

## Heavy Metals in Green Mussel (*Perna viridis*) and Oysters (*Crassostrea* sp.) from Trinidad and Venezuela

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**Abstract.** Heavy metal concentrations were monitored in edible soft tissues of shellfish from Trinidad and Venezuela. Oysters (*Crassostrea* sp.) and the green mussel (*Perna viridis*), which is a recently transplanted species to the Caribbean from the Far East, were collected at six locations in Venezuela and five in Trinidad, the latter along the coast line of the Gulf of Paria. Simple and low-cost methods of analysis were optimized and validated using standard reference materials. Cadmium, copper, lead, nickel, and zinc were analyzed by flame atomic absorption spectrometry. Mercury was determined by cold-vapor atomic absorption spectrometry. The present study has confirmed that oysters have a much greater capacity for accumulation of copper and zinc than does green mussel. In addition, concentrations of copper and zinc in oysters (*Crassostrea* sp.) at many of the sites in the Gulf of Paria exceeded local and international standards, whereas green mussel *P. viridis* contained generally acceptable levels for human consumption.

Fish and shellfish are important sources of dietary protein in Trinidad and neighboring Venezuela and provide a livelihood for many coastal communities. Although surveys of contaminant levels in edible species in these countries have been reported (Chopite *et al.* 1988; Singh *et al.* 1991, Singh *et al.* 1992; Guppy *et al.* 1994; Chang-Yen *et al.* 1999), no recent follow-up study has been done. The recent adoption of Hazard Analysis Critical Control Point (HACCP) procedures (FDA 1997) by local health authorities requires frequent monitoring of the quality of fish and shellfish offered for human consumption. To this end, local health regulations have been updated and enacted in Trinidad and Tobago under the Food and Drugs Act (1998), and World Health Organization standards are used in Venezuela (WHO 1982).

Though oysters have been harvested from local coastal waters for many years, the green mussel (*Perna viridis*) is a recently

introduced (possibly by international shipping) edible species from the Far East. First discovered in Trinidad in 1990 at the port of Point Lisas (Agard *et al.* 1992), it has proliferated and spread to Venezuela (Rylander *et al.* 1996) and has recently been reported in Jamaica and Miami. *P. viridis* has been found to grow at rates up to 9 cm shell length/annum in the Gulf of Paria (Phillip and Rooks 2001) and offers outstanding commercial potential because it is already being widely consumed (Siung-Chang 1994; Rylander *et al.* 1996).

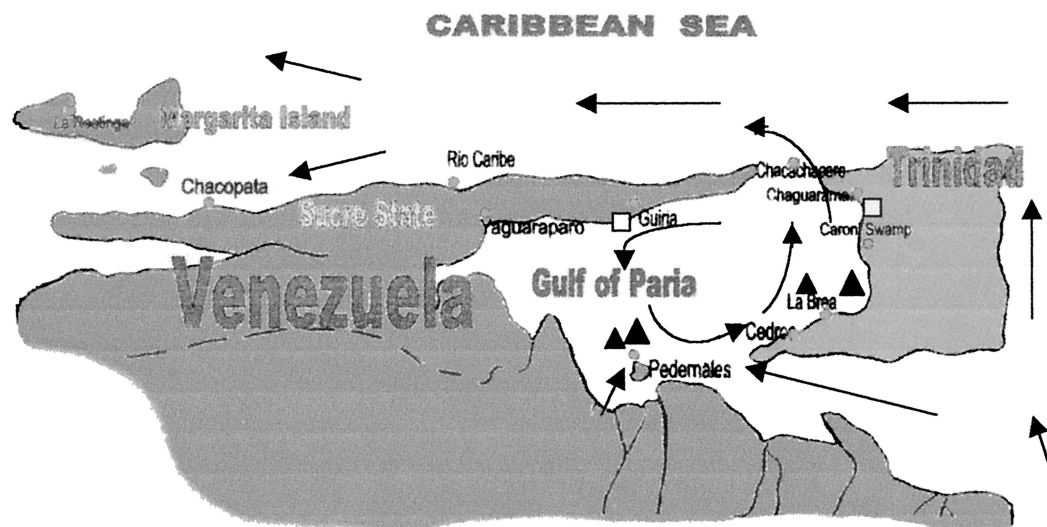
In addition, marine biota are being investigated as possible monitors of the coastal environment along the Gulf of Paria for heavy metals and other pollutants, which are regularly discharged from domestic and industrial sources. *P. viridis* and oysters (*Crassostrea* sp.) have been effectively used in this regard, due to their sedentary way of life and ability to accumulate a wide range of pollutants in proportion to the degree of environmental contamination (Goldberg *et al.* 1978; Martincic *et al.* 1984; Phillips 1985, 1991; Phillips and Segar 1986; Scanes 1996; Beliaeff *et al.* 1998; Ruangwises and Ruangwises 1998). However, little data on pollutants in shellfish are available in Trinidad and Venezuela, necessitating the generation of baseline data in these countries.

