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Research Article

Effect of aquaculture-agriculture sewage on the relation between iron and other trace element content in Venus clam from the coastal lagoons of the Gulf of California

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Abstract

In coastal systems, concentrations of trace metals in filter feeders such as shellfish may be affected by anthropogenic activities, including agriculture and aquaculture. Shellfish are a good source of iron, but can also be a potential source of toxic elements, such as cadmium and lead, when consumed by humans. The objective of this study was to determine the differences in iron, zinc, copper, manganese, nickel, lead, and cadmium levels in tissue of the clam *Chione gnidia* collected from a coastal lagoon influenced by agriculture (Lobos) or aquaculture (Tobari), using an atomic absorption spectrophotometer. The relationship of iron with all other trace elements in these organisms was explored using a generalized linear model (GLM). Iron, copper, manganese, and cadmium concentrations were significantly higher in shellfish collected from the coastal lagoon influenced by agriculture, while nickel was significantly higher in shellfish from the lagoon influenced by aquaculture. In these shellfish, cadmium and lead levels were the factors limiting the weekly intake of clam flesh. The GLM model explained 59 % of the iron concentration in Venus clam, suggesting that this element is directly related to zinc and manganese levels, but inversely related to cadmium content in Venus clam.

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1. Introduction

Malnutrition caused by disease, old age, or starvation is defined as a condition that results from low nutrient levels in food, affecting body composition and, ultimately, leading to impaired physical and mental function, hence fostering the progression of disease (Soeters et al., 2016). Inadequate intake of Trace Elements (TE) is one of the 3 major causes of malnutrition (WHO, 2000). Micronutrient malnutrition should be understood as a nutritional disorder and a major clinical risk factor (Soeters et al., 2016).

Inadequate iron intake is the most common micronutrient deficiency, affecting approximately one third of the population worldwide (Barany et al., 2005). In addition, an insufficient intake of zinc, manganese, and copper in the diet of people in developing countries has led to stunted growth, immunodeficiency, and skin lesions, among other health conditions (Bilandžić et al., 2014). Elements deemed essential, such as iron, zinc, copper, and manganese, are frequently found in diets also containing non-essential elements, such as cadmium and lead. In this context, the low dietary content of essential elements may be associated with increased absorption of non-essential elements (Barany et al., 2005; Mikolić et al., 2016).

Trace elements contained in food may interact with one another, either enhancing or restraining the uptake of essential or non-essential elements in humans; these aspects should be assessed (Freeland-Graves et al., 2015). Shellfish living in coastal lagoons are influenced by human activities that take place upstream (Sarma et al., 2013; Arumugam et al., 2020), such as agriculture and aquaculture (Satheeswaran et al., 2019; Jara-Marini et al., 2020). These activities use chemical supplies to mitigate issues or deficiencies in intensive systems, and may be enriched with trace elements (Ali et al., 2016; Prabhakaran et al., 2017). Agriculture is associated with the use of pesticides and mineral fertilizers such as phosphorite, a compound rich in cadmium and manganese (Mehmood et al., 2009). Lead is associated with the use of lead arsenate as a pesticide, and zinc is a minor constituent of some fungicides (Defarge et al., 2018; Jara-Marini et al., 2020). Emission factors for the Yaqui Valley have been estimated; agriculture emits 2277 to 6164 kg zinc and 10,465 kg copper annually (Jara-Marini et al., 2020). On the other hand, metals such as copper, zinc and nickel can be in feed pellets, feed additives, and fertilizers (Alshahri & Alqahtani, 2015; Ali et al., 2016; Hatje et al., 2016). Environmental loads of 598 ± 74 g copper ha^{-1} $year^{-1}$ and 5080 ± 328 g zinc ha^{-1} $year^{-1}$ from shrimp aquaculture have been estimated in Mexico (León-Cañedo et al., 2017).

Trace elements associated with aquaculture and agriculture add up to other trace elements already present in coastal lagoons where wild shellfish are commercially exploited for human consumption as food. Being filter-feeders, these shellfish accumulate metals in tissues from sources in filtered water (3 - 9 L h^{-1} g dry $mass^{-1}$) (Newell et al., 2005).

