



# Elemental Contamination in Brown Mussels (*Perna perna*) Marketed in Southeastern Brazil

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## Abstract

*Perna perna* mussels, abundant throughout the Brazilian coast, are routinely applied as bioindicators in environmental monitoring actions due to their sessile and filter-feeding characteristics. In addition, they are noteworthy for their food importance, especially for coastal populations. In this context, the aim of this study was to investigate elemental contamination in commercially marketed and highly consumed *P. perna* samples from the highly impacted Guanabara Bay, Rio de Janeiro, Brazil. A total of 30 mussels were sampled, and elemental concentrations (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) were determined in adductor muscle samples by inductively coupled plasma mass spectrometry (ICP-MS). Human consumption risks were assessed by comparisons to Brazilian and international legislations. No significant differences between sex were observed for all analyzed elements. Even when analyzing only the adductor muscle, all mussel samples exceeded the Brazilian limit for Cr, while 12 samples exceeded the limit for Se. When compared to other regulatory agencies, As and Zn levels were higher than the limits set by China, New Zealand, and the USA. Estimated daily dietary intake values were not above limits imposed by the Food and Agriculture Organization of the United Nations/World Health Organization for any of the assessed elements, although it is important to note that only the adductor muscle was assessed. Therefore, continuous metal and metalloid monitoring in bivalves in the study region is suggested, as metal transport and bioavailability, especially in coastal estuaries such as Guanabara Bay, which are currently undergoing significant changes due to anthropogenic activities.

**Keywords** Bivalves · Biomonitoring · Metals · Estuarine environment · Public health

## Introduction

Metal and metalloid inputs to different environmental compartments may occur through natural processes, such as volcanism and weathering, or due to anthropogenic activities, i.e., domestic and industrial effluents [1]. These chemical compounds are among the main classes of environmental pollutants, as they are not biodegradable and, depending on their environmental levels, may exhibit high toxicity [2, 3]. Some metals are essential to living organisms, while non-essential elements display no metabolic function. Each organism has a specific tolerance level for both types of metals which, when above a certain threshold, may cause toxic and/or lethal effects [2, 3].

Certain metals display the ability to bioaccumulate in aquatic ecosystems. This can occur either directly, through environmental exposure, or indirectly, by the ingestion of contaminated food items [4]. A classic example of the latter occurred in the 1950s, with the accumulation of the most toxic form of mercury (Hg), methylmercury, in Minamata Bay,

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Japan, leading to neurological symptoms and congenital toxicity cases in humans, cats, and waterfowl [5]. Later assessments reported cognitive development delays in preschool children who regularly consumed fish contaminated with Hg [6], so a limited consumption of fish and shellfish is recommended, as high methylmercury levels are proven to result in neurological impairment in newborns [7]. Other elements in this context are also of significant concern, such as As, Cd, Cr, and Pb, for example, which are known to bioaccumulate throughout the trophic web and may lead to carcinogenic effects in exposed organisms [3, 8].

Concerning wildlife, several studies have routinely reported deleterious biochemical, reproductive, and growth alterations in several organisms, such as bivalves and fish, due to exposure to high metal and metalloid concentrations [9, 10]. In addition, these organisms are routinely consumed by humans, which may lead to public health concerns. In Brazil, bivalve mollusks are widely applied in environmental monitoring actions [11–14], as they are excellent bioindicators, due to certain specific characteristics, such as their sessile nature, resistance to diverse environmental conditions, and filter-feeding habits, which result in the accumulation of diverse environmental contaminants in their tissues [11, 15, 16].

Bivalve mollusks are very representative in several countries due to high commercial sales and human consumption. For example, the 2016 mussel, oyster, and scallop production in Brazil comprised approximately 20.83 thousand tons, equivalent to 1.5% of the main products of Brazilian aquaculture [17]. In this regard, the brown mussel *Perna perna* (Linnaeus, 1758) is one of the most commercially cultivated bivalves in the country [18]. Originally from Africa, this species was introduced in South America and now thrives throughout the continent, establishing itself easily along the entire Brazilian coastline [16]. The success of its cultivation in Brazil is due to the fact that it exhibits fast growth rates due to the country's climate, reaches a larger size in comparison to other Brazilian mytilids, and is easy to handle [16, 19]. This species is also noteworthy for its food importance, especially for coastal populations [16], as it is an excellent alternative food source, highly nutritious, with high levels of proteins and vitamins [20]. However, as stated previously, mussel ingestion can lead to public health concerns, due to the presence of environmental contaminants, including metals and metalloids.

