Levels of Trace Metals in the Clam, *Polymesoda solida* (Philippi, 1846) (Bivalvia: Cyrenidae), from the Strait of Lake Maracaibo and Bahía El Tablazo, Venezuela[1]

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Abstract: This study determined concentrations of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), selenium (Se), vanadium (V), and zinc (Zn) in the clam *Polymesoda solida*, collected in nine different sites in the Strait of Lake Maracaibo and Bahía El Tablazo, to assess the spatial distribution of metals, and the safety of clam consumption by humans. Overall average concentrations ± standard deviations in µg/g dw were 8.34 ± 1.51 for As, 0.59 ± 0.28 for Cd, 13.80 ± 6.13 for Cu, 1.22 ± 0.51 for Pb, 2.29 ± 0.62 for Se, 2.59 ± 1.55 for V, and 24.70 ± 4.98 for Zn. No distinct trend in the spatial distribution of metals was observed, and levels in clams were below maximum permissible values for seafood consumption. Positive correlations of V with Pb, and Cd suggest common pollution sources, and their levels were comparable to other bivalve species from contaminated aquatic systems.

Key Words*:* *Polymesoda solida*, metals, bivalves, Venezuela, Lake Maracaibo

The Lake Maracaibo System (LMS) (which includes Lake Maracaibo, Strait of Lake Maracaibo, and Bahía El Tablazo) is located approximately from 12º N in the Gulf of Venezuela down to 8º N latitudes, and between 70º W and 73º W longitudes. Lake Maracaibo, with an area of 12013 km^2, connects to the estuary of Bahía El Tablazo through the Strait of Lake Maracaibo (Ávila et al. 2010). Both, Strait and Bahía El Tablazo, contribute another 1090 km^2 to the LMS, for a total area of 13103 km^2 (Ávila et al. 2010). The LMS is considered one of the largest oil-producing regions in Venezuela (Gundlach et al. 2001, Colina et al. 2005). The LMS has suffered from severe contamination problems caused by excessive inputs of pollutants from petroleum-derived waste; riverine, and agricultural sources; from treated and untreated domestic wastes, and industrial

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wastewaters (Gundlach et al. 2001, Ávila et al. 2010, Corona-Lisboa 2013, Marín-Leal et al. 2014). These anthropogenic activities in the lake and its surroundings result in the release of harmful pollutants, such as metals, that may reach the estuary of Bahía El Tablazo. Evidence of metal pollution has been well documented in water, sediments, and biota of the LMS (Colina et al. 2005, Salazar-Lugo 2009, Ávila et al. 2010, Corona-Lisboa, 2013, Marín-Leal et al. 2014). Benthic organisms would be the most directly impacted in terms of accumulation of metals from metal-contaminated aquatic environments (Gupta and Singh, 2011). An important public health concern arises when these benthic organisms contaminated with metals are consumed by humans. For instance, metals like Pb and Hg are known neurotoxicants, while As has been associated to various systemic effects (e.g., cardiovascular disease, skin disorders, neurotoxicity), and cancer (Liu et al. 2008). Cadmium has been associated with nephrotoxicity (Liu et al. 2008). The determination of metal pollution levels in benthic organisms, known as biomonitors, has been widely used to assess the degree of contamination in aquatic systems and the risks to public health (Rainbow 1995, Burger and Gochfeld 2006, Gupta and Singh 2011).

The benthic mollusk *Polymesoda solida* (Philippi, 1846) (Bivalvia: Cyrenidae) has a biogeographical range extending from Belize (east coast of Central America) through the Orinoco River (North coast of South America) (Severeyn et al. 1994). According to the *World Register of Marine Species (*http://www.marinespecies.org/index.php), the currently accepted name of *P. solida* (Figure 1) is *Polymesoda arctata* (Deshayes, 1854) (Bivalvia: Cyrenidae) (Bouchet 2015). However, to provide consistency and to facilitate the comparison with other studies, we retained the use of *P. solida*, as proposed by Severeyn et al. (1994).

Figure 1. The clam *Polymesoda solida* (Philippi, 1846) (Mollusca: Bivalvia: Cyrenidae).