Shellfish are major sources of iron and other essential elements such as zinc and manganese, but also of non-essential elements including lead and cadmium, a fact that may limit clam flesh intake. This study evaluates the levels of trace elements in clams from 2 coastal lagoons influenced by agricultural and aquaculture effluents to assess the nutritional value of shellfish in terms of iron content and estimate whether the content of these elements is related to other essential or non-essential elements in these organisms.

2. Materials and methods

2.1 Study area

The semiarid coastal lagoons of Lobos (27.0756° N, -109.5796° W) and Tobari (27.7045° N, -109.4903° W) are located on the central-east coast of the Gulf of California, separated by 43 km (**Figure 1**), and receive inland water from the Yaqui river basin. Lobos and Tobari lagoons receive effluents from agriculture and aquaculture, respectively, both of which are potential sources of trace elements (Jara-Marini et al., 2013; Ruiz-Ruiz, 2017). Both lagoons are permanently connected to the sea through 2 inlets, resulting in continuous circulation induced by semidiurnal mixed tides with a tidal range of 1 m (Jara-Marini et al., 2013; Ruiz-Ruiz, 2017). Lobos lagoon covers a surface area of 101.6 km^2, with an average depth of 1.65 m (Ruiz-Ruiz, 2017), and is affected by agricultural activities. Tobari lagoon has a surface area of 64.2 km^2, an average depth of 1.4 m, with a major axis parallel to the coast (Jara-Marini et al., 2013), and receives effluents from aquaculture farms (**Figure 1**).