In Southeastern Brazil, mussel farming activities are prominent at the east of the entrance to Guanabara Bay, Rio de Janeiro (RJ), producing about 6 T of mussels a month [21]. This bay is one of the most impacted environments on the Brazilian coast [22]. However, even though it is highly contaminated by metals and other chemical contaminants, it still displays significant economic and tourist importance. At Jurujuba Sound, in the municipality of Niterói, mussel farming is a significant activity, with an estimated production of up

to 800 kg of mussels a day in 2015, sustaining about 90 local families [23]. Cultivated mussels and those extracted from adjacent rocky shores serve as a significant source of income and consumption of local traditional fishing communities, where they are sold to local restaurants and other markets [24]. Studies concerning *P. perna* metal and metalloid contamination in Southeastern Brazil, however, are sorely lacking. In this context, the aim of the present study was to investigate the levels of elemental contamination in *P. perna* samples commercially marketed in the region and investigate associated human health risks through their consumption.

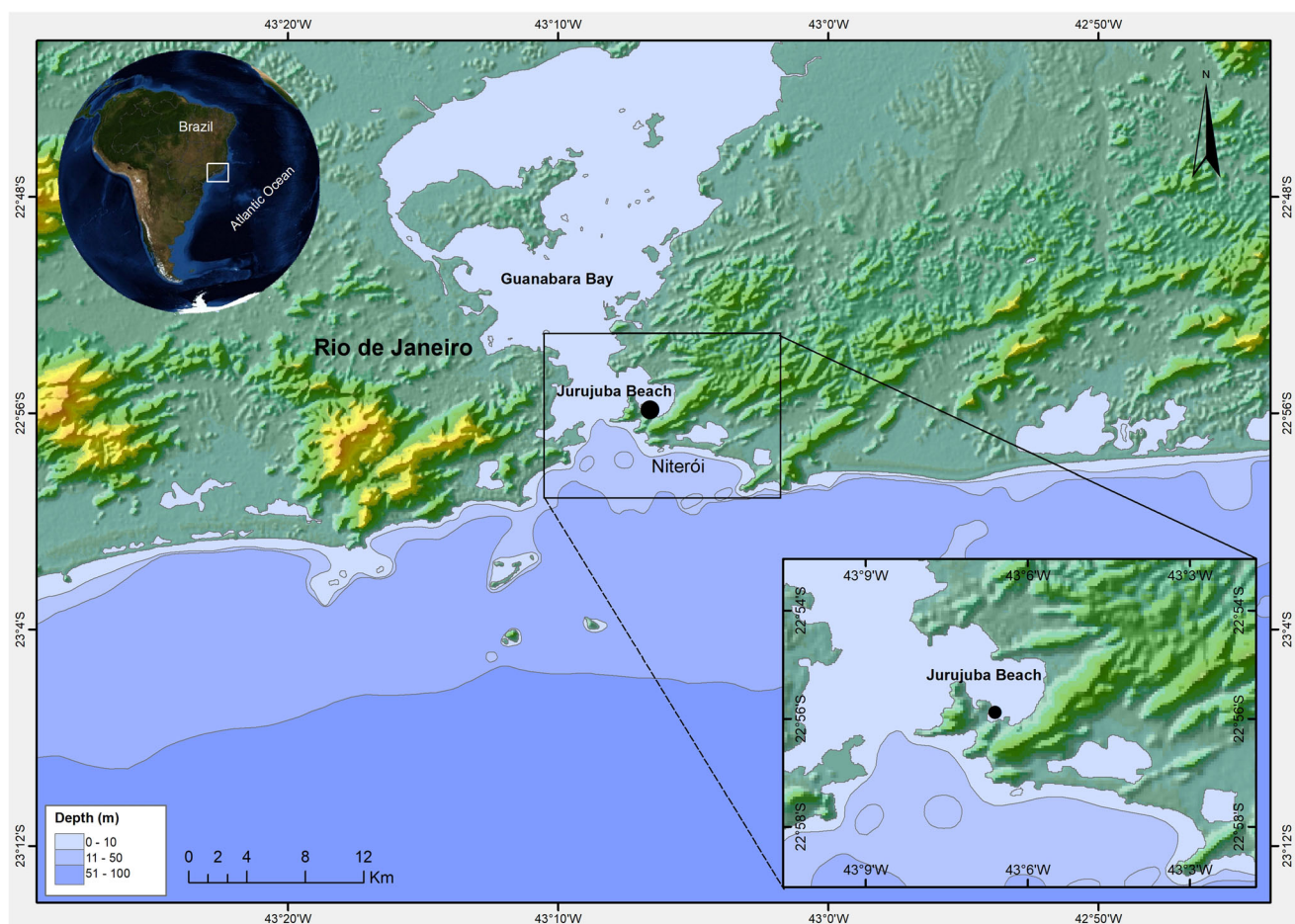
## Material and Methods

### Sample Collection and Processing

A total of 30 *P. perna* mussels were collected in 2019 by fishers from the Z8 Fishers Colony, located at the entrance of Guanabara Bay, RJ (22° 55' 53 "S, 43° 06' 35 "W) (Fig. 1). These mussels are routinely collected by fishers from this colony, both from surrounding rocky shores and from mussel farms located throughout the area, where mussels are rope-reared, so the number of fishers involved in the mussel samplings is unknown. This type of sampling is representative of mussels sold for consumption in the study area. The samples were packed in polystyrene boxes containing ice and transported to the laboratory for processing. At the laboratory, mussel shell lengths, weights (with shells), and sex were recorded, the latter through macroscopic gonad evaluations according to the literature [16, 25]. With the mussels on ice, the adductor muscles of each individual were removed, weighed (approximately 150 mg), and frozen (−20 °C) individually in 15 mL sterile screw-capped polypropylene tubes until analysis. All dissection tools were cleaned with alcohol between individual dissections, as recommended by the US Fish and Wildlife Services [26].