In this study, two species of oysters (*Crassostrea rizophorae* and *Crassostrea virginica*) and the green mussel *P. viridis*, common to both Trinidad and Venezuela, were collected along the coast of the Gulf of Paria, which is bordered by both countries (Figure 1). Oysters and mussels were also collected along the northern coastline (64°15'–63°06' N latitude, 10°42'–11°02' W longitude) of Venezuela for comparison of their heavy metal levels, with those in the Gulf of Paria (63°12'–61°30' N latitude, 9°57'–10°36' W longitude).

### Materials and Methods

#### Sampling

The samples were collected in Venezuela at six locations (Figure 1), three in the Gulf of Paria and three on the north coast of Venezuela. All five sample sites in Trinidad were located in the



- ▲ Petroleum Production + Refinery
- Industrial States/Port Activities
- Sampling Site
- ▶ Current flow

Fig. 1. Study area showing the location of sampling sites in Trinidad and Venezuela

Gulf of Paria, which has a mean current pattern in a cyclical gyre (Gopaul and Wolf 1996). Details about species collected at each location are shown in Table 1.

All sampling was carried out between June and December 1999. Shellfish were washed externally with a brush and distilled water to minimize contamination of tissue and then stored frozen until analysis was undertaken.

Identification of the different species of oysters was done at the Universidad de Oriente, Venezuela, in conjunction with a catalog of marine mollusks from the coastline of Venezuela (Lodeiros *et al.* 1999).

#### Sample Preparation for Chemical Analyses

*Determinations of Cadmium, Chromium, Copper, Lead, Nickel, and Zinc.* The whole soft tissues of 12 mussels of a similar shell length (9–12 cm) from each location were removed from their shells and weighed in Pyrex beakers (which had been washed in detergent, rinsed in distilled water, and then soaked in 5% nitric acid) and their wet weights recorded. Prior to removal of their tissue, byssal threads were removed.

Each composite sample was blended to produce a homogenized mixture. Three replicates of approximately 5 g of each slurry were weighed into boiling tubes and dried at 105°C overnight. Concentrated analytical-grade nitric acid (10 ml) was added to each sample and left to predigest overnight at room temperature in a fume hood. Reagent blanks were processed simultaneously. The boiling tubes were placed on a heating block to reflux at 130°C for 6 h. After cooling, the digests were diluted with 5 ml deionized water and filtered through Whatman # 542 filters, into 25-ml volumetric flasks. Each solution was made up to volume with deionized water rinses of the residues. The prepared solutions were analyzed for cadmium, chromium, copper, lead, nickel,

Table 1. Shellfish species collected at each location in Trinidad and Venezuela

Location	Species		
	<i>Perna viridis</i>	<i>Crassostrea rizophorae</i>	<i>Crassostrea virginica</i>
Venezuela			
La Restinga			
Margarita Island	X	X	
Chacopata	X	X	
Rio Caribe	X		
Guiria	X		X
Yaguaraparo			X
Pedernales			X
Trinidad			
Chacachacare			X
Chaguaramas	X	X	
Caroni	X	X	
La Brea	X	X	
Cedros	X	X	

and zinc, by flame atomic absorption spectroscopy with deuterium continuum background correction. In-date commercial metal calibration standards (BDH, Poole, UK) were used to prepare fresh calibration standards daily. A Varian Model SpectraAA-300 was used for all analyses.

