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Fractionation of rare earth and other trace elements in crabs, *Ucides cordatus*, from a subtropical mangrove affected by fertilizer industry

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ABSTRACT

Fractionation of rare earth elements (REE) and other trace metal concentrations (Th, U, Cd, Cr, Cu, Ni, Pb, and Zn) between mangrove sediments and claw muscles and shells of male crabs (*Ucides cordatus*) from a subtropical estuary highly impacted by fertilizer industry activities was investigated. This is the first record of REE distribution in these organisms, and the results showed higher accumulations of these metals, U and Th in shells, probably related to the replacement of Ca during molting. Contents of Cd, Cr and Ni were similar in both tissues, but Cu, Zn and Pb were mostly accumulated in the claw muscle with concentrations above those considered safe for human consumption according to the Brazilian legislation. REE fractionation was different in the analyzed tissues being softer in the shells. The results provided evidences that the water absorbed during molting controls the chemistry of REE in shells. In contrast, the chemistry of REE in the claw muscle, in which was observed preferential absorption of light REE, is controlled by diet. REE fractionation obtained for the claw muscles was closely correlated to the observed in the contaminated substrate and in materials related to the production of phosphate fertilizers (contamination source), which supports their transference to this *Ucides cordatus* tissue without fractionation by the ingestion of sediments. Our results showed the potential use of crab tissues for monitoring REE and trace element sources in mangrove areas, with claw muscle exhibiting the contaminant source fingerprint.

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Introduction

In the last few decades, geochemical studies have helped understand the contribution of natural and anthropogenic sources to the accumulation of certain elements in the

environment (Weiss et al., 2008). Chemical elements are potential tracers of the fate of a contaminant source in the environment. After introduction in a system, they can be followed through the course of the process, providing information about biological and physical-chemistry processes (Wiederhold, 2015).

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Trace metals, such as Cd, Cu, Zn, and Pb are widely used as tracers of environmental pollution in both physical and biotic environments (Sakan et al., 2010; Wiederhold, 2015).

Rare earth elements (REE; La–Lu) are now worldwide recognized as emerging micropollutants in aquatic systems (Hissler et al., 2014; González et al., 2014). Thanks to their low solubility and predictable and consistent behavior, REE have generated more complete information about sources and processes (Delgado et al., 2012; Hissler et al., 2014). These elements have become relevant in scientific research as excellent tracers of geochemical processes and anthropogenic disturbances in natural environments (Delgado et al., 2012; Agnan et al., 2014; Duvert et al., 2015). However, unlike what happens with other trace elements (e.g., Cd, Cu, Zn, Pb, Cr, Cd; Phillips, 1970; Viarengo and Nott, 1993; Wiederhold, 2015), little is known about REE uptake by aquatic organisms.

REE concentrations in fossil bones and teeth have received particular attention due to their relative abundances have allowed the reconstruction of the geochemical conditions during diagenesis. The fractionation observed within paleo-structures has permitted the use of REE signatures for paleoenvironmental reconstructions (Suarez et al., 2010; Trueman et al., 2011; Herwartz et al., 2011). When it comes to in vitro organisms, studies involving fish and shellfish have investigated REE distribution in the internal organs (e.g. gills > skeleton > muscle; Qiang et al., 1994; Yang et al., 1999; Mayfield and Fairbrother, 2015); bioconcentration factors between the organism, water and sediment (Moermond et al., 2001; Weltje et al., 2002), and more recently the ecotoxicity of these elements mostly in arthropods and fish (González et al., 2015). Therefore, the application of REE as source tracers in the current biosphere models is not common, but can be useful in tracing paths and processes in which these elements are involved, especially in contaminated environments and their biota.

The fertilizer industry is a potential REE source to the environment, as these elements are present as impurities in phosphate concentrate and phosphogypsum (a by-product of phosphate ore processing into fertilizer). Inappropriate processing, transportation and storage of these materials can result in potential contamination of sediment, soil and water bodies. Phosphate concentrate and phosphogypsum have REE geochemical signatures well documented and characterized (Borrego et al., 2004; Oliveira et al., 2007; Di Leonardo et al., 2009; Tranchida et al., 2011; Deepulal et al., 2012; Sanders et al., 2013; El-Didamony et al., 2013), which act as fingerprints that can be easily used as source tracers. In environments contaminated by REE, risk assessment and prevention of human exposure to these metals are issues that must be addressed, as the food chain, especially the consumption of seafood, may become the main route of contaminants transfer to humans (Plessi et al., 2001). Although REE biological effects are still poorly understood (Sneller et al., 2000; Andrès and Gérente, 2011), adverse effects of other trace metals (Cd, Cr, Cu, Ni, Pb, and Zn), which may also be associated with the fertilizer industry (Luiz-Silva et al., 2008; Saueia et al., 2013), are well known (Viarengo and Nott, 1993), and their concentrations are controlled by legislation.