### ICP-MS Analyses

A multielemental analysis was carried out by inductively coupled plasma mass spectrometry (ICP-MS). Each sample was thawed and mixed with 1 mL of concentrated subboiled bidistilled nitric acid (Merck, Rio de Janeiro). This mixture was then left to stand overnight at room temperature in the closed tubes. After 12 h, the acid decomposition was completed by heating the sample at 100 °C on a heating block, for 4 h in the closed vessels, avoiding volatilization of volatile elements, such as As, Hg, and Se [27]. The samples were then diluted with ultra-pure water (resistivity > 18.0 MΩ cm) obtained from a Merck Millipore purifying system (Darmstadt, Germany) to 10 mL. Metals and metalloids were determined, in quintuplicate, using multielemental external calibration, by



**Fig. 1** Map indicating the *Perna perna* mussel sampling area at the entrance of Guanabara Bay, Southeastern Brazil

appropriate dilutions of a mixed standard solution (Merck IV) and using  $^{102}\text{Rh}$  as the internal standard at  $20 \text{ mg L}^{-1}$ . The determinations were conducted on a NexIon 300X Perkin Elmer ICP-MS (Norwalk, CT, USA). Method accuracy was verified with procedural blanks and by the parallel analysis of a certified reference material (CRM) (ERM@-BB422, fish muscle), in triplicate. All CRM recovery values were within acceptable Eurachem standards [27] (Table 1), including for

**Table 1** Certified reference material recoveries in the ICP-MS analyses carried out herein

Element	Certified value	Observed value	% Recovery
As	$12.7 \pm 0.7$	$15.7 \pm 0.7$	124
Cd	$0.0075 \pm 0.0018$	$0.0049 \pm 0.0024$	66
Cu	$1.67 \pm 0.16$	$1.62 \pm 0.09$	97
Fe	$9.4 \pm 1.4$	$9.8 \pm 2.8$	104
Hg	$0.601 \pm 0.030$	$0.701 \pm 0.049$	116
Mg	1370	$1330 \pm 38.74$	97
Mn	$0.368 \pm 0.028$	$0.201 \pm 0.019$	55
Na	2800	$2665 \pm 114$	95
Zn	$16 \pm 1.1$	$16 \pm 0.7$	99

volatile elements, indicating that the sample preparation procedure is efficient and not prone to losses.

The limits of detections (LOD) for each investigated element were calculated as  $\text{LOD} = (10 * \text{SD} * \text{df}) / \text{slope of the line}$ , where SD is the standard deviation of the analytic signal ratio by the internal standard signal of 10 blank solutions and df is the sample dilution factor [28]. The calculated LODs ( $\text{mg kg}^{-1}$ ) were as follows: Ag, 0.017; Al, 1.082; As, 0.009; Cd, 0.009; Co, 0.004; Cr, 0.339; Cu, 0.146; Fe, 2.406; Hg, 0.020; Mn, 0.066; Ni, 0.486; Pb, 0.003; Se, 0.123; V, 0.008; and Zn, 0.916.

### Provisional Tolerable Weekly Intake Calculation

The Provisional Tolerable Weekly Intake (PTWI) was calculated for each investigated element, using the average seafood consumption rate for the state of Rio de Janeiro ( $173.18 \text{ g week}^{-1}$ ) [29], as no specific mussel consumption intake rates are available. The means for each element in mussels from Jurujuba Beach, as well as the highest elemental value, to estimate a for a worst-case scenario calculation, were multiplied by the average fish consumption in Brazil for a week

(173.18 g) and divided by the mean body weight of a Brazilian adult (70 kg).

### Statistical Analyses

The statistical analyses were performed using the Graphpad Prism v.8 software package. Metal and metalloid data normality was first verified by the Shapiro-Wilk test. As the data were non-normally distributed, the data were analyzed through the Mann-Whitney test to assess elemental differences between sexes.

## Results and Discussion

### Biometric Data

Total *P. perna* shell length ranged from 63 to 121 mm, averaging  $94.2 \pm 13.5$  mm, and mussel weight ranged from 21.62 to 113.60 g, averaging  $63.80 \pm 21.02$  g. All *P. perna* sampled were over 50 mm in length and, thus, classified as adults, according to the IBAMA [30], the Brazilian Ministry of the Environment's administrative arm, which established that 50 mm or larger sizes are suitable for commercialization and human consumption.

