

Trace metals in bivalves and seagrass collected from Venezuelan coastal sites

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Abstract: The bivalve mollusks *Tivela mactroides* and *Crassostrea rhizophorae* are abundant along the Venezuelan coast and are widely consumed as seafood. The globally important seagrass *Thalassia testudinum* is also abundant in Venezuelan. We report the concentrations of Cd, Cr, Cu, Ni, V and Zn in the soft parts of *Tivela mactroides* and *Thalassia testudinum*, and the concentrations of Cd, Cr, Cu and Ni in the soft parts of *Crassostrea rhizophorae*, in seven Venezuelan coastal sites. Metal concentrations were determined by Inductively Coupled Plasma Optical Emission Spectroscopy. Statistical analysis (one-way ANOVA) indicated that the organisms had different levels of metal bioaccumulation, and might be used as biomonitors for trace metals. Significant site-specific differences were found for the elements Cd, Cr, Ni and V in *Tivela mactroides*; Cd and Cr in *Crassostrea rhizophorae*; and V in *Thalassia testudinum* tissue. Rev. Biol. Trop. 56 (Suppl. 1): 215-222. Epub 2008 May 30.

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Bivalves have been extensively used as bio-indicators to assess the environmental conditions of coastal aquatic environments (Liang *et al.* 2004, Silva *et al.* 2006), since they can accumulate trace metals and other substances. *Tivela mactroides* and *Crassostrea rhizophorae* are two species of bivalves abundant along the Venezuelan coast and widely consumed by local populations. The clam *Tivela mactroides* has been considered to be a potential biomonitor of trace metals in Venezuelan marine ecosystems (LaBrecque *et al.* 2004a, Alfonso *et al.* 2005, Jaffe *et al.* 1995). It is well known that seagrasses are critical to the structure and function of many marine ecosystems (Klumpp *et al.* 1989). Seagrasses provide habitat, sediment stability, nutrients, and a food source. The seagrass, *Posidonia oceanica*, has been studied as a biomarker of

trace metal contamination in various parts of the world, especially the Mediterranean coast (Costantini *et al.* 1991, Catisiki and Panayotidis 1993). The globally important seagrass *T. testudinum* is a climax species (Zieman 1982) very abundant in Venezuelan coastal zones. Along the US Florida coast, *Thalassia testudinum* leaves were used to evaluate the As content in several estuaries (Fourqurean and Cai 1993). In the Laguna Madre of Texas, several studies of the trace metal content in *T. testudinum* and *Halodule wrightii* have been reported (Pulich 1980, Whelan *et al.* 2005). Only a few studies have been reported on trace metals in bivalves and seagrass from Venezuela, however, such studies have not dealt with bioaccumulation variations in different organisms and have mainly been undertaken in very restricted areas and for a limited number of metals.

In this work, we report concentrations of Cd, Cr, Cu, Ni, V and Zn in the soft bodies of *Tivela mactroides* and in the *Thalassia testudinum* tissue, and of Cd, Cr, Cu and Ni in the soft bodies of *Crassostrea rhizophorae*, which were collected at sites along the Venezuelan coast. Seagrass tissue was separated into blades and roots/rhizomes. This dataset is part of an ongoing study to evaluate temporal and spatial variation of potentially toxic metals in bivalves of commercial interest.

MATERIALS AND METHODS

Study sites: This study was carried-out in sampling seven Venezuelan coastal sites (Fig. 1): Los Pozones (10°55'32" N-68°16'42" W), in Golfete de Cuare, State of Falcon; Boca de Aroa (10°45'49" N-68°19'24" W), State of Falcon; Isla Buche (10°32'36" N-66°05'22" W), in Carenero, State of Miranda; Playa Chocolate (10°27'48" N-66°04'59" W), in Higuerote, State of Miranda; El Hatillo (10°08'34" N-65°10'21" W), State of Anzoategui; Pertigalete (10°14'38" N-64°34'35" W), State of Anzoategui and Isla Larga (10°20'28" N-64°19'56" W), in Mochima, State of Cumana. Each sampling site corresponds to an area of 30-60m². Samples of *Tivela mactroidea* were collected at Boca de

Aroa, Playa Chocolate and El Hatillo, while samples of *Crassostrea rhizophorae* at Los Pozones, Isla Buche and Isla Larga. Samples of *Thalassia testudinum* were collected at Isla de Buche, Pertigalete and Isla Larga.