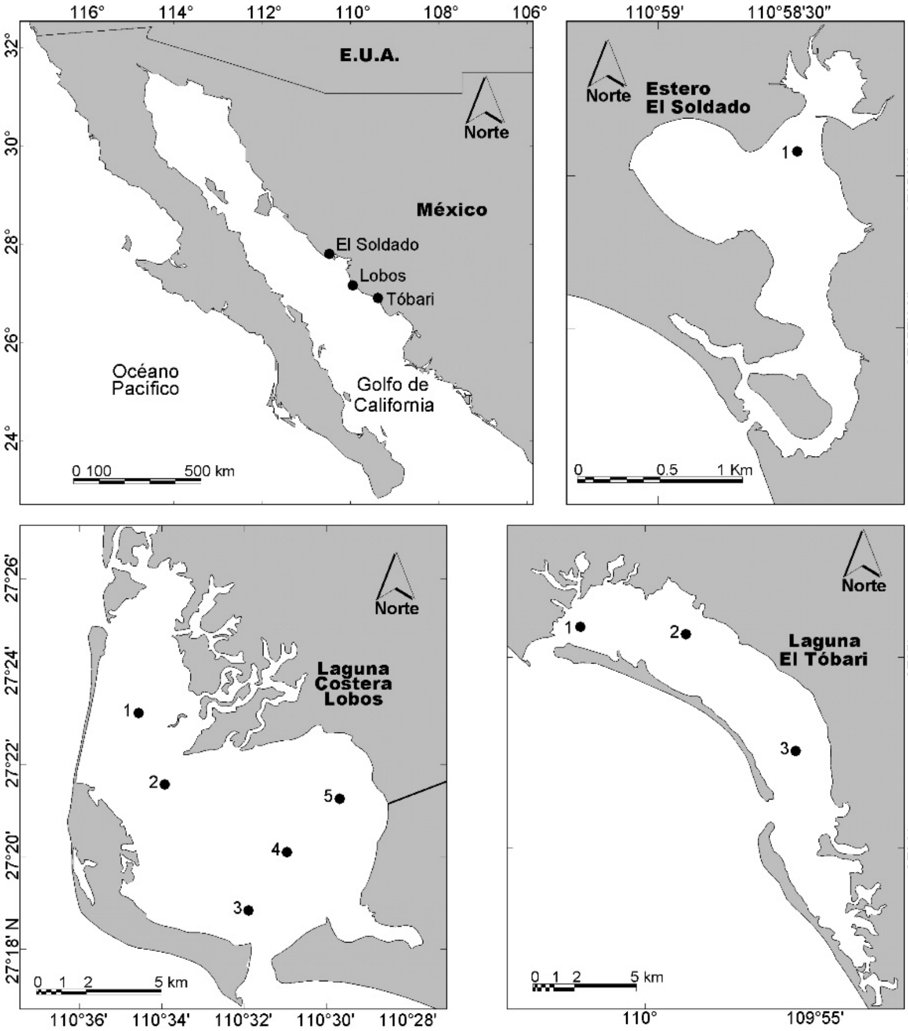

Figure 1 Collection stations of Venus clam, *Chione gnidia*, in Lobos lagoon and Tobari lagoon, Gulf of California, Mexico.

2.2 Trace element extraction and analysis

Five sampling stations were established in Lobos lagoon, and 3 in Tobari lagoon (**Figure 1**), to collect Venus clams, considering that Lobos and Tobari receive effluents from agriculture and aquaculture, respectively; the distance between sampling stations was similar in both lagoons. Ten specimens of Venus clam (*Chione gnidia*) were collected from each station. Shellfish of approximately the same size (shell length: 4.5 ± 0.8 cm; shell width: 49.2 ± 0.9 cm) were collected manually. Samples were placed in plastic bags and transported in a cooler (−4 °C) for laboratory analysis. The soft tissue of each organism was rinsed with Milli-Q water to remove sand and/or foreign particles (Méndez et al., 2002). All 80 samples of Venus clam were subjected separately to acid digestion with a 3:1 ratio of nitric acid and hydrogen peroxide (analytical grade; Mallinckrodt J.T. Baker, USA) in a microwave oven (Mars 5X, CEM; Matthews, USA). One mL of concentrated hydrochloric acid was added to each sample, bringing the volume to 25 mL in a volumetric flask with deionized water. Cadmium, copper, iron, manganese, nickel, lead, and zinc concentrations in each organism were determined using an atomic absorption spectrophotometer (GBC Scientific Equipment model XplorAA, Dandenong, Australia) with an air/acetylene flame (Méndez et al., 2002). In order to avoid possible impurities, all the glassware used for the experiments were drowned in diluted HNO_3 for 24 h and rinsed with distilled water before use, and analytical grade chemical reagents were used for the analysis (Cadena-Cárdenas et al., 2009). A quality control sample was analyzed at an interval of every 10 samples to ensure the quality of analyses in addition to blanks and calibration standard solutions. The standard reference material (TORT-2 National Research Council, Canada) was used to validate the processes and accuracy of the atomic absorption spectrophotometer. Metals recovered from TORT-2 (in parenthesis) were above 95 and 110 %: Fe:105±13(99.75±18); Zn:180±6(191±11); Cu:106±10(104.94±13); Mn:13.6±1.2 (13.05±1.9); Ni:2.50±0.19(2.42±0.21); Cd:26.7±0.6(27.8±0.1) and Pb:0.35±0.13(0.38±0.17). The linearity range (R^2) for all the elements was between 0.9994 and 0.9998. The limits of detection (µg g^{-1}) were 0.02 for cadmium, 0.02 for copper, 0.07 for iron, 0.08 for manganese, 0.03 for nickel, 0.07 for lead, and 0.02 for zinc. Concentrations are reported in µg g^{-1} wet weight.

2.3 Statistical analyses

Before each statistical analysis was run, the data sets were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and Levene tests, respectively. Log-transformation was performed to obtain normal distributions (Zar, 2010). Data were compared through one-way ANOVA to test for significant differences in *Chione gnidia* metal concentrations between sites, followed by the *post-hoc* Tukey's test. Comparisons in the metal content between shellfish from the 2 lagoons (average for shellfish from 3 stations in Tobari lagoon vs average for shellfish from 5 stations in Lobos lagoon) were conducted using the Student's *t* test. Data are shown as mean ± standard error.