A total of 15 females and 14 males were positively identified, according to the sexual dimorphism characteristics of *P. perna* mussels, where sexually mature males present a creamy white mantle color and females, orange [16, 25]. One individual sampled in the present study was classified as an indeterminate sex, due to a transparent mantle, probably in the partial or total follicle emptying stage [25].

### Elemental Concentrations

No significant differences were observed between male and female elemental concentrations. Thus, data for both sexes were combined and are exhibited in Table 2 (mg kg<sup>-1</sup> wet weight).

This lack of difference between sex for all determined elements contrast with previous reports by other researchers for the same species. For example, Campolim et al. [31], Carvalho et al. [32], and Ferreira et al. [33] all reported significant differences for Cd, Cu, and Ni, with highest values observed in female *P. perna* specimens. In another assessment, Kehrig et al. [34] reported significant differences for Hg concentrations, higher in female mussels. Orren et al. [35] suggested that metal accumulation in bivalves is associated to the reproductive season, after observing that female *Choromytilus meridionalis* mussels exhibit higher metal concentrations only in the pre-spawning period and exhibiting no inter-sex differences after the breeding period. In Brazil, *P. perna* mussel reproductive peaks occur from April to June, as well as in September and January [15]. Thus, as our

**Table 2** Elemental concentrations (mg kg<sup>-1</sup> wet weight) in *Perna perna* mussel samples from Guanabara Bay, Rio de Janeiro, Brazil (n=30)

Element	Minimum	Maximum	Means ± SD
Ag	0.003	0.051	0.013 ± 0.010
Al	<LOD	5.68	0.70 ± 1.19
As	0.27	1.38	0.69 ± 0.27
Cd	0.000	0.023	0.005 ± 0.006
Co	0.009	0.082	0.025 ± 0.016
Cr	0.38	1.06	0.68 ± 0.16
Cu	0.227	1.109	0.485 ± 0.191
Fe	0.49	20.51	6.12 ± 4.22
Hg	0.008	0.040	0.024 ± 0.008
Mn	0.41	12.68	2.35 ± 2.48
Ni	0.011	0.613	0.172 ± 0.178
Pb	0.012	0.101	0.032 ± 0.019
Se	0.148	0.468	0.284 ± 0.087
V	0.069	0.266	0.164 ± 0.047
Zn	6.66	38.16	17.00 ± 8.19

\*LOD limit of detection

samplings were carried out in February and March, reproductive effects are not a concern.

The mean Fe and Zn concentrations were the highest in the investigated bivalves (Table 2). Values below the LOQ were found in most samples for Ag (25/30, 83.3%), Al (26/30, 86.6%), Cd (23/30, 76.6%), and Ni (27/30, 90%). Some Fe and Hg concentrations were also below the LOQ, of 4/30 (13.3%) and 11/30 (36.6%), respectively. According to Baptista Neto et al. [36], the enrichment of certain elements of the environment, such as Zn, is usually indicative of urban development. These authors detected high Zn values in sediments near the area investigated in the present study, associating this with high levels of organic matter present in domestic effluents which may be the cause for the high levels observed herein. Fe is one of the most abundant elements in nature, present in rocks. However, it is also detected in solid residues, sewage, industrial waste, and fertilizers [1, 14]. One study concerning bottom Jurujuba Sound sediments demonstrated Fe enrichment, among other elements, towards the inner part of the sound [37], which may be the cause of the detected Fe concentrations observed herein, as Jurujuba Beach is located closer to the extremity of the sound. In addition, Ferreira et al. [33] suggested that high Fe concentrations in *P. perna* are due to the iron oxide-rich substrates on the northern coast of the state of Rio de Janeiro.

### Comparison of Elemental Concentrations in *P. perna* Mussels Worldwide

Not many studies have been carried out on metal contamination in *P. perna* mussels, both in Brazil and worldwide. A



comparison of elemental concentrations in *P. perna* to other studies is displayed in Table 3.

Significant sources of industrial effluents are present at Guanabara Bay, such as refineries, shipyards round, and fuel supply points [16]. The main elemental contamination sources at Jurujuba Sound comprise non-treated domestic and industrial effluents and urban runoff, with nautical activities and atmospheric depositions also contributing significantly [36].

The mean concentrations of most elements in this study were lower than those of the other studies also carried out in Guanabara Bay, except for the Cr concentrations reported by Lino et al. [38], which were similar to the concentrations observed herein. This element is considered one of the main industrial contaminants in the Guanabara Bay, although elemental concentrations in this region vary due to different environmental impacts and water quality [40]. Concerning other countries, the Cr mean concentrations observed herein were higher than those reported for *P. perna* from the Algerian and Senegalese coasts [41, 42], while the mean concentrations for the other elements analyzed herein were lower than in these countries.