The Santos–Cubatão estuarine system is located in a subtropical region in southeastern Brazil that hosts the largest industrial park of Latin America, including fertilizer industries.

Some studies have shown that significant REE contamination in sediments is linked to the processing of phosphate rock and its by-product (phosphogypsum) in the manufacture of fertilizers (Oliveira et al., 2007; Sanders et al., 2013). The impact of this activity in the surrounding area involves a number of other trace elements (e.g., Th, U, P) characterized in many materials related to the contaminant source (Santos et al., 2006; Oliveira et al., 2007; Saueia et al., 2013). This environment is therefore an excellent scenario to help understand the behavior of REE in the biosphere.

This is the first study that assesses the behavior of REE together with other trace elements in aquatic organisms (crabs) from a highly impacted subtropical environment, in order to identify contaminant sources. We used tools commonly applied in geologic studies to identify contaminant sources through REE signatures in *Ucides cordatus* tissues. The study compares fractionation models in biological tissues in respect to light rare earth elements (LREE; La–Nd), medium rare earth elements (MREE; Sm–Gd) and heavy rare earth elements (HREE; Tb–Lu) and their use as source tracers.

1. Material and methods

1.1. Study area and sampling

This study was conducted in the Morrão River mangrove, which is adjacent to the industrial complex of Cubatão, the largest Latin America industrial park, SE-Brazil (Fig. 1). The industrial activities are related especially to the manufacture of NPK (Nitrogen, Phosphorus, Potassium) fertilizers (since 1970) and steel (since 1964). Luiz-Silva et al. (2008) and Sanders et al. (2013) studied this area (intertidal mangrove and channel river substrates) and found high concentrations of REE, Cd, Cr, Cu, Ni, Pb, Zn, Th, and U in the first 20 cm of the sediment column. In general, studies in this area have shown that the phosphogypsum generated from the phosphate rock used in Cubatão fertilizer industries is a main source of contamination, contributing to an excessive accumulation especially of REE, U and Th in sediments (Oliveira et al., 2007; Sanders et al., 2013; Saueia et al., 2013). For this work, male *Ucides cordatus* specimens ($N = 20$) in intermolt stage (molting is the process of shell exchange) were hand-caught in site according to Fig. 1 in December 2012. After collection, the specimens were frozen and transported to analysis.

1.2. Analytical method

Initially, the width of the crab shells was measured and varied between 8 and 12 cm. The mud of the shells was removed with a clean brush and pure water, and the crabs were dissected with steel sterilized scissor and plastic hammer to remove the claw muscle. After that, the shells were dried at 80°C in an oven Tecnal, model TE-394/3, Brazil, for 36 hr, and the muscle tissues were freeze-dried at –80°C for 48 hr in a Freeze Dryer/Lyophilizer VIRTIS Benchtop 4 K Series model BT4KZL-105, United States. The frozen tissues were pulverized in a clean agate mill, and ca. 0.25 g of each sample were digested in 50 mL (perfluoroalkoxy) PFA (Saville® USA)

