations of major (percentage weight) and trace element (in $\mu g\ g^{-1}$) concentrations in the bottom sediments from the estuaries of Mocajuba and Pará rivers and from the Upper Continental Crust (UCC), based on Taylor and Mclennan (1985).

	Pará River	Mocajuba River	UCC
SiO_2	85 ± 11.42	57.4 ± 11	66
Al_2O_3	5.5 ± 5.06	15.3 ± 2.85	15
Fe_2O_3	2.41 ± 1.48	5 ± 1.01	4.3
MnO	0.02 ± 0.01	0.02 ± 0.00	0.07
Na_2O	0.13 ± 0.09	1.2 ± 0.60	3.9
K_2O	0.45 ± 0.37	1.5 ± 0.23	3.4
CaO	0.13 ± 0.11	0.28 ± 0.13	2.2
MgO	0.21 ± 0.20	1.4 ± 0.40	4.2
Cu	4.2 ± 3.65	7.6 ± 2.11	25
Zn	14 ± 11	28.4 ± 7.78	71
As	1.5 ± 0.71	9.3 ± 2.53	1.5
Se	0.5 ± 0.10	0.5 ± 0.10	
Sr	21 ± 15	87.38 ± 27	350
Cd	<0.10	<0.10	0.09
Ba	133 ± 93	210 ± 54	550
Pb	4 ± 2.58	12 ± 2.75	20

UCC: Upper continental crust (Taylor and Mclennan, 1985).

The Pará River estuary presents silty-sandy sediments (7% clay). The chemical composition of these sediments comprises SiO_2 (85.21 ± 11.42%), Al_2O_3 (5.46 ± 5.06%), Fe_2O_3 (2.40 ± 1.48%); Na_2O, K_2O, MgO and CaO (< 0.5%) and trace elements (Table 1). This estuary is primarily influenced by local rivers and presents acidic-to-neutral water pH (5.8–7.7). The highest pH level results from marine water inputs during the dry season. The water in this estuary is bicarbonate-dominated, sodic-calcic-magnesian ($Na^{+} > Ca^{2+} > Mg^{2+} > K^{+}$) and poor in alkalis. The Na^{+} content is 220 ± 127 mmol L^{-1}, whereas the Ca^{2+}, Mg^{2+}, and K^{+} content is < 0.11 mmol L^{-1}. Fe and Al are the major trace elements in the water (Table 2). The chemical concentrations of major and trace elements were significantly higher in the water and sediments of the Mocajuba River estuary than in those of the Pará River ($p < 0.05$).

3.2. Phytoplankton chemical composition

The microphytoplankton community is more diverse in the Mocajuba River estuary (total taxa 126). Among them, 85% belong to class Bacillariophyceae; 10%, to Cianophyceae; 3%, to Chlorophyceae; 1%, to Dynophycaea; and 1%, to order Tintinnidae. *Coscinodiscus oculus iridis*, *Amphiprora alata* and *C. janischii* were relatively abundant during the rainy season and rare in the less rainy months. *Coscinodiscus* was often found in most of the analyzed months.

A total of 60 species were found in the Pará River estuary, with the predominant species being *Aulacoseira granulata*, *Coscinodiscus rothii* and *Polymyxus coronalis* - these species are often found in the brackish water of rivers in the Amazon region (Paiva et al., 2006). Algal diversity increased during months recording lower rainfall rates: different taxa such as *Polymixus coronalis*, and genera such as *Aulacoseira* and *Coscinodiscus*, were highly abundant in this region (Fig. 2). Algal variety remained relatively high

Table 2
Cations, anions (mmol L^{-1}) and trace elements ($\mu g\ L^{-1}$) concentrations (mean and standard deviation) in the surface water. Trace elements concentrations in the phytoplankton and oysters (mean and standard deviation expressed in $\mu g\ g^{-1}$).