Lino et al. [38] reported high Cu and Fe concentrations in mussels at Jurujuba Beach, approximately 430-fold and 10-fold higher than those observed in our study, respectively. In 2013, the Clean Cove program was implemented in the sampled area with the aim of reducing Guanabara Bay pollution. The established actions include environmental education initiatives to improve solid waste management and expansion of the sewage collection network, and water quality analyses are carried out weekly or monthly to evaluate the efficiency of these actions. Based on these weekly and monthly monitoring, the Environmental Company of the State of São Paulo developed a Balneability Index which represents a synthesis of the quality of the monitored waters throughout the year [45], categorized as follows: excellent (classified as excellent at 100% of the year), good (classified as adequate for bathing activities during 100% of the year, except those classified as excellent in 100% of the year), regular (classified as inappropriate below 50% of the year), and bad (classified as inappropriate equal to or greater than 50% of the year). Balneability indices at Jurujuba Beach were 27.69% in 2013, increasing to 55.98% in 2018 [46, 47]. Although still inadequate for bathing, this is a significant difference, indicating increasing water quality in the area, which may be responsible for the decreased metal concentrations observed herein in *P. perna* mussels when compared to the study carried out in the same area by Lino et al. [38] in 2016.

The Se values reported by Diop et al. [42] were similar to those identified herein, while mean Hg and Mn concentrations herein were 2-fold and 3-fold higher than those reported by Legraa et al. [43] and Diop et al. [42], respectively. Higher elemental concentrations compared to present study were reported for *P. perna* from the Venezuelan coast. The authors,

however, do not mention whether the value represents dry or wet weight [44], impeding further discussions. As mentioned previously, studies on *P. perna* are scarce, and further comparisons in South America can only be made with other species, such as *Ameghinomya antiqua* (King & Brodery, 1932), *Aulacomya atra* (Molina, 1782), and *Mytilus chilensis* Hupe, 1854, from Argentina and Chile, which presented mean concentrations below those of this study only for Cr, between 0.129 and 0.135 mg kg<sup>-1</sup> wet weight [48, 49].

### Human Health Risks Associated to *P. perna* Consumption

Elemental comparisons to maximum permissible levels set by Brazilian and international regulatory agencies are displayed in Table 4.

Most of the means detected in this study were lower than permissible levels set by Brazilian and international regulatory agencies, with the exception of Cr and As. Concerning the former, all samples exceeded the limit of 0.10 mg kg<sup>-1</sup> determined by the Brazilian Health Regulatory Agency (ANVISA) [50] for Cr. With regard to As, the mean concentration observed herein was only higher than that established for the Ministry of Health of the People's Republic of China (MHPRC) [56], with 24 samples above 0.50 mg kg<sup>-1</sup>. On the other hand, despite the mean As concentration being below the established Brazilian legislation [50, 51] and Food Standards Australia New Zealand (FSANZ) [54] limits, four samples contained higher As values than these limits. Regarding Se, 12 samples exceeded the ANVISA [50] limit, while for Zn, three samples were above the value indicated by the US Environmental Protection Agency (USEPA) [57].

No specific legislation for Cr, Cu, Ni, Se, and Zn limits in aquatic animals is available in Brazil, so the legislation that establishes generic values for several foodstuffs was used. In fact, Campolim et al. [12] reinforce the need to include and update the concentration limits of these elements specifically for aquatic animals. With respect to the maximum concentrations established for As, Cd, Hg, and Pb, they are specific for bivalve mollusks. A lack of legal limits for many elements in both Brazilian and international regulations is noted. This reinforces the need to establish these limits in order to enable a more efficient control of contaminants in aquatic animals, while variations between agencies regarding the accepted limits of some elements are also observed, which raises human health concerns [12, 58].

Chromium is an essential element for human metabolism when present in small amounts [2], although some studies indicate that Cr can be carcinogenic in humans through ingestion [59, 60]. All mussel samples evaluated herein exceeded the limit established by the Brazilian legislation. However, the consumption of the evaluated mussels would be adequate, according to the Chinese and Japanese agencies. Lino et al.

**Table 3** Comparison of elemental concentrations in *Perna perna* mussels to studies carried out in different Guanabara Bay areas and other countries. Data are expressed as means  $\pm$  SD (when available) and range (mg kg<sup>-1</sup> wet weight<sup>®</sup>). - Data not reported