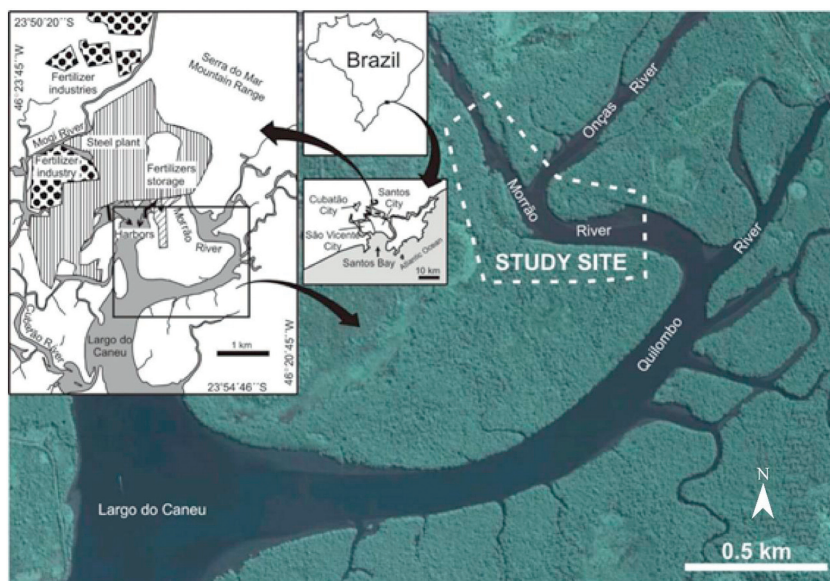


Fig. 1 – Landsat image with the location of the study area (specimen collection) and insets showing industries location. Modified after Sanders et al. (2013).

digestion vessels, by addition of 6 mL of concentrated HNO_3 (Merck, Germany) purified by sub-distillation, and then heat sealed for 3 hr at 80°C (modified from Agah et al., 2009). The water used was always ultrapure water ($18.2 \text{ M}\Omega \text{ cm}$), obtained from a Milli-Q system (Millipore, USA). All plastic materials were cleaned using a mixed solution of HNO_3 8% and HCl 2% (both from Merck, Germany), and rinsed with ultrapure water. The metal concentrations in solution were obtained using an ICP-MS, Thermo Fisher Scientific, Germany, XSERIES 2, equipped with CCT (Collision Cell Technology), conical chamber and concentric nebulizer Meinhard®. Before the analysis, the instrument was optimized according to the manufacturer recommendations. The instrument was calibrated using multi-elemental solutions prepared by mixing the necessary amounts from respective elemental 10 mg/L (High Purity Standards, USA). For analytical quality control, we analyzed the certified reference materials DOLT-4 (Dogfish Liver Reference Materials for Trace Metals) and DORM-3 (Dogfish Muscle Certified Reference Material for Trace Metals) produced by the National Research Council of Canada (NRCC). Calculated recovery was between 83% and 100% (generally better than 90%), and for elements that are not certified, including REE, the variation between recoveries ($N = 12$) was less than 20%, which confirms the good performance of the procedures adopted here. The detection limits for all metals are shown in Table 1.

1.3. Data analysis

One way analysis of variance was used to compare metal contents among tissues and the differences were considered significant at the level of $p < 0.05$. All statistical tests were carried out using the OriginPro (v.8.6) graphing and data analysis software.

Rare earth element pattern and fractionation were characterized by ratios (La/Yb , Sm/La , Yb/Sm) between element

concentrations normalized ($_n$) to the Post Archean Australian Shale (PAAS) (McLennan, 1989). As suggested by Noack et al. (2014) and Duvert et al. (2015), multi-elementary ratios were obtained according to Eqs. (1) and (2):

$$R_{(M/L)} = \log \frac{\text{MREE}_n}{\text{LREE}_n} = \log \left[\frac{(\text{Gd}_n + \text{Tb}_n + \text{Dy}_n) / 3}{(\text{La}_n + \text{Pr}_n + \text{Nd}_n) / 3} \right] \quad (1)$$

$$R_{(H/M)} = \log \frac{\text{HREE}_n}{\text{MREE}_n} = \log \left[\frac{(\text{Tm}_n + \text{Yb}_n + \text{Lu}_n) / 3}{(\text{Gd}_n + \text{Tb}_n + \text{Dy}_n) / 3} \right] \quad (2)$$

where, $R_{(M/L)}$ is the ratio between MREE and LREE, and $R_{(H/M)}$ is the ratio between HREE and MREE.