In order to identify the variables that make the greatest contribution to the variability of iron content in *C. gnidia*, a generalized linear model (GLM) was performed. The GLM is a flexible multiple linear regression analysis of variables that may or may not be normally distributed. Different distributions of the response variable (iron) were tested. The distribution which yielded the best fit was the normal distribution. The identity function was used for the relationship between the linear predictor and the mean of the distribution function. The variables included in the model were zinc, copper, manganese, nickel, lead, and cadmium as continuous variables, and the coastal lagoon sampled (Tobari or Lobos) was the categorical variable. The models were validated based on the analysis of the residual deviance and the observed and predicted values. Akaike's information criterion (AIC) was used (Akaike, 1973) to identify the model with the best fit to explain iron content in *C. gnidia*. Statistical analyses were performed using Statistica 13.3 (TIBCO software Inc., Tulsa, OK, USA)

2.4 Contribution and safety levels of trace elements

Clam tissue is an excellent source of iron; however, its cadmium and lead content may limit the amount that is safe for human consumption.

The Estimated Daily Intake (*EDI*, mg trace element kg^{-1} BW d^{-1}) was calculated as follows (Bilandžić et al., 2014; Anandkumar et al., 2019):

$$EDI = \frac{C_m \times CR}{BW} \tag{1}$$

where C_m = mean concentration (trace metals) in shellfish tissue, expressed as fresh weight (µg g^{-1}, fw).
CR = Mean *per capita* daily (30 mg d^{-1}) consumption rate of shellfish.

BW = mean body weight in the general population or subpopulation (70.43 kg for men, 62.9 kg for women, and 16 kg for children) (Alemán-Mateo et al., 2006; Guillette et al., 1998).

For each metal studied, the contribution of the mean daily consumption of shellfish to the recommended daily intake (% RDI) was calculated based on the recommended daily intake (RDI) set by the Food and Nutrition Board (2001) for the elements addressed in this work, which are as follows for men, women, and children, respectively: iron (8, 18, and 8 mg d^{-1} person), zinc (11, 8, and 8 mg d^{-1} person), copper (0.9, 0.9, and 0.7 mg d^{-1} person), manganese (2.3, 1.8, and 1.8 mg d^{-1} person). The lack of clear and consistent evidence that nickel plays a beneficial role in human health explains the lack of an RDI for this element (National Academies of Sciences, 2017).

Also, we calculated the contribution of clam consumption relative to the Provisional Tolerable Weekly Intake (PTWI) for each element in *C. gnidia* flesh for men, women, and children. PTWI is defined as the amount of a substance in food or drinking water that can be ingested per week (µg g^{-1} BW) throughout life with no appreciable health risk (Reilly, 2008). The values for the trace elements of interest (µg g^{-1} BW $week^{-1}$) were: 5.6 for iron (JEFCA, 1983); 7.0 for zinc (JECFA, 1982a); 3.5 for copper (JECFA, 1982b); 0.42 for manganese (WHO, 2004); 0.035 for nickel (WHO, 2004); 0.007 for cadmium (JECFA, 2010); and 0.0035 for lead (EFSA, 2010).

3. Results

Significantly lower ($p < 0.05$) iron and manganese concentrations were recorded in stations T1 and T3, both located at the mouth of Tobari lagoon (**Figure 1** and **Table 1**). Of the total number of shellfish analyzed, 20 and 47 % showed cadmium and lead contents below the limit of quantification of the method (cadmium < 0.02 µg g^{-1}; lead < 0.07 µg g^{-1}). The highest manganese and cadmium levels were recorded in stations L3 and L4, which are the closest to an agricultural drain emptying into Lobos lagoon (**Table 1**). Also, in station L4 of Lobos lagoon, shellfish showed significantly higher ($p < 0.05$) levels of lead relative to stations located at the mouth of both lagoons.

Table 1 Concentration, in µg g^{-1}, of trace elements in *Chione gnidia* in Lobos and Tobari coastal lagoons. Different letters indicate significant differences ($p < 0.05$) among stations (8 stations). Asterisks indicate significant differences ($p < 0.05$) between lagoons.