Region	Area	As	Cd	Cr	Cu	Fe	Hg	Reference
Guanabara Bay—Brazil	Jurujuba Beach	0.692 $\pm$ 0.270 (0.276–1.380)	0.005 $\pm$ 0.006 (0.000–0.023)	0.687 $\pm$ 0.162 (0.383–1.059)	0.485 $\pm$ 0.191 (0.227–1.109)	6.127 $\pm$ 4.226 (0.495–20.516)	0.024 $\pm$ 0.008 (0.008–0.040)	The present study
	Jurujuba Beach	-	-	0.84 (0.30–1.50)	209.40 (3.90–960.00)	59.10 (35.40–118.50)	-	Lino et al. [38]
	Santa Cruz Fortress	-	0.05 (0.04–0.07)	0.05 (0.03–0.10)	1.62 (1.10–2.20)	-	-	Francioni et al. [39]
	Boa Viagem Island	-	0.03 (0.02–0.04)	0.05 (0.03–0.07)	1.13 (0.08–1.40)	-	-	Francioni et al. [39]
	Rio-Niterói Bridge	-	0.04 (0.02–0.08)	0.08 (0.04–0.11)	1.96 (1.60–2.30)	-	-	Francioni et al. [39]
	Marina da Glória	-	0.03 (0.02–0.04)	0.10 (0.03–0.20)	1.80 (1.40–2.50)	-	-	Francioni et al. [39]
	Vermelha Beach	-	0.07 (0.05–0.10)	0.16 (0.08–0.26)	2.05 (1.40–2.40)	-	-	Francioni et al. [39]
	Maria da Glória, Boa Viagem Beach and Rio-Niterói Bridge	-	-	0.5 $\pm$ 0.1	1.4 $\pm$ 0.4	31.6 $\pm$ 22.1	0.038 $\pm$ 0.023 (0.017–0.068)	Kehrig et al. [34, 40]
	Diabo Beach	4.24 $\pm$ 0.22	0.090 $\pm$ 0.006	-	1.04 $\pm$ 0.09	-	-	Lavradas et al. [11]**
	Urca Beach	2.75 $\pm$ 0.16	0.036 $\pm$ 0.003	-	1.98 $\pm$ 0.19	-	-	Lavradas et al. [11]**
Other countries	Vermelha Beach	4.50 $\pm$ 0.31	0.115 $\pm$ 0.006	-	1.58 $\pm$ 0.19	-	-	Lavradas et al. [11]**
	Algeria	-	0.15 $\pm$ 0.03	0.56 $\pm$ 0.03	8.02 $\pm$ 0.74	279.61 $\pm$ 3.89	0.048 $\pm$ 0.009	Belabed et al. [41]
	Senegal	2.21 $\pm$ 0.71	0.91 $\pm$ 1.15	0.30 $\pm$ 0.14	2.82 $\pm$ 0.80	54.00 $\pm$ 20.01	-	Diop et al. [42]
	Mauritania	-	0.432 $\pm$ 0.177	-	1.785 $\pm$ 0.348	-	0.009 $\pm$ 0.003	Legraa et al. [43]
	Venezuela	-	4.60 $\pm$ 2.61	7.49 $\pm$ 3.50	7.17 $\pm$ 6.89	158.69 $\pm$ 78.26	-	Castillo et al. [44]
	Area	Mn	Ni	Pb	Se	V	Zn	Reference
	Jurujuba Beach	2.356 $\pm$ 2.481 (0.411–12.684)	0.172 $\pm$ 0.178 (0.011–0.613)	0.032 $\pm$ 0.019 (0.012–0.101)	0.284 $\pm$ 0.087 (0.148–0.468)	0.164 $\pm$ 0.047 (0.069–0.266)	17.003 $\pm$ 8.194 (6.660–38.163)	The present study
	Jurujuba Beach	6.60 (3.00–11.40)	0.69 (0.42–0.93)	< 0.18	-	-	18.60 (15.30–21.30)	Lino et al. [38]
	Santa Cruz Fortress	-	-	-	-	-	48.27 (30.97–59.23)	Francioni et al. [39]
	Boa Viagem Island	-	-	-	-	-	32.43 (17.81–49.33)	Francioni et al. [39]
Rio-Niterói Bridge	-	-	-	-	-	41.95 (25.79–61.42)	Francioni et al. [39]	
Marina da Glória	-	-	-	-	-	31.95 (26.68–37.05)	Francioni et al. [39]	
Vermelha Beach	-	-	-	-	-	30.49		

Table 3 (continued)

Region	Area	As	Cd	Cr	Cu	Fe	Hg	Reference
Other countries	Maria da Glória, Boa Viagem Beach and Rio-Niterói Bridge	-	1.8 ± 0.6	< 0.6	-	-	(20.81–40.52) 28.0 ± 14.0	Francioni et al. [39] Kehrig et al. [40]
	Diabo Beach	-	2.57 ± 0.34	0.10 ± 0.02	0.86 ± 0.06	-	48.21 ± 2.17	Lavradas et al. [11]**
	Urca Beach	-	2.30 ± 0.19	0.28 ± 0.06	0.79 ± 0.03	-	68.42 ± 5.09	Lavradas et al. [11]**
	Vermelha Beach	-	3.94 ± 0.81	1.19 ± 0.24	1.15 ± 0.12	-	70.64 ± 10.51	Lavradas et al. [11]**
	Algeria	6.68 ± 0.40	2.70 ± 0.68	0.50 ± 0.05	-	-	107.69 ± 1.62	Belabed et al. [41]
Senegal	0.78 ± 0.40	2.08 ± 1.55	0.41 ± 0.36	0.28 ± 0.08	0.59 ± 0.62	46.80 ± 12.30	Diop et al. [42]	
Mauritania	-	-	0.210 ± 0.054	-	-	35.712 ± 4.665	Legraa et al. [43]	
Venezuela	-	5.76 ± 2.68	9.76 ± 5.39	-	-	167.75 ± 149.58	Castillo et al. [44]	