	Water		Phytoplankton		Oysters		
	Pará River	Mocajuba River	Pará River	Mocajuba River	P1C, P2C, P3C *C. gasar*	P1B and P3B *P. ponderosus*	P5B-Control-sample *P. ponderosus*
Na^+	220 ± 127[a]	1099 ± 949[a]	–	–	–	–	–
K^+	0.03 ± 0.00[a]	3.29 ± 1.51[a]	–	–	–	–	–
Mg^{2+}	0.07 ± 0.03[a]	18 ± 9.51[a]	–	–	–	–	–
Ca^{2+}	0.01 ± 0.04[a]	3.57 ± 1.79[a]	–	–	–	–	–
Mn	6 ± 5.04[b]	79 ± 32[b]	510 ± 197	1064 ± 662	14 ± 7.35	2020 ± 681	3521 ± 259
Fe	196 ± 151[b]	5368 ± 4010[b]	21887 ± 9605	33435 ± 26437	374 ± 190	13199 ± 1432	15561 ± 2859
Cu	2.26 ± 0.27[b]	43.4 ± 37[b]	16 ± 3.0	17 ± 3.0	75 ± 28	66 ± 33	7.7 ± 1.03
Zn	11 ± 6.81[b]	164 ± 140[b]	38 ± 25	102 ± 55	8017 ± 4998	184 ± 40	201 ± 36
Se	1 ± 0.05[b]	1638 ± 1400[b]	1.4 ± 1.2	1.8 ± 1.1	3.50 ± 2.83	2.3 ± 0.6	–
Al	124 ± 119[b]	373 ± 298[b]	8111 ± 5820	4506 ± 2661	90 ± 58	1513 ± 207	96 ± 15
As	1 ± 0.13[b]	1514 ± 152[b]	6.5 ± 1.6	9.4 ± 4.0	8 ± 0.63	7.3 ± 0.6	3.7 ± 0.18
Cd	0.13 ± 0.01[b]	350 ± 257[b]	1.2 ± 0.6	0.5 ± 0.2	0.63 ± 0.17	2.2 ± 0.7	1.93 ± 0.13
Ba	22.6 ± 3.03[b]	48 ± 35[b]	100 ± 53	15.3 ± 5	1.70 ± 0.90	1286 ± 696	4293 ± 1975
Pb	0.8 ± 0.53[b]	2002 ± 1041[b]	10 ± 1.4	36 ± 18	1.85 ± 0.64	4.88 ± 0.65	–
Cl^-	0.4 ± 0.04[a]	191 ± 102[a]	–	–	–	–	–
Br^-	0.36 ± 0.07[a]	150 ± 103[a]	–	–	–	–	–

Phytoplankton and tissues of oyster ($\mu g\ g^{-1}$); P1C, P2C and P3C (samples of Mocajuba river); P1B and P3B (samples of Pará River); P5B (samples of Abaetetuba City). (–) not analyzed in this study.

[a] mmol L^{-1}.

[b] $\mu g\ L^{-1}$.

during the rainy season and recorded an increasing prevalence of *Coscinodiscus sp.*

The phytoplankton from the Mocajuba and Pará River estuaries was rich in essential micronutrients such as Fe, Mn, and Zn (Table 2). Fe recorded the highest concentrations in Mocajuba (33,435 ±26, 437 $\mu g\ g^{-1}$) and Pará Rivers (21,887 ±9, 605 $\mu g\ g^{-1}$); its concentration was also significantly higher in phytoplankton than in oysters and water ($p < 0.05$). Cu and Se recorded the lowest concentrations (<16 $\mu g\ g^{-1}$). On the other hand, concentrations of toxic elements such as Al and Ba were higher in phytoplankton from the Pará River (Table 2). As and Pb recorded the highest concentration levels in the Mocajuba River, besides being presumably associated with organic material and sulfates in the sediments.

3.3. Metal concentration in oysters

Mn, Fe and Zn concentrations were higher in *P. ponderosus* (Table 2). Fe concentration was approximately 27 times higher in *P. ponderosus* (13,199 ±1, 432 $\mu g\ g^{-1}$) than in *C. gasar*. Among the essential elements, Zn stood out in *C. gasar* with concentrations 27 times higher (8,017 ±4, 998 $\mu g.g^{-1}$) than the oyster *P. ponderosus*. The accumulated Zn, Fe, Cu, and Mn concentrations in *C. gasar* and in *P. ponderosus* were significantly higher than those found in water and sediments ($p < 0.05$). Zn and Cu accumulated at concentrations significantly higher than those found in phytoplankton ($p < 0.05$).

The concentrations of toxic elements such as Al, Cd, Ba, and Pb were relatively low in *C. gasar* tissues, unlike the high concentrations of metals found in the sediments and phytoplankton of the estuary of Mocajuba. On the other hand, Al (1, 513 ± 207 $\mu g\ g^{-1}$) and Ba (1,286 ± 696 $\mu g\ g^{-1}$) concentrations were relatively high in *P. ponderosus*, approximately 14 to 756 times higher than those recorded for *C. gasar* and 14 times higher than the control sample (96 ± 15 $\mu g\ g^{-1}$ Al; Table 2). As concentration (8 ± 0.63 $\mu g\ g^{-1}$) in oysters species *C. gasar* and *P. ponderosus* was higher than that in control oysters.

3.4. Oyster-sediment metal bioaccumulation factor (BSAF)

The highest BSAF values were calculated for Cu (6.21 and 8.65), Mn (4.63), Zn (15.49 and 171.64), Se (2.19 and 5.17), Sr (6.26) Ba (9.83) and Cd (16.83 and 6.79), respectively, following the order Cd > Zn > Ba > Sr > Cu > Mn > Se, for *P. ponderosus* and Zn > Cu > Se > Cd for *C. gasar*. BSAF values recorded for Mn, Fe, Al, Ba and Pb remained lower in *C. gasar* (Fig. 3).