This clam species is tolerant to variable salinity values, and it lives buried preferably in fine sandy sediments of the intertidal zone of estuaries, or in muddy anoxic sediments of mangrove roots (Severeyn et al. 1994). *Polymesoda solida*, commonly distributed through the LMS, is an important subsistence and commercial bivalve mollusk harvested in Venezuela, as well as in other countries like Colombia, for human consumption (Sarcos and Botero, 2005, De La Hoz-Aristizábal, 2010). Metal pollution in this bivalve species has not been extensively studied. Only one recent published study reported metal levels in *P. solida* focusing in Cd and Pb (Marín-Leal et al. 2014). Therefore, *P. solida* could serve as an ideal indicator species of metal pollution in the LMS, especially in Bahía El Tablazo. The objectives of this study were to determine the spatial distribution of metal concentrations (specifically for As, Cd, Cu, Pb, Se, V and Zn) in the clam *P. solida*, which can be used for future comparisons and/or monitoring studies in the LMS, and to compare metal levels in clams to permissible values for seafood consumption in order to evaluate potential human health impacts.

Methods

Polymesoda solida clams, buried in surface bottom sediments, were collected by hand, using powder-free rubber gloves, on March 2006, from nine different sites: two in the Strait of Lake Maracaibo (Isla Dorada and Punta de Leiva) and seven in Bahía El Tablazo, Venezuela (Figure 2). Clams from each site were placed in plastic bags and stored in an ice-box (< 5^0C) for transportation. Then, they were washed with distilled deionized water (ddw), temporarily stored in a freezer, and later shipped frozen to the Laboratory of the Department of Environmental Health of the University of Puerto Rico. Clams were measured in length and weighed. Soft tissues were removed using Teflon-coated spatulas and plastic tweezers, and washed with ddw to discard any sediments associated with it. Excess ddw was removed with Kimwipes (Kimberly-Clark, Roswell Georgia, USA). In order to obtain sufficient clam soft tissue for metal analyses, a composite of each sampling site, consisting of an average of 10 clams (6-12) per site, was placed in a pre-weighed 250 mL beaker, and oven-dried at 60°C, until constant weight. Each dried tissue composite sample was homogenized using a ceramic mortar and pestle, and transferred into plastic bags.

Figure 2. Sampling sites in the Strait of Lake Maracaibo and at the Bahía El Tablazo in Venezuela. 1 = El Moján; 2 = Isla de Toas; 3 = Isla de San Carlos; 4 = Playa Apuz; 5 = Punta de Palmas-I; 6 = Punta de Palmas-II; 7 = Los Jobitos; 8 = Isla Dorada; 9 = Punta de Leiva. Satellite image generated by the USGS Earth Resources Observation and Science (EROS) Center. For geographical context, larger insert includes portions of northern Venezuela and Colombia; smaller insert, portions of The Americas. Attribution of inserts: By No machine-readable author provided. NormanEinstein assumed (based on copyright claims). [GFDL (http://www.gnu.org/copyleft/fdl.html) or CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0/)], via Wikimedia Commons. URL of insert: https://upload.wikimedia.org/wikipedia/commons/c/c9/Lake_Maracaibo_map.png .

The digestion of approximately 0.5 g dry weight (dw) of each composite homogenized clam tissue sample per site was conducted in duplicates in a mixture of 2 mL ddw with 5 mL HNO_3 (CEM Corporation 1996, Pérez et al. 2001). Acidified samples were digested in a Model 1000 CEM microwave oven (CEM Corporation, Matthews, North Carolina, USA) for 32 min and digested again with 2 mL of 30% H_2O_2 for an additional 10 min (CEM Corporation, 1996). Samples were then filtered using Whatman 41 paper-filters, diluted to 50 mL with ddw, and transferred to 50-mL polypropylene tubes (Corning Incorporation, Corning, New York). Metals were analyzed using a Perkin Elmer Atomic Absorption Spectrometer (AAS) Model AAnalyst 800 (Pérez et al. 2001). Average metal concentrations (n = 2) were calculated from each composite homogenized sample site (except for site 7, n = 1). Quality control (QC) included the standard reference material (SRM) 1566b-Oyster tissue (National Institute of Standards and Technology, Gaithersburg, Maryland, USA), and spiked blank solution (9 mL aqueous HNO_3 digestion solution in the absence of clam tissue) samples to determine percent recoveries and evaluate the analytical method. The minimum correlation coefficient of the AAS calibration curve accepted was 0.995. Statistical analyses were performed using STATA™ v. 12.0 (StataCorp, College Station, Texas, USA). Kruskal-Wallis nonparametric tests (χ^2 statistic) were used to determine spatial differences in metal concentrations, while Spearman correlation analyses were conducted to determine relationships between metal concentrations.