Sampling and sample pretreatment:

The sampling was carried out between January and February during 2005 and 2006. Samples of *Tivela mactroidea* and *Thalassia testudinum* were collected by hand from the marine sediment, while samples of *Crassostrea rhizophorae* were collected using a stainless steel knife. It is well known that the accumulation of heavy metals in bivalves is affected by age, size and weight of the mollusk (Rainbow 2000, Ruelas-Inzunza and Paez-Ozuna 2000). As these factors were not the objective of our study, they were kept as constant as possible, in order to minimize their influence on the measured concentrations. Each *Tivela mactroidea* sample consisted of a group of at least 20 clams 3-4 cm in size, whereas *Crassostrea rhizophorae* sample of a group of at least 15 oysters 6-7 cm in size. Seagrass tissue was separated into leaf blades and roots/rhizomes. Triplicate samples were collected at each sampling site was taken three samples. All samples were rinsed with seawater after collection to remove marine

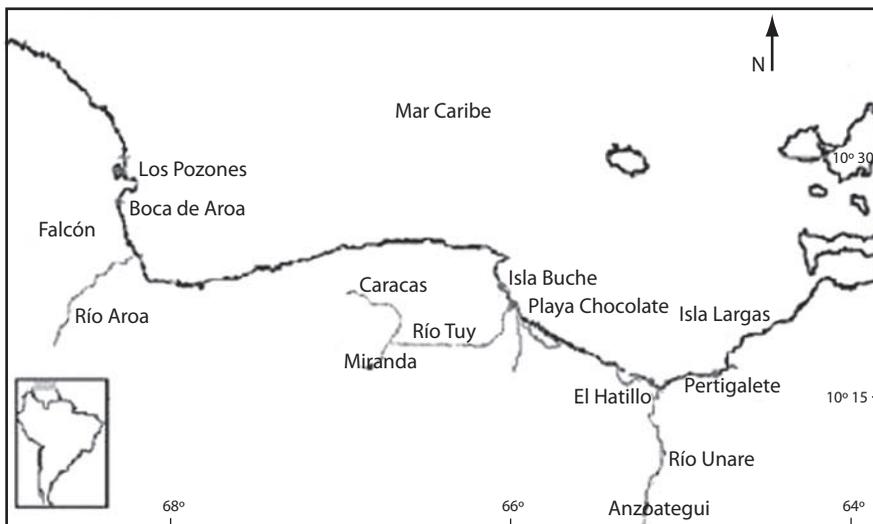


Fig. 1. Sampling sites, states and rivers; Venezuela.

sediments, shells and other debris, placed in plastic bags and transported to the laboratory on ice.

In the laboratory, the complete soft tissue of mollusks was removed from the shell and washed with distilled deionized water to remove marine sediment and others impurities. The bivalves tissues were transferred to clean one-liter beakers and washed several times with distilled deionized water until the solution was clear. After discarding the excess liquid, the beakers were placed in a drying oven at about 65 °C until most of the liquid was evaporated, then the temperature was increased to 80 °C for 24 hours, then cooled in desiccators. Previous studies showed that rinsing seagrasses with distilled water caused premature leaching of metals and other cations (Ledent *et al.* 1995). Thus, seagrass tissues were blotted with paper towels and set out at room temperature until partially dry in the laboratory. Epiphytes were easily removed from the partially dry leaves by scraping. After partial drying at room temperature, all of the tissue was oven dried at 80 °C for 24 h and cooled in desiccators.

The dried tissue samples were ground into a fine powder with an agate mortar and pestle and well mixed to prepare homogenous samples. One gram portions of the dried tissues were dissolved in 8 ml of concentrated super

pure nitric acid (Merck) by heating on a hot plate at about 70 °C for about 8 hours. When the solution cooled, 2 ml of hydrogen peroxide (30 %) (Merck) was added and heated again at 70° C for another 4 hours. Finally, the resulting solution was diluted to 50 ml with distilled deionized water. Duplicate digestions were performed for each sample. Method blanks were always low indicating minimal contamination during sample processing, and they were subtracted from sample readings to give final values of metal concentration.