	Iron	**Zinc**	**Copper**	**Manganese**	**Nickel**	**Cadmium**	**Lead**
Stations Lobos lagoon							
L1	145±26.8a	12.9±0.6a	4.8±0.34ab	6.2±0.98a	0.07±0.03a	0.04±0.02a	0.12±0.02a
L2	113±19.2a	10.7±0.6ab	3.0±0.34a	4.6±0.73a	1.61±0.22b	0.27±0.09a	0.44±0.16ab
L3	188±25.0a	9.1±0.3b	9.3±1.68b	65.7±4.7b	0.44±0.11a	2.19±0.35b	0.28±0.13ab
L4	163±45.2a	9.2±0.5b	16.4±4.03b	69.9±6.7b	0.23±0.08a	2.17±0.38b	0.81±0.26b
L5	210±10.6a	11.1±0.3b	3.6±0.17a	5.1±0.2a	0.39±0.09a	0.05±0.02a	0.10±0.02a
Lobos lagoon	164±12*	10.6±0.3	7.42±0.89*	30.3±3.7*	0.55±0.106*	0.94±0.14*	0.35±0.07
Stations Tobari lagoon							
T1	20±2.2b	9.5±0.3b	3.3±0.67a	0.14±0.05c	0.43±0.13a	0.11±0.04a	0.09±0.01a
T2	165±15.4a	11.0±0.5ab	3.8±0.62ab	6.30±0.51a	1.77±0.12b	0.19±0.04a	0.45±0.17ab
T3	39±4.0b	9.6±0.3b	3.1±0.39a	2.11±0.11d	2.23±0.15b	0.53±0.08b	0.68±0.13ab
Tobari lagoon	74±15.34*	10.0±0.3	3.4±1.148*	2.85±4.78*	1.48±0.137*	0.28±0.18*	0.41±0.09
Lobos and Tobari lagoons	130.8±10.5	10.4±0.2	5.9±0.73	20.01±3.27	0.90±0.10	0.69±0.12	0.37±0.06
Certificate Reference Material (TORT-2)	99.75±18	191±11	104.94±13	13.05±1.9	2.42±0.21	27.8±0.1	0.38±0.17

When comparing both coastal lagoons (**Table 1**), shellfish from Lobos contained significantly higher levels of iron ($p = 0.0002$), copper ($p = 0.016$), manganese ($p = 0.0002$), and cadmium ($p = 0.011$), and lower levels of nickel ($p = 0.0001$), versus Tobari. No significant differences ($p > 0.05$) were observed in zinc and lead content.

The contribution of elements from the consumption of this clam also differed between the samples obtained from both lagoons. In Lobos, the consumption of a 30-gram serving of clam flesh (EDI) provides around 60 % of the daily iron requirements (RDI) for men and children, but only about 27 % for women (**Table 2**). Shellfish collected from Tobari deliver almost half of the iron, relative to Lobos. Shellfish from both lagoons are a poor source of zinc, with less than 4 % of the recommended daily intake of this element for men, women, and children. With respect to manganese, shellfish from Lobos lagoon provide twice the daily requirement, while those from Tobari contribute less than 5 % of the RDI. The shellfish from both lagoons did not exceed the Provisional Tolerable Weekly Intake (PTWI) of cadmium and lead for adults, but they did in the case of children (**Table 2**).

Table 2 Contribution of trace elements from the consumption of Venus clams, *Chione gnidia*, in a fishing community. RDI: Recommended Daily Intake (mg day^{-1}); EDI: Estimated Daily Intake (mg trace element kg-1 day-1 fw); %RDI; Mean contribution to RDI; PTWI (Provisional Tolerable Weekly Intake): mg of trace element kg^{-1} BW week^{-1}, considering the mean body weight of the general population: men: 70.43 kg; women: 62.90 kg; children: 16 kg. % EWI: percentage of the Estimated Weekly Intake (mg day^{-1}; EDI×7) met; % PTWI = Mean contribution to PTWI.