Results and Discussion

The overall average percent water content for clams was 88.0 ± 1.2%, while the remaining average 12% represents the solid tissue material. The overall average size length and weight ± sd for clams analyzed were 30.8 ± 4.0 mm and 1.38 ± 0.23 g wet weight, respectively. Percent recoveries for all metals from triplicate spiked blanks were above 90%, while average percent (%) recoveries ± standard deviation (sd) (n = 4) from the SRM 1566-Oyster tissue samples were: 85.8 ± 3.1 for As, 86.5 ± 4.2 for Cd, 90.3 ± 2.8 for Cu, 91.6 ± 6.2 for Pb, 80.5 ± 0.8 for Se, 93.2 ± 9.7 for V, and 95.0 ± 0.9 for Zn. Satisfactory recoveries (≥80%), as well as precision (relative standard deviations ≤ 10%), were obtained by the microwave digestion method used for the oyster SRM.

Overall average metal concentrations showed a large degree of spatial variability, as shown by the large coefficients of variation (CV) (18.1% to 59.8%) (Table 1). Despite this heterogeneous spatial distribution, none of the sampling sites obtained the highest concentration for more than two different metals (Table 1). There were significant ($p < 0.05$) inter-spatial differences for Cd and Cu, while the rest of the metals showed moderately significant ($0.047 \leq p < 0.080$) locational differences (Table 1).

Table 1. Average metal concentration ± standard deviation (µg/g dw)* in the bivalve *Polymesoda solida* for Strait of Lake Maracaibo and Bahía El Tablazo.

Site Number	Site Name	As	Cd	Cu	Pb	Se	V	Zn
1	El Moján	9.95 ± 0.46	0.72 ± 0.01	17.16 ± 2.08	2.47 ± 0.00	2.56 ± 0.08	4.98 ± 0.73	24.29 ± 0.33
2	Isla de Toas	8.26 ± 0.03	0.66 ± 0.03	10.78 ± 0.54	1.03 ± 0.01	2.42 ± 0.08	2.76 ± 0.12	20.75 ± 0.74
3	Isla de San Carlos	7.99 ± 0.05	0.49 ± 0.01	8.63 ± 0.16	1.20 ± 0.10	3.71 ± 0.32	4.48 ± 0.49	21.25 ± 0.82
4	Playa Apuz	5.96 ± 0.21	0.66 ± 0.01	9.19 ± 0.22	1.33 ± 0.05	1.80 ± 0.00	3.60 ± 0.03	21.16 ± 0.34
5	Punta de Palmas-I	8.23 ± 0.14	0.43 ± 0.01	10.28 ± 0.12	0.80 ± 0.07	2.26 ± 0.00	0.99 ± 0.26	24.99 ± 0.42
6	Punta de Palmas-II	10.04 ± 0.18	0.26 ± 0.00	8.90 ± 0.47	0.87 ± 0.37	2.06 ± 0.05	1.21 ± 0.07	26.86 ± 0.07
7	Los Jobitos	8.44	1.56	11.38	1.03	2.37	3.23	22.36
8	Isla Dorada	6.29 ± 0.12	0.57 ± 0.03	24.99 ± 1.59	1.24 ± 0.08	1.76 ± 0.13	1.13 ± 0.05	36.74 ± 0.26
9	Punta de Leiva	9.93 ± 0.20	0.46 ± 0.00	21.67 ± 1.76	0.96 ± 0.04	1.74 ± 0.06	1.25 ± 0.05	22.69 ± 1.08
	Overall average	8.34 ± 1.51	0.59 ± 0.28	13.80 ± 6.13	1.22 ± 0.51	2.29 ± 0.62	2.59 ± 1.55	24.70 ± 4.98
	χ^2 (p)	15.098 (0.057)	15.69 (0.047)	15.57 (0.049)	14.06 (0.080)	15.37 (0.052)	15.02 (0.059)	15.28 (0.054)
	CV (%)	18.1	47.5	44.4	41.8	27.1	59.8	20.2
	MRL	0.1	0.01	0.1	0.1	0.1	0.2	2

* For each site, n = 2, except for site 7, where n = 1; χ^2 = Kruskal-Wallis nonparametric statistic; (p) = p-value; CV = coefficient of variation; MRL = minimum reporting limit of the AAS instrument based on a 0.5 g dw of clam tissue.