3.5. $\delta^{13}C$ and $\delta^{15}N$ values

The $\delta^{13}C$ signatures in the sediments ranged from −27.5‰ to −21‰, whereas the $\delta^{15}N$ ones ranged from 1.5‰ to 5.6‰. The $\delta^{13}C$ isotope values in the phytoplankton ranged from −33.5‰ to −21‰, whereas the $\delta^{15}N$ ones ranged from 1.8‰ to 6.5‰. High $\delta^{13}C$ values were found in the sediments and phytoplankton from the Pará River estuary, whereas the highest $\delta^{15}N$ values were recorded for phytoplankton from Mocajuba River. The oysters recorded significant $\delta^{13}C$ (-35‰ to −28 ‰) and $\delta^{15}N$ (4.4‰ to 8.7‰) isotope variation. The highest $\delta^{13}C$ values were found in oysters of the species *P. ponderosus*, whereas the highest $\delta^{15}N$ values were recorded for *C. gasar* (Table 3 and Fig. 4).

3.6. Relation between trace elements and $\delta^{13}C$ and $\delta^{15}N$

The relation between $\delta^{15}N$ and trace element concentrations was used to investigate metal transfer to and accumulation in oysters and phytoplankton from the Mocajuba and Pará River estuaries (Fig. 5, Table 4). In the Mocajuba River significant negative slopes ($p < 0.05$) were recorded for Fe, Mn, Al, Ba, and Pb, in the Para River only Al and Pb. Weak, but significant positive ($p < 0.05$) correlations to Mn ($r^2 = 0.41$), Zn ($r^2 = 0.51$), Ba ($r^2 = 0.56$) were recorded in the Pará River. In the Mocajuba River the only correlation found was Zn ($r^2 = 0.23$).

In the Mocajuba River the relations between $\delta^{13}C$ and Mn, Fe, Al, Ba and Pb were positive and highly significant ($p < 0.001$), whereas in the Pará River, only Al and Pb were correlated with $\delta^{13}C$. Negative correlations with $\delta^{13}C$ were observed in the Pará River for Mn, Zn and Ba ($p < 0.05$) and in the Mocajuba River, only for Zn. No relation between $\delta^{13}C$ and Cu, Se, As, or Cd was recorded in either estuary (Table 4 and Fig. 6).

4. Discussion

Metal concentrations found in the Mocajuba and Pará River estuaries result from the strong tropical weathering affecting rocks