Metal		RDI	EDI Lobos	EDI Tobari	%RDI Lobos	%RDI Tobari	PTWI	EWI Lobos	EWI Tobari	%PTWI Lobos	%PTWI Tobari
Iron	Men	8	4.930	2.247	61.619	28.083	394	34.507	15.727	8.758	3.992
	Women	18	4.930	2.247	27.386	12.481	352	34.507	15.727	9.803	4.468
	Children	8	4.930	2.247	61.619	28.083	90	34.507	15.727	38.341	17.474
Zinc	Men	11	0.318	0.301	2.893	2.739	493	2.228	2.109	0.452	0.428
	Women	8	0.318	0.301	3.978	3.766	440	2.228	2.109	0.506	0.479
	Children	8	0.318	0.301	3.978	3.766	112	2.228	2.109	1.989	1.883
Copper	Men	0.9	0.223	0.102	24.725	11.355	246	1.558	0.715	0.633	0.291
	Women	0.9	0.223	0.102	24.725	11.355	220	1.558	0.715	0.708	0.325
	Children	0.7	0.223	0.102	31.789	14.599	56	1.558	0.715	2.782	1.277
Manganese	Men	2.3	0.909	0.086	39.528	3.718	29.6	6.364	0.599	21.500	2.022
	Women	1.8	0.909	0.086	50.508	4.751	26.4	6.364	0.599	24.106	2.268
	Children	1.8	0.909	0.086	50.508	4.751	6.7	6.364	0.599	94.986	8.935
Nickel	Men	-	-	-	-	-	2.46	0.116	0.310	4.696	12.605
	Women	-	-	-	-	-	2.21	0.116	0.310	5.228	14.031
	Children	-	-	-	-	-	0.56	0.116	0.310	20.631	55.371
Cadmium	Men	-	-	-	-	-	0.493	0.198	0.058	40.216	11.750
	Women	-	-	-	-	-	0.441	0.198	0.058	44.958	13.136
	Children	-	-	-	-	-	0.112	0.198	0.058	177.023	51.723
Lead	Men	-	-	-	-	-	0.246	0.073	0.085	29.774	34.663
	Women	-	-	-	-	-	0.221	0.073	0.085	33.142	38.584
	Children	-	-	-	-	-	0.056	0.073	0.085	130.791	152.268

The analysis of the relationship between iron and the content of the other elements tested accounted for 59 % of iron content (**Table 3**). This shows that higher iron levels are associated with higher manganese and zinc levels. The relationship between iron and cadmium concentrations seems to be antagonistic, that is, as iron content increases, cadmium content decreases, and vice versa. However, although the relationship between iron and copper did not reach statistical significance, the model shows a significant inverse relationship between iron and [Copper*Manganese], and a significant direct interaction between iron and [Copper*Cadmium] (**Table 3**).

Table 3 Generalized linear model of iron concentration as a function of trace elements concentrations (µg g^{-1}) in Venus clam *Chione gnidia.*

Model		**Iron**	
Error		Normal	
Link		Identity	
N		80	
Deviance		287151.3	
AIC		903.9	
DF		70	
% explained deviance		59 %	
Level of effect	Estimate	Error Standard	p
Intercept	22.25	51.29	0.66
Zinc	11.08	4.59	0.02
Copper	0.66	4.12	0.87
Manganese	4.33	0.96	0.00
Nickel	12.11	11.43	0.29
Cadmium	-102.60	26.09	0.00
Lead	13.50	19.77	0.49
Copper*Manganese	- 0.39	0.09	0.00
Copper*Cadmium	9.49	1.61	0.00
Lobos vs Tobari	27.50	9.21	0.00