Oyster tissues were analyzed by the same procedure, using oysters of 4–10 cm shell length.

*Determination of Mercury.* Samples were prepared for mercury analysis using a modification of the method of Singh (1988). Triplicate 5-g

**Table 2.** Recoveries of metals from certified reference materials

Element	% Recovery		
	SRM 1566a (NIST) Oyster	DORM-2 (NRC) Dogfish Muscle	TORT-2 (NRC) Lobster Hepatopancreas
Cadmium	107.3 ± 1.2	ND	95.1 ± 2.2
Chromium	109.1 ± 4.3	94.6 ± 2.3	111 ± 2.7
Copper	94.5 ± 1.0	104 ± 0.3	100 ± 1.6
Mercury	107.0 ± 5.1	97.6 ± 2.4	99.6 ± 0.7
Nickel	103 ± 1.4	91.7 ± 0.9	94.4 ± 1.8
Zinc	98.6 ± 1.0	98.8 ± 0.8	99.4 ± 2.1

ND: Not detected (< 0.1 µg g<sup>-1</sup> dry weight for 0.5-g sample).

**Table 3.** Mean concentrations ± SEs of heavy metals in green mussels (*Perna viridis*) from Trinidad and Venezuela

Location	Concentration (µg g <sup>-1</sup> wet weight)					
	Cd	Cu	Cr	Hg	Ni	Zn
Margarita	0.02 ± 0.00	1.68 ± 0.13	0.12 ± 0.2	0.022 ± 0.00	1.00 ± 0.06	10.69 ± 0.47
Chacopata	0.03 ± 0.00	1.55 ± 0.04	0.15 ± 0.01	0.021 ± 0.00	0.22 ± 0.07	8.77 ± 0.63
Rio Caribe	0.03 ± 0.00	1.42 ± 0.05	0.13 ± 0.00	0.02 ± 0.00	0.48 ± 0.01	8.75 ± 0.16
Guiria	0.05 ± 0.02	3.43 ± 0.07	0.16 ± 0.03	0.08 ± 0.01	1.30 ± 0.03	16.38 ± 0.54
Chaguaramas	0.53 ± 0.10	1.02 ± 0.03	0.11 ± 0.01	0.07 ± 0.01	0.53 ± 0.13	13.63 ± 0.72
Caroni	< 0.01	1.43 ± 0.01	0.09 ± 0.01	0.04 ± 0.00	0.75 ± 0.02	89.23 ± 8.9*
La Brea	0.61 ± 0.08	1.98 ± 0.01	0.06 ± 0.01	0.17 ± 0.02	0.61 ± 0.08	11.30 ± 2.27
Cedros	< 0.01	1.35 ± 0.00	0.20 ± 0.01	0.03 ± 0.00	0.30 ± 0.01	40.37 ± 3.92

Exceeds maximum permissible level of 50 µg g<sup>-1</sup> Zn wet weight.

aliquots of oyster and mussel macerates were weighed into 50-ml conical flasks. To each macerate, concentrated nitric acid (10 ml) was added and left to predigest overnight at room temperature in a fume hood. A small glass funnel was placed in the mouth of each conical flask to prevent loss of samples during digestion and the samples refluxed on a hot plate at 130°C for 3 h. Concentrated sulfuric acid (2.5 ml) and concentrated hydrochloric acid (1.0 ml) were then added to each sample, which were allowed to reflux at 130°C for 3 h more. Potassium manganate (VII) solution (5% w/v) was added drop-wise to each sample until a permanent pink coloration resulted. Hydroxylamine solution (50 µl, 10% w/v) was added to destroy the excess potassium manganate (VII). The digests were filtered into 25-ml volumetric flasks, through Whatman 542 filters, and made up to volume with deionized water rinses of the residues. Mercury in mussels and oysters was determined by cold-vapor atomic absorption.