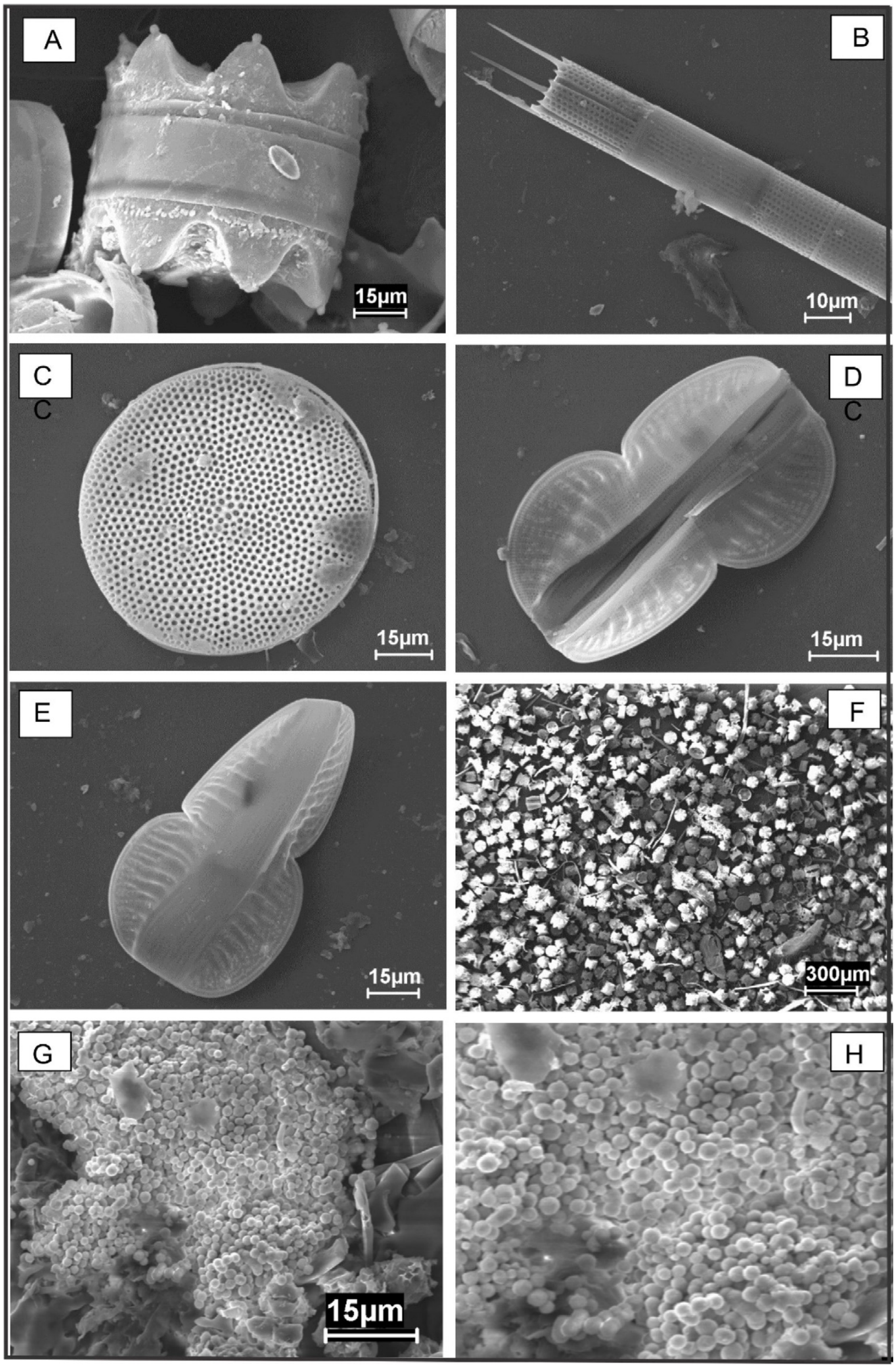

Fig. 2. Scanning electron microscopy image (SEM) depicting phytoplanktonic species from the estuaries of the Pará river and the Mocajuba river: (A) *Polymyxus coronalis*; (B) *Aulacoseira granulata*; (C) *Coscinodiscus* sp. (D and E) *Amphiprora* spp; (F) abundance of diatomaceas and phosphate granules identified in the oyster *Paxyodon ponderosus* (G) Phosphate granules and (H) phosphate granule detail.

and sediments in the region, which are poor compared to the Upper Continental Crust (Costa et al., 2004; Vilhena et al., 2010). These trace elements are primarily absorbed by phytoplankton (mainly diatoms), which are abundant in the water and sediments analyzed. The excess of metals is probably transferred to younger diatom specimens (Sunda and Huntsman, 1992; Takeda, 1998), which may account for the high diatom densities and for the associated metal concentrations found in our study.

The phytoplankton analyzed in this study accumulated trace and toxic metals at concentrations up to 10,000 times higher than those found in the water as reported by some studies (Price and Morel, 1990; Loscher et al., 1998; Vilhena et al., 2016). This finding reinforces phytoplankton's potential for accumulating metals (Rainbow, 1995; Denton et al., 2006; Fowler and Villeneuve, 2007). Fe, Mn, Zn, and Se concentrations were higher than those recorded for phytoplankton from the Rhine River in Germany

Table 3
Mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions in sediments, phytoplankton, and oysters.

Sample name	Station	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
C. gasar ($n = 10$)	Mocajuba river	−35.5 to −30.7 (−33.6 ± 0.45)	5.8–8.7 (7.1 ± 1.04)
P. ponderrosus ($n = 10$)	Pará river	−30.1 to −28.1 (−29.8 ± 0.1.6)	4.4–7.1 (5.9 ± 0.83)
Phytoplankton ($n = 10$)	Mocajuba river	−33.5 to −22.5 (−30.6 ± 5.36)	5.5–6.5 (6.1 ± 0.43)
Phytoplankton ($n = 10$)	Pará river	−27.3 to −21 (−25.1 ± 2.2)	1.8–5.2 (3.8 ± 1.13)
Sediment ($n = 8$)	Mocajuba river	−27.5 to −25.5 (-27 ± 0.7	1.50–5 (3.1 ± 1.3)
Sediment ($n = 8$)	Pará river	−26.5 to −22 (−25.1 ± 1.0)	3.7–5.9 (4.6 ± 0.71)

Table 4
Linear regression equations between log-metal concentrations (Mn, Fe, Cu, Zn, Se, Al, As, Cd, Ba, and Pb) and isotopes ($\delta^{15}N$ and $\delta^{13}C$) in the Phytoplankton and oysters ($n = 10$).

	Regression coefficients for log-linear, relationship between trace elements in oysters (*P. ponderosus and C. gasar*) and phytoplankton (Log elements μg g^{-1}) *vs* . $\delta^{15}N$ and $\delta^{13}C$ (‰)							
	Oysters *P. ponderosus* (Pará river)				Oysters *C. gasar* (Mocajuba river)			
	R^2	*p*-Value	Slope	Intercept	R^2	*p*-Value	Slope	Intercept
Elements *vs.* $\delta^{15}N$								
Log Mn	0.418	0.002**	2.267	0.136	0.426	0.001**	6.641	−0.702
Log Fe	0.098	0.177 ns	4.375	−0.057	0.430	0.001**	7.901	−0.683
Log Cu	0.012	0.638 ns	1.226	−0.017	0.124	0.127 ns	0.672	0.130
Log Zn	0.519	<0.0001***	0.857	0.222	0.237	0.029*	−0.429	0.505
Log Se	0.014	0.62 ns	−0.242	0.026	0.037	0.414 ns	−0.130	0.068
Log Al	0.218	0.037*	4.074	−0.159	0.434	0.001**	6.726	−0.612
Log As	0.008	0.703 ns	0.791	−0.005	0.007	0.714 ns	0.845	0.011
Log Cd	0.306	0.010*	−0.357	0.097	0.000	0.937 ns	−0.241	−0.003
Log Ba	0.567	0.0001***	0.975	0.318	0.431	0.001**	3.335	−0.405
Log Pb	0.400	0.001**	1.492	−0.172	0.400	0.002**	1.492	−0.172
Elements *vs.* $\delta^{13}C$								
Log Mn	0.367	0.005**	1.145	−0.064	0.441	0.001**	6.456	0.145
Log Fe	0.160	0.080 ns	5.079	0.035	0.400	0.002**	7.533	0.134
Log Cu	0.005	0.753 ns	1.295	0.005	0.270	0.018*	0.257	−0.041
Log Zn	0.610	<0.0001***	−1.413	−0.122	0.319	0.001**	−1.048	−0.127
Log Se	0.000	0.943 ns	−0.733	−0.027	0.019	0.558 ns	−0.074	−0.011
Log Al	0.347	0.006**	6.183	0.107	0.330	0.008**	6.084	0.110
Log As	0.031	0.451 ns	0.913	0.005	0.039	0.400 ns	0.743	−0.005
Log Cd	0.269	0.019*	−1.511	−0.058	0.168	0.072 ns	−0.929	−0.020
Log Ba	0.607	<0.0001***	−1.947	−0.162	0.314	0.010*	2.804	0.070
Log Pb	0.492	<0.001**	2.426	0.061	0.313	0.010*	3.368	0.086