Chemical analysis: The samples were then analysed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a Perkin Elmer, Optima 3000. Determination of Cd, Cr, Cu, Ni, V and Zn was carried out in the automatic mode, with background correction employing the ICPWinLab Optima 3000 software package. The following wavelengths were used: 226.502 nm for Cd, 205.552 nm for Cr, 324.754 nm for Cu, 231.604 nm for Ni, 292.402 nm for V and 202.548 nm for Zn. A comparison of the trace metals determination using the above described methodology and reported values for mussel tissue reference materials NIST-2977, NIST-2977 and aquatic plant reference material BCR-60 are presented in Tables 1 and 2. It can

TABLE 1

The comparison of the determined mean and standard deviation values (n=6) in mussel tissue standard reference materials NIST-2976 and NIST-2977 in this study with the certified (CV), recommended (RV) and information (IV) values

Element	NIST-2976		NIST-2977	
	Reference material values (µg/g)	This study (µg/g)	Reference material values (µg/g)	This study (µg/g)
Cd	0.82 ± 0.16 (CV)	0.81 ± 0.05	0.179 ± 0.003 (CV)	<dl
Cr	0.50 ± 0.16 (RV)	<dl	3.91 ± 0.47 (RV)	3.92 ± 0.07
Cu	4.02 ± 0.33 (CV)	4.08 ± 0.32	9.42 ± 0.52 (CV)	9.40 ± 0.13
Ni	0.93 ± 0.12 (RV)	<dl	6.06 ± 0.24 (CV)	6.78 ± 0.49
V	-	-	1.1 (IV)	1.06 ± 0.05
Zn	137 ± 13 (CV)	134.8 ± 5.0	135 ± 5 (RV)	129.5 ± 5.8

<dl = below detection limit.

TABLE 2

The comparison of the determined mean and standard deviation values (n=6) in aquatic plant tissue standard reference material BCR-60 in this study with the certified (CV) values

Element	BCR-60 Reference material values (µg/g)	This study (µg/g)
Cd	2.20 ± 0.10 (CV)	2.17 ± 0.12
Cu	51.2 ± 1.9 (CV)	51.3 ± 1.1
Zn	313 ± 8 (CV)	311.5 ± 6.3

be seen that determined values were very similar to the certified and recommended values, thus the method employed (LaBrecque *et al.* 2004b) can be considered accurate. Standard deviations (1σ) of the element determinations (n=6) are small, indicating good precision. The detection limits were determined as three times the standard deviation of the background under the emission lines of the selected trace elements for the blank solution. The detection limits of Cd, Cr, Cu, Ni, V and Zn were 0.79, 0.93, 0.25, 1.1, 0.32 and 0.16 µg/g.

Statistical analysis: A one way ANOVA was performed in order to evaluate the statistical significance of variations between the two sampling times and the sites, for each species and the metals considered. A value of $p < 0.05$ was considered to indicate a significant difference. Statistical analyses were carried out using Statistica 6.0 software (StatSoft, USA).

RESULTS

Results of the one-way ANOVA of comparison between the two sampling times for metal concentrations are presented in Table 3. In all sampling sites, the difference in the mean metal concentrations between the two sampling times was no significant ($p > 0.05$), so a combination of 2005 and 2006 data will be considered.

Mean and standard deviation of the trace metal concentrations in *Tivela mactroides*, *Crassostrea rhizophorae* and *Thalassia testudinum* tissues from the two sampling times are presented in Tables 4, 5 and 6, respectively. Significance levels were fixed at $p < 0.05$ on 95 % confidence level. For each organism studied, mean values of metals with significant concentration differences between the sampling sites are marked by an asterisk.

DISCUSSION

The mean concentrations of Cd, Cr and Ni in *Tivela mactroides* samples were significantly higher in Playa Chocolate. This was not unexpected, considering the fact that this site are located west of the mouth of the Tuy River, which has a plume known to move in a north-westerly direction and receives the waste water effluents from the metropolitan area of Caracas, a city of approximately 6 million inhabitants, via the Guaire River. Most of this waste water undergoes only minor treatment. In addition, the Tuy River drainage basin, of approximately 6600 km², covers an active industrial and agricultural area which also affects water quality (Jaffe *et al.* 1995).

Vanadium in *Tivela mactroides* was significantly higher in El Hatillo. This coastal area contains very extensive petroleum refining and transportation activities and is located near of the mouth of the Unare River, which flows along the one of the most important oil producing areas for Venezuela. Vanadium is typically associated to oil (Macías-Zamora *et al.* 1999; Agusa *et al.* 2004).