Methods were validated with certified reference materials (CRMs) namely, SRM 1566a oyster tissue (National Institute of Standards and Technology, MD) and lobster hepatopancreas (TORT-2) and dogfish muscle (DORM-2) from the National Research Council of Canada (Nova Scotia, Canada). Five 0.5–0.7-g replicates of each CRM were analyzed for Cd, Cr, Cu, Hg, Ni, and Zn. A sample of each of these CRMs was subsequently analyzed with each batch of samples to monitor the quality of the analyses.

### Statistical Analysis

Parametric and nonparametric statistical methods were used for data analysis using Minitab Statistical Software (Minitab Statistical Software 2000).

## Results and Discussion

The results of analysis of the oyster, dogfish muscle, and lobster hepatopancreas CRMs agreed well with certified values, with recoveries ranging from 92% to 111% for the six metals studied (Table 2). Our optimized method for all heavy metals (except mercury), using overnight predigestion in 10 ml of HNO<sub>3</sub> per 5 g wet sample followed by a 6-h digestion time, allowed for large batches of samples (up to 20 in triplicate) to be processed at a time. For mercury, the addition of sulfuric and hydrochloric acids after predigestion with nitric acid prevented sample charring and resulted in faster and more efficient sample preparation.

In none of the samples of CRM, oysters, or mussels was lead detected (< 0.05 µg g<sup>-1</sup> wet weight). However, the use of 5-g aliquots of sample, as opposed to 0.5 g used for CRMs, allowed the detection of metals in samples, *e.g.*, Cd, at levels 10× lower than in CRM. Table 3 shows that the highest level of copper in green mussels was 3.43 µg g<sup>-1</sup>, from Guiria in the Gulf of Paria in Venezuela. With the exception of the mussel samples from Caroni in Trinidad, where the concentration of zinc was 89.23 µg g<sup>-1</sup>, the concentrations of heavy metals in *P. viridis* did not exceed the maximum permissible levels (MPLs) of > 50 µg g<sup>-1</sup> wet weight defined by the Food and Drug Act of Trinidad and Tobago (1998) and WHO (1982).

For oysters, Table 4 shows that the highest concentrations of cadmium, chromium, and nickel were found in oysters from Cedros, whereas those with the highest mercury concentrations were from Chaguaramas, both sites located in the Gulf of Paria in Trinidad. Fortunately, none exceeded the MPL for human