r^2, coefficient determination. ns, not significant.
*r^2 to the level of significance $p<0.05$.
**r^2 to the level of significance $p<0.01$.
***r^2 to the level of significance $p<0.001$.

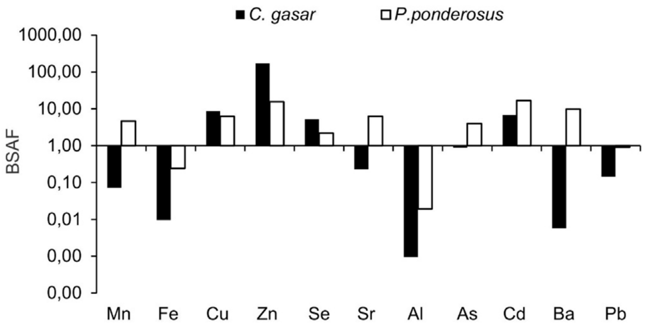

Fig. 3. Biota–sediment accumulation factor (BSAF) recorded for Mn, Fe, Cu, Zn Se, Sr, Al, As, Cd, Pb and Ba between sediments of Mocajuba and Pará rivers and oyster species *C. gasar* and *P. ponderosus*.

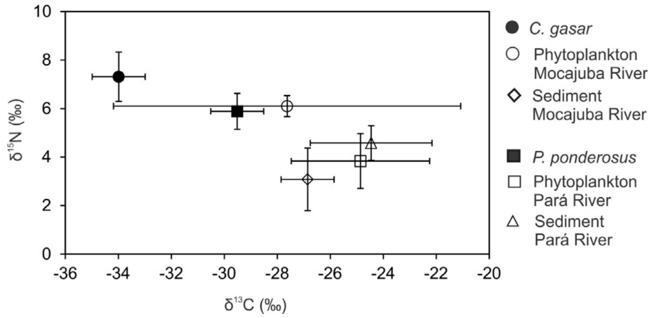

Fig. 4. Mean $\delta^{15}N$ and $\delta^{13}C$ in sediments, phytoplankton, and oysters (*Paxyodon ponderosus* and *Crassostrea* gasar).

(Admiraal et al., 1995) and in Kuwait (Bu-Olayana et al., 2001). Al, As, Ba, and Pb concentrations in this study were higher than phytoplankton levels recorded by Cullen and Sherrell (1999) and Bu-Olayana et al. (2001) (except for As and Pb), although they were similar to those recorded for natural phytoplankton by Martin and Knauer (1973).

The hypothesis that phytoplankton are at the base of the oyster food chain is substantiated by the high metal concentrations found in these bivalves. These metals may have been transferred to the oysters through microalgae impregnated with clay from suspended and bottom sediments, through oxides and hydroxides formed in the phytoplankton, or through the natural senescence of microphytoplankton, which tend to absorb high concentrations of metals such as Al, Fe, Mn, and Zn (Riley and Roth, 1971; Martin and Knauer, 1973; Vilhena et al., 2014).

