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continued on the back inside cover

Cover: The critically endangered *Lilium polyphyllum* in watercolour and acrylics. © Aishwarya S Kumar.

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COMMUNICATION

Nonessential elements (Al, As, Cd, & Pb) in shrimps and mussels from southeastern Brazil

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Abstract: The bioaccumulation of nonessential elements (Al, As, Cd, & Pb) in shrimps and mussels from southeastern Brazil (21°S–23°S) were compared. The objective was to verify and confirm the differential responses of elemental assimilation at both the taxonomic and spatial level. Two hypotheses were predicted: i) shrimps have lower element concentrations than mussels, and ii) both shrimps and mussels from the highly polluted site have higher element concentrations. The results confirmed the first hypothesis. The intense filter feeding activity of mussels explains the taxonomic difference. The second hypothesis was not validated. Both shrimps and mussels from the highly polluted site (Guanabara Bay) have lower elemental concentrations than individuals from the less polluted site. This finding is explained by the large inputs of sewage that result in partially reducing conditions of the water and high sedimentation rates, maintaining elements buried in anoxic sediment and making them unavailable for biological uptake. To understand what drives the bioaccumulation of chemical elements in marine animals it is necessary to know the species feeding habits and physiology, and the habitat characteristics in each region.

Keywords: *Artemesia longinaris*, Atlantic Ocean, Brazilian coast, hazardous elements, *Penaeus brasiliensis, Penaeus paulensis, Perna perna*, pollution, Rio de Janeiro State, *Xiphopenaeus kroyeri*.

Portuguese abstract: A bioacumulação de elementos não essenciais (Al, As, Cd, e Pb) em camarões e mexilhões do Sudeste do Brasil (21°S–23°S) foi comparada. O objetivo foi verificar e confirmar as respostas diferenciais de assimilação elementar tanto em nível taxonômico quanto espacial. Duas hipóteses foram previstas: i) os camarões têm concentrações de elementos mais baixas do que os mexilhões, e ii) tanto os camarões como os mexilhões do local altamente poluído têm concentrações de elementos mais elevadas. Os resultados confirmaram a primeira hipótese. A intensa atividade de filtração dos mexilhões explica a diferença taxonômica. A segunda hipótese não foi validada. Tanto os camarões quanto os mexilhões do local altamente poluído (Baía de Guanabara) apresentam concentrações elementares mais baixas do que os indivíduos do local menos poluído. Esse achado é explicado pelos grandes aportes de esgoto que resultam na redução parcial das condições da água e nas altas taxas de sedimentação, mantendo elementos soterrados em sedimentos anóxicos e tornando-os indisponíveis para captação biológica. Para compreender o que impulsiona a bioacumulação de elementos químicos nos animais marinhos é necessário conhecer os hábitos alimentares e a fisiologia das espécies, e as características do habitat em cada região.

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INTRODUCTION

Fish and shellfish are important to food security because they are readily available sources of animal protein that people can self-harvest throughout the year (Henchion et al. 2017). Thus, it is important to determine if the target species are safe for consumption regarding the presence of harmful agents (bacteria, viruses, parasites) and / or the concentration of chemicals (nonessential elements and other pollutants) (WHO 2019). In the aquatic environment, nonessential elements can concentrate in all compartments (water, sediment, and biota), reaching consumers via trophic transfer (Ali et al. 2019). The concentrations of chemical elements tend to be higher in more industrialized and populous areas than in areas with lower anthropic influence (Wang et al. 2013; Delgado et al. 2023).

Aluminum (Al), arsenic (As), cadmium (Cd), and lead (Pb), for instance, are biologically nonessential elements with known adverse effects. Toxic effects of Al, for instance, induce oxidative stress, immunologic alterations, and other metabolic disorders (Igbokwe et al. 2019). Arsenic is responsible for several types of cancer, especially those affecting the skin (Palma-Lara et al. 2020). Cadmium and Pb are related to neurological and kidney damage (WHO 2019). The concentrations of these elements in the fishery resources are highly variable among species (Wang et al. 2013; Silva et al. 2021).