2. Results and discussion

2.1. Levels of REE and trace metals in *Ucides cordatus*

Mean concentrations (dry weight), standard deviation and detection limits for Cd, Cr, Cu, Ni, Pb, Zn, Th, U, and REE obtained for shell and claw muscle are shown in Table 1. This table also lists analysis of variance (F-values) for metal concentrations in tissues and data reported in the literature on contents of some trace metals in the study area with respect to sediments and materials related to the fertilizer industry. Concentrations of metals other than REE in crab tissues followed the order $\text{Zn} > \text{Cu} > \text{Ni} > \text{Pb} > \text{Cr} > \text{U} > \text{Th} > \text{Cd}$. These elements are in relative high concentrations in surface sediments from the study area, being up to four times higher than the local background (Table 1). The order of decreasing metal content in crab tissues was similar to the sequence in sediments from the study area obtained by extraction with HCl 6 mol/L (U and Th not analyzed; Nizoli and Luiz-Silva, 2012). Thus, metal solubility

Table 1 – Mean metal concentrations in *Ucides cordatus* shell and claw muscle and corresponding standard deviation (unit: mg/kg).

Element	Crabs (this study)				Sediments		Materials related to the fertilizer industry	
	Shell (N = 20)	Muscle (N = 20)	Detection limits	F	Geochemical background ^{a,b}	Contaminated sediments ^{c,d}	Phosphogypsum/phosphate rock concentrate ^a	Phoscorite ^b (range)
Cd	0.004 ± 0.01	0.006 ± 0.004	0.001	2.3	0.2 ± 0.1 ^a	0.73 ± 0.61 ^c	–	–
Cr	0.15 ± 0.07	0.065 ± 0.08	0.01	8.97	75 ± 2 ^a	127 ± 10 ^c	–	27.4/61.6
Cu	3.2 ± 1.8	52.8 ± 9.9	0.05	505.5	18.8 ± 0.2 ^a	91 ± 21 ^c	–	168/33.1
Ni	0.42 ± 0.23	0.29 ± 0.23	0.04	3.41	27.1 ± 0.6 ^a	36 ± 2.9 ^c	–	63.5/28.4
Pb	0.26 ± 0.20	1.25 ± 0.5	0.002	36.23	29.5 ± 3.7 ^a	104 ± 16 ^c	–	14.4/2.3
Zn	6.8 ± 1.6	327.6 ± 24.8	0.1	3496	92 ± 5 ^a	440 ± 31 ^c	–	117/102
Th	0.08 ± 0.02	0.011 ± 0.002	0.005	121.6	15.8 ± 0.4 ^a	19 ± 5 ^c	54 ± 14/103 ± 19	162/155
U	0.10 ± 0.04	0.02 ± 0.01	0.002	74.78	5.3 ± 0.4 ^a	17 ± 4 ^c	–	192/354
La	0.46 ± 0.21	0.12 ± 0.07	0.006	47.22	41 ± 3.5 ^b	141 ± 48 ^d	1210 ± 360/2220 ± 530	1202/664
Ce	0.75 ± 0.34	0.18 ± 0.1	0.007	46.93	79 ± 11.5 ^b	318 ± 116 ^d	2650 ± 730/5310 ± 1160	2477/1543
Pr	0.10 ± 0.04	0.02 ± 0.01	0.001	56.67	–	38 ± 13 ^d	–	341/206
Nd	0.34 ± 0.15	0.07 ± 0.04	0.005	47.45	48.2 ± 2.6 ^b	146 ± 53 ^d	1100 ± 340/1490 ± 410	1235/776
Sm	0.06 ± 0.04	0.01 ± 0.005	0.001	43.92	7.7 ± 0.7 ^b	19 ± 6 ^d	138 ± 40/256 ± 24	151/99.3
Eu	0.015 ± 0.008	0.003 ± 0.001	0.0005	14.88	0.9 ± 0.15 ^b	4.7 ± 1.6 ^d	36 ± 9/68 ± 8	38.1/25.6
Gd	0.66 ± 0.03	0.01 ± 0.006	0.001	55.74	–	13 ± 4 ^d	–	66.3/46.5
Tb	0.012 ± 0.03	0.005 ± 0.0004	0.0003	1.5	0.55 ± 0.07 ^b	1.6 ± 0.5 ^d	6 ± 1/13 ± 4.9	9.6/6.5
Dy	0.03 ± 0.01	0.005 ± 0.003	0.001	19.8	–	8 ± 2 ^d	–	37.2/25.6
Ho	0.01 ± 0.02	<0.005	0.0002	–	–	1.2 ± 0.3 ^d	–	4.7/3.2
Er	0.02 ± 0.008	0.004 ± 0.002	0.0005	17.33	–	3 ± 0.6 ^d	–	8.8/6.1
Tm	0.003 ± 0.001	<0.006	0.0002	–	–	0.35 ± 0.1 ^d	–	1.0/0.7
Yb	0.012 ± 0.004	0.003 ± 0.001	0.0003	22.17	2.25 ± 0.35 ^b	2.3 ± 0.3 ^d	5 ± 2.7/14.8 ± 3.0	5.4/3.5
Lu	0.002 ± 0.001	<0.003	0.0002	–	0.4 ± 0.03	0.35 ± 0.05 ^d	0.22 ± 0.06/0.65 ± 0.13	0.6/0.4
(La/Yb) _n	2.53 ± 0.8	2.66 ± 1.67	–	–	1.4 ± 0.32	4.4 ± 1.2	24.4 ± 16.4/11.5 ± 3.9	16.4/14.0
(Sm/La) _n	0.85 ± 0.12	0.57 ± 0.12	–	–	1.3 ± 0.04	0.97 ± 0.06	0.79 ± 0.08/0.82 ± 0.12	0.86/1.02
(Yb/Sm) _n	0.50 ± 0.11	0.88 ± 0.42	–	–	0.58 ± 0.13	0.26 ± 0.11	0.07 ± 0.03/0.11 ± 0.02	0.07/0.07
R _(M/L)	–0.05 ± 0.04	–0.42 ± 0.11	–	–	–	–0.25 ± 0.06	–	–0.49/–0.43
R _(H/M)	–0.28 ± 0.09	–0.32 ± 0.28	–	–	–	–0.41 ± 0.09	–	–0.80/0.80