The BSAF factor refers to bioavailable concentrations and reflects the efficiency of organisms as bioconcentrators of any metal

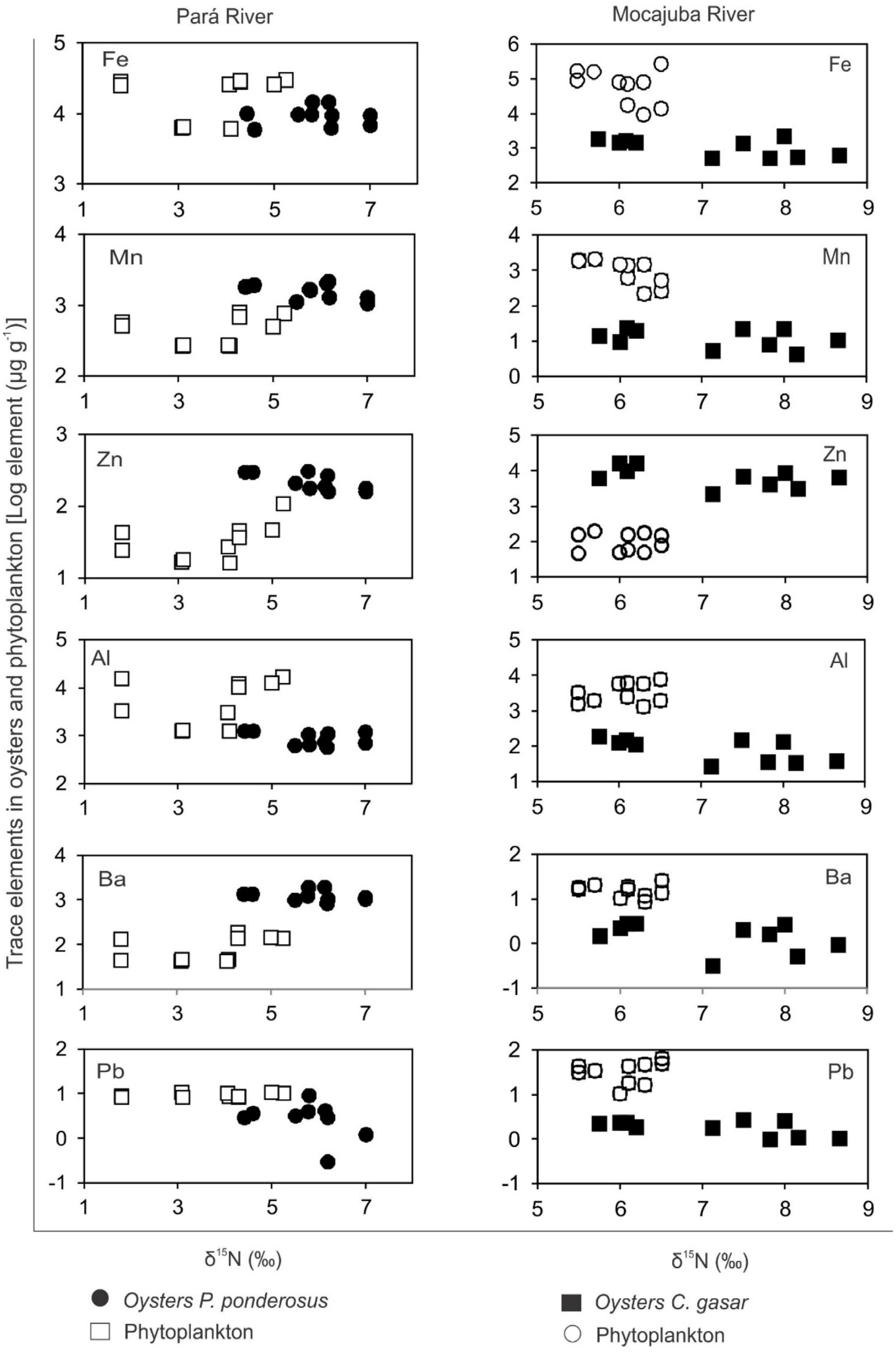

Fig. 5. Relation between trace elements (log-transformed µg g^{-1}) and δ^{15}N in oysters (*Paxyodon ponderosus* and *Crassostrea gasar)* and in the phytoplankton. The linear regression equations and significance levels are shown in Table 4.

(Soto-Jiménez and Páez-Osuna, 2001). The (BSAF) values calculated for Mn, Cu, Zn, Se, Sr, As, Cd and Ba remained higher than 1.0 in oysters (*P. ponderosus* and *C. gasar*). Such an outcome indicates that these elements are easily bioconcentrated in oysters, except for Fe, Al and Pb (Fig. 3). According to Soto-Jiménez and Páez-Osuna (2001), BSAF values recorded for Cu, Zn and Cd were quite high in *Crassostrea iridescens*.

However, the high Al and Ba concentrations found in *P. ponderosus* may have not derived from sediments (Fig. 3), since local substrates in the Pará River estuary were poor in clay minerals (7%). Al concentrations in these oysters were much higher than those in the control sample of the same species, thus suggesting contamination by external sources and Al transfer to oysters through their own food chain.