Shrimps of the Penaeidae family are targeted by marine fisheries worldwide (FAO 2020). In Brazil, they are key resources for the economy of coastal communities (Boos et al. 2016). In southeastern Brazil, *Xiphopenaeus kroyeri* Heller, 1862, *Artemesia longinaris* Bate, 1888, *Litopenaeus schmitti* Burkenroad, 1936, *Penaeus brasiliensis* Latreille, 1817, and *P. paulensis* Perez Farfante, 1967 are the main target species (Boos et al. 2016). Shrimps are omnivorous secondary consumers with high feeding plasticity, ingesting mainly other benthic invertebrates, particulate organic matter, and benthic algae (Albertoni et al. 2003; Di Beneditto et al. 2012; Willems et al. 2016). Shrimps accumulate chemical elements mainly from feeding, whether essential or nonessential for their metabolism (Boudet et al. 2019; Di Beneditto et al. 2023).

In Brazil, the mussel *Perna perna* (L.) (Mytilidae family) is a naturalized exotic species that has become the main species of Brazilian mussel farming (Resgalla et al. 2008; Silva et al. 2018). The high abundance of *P. perna* off the Brazilian coast has made it a key resource for traditional communities that practice extractive fishing (Antunes & Mesquita 2018). Mussels are suspension-feeding organisms that obtain nutrition by filtering particulate organic matter, comprising algae, detritus, and bacteria, out of the water column (Berry & Schleyer 1983). Due to their intense filtering activity, bivalve mollusks have a well-known capacity to accumulate chemical elements in different tissues, with overall higher concentrations than other marine organisms, such as fish, shrimp, crabs, and cephalopods, from the same area (Wang et al. 2013; Catry et al. 2021).

This study compares the concentrations of the nonessential elements Al, As, Cd and Pb in the edible portion of shrimps and mussels from southeastern Brazil (22^{0}S, 43^{0}W and 23^{0}S, 41^{0}W) to verify taxonomic and spatial patterns regarding element assimilation. We predicted two hypotheses: i) shrimps have lower element concentrations than mussels, and ii) shrimps and mussels from highly polluted sites have higher element concentrations.

METHODS

The samplings were performed between 2020–2022 in the coastal waters of Rio de Janeiro State, Southeast Brazil (Figure 1). The sampling sites were named sites I and II. Site I is less polluted, facing an open sandy beach in northern Rio de Janeiro State (Figure 1). At this site, we sampled the shrimps *X. kroyeri* and *A. longinaris* and the mussel *P. perna*. Site II is highly polluted, located inside the Guanabara Bay (Rio de Janeiro municipality), which is a semi enclosed oceanic bay with 400 km^2, densely populated (~12 million people live around it) and industrialized (~6,000 industries around it) (Figure 1). At this site, we sampled the shrimps *P. brasiliensis* and *P. paulensis* and the mussel *P. perna*. All shrimps were sampled from local fisheries, while mussels were sampled directly from rocky intertidal zones.

After sampling, the individuals were stored in clean plastic bags inside an icebox and transported to the laboratory. The abdominal muscle (edible portion) of each shrimp and the soft tissue of each mussel (edible portion) were removed, stored in a dry sterile bottle, frozen (-20 °C), freeze-dried and homogenized to a fine powder using a mortar and pestle. The nonessential elements Al, As, Cd, & Pb were determined in each individual using ICP–OES (Inductively Coupled Plasma-Optical Emission Spectrometry, Model 720 ES, Varian Liberty Series II, USA). Freeze-dried muscle (0.5 g) was solubilized in 10 mL of 65% HNO_3 and heated in a digester block. Subsequently, samples were resuspended in 5 mL