Variance values (F) between the concentrations in tissues for each element are in bold for $p < 0.05$. Mean contents and standard deviation (or range when indicated) of some trace metals in the study area with respect to sediments and materials related to the fertilizer industry, and $R_{(H/M)}$, $R_{(M/L)}$, $(La/Yb)_n$, $(Sm/La)_n$ and $(Yb/Sm)_n$ values are also listed. Values below the analytical detection limit are represented by the symbol (–).

^a Phosphogypsum from the study area ($n = 8$) and phosphate rock concentrate ($n = 6$) from the Catalão Mine (Oliveira et al., 2007).

^b Two phoscorite samples from the Catalão Mine (Goiás State, Brazil) used as raw material in fertilizer production (Cordeiro et al., 2011).

^c Background geochemical data based on sediment records from the Morrão River (200–220 cm depth, $N = 4$; Luiz-Silva et al., 2008)^a and Moji River (26–34 depth cm, $N = 4$; Oliveira et al., 2007)^b.

^d Intertidal mangrove and channel river sediments (0–20 cm depth) related to the study area (Luiz-Silva et al., 2008^c, $N = 4$; Sanders et al., 2013^d, $N = 9$).

in contaminated sediments seems to play an important role to metal transfer for crab tissues.

In respect to REE, a common tendency of decreasing concentrations, such as LREE > MREE > HREE, was observed in both tissues, although these decreases with increasing atomic number were not systematically followed (Table 1). The results for shell showed the REE ordered according to Ce > Gd > La > Nd > Pr > Sm, while claw muscle presented Ce > La > Nd > Pr > Gd > Sm. For other REE (Eu to Lu), the concentrations were low in both tissues and varied very little. The REE concentrations verified in the crab tissues reflected the contamination levels showed by sediments, which were strongly enriched in Ce, La, Nd, Pr, Sm, and Gd, with minor contents of other REE (Table 1). In general, metal concentrations from the sediment rather than from the overlying waters is seen as having a more significant influence on the accumulation of metals by the crabs (Weimin et al., 1994), as discussed below.

Cadmium, Cr, Cu and Pb contents in *Ucides cordatus* claw muscle contrasted with those reported by Pinheiro et al. (2012), who studied the same species in other sectors of the same estuarine system. These authors showed higher Cd (between 0.06 and 0.14 mg/kg) and Cr (between 0 and 0.57 mg/kg) and lower Cu (between 3.85 and 6.72 mg/kg) and Pb (<0.05 mg/kg) concentrations, when compared to our results (Table 1).