For As, the highest average concentration in *P. solida* clam was obtained in Punta de Palmas-II (10.04 µg/g dw) (Table 1). The lowest average level of As recorded was in Playa Apuz (5.96 µg/g dw), followed by Isla Dorada (6.29 µg/g dw). In comparison to other studies, the overall average concentration of As in *P. solida* was similar (less than two-fold differences) to levels observed in other three different species of bivalve, *Brachiodontes exustus* (Linnaeus, 1758; Mytilidae) (Sastre et al. 2015), *Mercenaria mercenaria* (Linnaeus, 1758; Veneridae) (Trocine and Trefry 1996), and *Ruditapes philippinarum* (Adams and Reeve, 1850; Veneridae) (Haiqing et al. 2009) (Table 2).

The highest concentrations of Cd were obtained in clam tissues from Los Jobitos (1.56 µg/g dw), whereas the lowest was measured in clams from Punta de Palmas-II (0.26 µg/g dw) (Table 1). Marín-Leal et al. (2014) collected *P. solida* from eleven sites in the Lake Maracaibo System. Although they reported higher average levels (0.963 µg/g dw), Cd was not detected in clams from six sampling sites within the Strait and Bahía El Tablazo that included El Moján, Playa Apuz, and Las Palmas (area of Punta de Palmas). Marín-Leal et al. (2014) showed only one sampling site located in the south east side within the Lake Maracaibo that exhibited a higher Cd concentration (1.267 µg/g dw) in *P. solida* than our overall average value of 0.59 µg/g dw (Table 2). Our study detected Cd in *P. solida* in all sampling sites (Table 1). The overall average level of Cd in *P. solida* was three to five-fold higher than levels reported for other bivalves from Puerto Rico (Table 2). Comparable levels were observed with bivalves like *Corbicula fluminea* (O. F. Müller, 1774; Cyrenidae) (Ruelas-Inzunza et al. 2009), *M. mercenaria*, *Isognomon alatus* (Gmelin, 1791; Pteriidae; Jaffé et al. 1998), and *R. philippinarum* (Table 2). However, when compared to *Tivela mactroidea* (Born, 1778; Veneridae) (Alfonso et al. 2005), *Polymesoda caroliniana* (Bosc, 1801; Cyrenidae) (Ruelas-Inzunza et al. 2009), and *Perna viridis* (Linnaeus, 1758; Mytilidae) (Lemus et al. 2010), average levels of Cd in *P. solida* were about two-fold lower (Table 2).

Average levels of Cu were higher in clams from Isla Dorada (24.99 µg/g dw) and Punta de Leiva (21.67 µg/g dw), both sites from the Strait of Lake Maracaibo, possibly reflecting the proximity to urbanized land such as Maracaibo city that covers the western shore of the Strait (Figure 2). Isla de San Carlos in Bahía El Tablazo exhibited the lowest average Cu concentration (8.63 µg/g dw) (Table 1). *Polymesoda solida* exhibited higher overall average Cu concentrations in comparison to *Mytilopsis domingensis* (Récluz, 1852; Dreissenidae) (Pérez et al. 2001), *P. caroliniana*, *C. fluminea*, *P. viridis*, and *R. philippinarum* (Table 2). The overall average Cu concentration observed in *P. solida* was similar to levels measured in *B. exustus* and *M. mercenaria*, but two-fold lower when compared to *I. alatus* (Table 2).

Table 2. Comparison of average (and/or range) of metals concentrations (μg/g dw) with different species of bivalves.

Site	Species Authorship Family	As	Cd	Cu	Pb	Se	V	Zn	Reference
Lake Maracaibo System*, Venezuela	*Polymesoda solida*[1] (Philippi, 1846) Cyrenidae	8.34 (5.8-10.3)	0.59 (0.26-1.56)	13.80 (8.5-26.1)	1.22 (0.61-2.5)	2.29 (1.67-3.94)	2.59 (0.81-5.50)	24.70 (18.7-36.9)	This study
Lake Maracaibo Venezuela	*Polymesoda solida* (Philippi, 1846) Cyrenidae	na	0.963 0.483-1.267	na	6.053 0.076-38.41	na	na	na	Marín-Leal et al. 2014
SJBEP[a], Puerto Rico	*Mytilopsis domingensis*[2] (Récluz, 1852) Dreissenidae	2.7 (1.8-3.6)	0.16 0.11-0.24	9.4 6.9-13.9	0.80 0.4-1.8	3.8 1.5-8.0	na	65.9 40.0-86.3	Pérez et al. 2001
Jobos Bay, Puerto Rico	*Brachiodontes exustus* (Linnaeus, 1758) Mytilidae	12.4 10.4-15.2	0.11 0.08-0.12	14.3 8.3-20.9	0.59 0.43-0.67	2.2 1.3-2.9	na	56.2 51.6-64.9	Sastre et al. 2015
Venezuela coast[b]	*Tivela mactroidea* (Born, 1778) Veneridae	na	1.02-1.53	9.98-19.71	na	na	1.64-3.78	98.5-126.3	Alfonso et al. 2005
Coatzacoalcos estuary, Mexico	*Polymesoda caroliniana* (Bosc, 1801) Cyrenidae	na	1.01	9.39	0.91	na	na	112.7	Ruelas-Inzunza et al. 2009
	Corbicula fluminea (O. F. Müller, 1774) Cyrenidae	na	0.45	9.0	0.09	na	na	65.9	