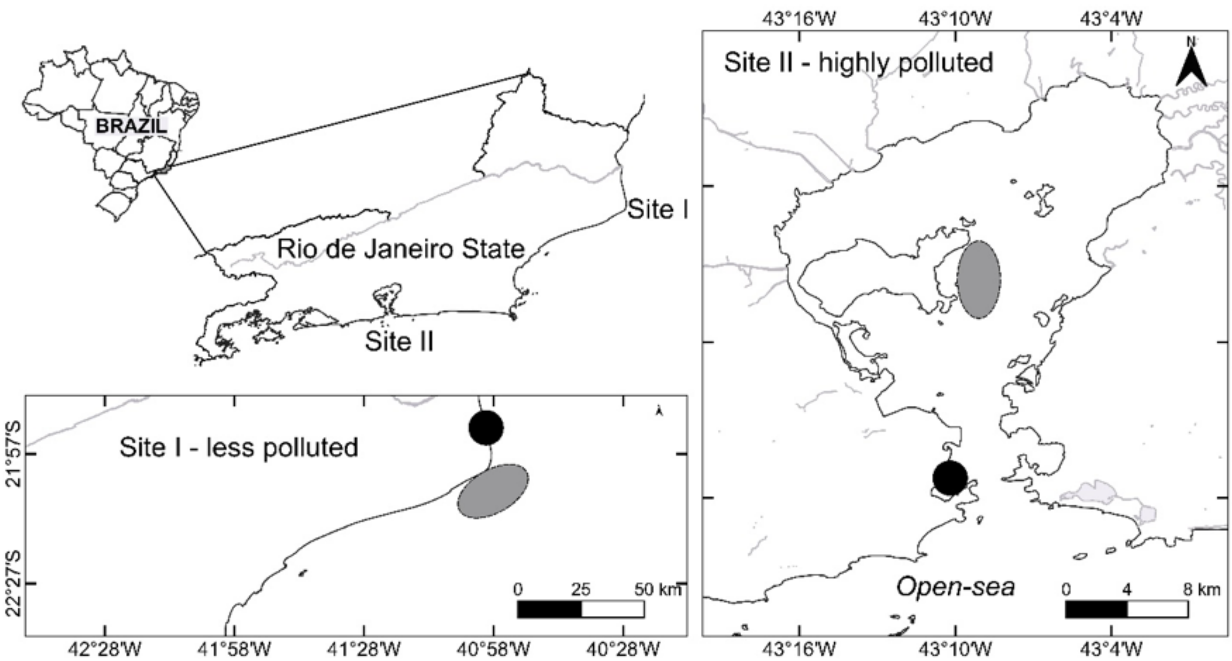

Figure 1. Map of Rio de Janeiro State, southeastern Brazil, with sites I (less polluted) and II (highly polluted) where shrimps (gray ellipses) and mussels (black circles) were sampled.

of 0.5% HNO_3 at 60 °C, filtered and brought to a final volume of 20 mL with 0.5% HNO_3. An analytical control solution was prepared to check for contamination, and a reference material (*DORM-4* fish protein, National Research Council of Canada) was analyzed to test the precision and accuracy (recovery values above 90%). The coefficients of variation among analytical replicates were <10%. All concentrations were determined in µg g^{-1} of dry weight.

Statistical analyses were performed using the R program (R Core Team 2023) considering a type I error of 5% ($\alpha = 0.05$). Descriptive statistics are reported as the median and interquartile range. An analysis of variance (ANOVA) followed by Tukey's test was used to evaluate the differences in the element concentrations regarding taxonomic groups and sampling sites. Mathematical transformations were used whenever necessary to meet the assumptions of normality, linearity, and homoscedasticity of residues using a maximum likelihood function (Venables & Ripley 2002). ANOVA assumptions were validated using diagnostic plots (Altman & Krzywinski 2016).

In addition to comparing each element separately, we also calculated and compared the normalized total load between taxonomic groups and sampling sites. It provides a holistic view of the elements' pathways, as detailed in Agostinho et al. (2021). The normalized total load represents the sum of element concentrations in each individual weighted by the number of elements detected in that individual (element load), as follow:

$$\textbf{Normalized total load } (\mu g \cdot g^{-1}) = \sum_{1}^{n=4} \frac{\textbf{Element concentration } (\mu g \cdot g^{-1})}{\textbf{N of Elements}}$$

RESULTS AND DISCUSSION

Samplings included two shrimp species from each site (site I: *A. longinaris* and *X. kroyeri*; and site II: *P. brasiliensis* and *P. paulensis*), and to avoid biased interpretation, we tested whether the element concentration was species dependent. The ANOVA results showed that in most cases (75%), the species from the same site did not show significant differences ($p > 0.05$) regarding element concentrations. Therefore, we grouped them only as 'shrimps' for further comparisons.

The results confirmed the first hypothesis that shrimps do have lower element concentrations than mussels, except for As at site I (Table 1 & Figure 2). This finding was corroborated by the normalized total load of nonessential elements, which was 13 times and 25 times lower in shrimp than in mussels at sites I and II, respectively (Table 1). The higher elemental concentration in the tissues of bivalve mollusks concerning other marine organisms (invertebrates and vertebrates) that share the environment is well documented elsewhere (e.g., Wang et al. 2013; Suami

Table 1. Concentration (µg·g^{-1} dry weight) of nonessential elements (Al, As, Cd, & Pb) and normalized total load in the edible portion of shrimps and mussels from two sampling sites in Rio de Janeiro State, southeastern Brazil. Data are presented as the median ± interquartile range, and n values are the sample size.