Although there are no records of REE in Crustaceans Decapods, as *Ucides cordatus*, Mayfield and Fairbrother (2015) presented reference data for these elements in fish of different trophic levels from a reservoir in the state of Washington (USA). Total concentrations (Σ REE) between 0.014 and 3.0 mg/kg (dry weight), being higher in benthic species (exposed to sediment), were lower than the concentrations in the *Ucides cordatus* from the Morrão River, which yielded Σ REE between 0.2 and 4.6 mg/kg (dry weight).

Metal concentrations in muscle tissue deserve special attention due to human consumption. Cadmium, Cr, and Ni

concentrations in *Ucides cordatus* claw muscle were lower than the limit for consumption established by the Brazilian legislation, respectively 0.5 mg/kg (BRAZIL, 2013), 0.1 and 5.0 mg/kg (BRAZIL, 1998). However, Pb, Cu and Zn contents were above the Brazilian limit for inorganic contaminants in food, respectively 5 mg/kg (BRAZIL, 2013), 30 and 50 mg/kg (BRAZIL, 1998). Limits for U, Th and REE are not included in the Brazilian legislation for food. However, Jiang et al. (2012) analyzed REE in different types of fresh seafood, including crustaceans from six Chinese provinces. These authors reported mean Σ REE concentrations of 0.283 mg/kg, whereas the Morrão River specimens yielded mean Σ REE concentrations of 0.44 mg/kg in claw muscles.

2.2. Distribution between tissues and transfer routes of metals

Internal trace metal distribution was observed and significant variance (F) between the concentrations of each element analyzed in the tissues is highlighted ($p < 0.05$) in Table 1. Copper, Zn (essential metals) and Pb (non-essential metal) contents were higher in the claw muscle, whereas Th, U and REE (minor or non-essential metals; Smith and Huyck, 1999) contents were higher in the shells (Table 1). In contrast, other minor or non-essential metals (Smith and Huyck, 1999) such as Cd, Cr and Ni showed contents statistically similar in both tissues (Table 1). Higher Cu, Zn and Pb concentrations in the muscle tissue suggest that the contaminants are transferred to this tissue via hemolymph (Viarengo and Nott, 1993), after intake of sediments enriched in such metals (Table 1). Nordhaus and Wolff (2007) analyzed *Ucides cordatus* stomach contents and the following food items were found: mangrove leaves (61.2%), unidentified plant material and detritus (28%), roots (4.9%), sediments (3.3%), bark (2.5%), and animal material (0.1%). Therefore, the *Ucides cordatus* diet is highly correlated with the substrate, which in the study area is characterized by Cu, Zn and Pb enrichment (Table 1).

Despite high amounts of REE (especially LREE) and moderate enrichment of Th and U in sediments from the study area (Table 1), their preferred accumulations in shell than muscle indicate that a process different than absorption via food was implicated in their transfer from the physical environment to organisms. It has observed in *in vitro* fishes (*Cyprinus carpio* L.) that the skeleton concentrates more REE than the muscle, whereas Weltje et al. (2002) showed the opposite behavior in seven different mollusk species from industrial areas in the Netherlands. These previous works allow us to observe that so far only in the filter feeding organisms REE accumulate preferably in the muscles. Another explanation for the observed differences in REE concentrations between tissues can be associated with the internal transfer of these contaminants. In this sense, Bergey and Weis (2007) studied the distribution of some metals between soft tissues and the shell of *Uca pugnax* and observed, in a population from a contaminated site in New Jersey, internal shifts between tissues in the period prior to molting. The authors observed that while Pb is transferred to the shell that will be eliminated by molting, Cu and Zn transfer from the shell into soft tissues occurs. This may explain why Pb amounts in claw muscle were, on average, 4.8 times higher than observed in shell, a value significantly less than the observed for Cu (16.5 times)

and Zn (48.2 times). Although the internal transport of REE was not addressed in our study, our results lead us to suppose that there is a transfer of these contaminants and, the process of molting can be a depuration mechanism of these metals from the soft tissue to the shell. This seems to not occur for the essential metals Cu and Zn, the most concentrated elements in muscle samples analyzed here. If proven, this can be extremely important to the understanding of the processes involving REE in the biosphere, especially in relation to crustaceans.