4. Discussion

Mean concentrations of nickel (0.90 ± 0.10), cadmium (0.69 ± 0.12 µg g^{-1}) and lead (0.37 ± 0.06 µg g^{-1}) in clam soft tissue from both coastal lagoons were below the maximum recommended intake in humans (nickel: 80 µg g^{-1}; cadmium: 2.0 µg g^{-1}; lead: 1.7 µg g^{-1}) set by international regulations (FDA, 2007; FAO, 2015). However, 11.2 and 1.25 % of the organisms tested exceeded the limits for cadmium and lead. No organism exceeded the limit for nickel, mentioned above. Also, other studies performed on the east coast of northwestern Mexico in shellfish have reported levels of cadmium and lead over the permissible limits for human consumption and the range recorded for nickel in this study (Méndez et al., 2002; Góngora-Gómez et al., 2018; Sepúlveda et al., 2020). When stations were compared, significantly higher manganese, cadmium, and lead concentrations were recorded at sites that receive agricultural discharges in Lobos lagoon. These discharges carry excess fertilizers that are frequently based on phosphorite, a mineral that contains cadmium and manganese (Mehmood et al., 2009; Arumugam et al., 2020; Jara-Marini et al., 2020). Also, these sites may have received sediments loaded with wastes containing lead from fuel used in vehicles from the 1940s until 1997 (Soto-Jimenez et al., 2008).

Although nickel concentration in shellfish from both lagoons is typical of sites unpolluted by this element (Méndez et al., 2002; Cadena-Cárdenas et al., 2009), this element showed a significantly higher concentration ($p < 0.05$) in shellfish from Tobari lagoon compared to Lobos. This difference may be associated with aquacultural practices, which use various types of inputs, such as flour from plant or animal sources used in the elaboration of feed in the form of pellets or granules (Ali et al., 2016). Animal meals that include crustaceans and mollusks in their manufacture may show a higher nickel content because these organisms tend to accumulate more nickel than fish (Arias et al., 2015). This element may also derive from the plant compost used to fertilize ponds in aquaculture farms (Ali et al., 2016). On the southeast coast of India, increased levels of Ni have been found in sediments of sites influenced by sewage effluents from agricultural, aquaculture, and industrial activities (Satheeswaran et al., 2019).

The FDA considers shellfish as an excellent source of iron; however, iron content varies broadly between shellfish. The baby manila (*Venerupis philippinarum*) contains around 300 $\mu g\ g^{-1}$ fresh weight (approximately 1,500 $\mu g\ g^{-1}$ dry weight) (Lai et al., 2012), being nearly 10-fold higher than the concentration in *C. gnidia*, while the latter provide 7 times as much iron as other shellfish, such as cockle shells (*Arca granosa*, 17.6 ± 2.8 $\mu g\ g^{-1}$) and green mussels (*Perna viridis*, 14.7 ± 3 $\mu g\ g^{-1}$) (Kongkachuichai et al., 2002). Iron levels in *C. gnidia* and other seafood species are similar to levels in other meat sources, such as beef loin (11.6 ± 1.5 $\mu g\ g^{-1}$), and higher than chicken breast (0.4 ± 0.0 $\mu g\ g^{-1}$), pork sausages (1.6 ± 0.4$\mu g\ g^{-1}$), various prawn species such as tiger prawn (0.7 ± 0.1 $\mu g\ g^{-1}$), or marine fish such as mackerel (2.5 ± 0.6 $\mu g\ g^{-1}$), which are used as references for animal sources of iron (Lai et al., 2012).