Table 2. Comparison of average (and/or range) of metals concentrations (µg/g dw) with different species of bivalves (continuation).

Site	Species Authorship Family	As	Cd	Cu	Pb	Se	V	Zn	Reference
Indian River Lagoon estuary, Florida, USA	*Mercenaria mercenaria* (Linnaeus, 1758) Veneridae	12 6 - 18	0.41 0.07 - 0.95	15 6.3-27	3.5 0.3-12	0.8 0.15-3.2	0.82 0.16-3.7	127 12-353	Trocine and Trefry 1996
Sucre, Venezuela	*Perna viridis* (Linnaeus, 1758) Mytilidae	na	1.20	2.64	0.41	na	na	28.54	Lemus et al. 2010
Bay of Biscay, France	*Mytilus edulis* Linnaeus, 1758 Mytilidae	na	na	na	na	na	1.4 0.6-4.1	na	Chiffoleau et al. 2004
	Crassostrea gigas (Thunberg, 1793) Ostreidae	na	na	na	na	na	1.3 0.5-2.7	na	
Morrocoy National Park, Venezuela[Ψ]	*Isognomon alatus* (Gmelin, 1791) Pteriidae	na	0.61 0.33-0.91	26.9 14-49	0.56 0.4-0.71	na	na	0.91 0.25-2.1	Jaffé et al. 1998
Jiaozhou Bay, China[δ]	*Ruditapes philippinarum* (Adams and Reeve, 1850) Veneridae	8.3-14.3	0.39-0.68	7.7-9.1	0.53-1.4	na	na	62.2-87.1	Haiqing et al. 2009
Persian Gulf, Iran (north)	*Saccostrea cucullata* (Born, 1778) Ostreidae	na	na	na	na	na	1.91 0.55-4.97	na	Moradi et al. 2011

*Strait of Lake Maracaibo and Bahía El Tablazo; [*]SJBEP =San Juan Bay Estuarine Program; [δ]range of average concentrations; na = not available; [Ψ]Average concentrations calculated from 10 stations.

[1] The currently accepted name and authorship for *Polymesoda solida* are *Polymesoda arctata* (Deshayes, 1854). This species has been placed in the family Corbiculidae but, herein, we are following the placement of *Polymesoda* in the Cyrenidae.

[2] According to WoRMS, the currently accepted name and authorship for *Mytilopsis domingensis* are *Mytilopsis sallei* (Récluz, 1849), see Marelli and Gray (1983).

The highest average Pb concentration in *P. solida* corresponded to El Moján (2.47 µg/g dw) (Table 1). This highest value differed from average levels detected in the other sites (0.87 - 1.33 µg/g dw), which were similar to the lowest level obtained of 0.80 µg/g dw in Punta de Palmas-I. Marín-Leal et al. (2014) reported average Pb concentrations in *P. solida* collected in the Lake Maracaibo System that were six-fold higher than the overall average Pb level in *P. solida* from the current study (Table 2). However, in their study, Pb was not detected in El Moján, while *P. solida* in Playa Apuz showed similar average Pb concentrations (about 1 µg/g dw) (Marín-Leal et al. 2014). They attributed the higher Pb concentrations in *P. solida* collected within the Lake Maracaibo to the extraction activities of petroleum (Marín-Leal et al. 2014). In comparison to other species of bivalves, *P. solida* showed comparable levels with *P. caroliniana*, and *R. philippinarum* (Table 2). Higher Pb levels in *P. solida* were observed when compared to *M. domingensis*, *B. exustus*, *C. fluminea*, *P. viridis*, and *I. alatus* (Table 2). In comparison to *M. mercenaria*, *P. solida* exhibited about three-fold lower Pb concentrations (Table 2).