**Table 4.** Mean concentrations  $\pm$  SEs of heavy metals in oysters (*Crassostrea* sp.) from Trinidad and Venezuela

Location	Concentration ( $\mu\text{g g}^{-1}$ wet weight)					
	Cd	Cu	Cr	Hg	Ni	Zn
Margarita						
<i>C. rhizophorae</i>	< 0.01	3.28 $\pm$ 0.03	< 0.02	0.02 $\pm$ 0.00	0.14 $\pm$ 0.02	24.87 $\pm$ 0.66
Chacopata						
<i>C. rhizophorae</i>	0.22 $\pm$ 0.01	1.38 $\pm$ 0.02	0.08 $\pm$ 0.01	0.05 $\pm$ 0.00	< 0.01	40.00 $\pm$ 0.22
Guiria						
<i>C. virginica</i>	0.08 $\pm$ 0.02	36.11 $\pm$ 0.03*	0.08 $\pm$ 0.01	0.01 $\pm$ 0.00	0.18 $\pm$ 0.04	776.59 $\pm$ 0.92*
Yaguaraparo						
<i>C. virginica</i>	0.03 $\pm$ 0.00	11.96 $\pm$ 0.05	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.06 $\pm$ 0.01	168.20 $\pm$ 8.50*
Pedernales						
<i>C. virginica</i>	0.04 $\pm$ 0.00	26.86 $\pm$ 0.57*	0.10 $\pm$ 0.01	0.05 $\pm$ 0.01	0.09 $\pm$ 0.01	505.03 $\pm$ 41.37*
Chacachacare						
<i>C. virginica</i>	< 0.01	13.76 $\pm$ 0.41	0.13 $\pm$ 0.02	0.04 $\pm$ 0.01	0.25 $\pm$ 0.07	210.58 $\pm$ 7.12*
Chaguaramas						
<i>C. rhizophorae</i>	0.22 $\pm$ 0.06	28.12 $\pm$ 0.04*	0.10 $\pm$ 0.01	0.08 $\pm$ 0.00	0.20 $\pm$ 0.06	540.12 $\pm$ 14.05*
Caroni						
<i>C. rhizophorae</i>	< 0.01	5.73 $\pm$ 0.07	0.10 $\pm$ 0.01	0.02 $\pm$ 0.00	0.09 $\pm$ 0.01	138.47 $\pm$ 10.23*
La Brea						
<i>C. rhizophorae</i>	0.52 $\pm$ 0.01	52.10 $\pm$ 0.93*	0.64 $\pm$ 0.01	0.07 $\pm$ 0.00	0.31 $\pm$ 0.02	416.42 $\pm$ 8.27*
Cedros						
<i>C. rhizophorae</i>	0.53 $\pm$ 0.02	33.32 $\pm$ 0.80*	1.23 $\pm$ 0.02	0.04 $\pm$ 0.00	0.48 $\pm$ 0.04	289.18 $\pm$ 21.28*

\* Exceeds maximum permissible levels (Cu 20; Zn 50  $\mu\text{g g}^{-1}$  wet weight).

**Table 5.** Comparison of mean heavy metal concentrations  $\pm$  SEs in green mussel (*Perna viridis*) from the Gulf of Paria (Trinidad and Venezuela) and the north coast of Venezuela

Location	Concentration + SE ( $\mu\text{g g}^{-1}$ wet weight)					
	Cd*	Cr <sup>ns</sup>	Cu <sup>ns</sup>	Ni <sup>ns</sup>	Hg <sup>ns</sup>	Zn**
North coast	0.02 $\pm$ 0.01	0.13 $\pm$ 0.02	1.61 $\pm$ 0.19	0.61 $\pm$ 0.36	0.02 $\pm$ 0.00	9.73 $\pm$ 1.19
Gulf of Paria	0.24 $\pm$ 0.30	0.13 $\pm$ 0.06	1.84 $\pm$ 0.89	0.70 $\pm$ 0.36	0.08 $\pm$ 0.10	34.1 $\pm$ 30.7

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

NS:  $p > 0.05$ .

consumption. The highest levels of copper (52.10  $\mu\text{g g}^{-1}$ ) in oysters were found in Cedros, Trinidad, and zinc was highest (776.59  $\mu\text{g g}^{-1}$ ) in those from Guiria, Venezuela, again both sites in the Gulf of Paria.

In oysters, the concentrations of copper at five sites and zinc at all sites in the Gulf of Paria exceeded the MPL of 20  $\mu\text{g g}^{-1}$  Copper and 50  $\mu\text{g g}^{-1}$  Zinc. These results strongly suggest the need for regular monitoring and control of shellfish harvested for human consumption in both Trinidad and Venezuela in the Gulf of Paria. The clockwise circulatory current pattern identified in the Gulf (Gopaul and Wolf 1996) is likely to result in pollutants being distributed between Trinidad and Venezuela.

Similarly elevated levels of copper and zinc have been reported in oysters (Martincic *et al.* 1984; Han and Hung 1990; Han *et al.* 1993; Páez-Osuna *et al.* 1995; Beliaeff *et al.* 1998) due to anthropogenic sources. However, depuration may be used to reduce these levels, to render such organisms suitable for human consumption (Han *et al.* 1993; Chan *et al.* 1999), and similar measures are being recommended locally.