Site I **less polluted**	***A. longinaris*** **(n = 58)**	***X. kroyeri*** **(n = 57)**	**Shrimps grouped** **(n = 115)**	***Perna perna*** **(n = 13)**
Al	86.1 ± 35.6	84.4 ± 125.3	85.5 ± 58.5	1,781.5 ± 846.9
As	25.8 ± 9.6	20.3 ± 11.6	23.6 ± 12.4	14.4 ± 6.0
Cd	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
Pb	0.8 ± 0.4	0.7 ± 0.4	0.7 ± 0.4	1.0 ± 1.0
Normalized total load			35.4 ± 19.3	449.9 ± 212.8
Site II **highly polluted**	***P. brasiliensis*** **(n = 41)**	***P. paulensis*** **(n = 39)**	**Shrimps grouped** **(n = 80)**	***Perna perna*** **(n = 17)**
Al	13.3 ± 18	28.1 ± 29.6	16.8 ± 25.5	614.3 ± 345.7
As	3.8 ± 2.7	2.7 ± 1.8	3.1 ± 2.1	6.9 ± 2.1
Cd	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
Pb	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	3.2 ± 2.1
Normalized total load			6.3 ± 7.5	157.1 ± 86.3

et al. 2019; Catry et al. 2021). The higher concentrations are explained by the suspension-feeding habit of bivalves, with intense filtering activity, the elements are transferred to the tissues through phytoplankton, which is at the base of the marine food chains (Santos & Boehs 2023).

Conversely, the results did not support the second hypothesis predicted in this study. Both shrimps and mussels from the highly polluted site (site II) had lower elemental concentrations than individuals from the less polluted site (site I) (Table 1 & Figure 2). The normalized total load of nonessential elements followed the same trend: six times lower in shrimps from site II and three times lower in mussels from site II (Table 1). The only exception was the Pb concentration in mussels, which was higher in individuals from site II.

Site II is Guanabara Bay (Figure 1). This semi-enclosed coastal bay suffers from several forms of anthropogenic impact threats. The edge and surroundings of this bay are heavily urbanized, receiving inputs from industrial and domestic sewage and residuals of crops (Soares-Gomes et al. 2016). Thus, a higher nonessential element concentration in organisms at site II would be expected. Site I, in turn, is an open coastal area of northern Rio de Janeiro State, sparsely populated, and whose only noteworthy anthropogenic activity in coastal waters is the Açu Harbor cargo handled (solid and liquid bulk, iron ore and oil) that began in 2014 (Zappes et al. 2016).

The unexpected result regarding the spatial pattern of element assimilation by the target species can be explained by the geochemistry of Guabanara Bay. Carvalho & Lacerda (1992) stated that element (Zn, Cu, Cd, Pb, Mn, & Ni) concentrations determined in the marine organisms (benthic algae, crustaceans, and mollusks) of Guanabara Bay were very low compared to those in other contaminated sites along Rio de Janeiro State, and they were even comparable with those in noncontaminated sites. The authors concluded that the large sewage inputs reduce conditions in the bay's water. These conditions, combined with high sedimentation rates, result in the immobilization of elements in the sediment. Consequently, these elements become unavailable for biological uptake. The same geochemistry pattern and its influence in the elements' bioavailability is reported elsewhere, as presented in the review done by Zhang et al. (2014).

The difference in the element concentrations was driven by the environment in which the target species (shrimp and mussel) belonged. The preliminary ANOVA that compared the elemental concentration in different shrimp species (site I: *A. longinaris* and *X. kroyeri*; and site II: *P. brasiliensis* and *P. paulensis*) showed that the difference in element concentrations is not species dependent, supporting this affirmation. The target species from a polluted site did not contain a necessarily high load of nonessential elements compared with those from a less polluted site due to spatial variation in the elements' bioavailability (Carvalho & Lacerda 1992; Zhang et al. 2014).