The high Ba concentrations found in the oyster species *P. ponderosus* and in the control sample may result from the influence of marine-water access to the estuary (Vilhena et al., 2016). As Ba has no known biological function it is possible to assume that high concentrations of this element in *P. ponderosus* result from the intake of Ba-enriched diatoms adsorbed as iron

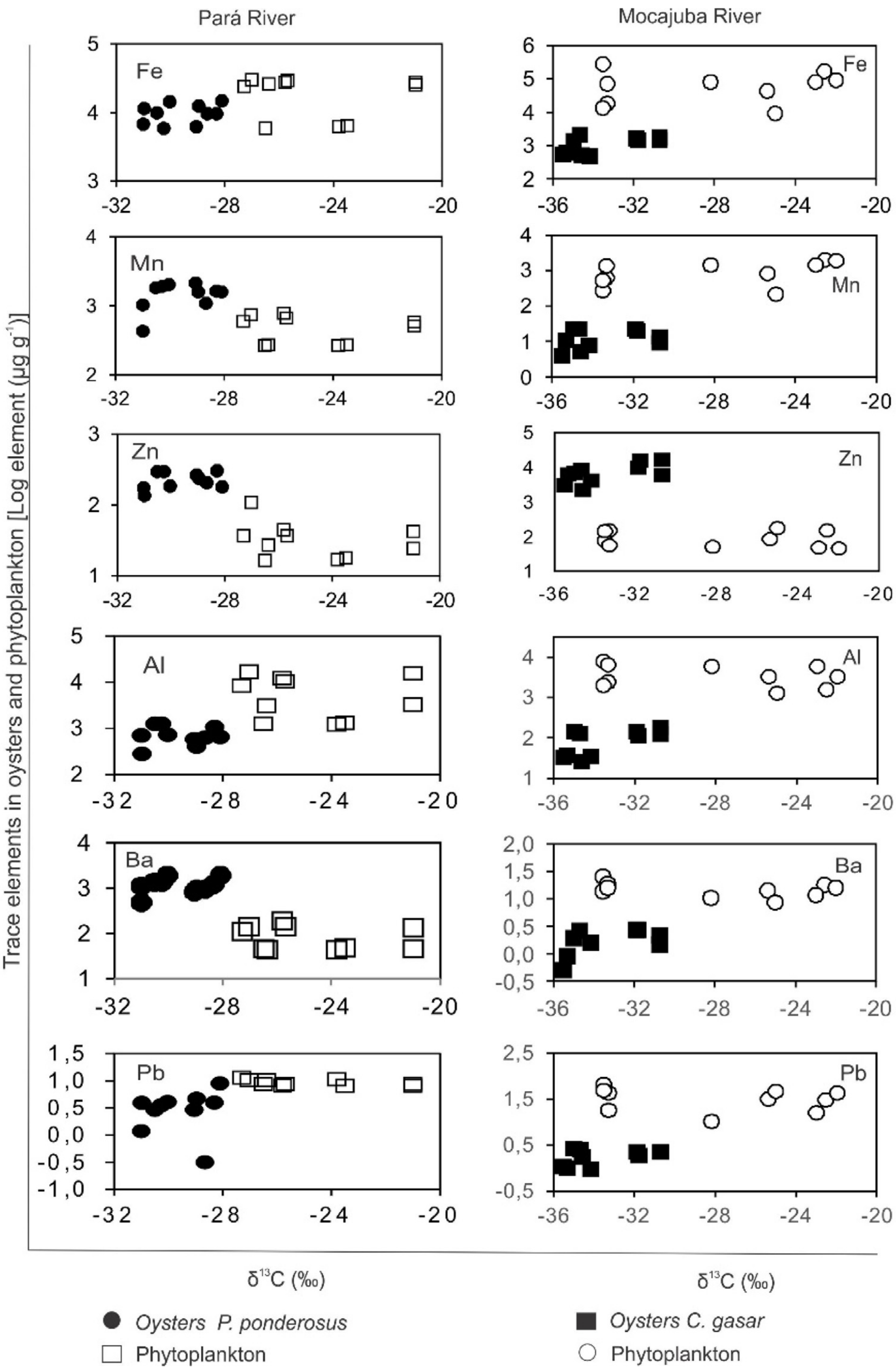

Fig. 6. Relation between trace elements (log-transformed µg g^{-1}) and $\delta^{13}C$ in oysters (*Paxyodon ponderosus* and *Crassostrea gasar)* and in the phytoplankton. The linear regression equations and significance levels are shown in Table 4.

oxyhydroxides associated with frustules (Thébault et al., 2009; Hatch et al., 2012).

For this study, the high BSAF values confirm that *P. ponderosus and C. Gasar* oysters can accumulate metals; however, some of them do not reflect the fractions that are bioavailable in the environment (Fig. 3). The metal concentration variability in *C. gasar* and *P. ponderosus* may be explained by different bioaccumulation dynamics between species, as well as by other factors such as diet and metal availability in the studied sites (Croteau et al., 2005).

A significant portion of the energy demanded by oysters comes from marine ($\delta^{13}C$, between −21‰ and -25‰) (Brito et al., 2006) and terrestrial ($\delta^{13}C$, between −25.5‰ and -27‰) sources (Garcia et al., 2006). These results indicate carbon and nitrogen assimilation from different sources, as well as a low trophic level in the food web ($\delta^{15}N$ 6.5‰ ± 1) (Vander Zanden et al., 1999; Post, 2002). The $\delta^{13}C$ values recorded for *C. gasar* and *P. ponderous* were close to the phytoplankton signature, thus confirming that phytoplankton are their primary food source (Fukumori et al., 2008).