Conversely, the question arises as to the relevance of the 50  $\mu\text{g g}^{-1}$  wet weight MPL for zinc in shellfish established in Trinidad and Venezuela, because zinc is used as a supplement in many vitamin preparations, at 15 mg/day for men and 12

mg/day for women (Recommend Dietary Allowances 1989). In addition, zinc in shellfish is likely to be bound partially as metalloproteins (Beek and Baars 1990) and thus even less bioavailable than in pharmaceutical preparations, and only 20% to 40% of zinc in food is absorbed in humans (Murray 1996). It is suggested that a revision of the MPL for zinc in shellfish be made to take these factors into account.

Heavy metal concentrations in *P. viridis* from the Gulf of Paria along the coastline of Trinidad in this study were similar to those previously reported by Chang-Yen *et al.* (1999). In our study, cadmium and zinc levels in *P. viridis* from Margarita Island and Chacopata on the north coast of Venezuela were significantly lower for Cadmium ( $p < 0.05$ ) and zinc ( $p < 0.01$ ) than mean values of those in the Gulf of Paria, but chromium, copper, and nickel and mercury levels were similar (Table 5).

Comparison of metal concentrations in *C. rhizophorae* from Trinidad and *C. virginica* from Venezuela in the Gulf of Paria showed that only Cd and Cr differed significantly ( $p < 0.05$ ) while copper, nickel, mercury, and zinc levels were similar. In contrast, concentrations of all metals except mercury were significantly higher ( $p < 0.05$ ) in *C. rhizophorae* from the Gulf of Paria, which receives industrial and domestic effluent from

**Table 6.** Comparison of mean heavy metal concentrations  $\pm$  SEs in oysters (*Crassostrea rizophorae*) from the Gulf of Paria (Trinidad and Venezuela) and the north coast of Venezuela

Location	Concentration $\pm$ SE ( $\mu\text{g g}^{-1}$ wet weight)					
	Cd*	Cr**	Cu**	Ni**	Hg <sup>ns</sup>	Zn**
North coast	0.11 $\pm$ 0.11	0.04 $\pm$ 0.04	2.33 $\pm$ 1.05	0.07 $\pm$ 0.10	0.04 $\pm$ 0.02	32 $\pm$ 8
Gulf of Paria	0.31 $\pm$ 0.24	0.52 $\pm$ 0.48	29.8 $\pm$ 17.3	0.27 $\pm$ 0.16	0.05 $\pm$ 0.02	346 $\pm$ 157

\*  $p < 0.05$ .\*\*  $p < 0.01$ .ns:  $p > 0.05$ .**Table 7.** Comparison of mean metal levels in (*Crassostrea sp*) and *Perna viridis* at sites of common occurrence

Source of Variation	Metal					
	Cd	Cr	Cu	Ni	Hg	Zn
Oyster versus Mussel	**	***	***	**	ns	***

\*\*  $p < 0.01$ .\*\*\*  $p < 0.001$ .ns:  $p > 0.05$ .

both Trinidad and Venezuela (Table 6), than those from the north coast of Venezuela, which may be regarded as a pristine environment.

Similarly, at the sites from which both *P. viridis* and oysters were collected (Table 1), all metal levels except mercury in oysters were significantly higher ( $p < 0.01$ ) than those in *P. viridis* (Table 7). While *P. viridis* has been used as an indicator of cadmium pollution (Chidambaran 1996), it appears to regulate metals in its tissues to greater degrees than oysters, by means of mucus secretion (Sze and Lee, 1995) and metalloprotein production (Yang and Thompson 1996). The results of our study confirm that oysters (*Crassostrea sp.*), which have been reported to have special mechanisms for accumulating exceptionally high levels of copper and zinc (Pirie *et al.* 1984; Deb and Fukushima 1999) and are likely to be better indicators of heavy metal pollution than *P. viridis* in the Caribbean. Effective use of oysters or mussels as biological indicators of environmental pollution in the Caribbean will need to take these factors into consideration.

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