In conclusion, mussels had higher nonessential elements load than shrimps due to differences in their feeding habits and, consequently, bioaccumulation of these elements. The spatial approach showed that the geochemistry pattern of the sampling sites was probably

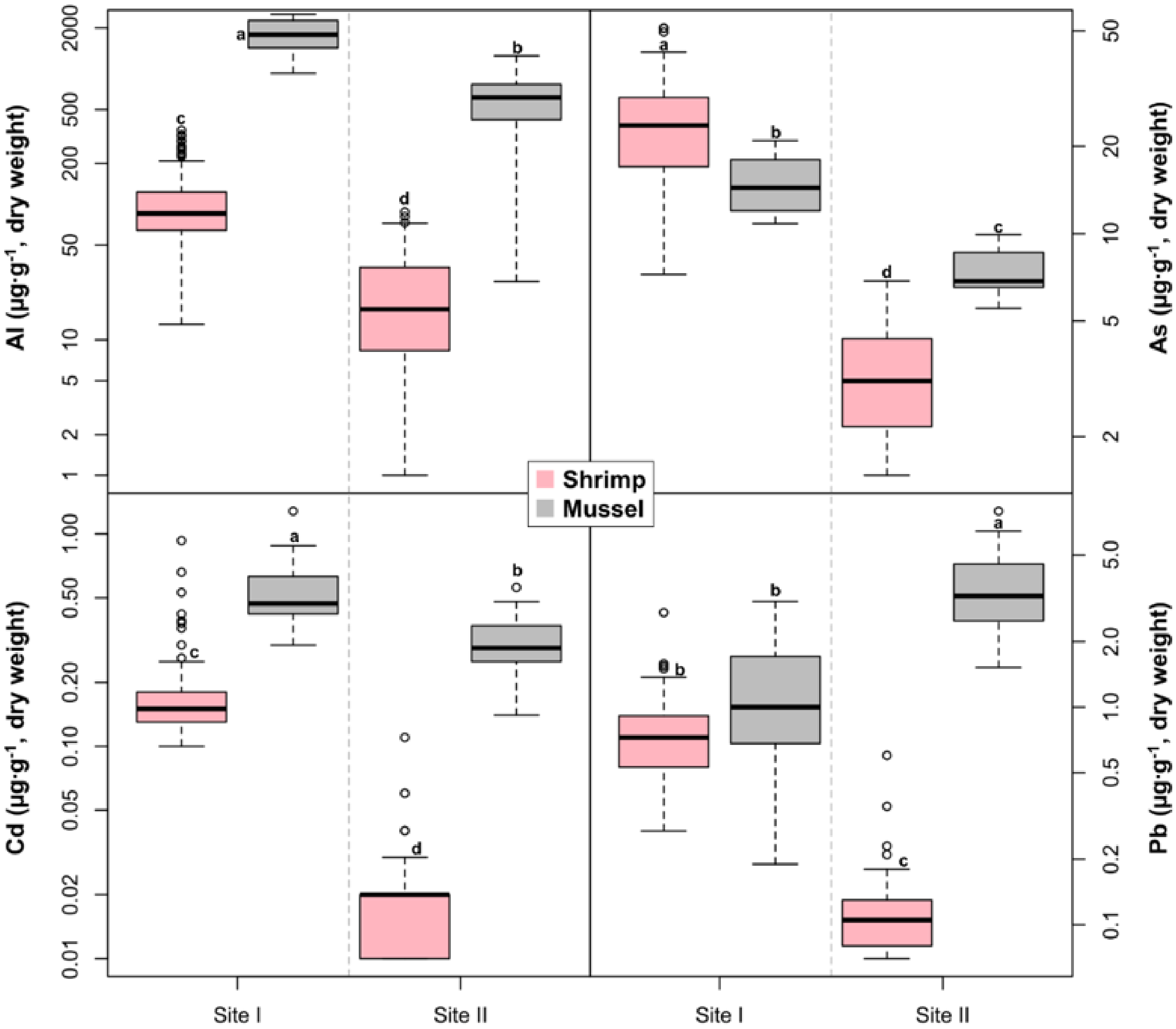

Figure 2. Boxplots representing median (bar inside the box), interquartile range (box: 1st to 3rd quartile), minimum and maximum concentrations of the nonessential elements (Al, As, Cd, & Pb) in the edible portion of shrimps (grouped species) and mussels from two sampling sites in Rio de Janeiro State, southeastern Brazil. Lowercase letters differentiate the concentration of each element (p <0.05) between sampling sites and taxonomic groups. Open circles are outliers. The y-axis distances were log-transformed to optimize data visualization.

the major influence for the elements' bioavailability, regardless of the target species. To understand what drives the bioaccumulation of chemical elements in marine organisms, it is necessary to know their feeding habits and physiology, besides the habitat characteristics in each region. This understanding of species for commercialization and human consumption, such as the shrimps and mussels analyzed in this study, is even more important since it affects both the local economy and public health.

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