The elements (Fe, Mn, Al, Ba, Pb) presented negative and significant correlation ($p < 0.05$) with δ^{15}N in the oysters and in the phytoplankton from the Mocajuba River and, with Al and Pb in oysters from the Pará River (Figs. 5 and 6, Table 4). That may be explained by the accumulation of metals in phytoplankton or by the biodilution of those metals through the trophic chain (Asante et al., 2008). Campbell et al. (2005a) and Pereira et al. (2010) recorded similar results in studies of other marine food webs. The aforementioned authors also suggested that some elements may be biodiluted by increased δ^{15}N, or bioaccumulated by the organisms themselves, although they are not biomagnified in the web as a whole.

These negative correlations reinforce the hypothesis that trophic transfer did not occur in the organisms, despite the high concentrations of some elements (Adewunmi et al., 1996; Maher and Butler, 1998). This process may have happened due to biochemical aspects associated with the oyster-detoxification process performed by specific organs that block metal accumulation in and transfer to the environment (Marsden and Rainbow, 2004; Pereira et al., 2010). This detoxification process may result from the binding of metals to insoluble phosphate and carbonate granules, which are abundant in tissues of oysters belonging to the species *P. ponderosus* (Fig. 2, G and H; (Vilhena et al., 2016)). This species acts as a natural biofilter (Jones et al., 2002).

The elements (Mn, Zn and Ba) that presented positive and significant correlation ($p < 0.05$) with δ^{15}N in the oysters and in the phytoplankton probably indicate their trophic magnification (biomagnification) throughout the food chain (Guo et al., 2016; Xia et al., 2019). Biomagnification of Zn has been reported in several studies on different aquatic organisms (Liu et al., 2018), and δ^{13}C (Figs. 5 and 6, Table 4) and can be attributed to similar δ^{13}C signatures between phytoplankton and sediments. This finding suggests that oysters feed on particulate organic matter (including phytoplankton), which is mostly derived from terrestrial sources. Marine inputs may also be associated with the link between these elements and clay minerals or iron sulfates that become available in phytoplankton and in oyster tissues, after they are ingested. Both the positive correlation between Al and δ^{13}C, and the negative correlation between Al and δ^{15}N, appear to confirm the initial assumption that this element derives from non-natural sources and that it bioaccumulates in *P. ponderosus* through phytoplankton ingestion.

The low positive correlation between the chemical elements and isotopes (δ^{13}C and δ^{15}N) confirmed that Mn, Fe, Cu, Zn, Al, Ba, and Pb were bioaccumulated, but not biodiluted (Watanabe et al., 2008; Asante et al., 2008), likely because Fe, Cu, Zn, and Se are essential elements given their important metabolic functions in the detoxification process. However, a number of studies have confirmed Zn, Se, and Cd biomagnification in bivalves due to diets rich in phytoplankton (Bayne, 1993; Wang, 2002; Quigg et al., 2003), whereas As is bioaccumulated by the organisms, but not biomagnified in the food chain (Maher and Butler, 1998).

A number of hypotheses have been proposed to explain the lack of trophic transfer of trace elements in oysters (*C. gasar* and *P. ponderosus*) and phytoplankton. Some of these elements (e.g., Cu and Zn) are actively regulated (Van Hattum et al., 1991; Viarengo and Nott, 1993), excreted or detoxified, and deposited as inert storage molecules (granulated phosphates), which makes them unavailable to the trophic web (Blackmore, 2001; Wang, 2002; Campbell et al., 2005b). This indicates that both oysters and phytoplankton are excellent bioindicators suitable for evaluating contamination caused by metals, as well as by nitrogen and carbon sources, in Amazonian ecosystems.

5. Conclusions

Phytoplankton are responsible for absorbing high concentrations of metals in Amazonian waters. Those metals are transferred through the food chain to oyster species such as *C. gasar* and *P. ponderosus* and bioaccumulate in them. Specimens of *C. gasar* found in the Mocajuba River estuary were shown to have increased levels of Zn, Fe, Cu, Mn and As, whose concentrations are associated with local background levels, given that they can be found in clay minerals and sulfides formed in mangroves. *P. ponderosus* found in the Pará River estuary bioaccumulated Fe, Mn, Ba and Pb in natural concentrations.

The BSAF confirmed that *C. gasar* and *P. Ponderosus* oysters are highly capable of accumulating metals. Phytoplankton and oysters from the region presented high concentrations of Al, which are positively correlated with δ13C, indicating the possible presence of contaminated materials in the food chain for oysters. The correlations of metals with isotopes (δ^{15}N and δ^{13}C) confirmed bioaccumulation as well as biodilution and biomagnification of metals in oysters and phytoplankton. That confirms that oysters may be used as bioindicators for detecting metal contamination in the environment.

Declaration of competing interest

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

Acknowledgments

The authors are grateful to National Council for Technological and Scientific Development-CNPq, Brazil, for granting a scholarship to the first author, and a productivity scholarship to the second one; to Coordination of Earth Sciences and Ecology (MPEG), for providing the infrastructure; to the Isotope Ecology Laboratory of the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), for the isotope analyses.

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