\*Dry weight results were converted to wet weight estimating 70% water content [32]

\*\*The authors did not present the results of the minimum and maximum concentrations of the three areas separately

[38], when assessing Cr in *P. perna* sampled from four different locations throughout the Rio de Janeiro coast, observed that the mean concentrations exceeded the tolerable limit for human consumption set by ANVISA for this element, even in less impacted areas. Similar results were reported for Santos Bay and Ubatuba Bay, both in the state of São Paulo [12, 61].

With regard to Se, also an essential element, the mean concentration of this element, was very close to the ANVISA [50] limit, with almost half of the samples exceeding this value, indicating potential human health risk concerns. MacFarquhar et al. [8] reported a Se toxicity outbreak in the USA in 2008, associated with a liquid dietary supplement, resulting in diarrhea, nausea, tiredness, hair loss, headaches and joints, nail discoloration or fragility, skin rashes, and bad breath, and some symptoms persisted for 90 days or more. Thus, concerns regarding Se content in the *P. perna* mussels analyzed herein are noted.

Arsenic is a non-essential potentially toxic metalloid [3, 62], depending on its environmental speciation. The International Agency for Research on Cancer (IARC) [63] in France classifies As as Group 1 carcinogen for humans, affecting the lungs, bladder, and skin. Although few samples presented higher concentrations than that established by the Brazilian agency for this element, this result cannot be neglected. Considering the maximum tolerable level of the Chinese agency, 80% of the samples would exceed the limit.

High As and Se concentrations were also identified in *P. perna* samples from three different Guanabara Bay areas, in addition to Ilha Grande Bay and coastal regions of São Paulo State [11, 64]. Barbosa et al. [13] also detected As and Se concentrations above ANVISA limits in different bivalves such as *Anomalocardia brasiliensis* (Gmelin, 1791), *Iphigenia brasiliensis* (Lamarck, 1818), *Lucina pectinata* (Gmelin, 1791), and *Trachycardium muricatum* (Linnaeus, 1758) from Todos os Santos Bay, Bahia. In Brazil, however, As and Se evaluations in bivalves are less frequent, and a lack of data concerning these elements, especially in *P. perna*, is noted.

Zinc concentrations in this study were within the levels permitted by the Brazilian agency, as observed in the other studies carried out at Guanabara Bay for the same species [38, 39]. For the American agency, which sets a more restricted limit for this element, mussel consumption would not be considered safe as some samples were above the established limit and excess Zn consumption in the long term may lead to anemia, leukopenia, and neutropenia in humans [65, 66].

Cd, Cu, Hg, Ni, and Pb do not seem to be worrying pollutants for the investigated area, since the detected concentrations in the *P. perna* samples were well below the maximum tolerable values. Further studies employing higher sample sizes shall be performed to better evaluate the risks associated to mussel consumption in the study area.

**Table 4** Mean concentrations of elements in the adductor muscle of *Perna perna* mussel samples from Guanabara Bay, Rio de Janeiro, Brazil in comparison with Brazilian and international legislations (mg kg<sup>-1</sup> wet weight)

	Mean	ANVISA [50]	ANVISA [51]	EC [52]	FAO/WHO [53]	FSANZ [54]	MAFF [55]	MHPRC [56]	USEPA [57]
Ag	0.01	-	-	-	-	-	-	-	-
Al	0.698	-	-	-	-	-	-	-	-
As	0.69	1.00	1.00	-	-	1.00	-	0.50	-
Cd	0.005	1.00	2.00	1.00	1.00	2.00	1.00	2.00	2.00
Co	0.025	-	-	-	-	-	-	-	-
Cr	0.68	0.10	-	-	-	-	20.00*	2.00	-
Cu	0.48	30.00	-	-	-	-	-	-	20.00
Fe	6.12	-	-	-	-	-	-	-	-
Hg	0.024	0.50	0.50	0.50	1.00	0.50	0.30	0.50	-
Mn	2.35	-	-	-	-	-	-	-	-
Ni	0.172	5.00	-	-	-	-	-	-	-
Pb	0.032	2.00	1.50	1.50	-	2.00	5.00	1.50	0.80
Se	0.284	0.30	-	-	-	-	-	-	-
V	0.164	-	-	-	-	-	-	-	-
Zn	17.00	50.00	-	-	-	-	50.00*	-	30.00