2.3. REE fractionation, pattern and tracer between physical environment and biota

During molting, crustaceans absorb salts from the old shell and absorb elements from the external environment, especially Ca^{2+} (from water) to biomineralize the new shell inside their burrows (Luquet, 2012). It is likely that in this process, elements that have ionic radius similar to Ca^{2+} (1.12 Å), such as REE^{3+} (ionic radii decrease from $\text{La}^{3+} = 1.16$ Å to $\text{Lu}^{3+} = 0.98$ Å), Th^{4+} (1.05 Å), and U^{4+} (1.00 Å), are likely to structurally replace it in the shells (ionic radii related to eight-coordination sites; Brookins, 1989). In minerals, replacement of Ca^{2+} and Sr^{2+} (1.26 Å in eight-coordination site; Brookins, 1989) by REE is accepted and well documented (Lipin and McKay, 1989), which makes it possible to extrapolate this exchange to mineralization processes occurring in aquatic organisms (biomineralization of calcic exoskeletons). In vertebrates, this process was documented by Koch et al. (1992) and Tütken et al. (2011), where REE are incorporated in structures composed of hydroxyapatite (crystalline calcium phosphate– $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) in bones and teeth by adsorption of cations (Trueman, 2013). Therefore, the same process may occur in crab shells, since this structure is very similar to that of hydroxyapatite, being constitute by calcium carbonate (CaCO_3) and chitin ($\text{C}_8\text{H}_{13}\text{O}_5\text{N}$) associated with protein and minerals (Vijayaraghavan et al., 2005). This assumption is supported by the fact that crab shells show great affinity to REE and U absorption (Vijayaraghavan and Balasubramanian, 2010; Muzzarelli, 2011).

Information generated from the concentrations of REE and other trace elements, such as REE pattern and fractionation, may be used as tracers to evaluate whether the impacted sediment and the contaminant sources are exerting some influence on the chemistry of the local biota. Our data show that $(\text{La}/\text{Yb})_n$ ratios range between 8.0 and 1.0, which indicates HREE-depleted fractionation (Table 1). This was more pronounced in muscles ($1.0 > (\text{La}/\text{Yb})_n < 8.0$) when compared to shells ($1.4 > (\text{La}/\text{Yb})_n < 5.1$) (Table 1). The values of $(\text{Sm}/\text{La})_n$ were lower than 1 in all samples for both tissues, whereas $(\text{Yb}/\text{Sm})_n$ were lower than 1 in shells and in 85% of the muscle samples (Table 1). These ratios indicate a downward pattern, which is mostly evident in the muscles, with concentrations of LREE > MREE > HREE (normalized by (Post-Archean Australian Shale) PAAS). This suggests preferential absorption of LREE in the muscles. The mangrove sediments where these crabs live are also enriched in LREE. In the substrate, this feature was inherited from the contaminant source related to the phosphate rock used in the fertilizer industry, as can be seen in Fig. 2, which shows the corresponding REE pattern (Sanders et

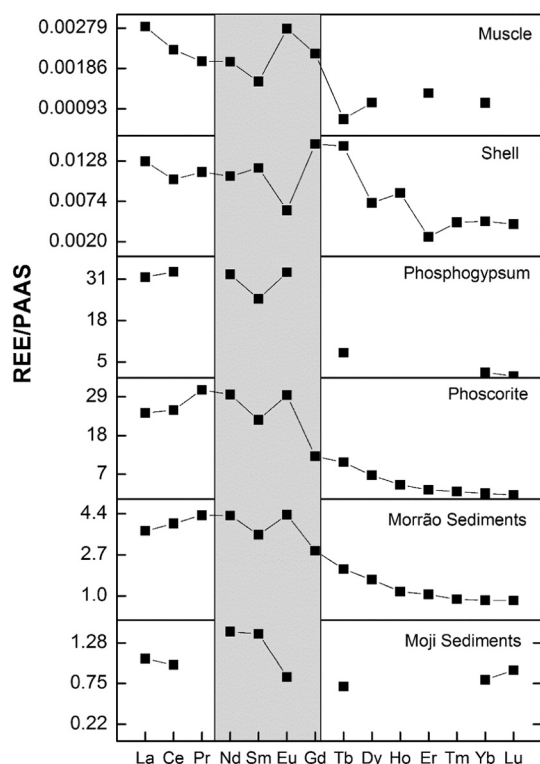


Fig. 2 – Rare earth elements/Post-Archean Australian Shale (REE/PAAS) patterns obtained for *Ucides cordatus* claw muscle and shell samples from the Morrão River; for phosphogypsum from Cubatão (Oliveira et al., 2007), for phoscorites from Catalão (Cordeiro et al., 2011), for Morrão River surface sediments (up to 10 cm depth) (Sanders et al., 2013), and for deeper sediments (below the 26 cm) of the Moji River (Oliveira et al., 2007).

al., 2013) also for the phoscorites used as raw material for fertilizers production (Cordeiro et al., 2011). In the pore water of these sediments, MREE and HREE are more soluble than LREE (Bosco-Santos, unpublished data), which indicates for REE that as the atomic number increases, the bioavailability decreases.