The mean concentrations of Cd and Cr in *Crassostrea rhizophorae* samples were significantly higher in Los Pozones. This region is one of the most important tourist (boats and jet skis) zones from Venezuela. Cd and Cr are typically associated to coatings (anti-corrosives), fossil fuel combustion, paints and pigments, and others industrial processes (Siegel 2002).

In contrast to the others elements with significant differences between sites, the difference in the mean concentrations of Cu and

TABLE 3
Results of the one-way ANOVA of comparison between the two sampling times for metal concentrations

Sampling site (organism)	Cd	Cr	Cu	Ni	V	Zn
Boca de Aroa (<i>T. mactroides</i>)	0.062	0.207	0.103	0.189	0.096	0.384
Playa Chocolate (<i>T. mactroides</i>)	0.056	0.162	0.078	0.069	0.281	0.212
El Hatillo (<i>T. mactroides</i>)	0.260	0.399	0.114	0.223	0.192	0.321
Los Pozones (<i>C. rhizophorae</i>)	0.102	0.083	0.219	0.312	-	0.241
Isla Buche (<i>C. rhizophorae</i>)	0.241	0.221	0.384	0.351	-	0.281
Isla larga (<i>C. rhizophorae</i>)	0.401	0.198	0.219	0.254	-	0.199
Isla Buche (root- <i>T. testudinum</i>)	0.111	0.088	0.241	0.165	0.243	0.058
Isla Buche (leaf- <i>T. testudinum</i>)	0.201	-	0.301	0.098	0.261	0.079
Pertigalete (root- <i>T. testudinum</i>)	0.251	0.185	0.152	0.231	0.152	0.066
Pertigalete (leaf- <i>T. testudinum</i>)	0.304	-	0.212	0.259	0.185	0.126
Isla Larga (root- <i>T. testudinum</i>)	0.326	-	0.367	0.127	0.208	0.201
Isla Larga (leaf- <i>T. testudinum</i>)	0.264	-	0.184	0.210	0.136	0.084

Zn in *Tivela mactroides* and Cu and Ni in *Crassostrea rhizophorae*, between the sampling sites where these bivalves were studied is negligible. In contrast to Zn, Ni bioaccumulation was examined in both species of bivalves. Ni concentrations found in *C. rhizophorae* at sampling sites are low compared to those obtained for *T. mactroides*. Some mussel species were found to be able to regulate the level of

these biologically essential elements (Phillips 1985, Malinovskaya and Khristoforova 1997, Gunther *et al.* 1999), if the concentration in the aquatic environment does not exceed certain levels. As a consequence of this effect, a direct relationship between biologically essential element concentrations in the environments, on the one hand, and in the biomonitors, on the other hand, would be impossible to observe.

TABLE 4
Mean concentration of metals in Tivela mactroides (standard deviation in brackets)

Sampling site	Cd ($\mu\text{g/g}$)	Cr ($\mu\text{g/g}$)	Cu ($\mu\text{g/g}$)	Ni ($\mu\text{g/g}$)	V ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)
Boca de Aroa	3.67 (0.13)	2.51 (0.16)	26.32 (1.32)	11.63 (0.28)	1.31 (0.09)	78.13 (9.7)
Playa Chocolate	4.39* (0.28)	3.67* (0.17)	26.20 (1.35)	13.26* (0.33)	1.21 (0.07)	87.89 (7.3)
El Hatillo	3.68 (0.16)	2.69 (0.11)	24.70 (2.32)	11.75 (0.37)	4.03* (0.14)	80.62 (6.8)

* = mean values of elements with significant concentration differences between the sampling sites.

TABLE 5
Mean concentration of metals in Crassostrea rhizophorae (standard deviation in brackets)

Sampling site	Cd ($\mu\text{g/g}$)	Cr ($\mu\text{g/g}$)	Cu ($\mu\text{g/g}$)	Ni ($\mu\text{g/g}$)
Los Pozones	2.5* (0.6)	2.4* (0.5)	38 (4.73)	1.70 (0.38)
Isla Buche	1.0 (0.1)	1.7 (0.3)	40 (4.67)	1.51 (0.09)
Isla larga	1.6 (0.2)	1.8 (0.6)	35 (4.51)	1.49 (0.20)

* = mean values of elements with significant concentration differences between the sampling sites.