ANVISA Brazilian Health Regulatory Agency, EC European communities, FAO/WHO Food and Agriculture Organization of the United Nations/World Health Organization, FSANZ Food Standards Australia New Zealand, MAFF Ministry of Agriculture, Forestry and Fisheries (Japan), MHPRC Ministry of Health of the People's Republic of China, USEPA United States Environmental Protection

\*Higher levels in shellfish are permitted if Cu and Zn are of natural occurrence, reaching up to 500 mg kg<sup>-1</sup> and 100 mg kg<sup>-1</sup> of wet weight, respectively

Mussels are usually ingested whole. In this study, only the adductor muscle of the animal was analyzed, and, even so, concentrations higher than the levels allowed by ANVISA were found. Although a dilution effect is observed in some shellfishes when entire tissue is analyzed, higher concentrations could potentially be detected when analyzing the entire animals from our study area. Therefore, *P. perna* consumption in the investigated area could result in public health concerns for some of the evaluated elements. Taking this into account, the Provisional Tolerable Weekly Intake (PTWI), comprising the calculation of the amount of a specific food item that can be safely consumed without risks, was also calculated for each element, as described in the Material and Methods section and compared to the reference values proposed by the World Health Organization (WHO) (Table 5).

In the present study, the estimated daily dietary intake values in *P. perna* adductor muscle were not above any of the limits imposed by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) for any of the assessed elements. It is important to note, however, that only the adductor muscle was assessed herein. In addition, certain populations, such as fishers, consume significantly high amounts of their catch items, thus becoming highly vulnerable to environmental degradation and contamination [72]. In fact, these populations are considered vulnerable by the Brazilian Ministry of the Environment [73, 74]. Therefore, continuous biomonitoring of *P. perna* mussels at the study area is recommended, especially when taking into

**Table 5** Estimated Provisional Tolerable Weekly Intake (PTWI) of *Perna perna* mussel samples from Guanabara Bay, Rio de Janeiro, Brazil compared with recommended PTWI from FAO/WHO (µg kg<sup>-1</sup> body weight)

Element	Mean PTWI	Worst-case PTWI	FAO/WHO PTWI
Ag	0.07	0.13	-
Al	7.77	14.06	2000 <sup>c</sup>
As	1.71	3.47	Withdrawn*
Cd	0.04	0.06	7 <sup>a</sup>
Co	0.06	0.20	700 <sup>d</sup>
Cr	3.45	5.37	-
Cu	1.31	4.52	3500 <sup>a</sup>
Fe	16.64	50.76	5600 <sup>a</sup>
Hg	0.07	0.10	1.6 (MeHg) <sup>a</sup>
Mn	5.83	31.38	980 <sup>a b</sup>
Ni	1.43	1.52	35 <sup>a b</sup>
Pb	0.08	0.25	Withdrawn*
Se	0.73	1.56	-
V	0.40	0.66	-
Zn	41.47	94.41	7000 <sup>a</sup>

<sup>a</sup> FAO/WHO [67]

<sup>b</sup> Calculated for a week

<sup>c</sup> FAO/WHO [68]

<sup>d</sup> EVM [69]

\*Previously established PTWIs for As and Pb of 15 and 25 µg kg<sup>-1</sup> body weight, respectively, were withdrawn by the WHO as they were no longer considered adequate, and new PTWIs have not yet been established [70, 71]



account increasing climate changes, more devastating in coastal areas [75] and comprising accelerating factors regarding metal leaching from soil systems to surface waters [76], and affecting estuaries, in particular, modifying metal bioavailability [77].

## Conclusions

The consumption of *P. perna* mussels in the investigated area seems to indicate potential consumer risks concerning As, Cr, Se, and Zn, since levels exceeded Brazilian and international regulatory agents, even when assessing only adductor muscle samples. Estimated daily dietary intake values, however, were not above any of the limits imposed by the FAO/WHO for the assessed elements. As coastal human populations are expected to consume much more seafood than populations living inland, including mussels, continuous metal monitoring in bivalves in the study region is suggested, in order to assess human health risks, especially when taking into account the increasing anthropogenic activities worldwide, which are altering metal transport and bioavailability, especially in coastal estuaries such as Guanabara Bay.

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**Availability of Data and Material** All data will be made available upon reasonable request.

**Code Availability** Not applicable.

## Declarations

**Ethics Approval** This study was authorized by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA, license no. 68263-1 and 68263-2) and National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, no. A20BC45) and approved by the Animal Ethics Committee of the

Oswaldo Cruz Foundation (CEUA, Fiocruz no. L-008/2018) in accordance with the guidelines of the Brazilian College for Animal Experiments (COBEA).

**Consent to Participate** Not applicable.

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**Conflict of Interest** The authors declare no competing interests.

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