The difference between REE fractionation in both analyzed tissues, which is less pronounced in the shells in relation to the claw muscles, can be seen in Fig. 3, which shows the results of multi-elemental ratios $R_{(M/L)}$ and $R_{(H/M)}$. With less variation of $R_{(M/L)}$ and larger $R_{(H/M)}$ values, REE fractionation in the shell samples is lower (Table 1, Fig. 3) compared to claw muscle samples and to contaminated sediments from the mangrove (Sanders et al., 2013). The contrast between fractionation of shell and claw muscles is probably related to the fact that the chemistry of the shells is largely controlled by the water chemistry in the molting period, as previously mentioned.

In contrast, REE fractionation in claw muscles closely correlates with the fractionation observed in the contaminated sediments of the Morrão River (Table 1, Fig. 3). Indeed, REE pattern obtained for the muscle tissues has the same signatures found in surface sediments impacted by the contaminant source, represented in Fig. 2 by the phosphogypsum and the phoscorites of Catalão (mineral deposit located in the State of Goiás, Central Brazil), including the

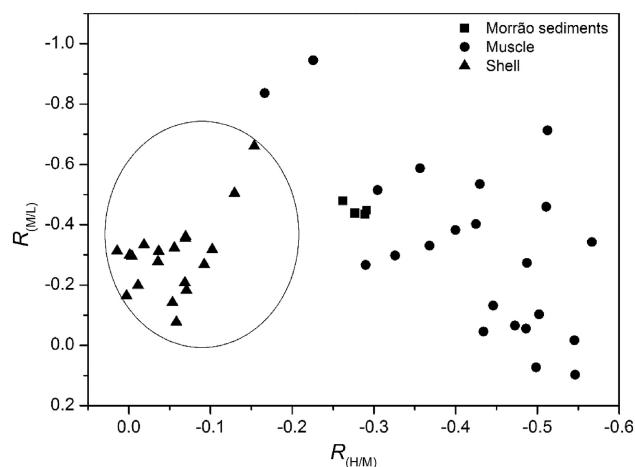


Fig. 3 – Rare earth elements-fractionation ($R_{(M/L)}$ and $R_{(H/M)}$) in *Ucides cordatus* claw muscle and shell ($N = 20$) and in the Morrão River sediments.

convex Nd-Sm-Eu-Gd trend (highlighted in Fig. 2). This convexity seems to be an important signature of contaminant sources related to the Cubatão fertilizer industry. Therefore, it is possible to propose that the contaminant source fingerprint (e.g. phosphogypsum–Oliveira et al., 2007; Sanders et al., 2013) is being transferred to the crab muscles via diet, virtually with no fractionation between the physical environment and biota, through the consumption of sediments by *Ucides cordatus*.

3. Conclusions

Differentiated internal distribution of contaminants was identified in *Ucides cordatus* tissues as a consequence of uptake pathways, sediments and water. In the shells, higher REE, Th and U concentrations were observed. In contrast, higher concentrations of other trace metals were observed in muscle tissues and may be related to their high bioavailability in sediments. The claw muscles of the analyzed specimens show Cu, Zn and Pb concentrations exceeding the maximum limits for inorganic contaminants in food set by the Brazilian legislation, representing risk for human consumption. Both tissues showed HREE-depleted fractionation, which is softer in shells due to their chemical composition, be controlled by the water absorbed for molting. REE fractionation in *Ucides cordatus* claw muscles is strongest and similar to that found in the sediments impacted by fertilizer production activities. These results can be associated with eating habits highly related to the substrate. For this reason, metals accumulated in the muscles appear to be the most appropriate to be used as environmental contamination markers, when crabs are used in environmental monitoring studies.

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