TABLE 6
Mean concentration of metals in Thalassia testudinum (standard deviation in brackets)

Sampling site		Cd ($\mu\text{g/g}$)	Cr ($\mu\text{g/g}$)	Cu ($\mu\text{g/g}$)	Ni ($\mu\text{g/g}$)	V ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)
Isla Buche	root-rhizome	1.52 (0.53)	2.14 (0.30)	5.86 (0.48)	4.62 (2.53)	6.51 (0.25)	25.11 (5.93)
	leaf	1.79 (0.89)	<dl	3.80 (2.61)	3.88 (0.25)	1.60 (0.61)	16.11 (3.48)
Pertigalete	root-rhizome	1.49 (0.24)	1.81 (0.48)	4.73 (1.28)	4.27 (0.18)	15.14* (1.65)	32.47 (4.68)
	leaf	1.6 (0.37)	<dl	6.01 (1.62)	4.83 (2.34)	2.20 (0.81)	14.07 (2.14)
Isla Larga	root-rhizome	1.70 (0.40)	<dl	6.26 (2.22)	3.14 (1.17)	4.49 (0.15)	32.58 (7.02)
	leaf	1.62 (0.26)	<dl	5.41 (0.52)	2.80 (1.44)	1.09 (0.92)	12.31 (3.10)

* = mean values of elements with significant concentration differences between the sampling sites.

<dl = below detection limit.

At Pertigalete, the mean concentration of V in the seagrass root/rhizome tissue was significantly higher and the seagrass leaf tissue contained the highest values for this metal of the three sites where the seagrass was studied. This coastal area contains very extensive petroleum refining and transportation activities, and so, was not unexpected the higher value of V. Thus, the present results are consistent with the use of V as possible marker for oil transportation and exploitation activities. The values of the other metals studied did not show significant differences between leaf and root/rhizome tissue.

T. testudinum is complex in trace metal distribution and appears to vary in concentration for each metal and morphological unit. These results suggest that if *T. testudinum* is to be used as a biomonitor, care must be taken to analyze the morphological units. The variability in trace metal content between the morphological units is highly dependent upon the metal in question. For example, V and Zn concentrations were found to be highest in root/rhizome tissue by a factor of 2 to 7 compared to leaf tissues (Table 6).

The results of this study indicate that organisms considered selectively can accumulate certain elements. This selectivity is the most important criterion in choosing organisms as biomonitors (Duquesne *et al.* 2000). Sampling of *Tivela mactroides*, *Crassostrea rhizophorae* and *Thalassia testudinum* at sites along the Venezuelan coast revealed significant site-specific concentration differences for the elements Cd, Cr, Ni and V in *Tivela mactroides* tissue, Cd and Cr in *Crassostrea rhizophorae* tissue, and V in *Thalassia testudinum* tissue.

The mean concentrations in soft *Tivela mactroides* tissue found in Playa Chocolate are within the range of values determined in previous works for the same species of bivalve at the same sampling area (LaBrecque *et al.* 2004a, Alfonso *et al.* 2005). No known values have been reported for trace metals for *Crassostrea rhizophorae* and *Thalassia testudinum* in Venezuela.

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RESUMEN

Tivela mactroides y *Crassostrea rhizophorae* son dos especies de bivalvos abundantes a lo largo de la costa venezolana y de alta demanda local como mariscos. La hierba marina *Thalassia testudinum*, de interés en diferentes partes del mundo, es muy abundante en zonas costeras de Venezuela. Aquí informamos las concentraciones de Cd, Cr, Cu, Ni, V y Zn en muestras de partes blandas de *Tivela mactroides* y *Crassostrea rhizophorae* y de tejidos de *Thalassia testudinum*, recolectadas a lo largo de la costa venezolana. Estas concentraciones metálicas fueron determinadas utilizando espectrometría de emisión óptica con plasma inductivamente acoplado (ICP-OES). Los análisis estadísticos (ANDEVA de una vía) indicaron que las especies examinadas presentan características diferentes frente a la bioacumulación de metales y podrían ser utilizadas como potenciales indicadores para investigar la contaminación por metales trazas.

Palabras clave: bioacumulación metálica, *Tivela mactroides*, *Crassostrea rhizophorae*, *Thalassia testudinum*, bivalvos, hierba marina.

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