Shellfish from the 2 lagoons are poor sources of zinc and copper, compared to their iron content (**Table 1**), but they can also be a source of cadmium and lead, both of which are potentially hazardous for human health, especially if we considered that the half-life of the lead in soft tissue of clams is about 2.5 ± 0.7 months (Boisson et al., 1998), and cadmium about 52 days (Hervé-Fernández et al., 2010). The presence of these 2 metals significantly limits the amount of clam that should be consumed as food in order not to exceed the maximum PTWI for cadmium (0.007 $\mu g\ g^{-1}$ BW week^{-1}) and lead (0.0035 $\mu g\ g^{-1}$ BW week^{-1}). Lead is a limiting trace element that restrains to a greater extent the amount of clam tissue to be consumed, followed by cadmium (**Table 2**). For men and women, a shellfish consumption of about 0.70 kg per week does not exceed the 100 % PTWI of lead and cadmium. Taking these limiting elements into account, the amount of *C. gnidia* flesh usually consumed by men and women is below the maximum recommended intake of clam tissue (**Table 2**). In the case of children, the cadmium content associated with the consumption of clam flesh varies between lagoons, with shellfish from Lobos providing 3 times as much cadmium as those from Tobari, exceeding by 77 % the PTWI established for children. As for lead, shellfish from both lagoons exceeded the recommended intake of this element. As children are particularly vulnerable, a more conservative approach needs to be adopted regarding serving sizes. For children living in this area, an average daily consumption not to exceed 20 g of shellfish, equivalent to 120 g week^{-1}, or 1.2 servings, is hereby proposed. The PTWI limit established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for lead prior to 1986 was 0.025 $\mu g\ g^{-1}$ BW week^{-1}. However, JECFA acknowledged that this figure was not protective enough and, on the contrary, may result in a reduction of 3 IQ points in children, as well as in increased systolic blood pressure in adults (Jović and Stanković, 2014). Lead has been considered as the most harmful element to the central nervous system in small children, and to the cardiovascular system in adults, and 3 baseline dietary intake figures (0.63, 1.50, and 0.50 $\mu g\ kg^{-1}$ BW d^{-1}) have been recommended to prevent nephrotoxic and cardiovascular effects in adults, as well as neurodevelopmental effects in children (EFSA, 2010). Based on the above, precaution is advised in setting PTWI figures for children; hence, the PTWI figure recommended from our findings is 0.0035 $\mu g\ g^{-1}$ BW week^{-1}.

Shellfish accumulate from the environment trace elements that are antagonistic between them, e.g., iron, zinc, and manganese decrease cadmium uptake in the body (Barany et al., 2005;

Copes et al., 2008; Freeland-Graves et al., 2015). Therefore, the inverse relationship between iron and cadmium (**Table 3**) is clinically relevant to achieve a balance between nutrition and toxicity. The lack of correlation between iron and copper (**Table 3**) does not necessarily translate into a lack of an association between both elements, as correlations are no evidence of causality; in addition, interactive mechanisms at the molecular level may be complex and involve more than 2 metals (Wang et al., 2012). In fact, it has been suggested that the relationship between iron, zinc, copper, manganese, and cadmium may involve common carriers that control the uptake and transport of metals, called Divalent Metal Transporters (DMT1) (Au et al., 2008). The existence of DMT1 and other carriers could explain not only the significant inverse correlation between iron and cadmium, but also the correlations between iron and zinc, iron, and manganese, and the iron-copper-manganese interactions observed in this study.

Shellfish show a direct relationship between iron and zinc content. In this study, the molar ratio of these elements was around 10:1 (**Table 1**), but other studies have found that a molar ratio of iron to zinc intake of 25:1 indicates antagonism (Lönnerdal, 2000). Zinc may stimulate the presence of metallothioneins - cysteine-rich proteins that possess a sulfhydryl group (Nordberg & Nordberg, 2016), thus favoring higher zinc levels in the body. Zinc is necessary for growth, as it is involved in immune functions and, together with iron, helps fight infections (Black, 2003). These 2 elements share some sites from which both are absorbed, as well as transport mechanisms. When zinc and iron from food reach levels in excess of those that organisms can regulate, an antagonism between them may arise (Sandstrom, 2001); however, this is not the case in the shellfish analyzed in the present study.

5. Conclusions

As filter-feeders, shellfish are highly sensitive to trace elements available in the surrounding environment. The anthropogenic activities that develop in coastal lagoons can influence the metal content in the organisms that live there. In this study, clams associated with agricultural effluents tend to contain a higher amount of iron, copper, manganese, and cadmium. At the same time, those that live in a water body receiving aquaculture effluents show a higher nickel content. The presence of nickel does not influence the levels of iron in Venus clams. Venus clams are a good source of iron, as well as copper, although the presence of cadmium and lead limit the daily consume of clams, especially for children. Therefore, the processes of depuration of shellfishes to reduce the concentration of non-essential elements, and continuous monitoring systems for contaminants, especially in coastal organisms, are necessary to ensure that shellfish consumption is healthy.

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