Clams collected from Isla de San Carlos had higher average Se concentrations (3.71 µg/g dw) in comparison to the rest of the sampling sites (Table 1). The site with the lowest average concentration was Punta de Leiva, with 1.74 µg/g dw (Table 1). The comparison of Se levels with other investigations was limited to studies reported from Puerto Rico and USA (Table 2). Overall average Se concentrations in *P. solida* were similar to levels reported for *B. exustus,* but lower than *M. domingensis* (Table 2). When compared to levels reported in *M. mercenaria*, *P. solida* obtained about three-fold higher average Se concentrations (Table 2).

There was a 5-fold difference between the highest (4.98 µg/g dw) and the lowest (0.99 µg/g dw) average concentration of V, corresponding to El Moján and Punta de Palmas-I, respectively (Table 1). Another site with similar V levels to El Moján was Isla de San Carlos with 4.48 µg/g dw (Table 1). In comparison to other bivalves, *P. solida* showed higher average V concentrations than *Saccostrea cucullata* (Born, 1778; Ostreidae) collected from an oil production zone in Iran (Moradi et al. 2011), and *M. mercenaria* from the USA (Table 2). Average levels of V in *Mytilus edulis* (Linnaeus, 1758; Mytilidae) and *Crassostrea gigas* (Thunberg, 1793; Ostreidae) from France (Chiffoleau et al. 2004) displayed almost two-fold lower concentrations than *P. solida* (Table 2). After the "Erika" shipwreck, resulting in fuel oil contamination in the Bay of Biscay, France, V increased to maximum levels of 4.6 and 3.2 µg/g dw in *M. edulis* and *C. gigas,* respectively (data not shown on Table 2) (Chiffoleau et al. 2004). Alfonso et al. (2005) reported that the highest average level of V observed in *T. mactroidea* (3.78 µg/g dw) from six sampling sites along the coast of Venezuela was associated to the presence of petroleum refining and transportation activities. The higher levels of V in bivalves from France and Venezuela, obtained as a result of fuel oil contamination and proximity to oil producing activities, are comparable to values obtained in *P. solida* in sites like El Moján and Isla de San Carlos (Tables 1 and 2).

Clams from most of the sites had similar average concentrations of Zn (Table 1). The only exception were clams collected from Isla Dorada, where the highest Zn concentration (36.74 µg/g dw) was measured (Table 1). Most probably, this metal is partially regulated by this bivalve organism, as it has been observed with other bivalve species (Rainbow 1995, Alfonso et al. 2005). Average Zn concentrations were similar to *P. viridis* (Table 2). Most other bivalves (except *I. alatus* from Venezuela) exhibited higher Zn levels (Table 2).

Spearman correlation analyses between every possible pair of metals are shown on Table 3. We obtained a significant negative correlation between V and Zn. This negative relationship between V and Zn suggests a different source for Zn or dissimilar metal uptake/control mechanisms. In contrast, V exhibited significant positive correlations ($p < 0.05$) with Cd, Pb, and Se. The positive relationship between metals in *P. solida* could be due to similar metal uptake mechanisms, and sources (natural and/or anthropogenic) (Preston et al. 1972, Phillips 1976, Broman et al. 1991). In the LMS, the presence of metals like V, Pb and Cd has been associated with anthropogenic sources such as petroleum-related industrial activity zones (Colina et al. 2005, Salazar-Lugo 2009, Ávila et al. 2010, Marín-Leal et al. 2014). Also, the extraction of petrochemical products is a well-known anthropogenic source of metal contamination, particularly for V, to the LMS as well as to other marine systems (Chiffoleau et al. 2004, Alfonso et al. 2005, Colina et al. 2005, Moradi et al. 2011). Petroleum refinery effluents are also known sources of Se (Reyes et al. 2009). In addition to urban run-off, other potential sources of metal pollution in LMS include petroleum and chemical industrial activities (Figure 2), spillage from ocean-going ships, and waste discharges from mining activities that are transported into tributaries of LMS (Colina et al. 2005, Salazar-Lugo 2009, Ávila et al. 2010).

In this study, no distinct trend in the spatial distribution of metals was observed. However, clams from El Moján exhibited the highest and second highest average metal concentrations for most metals (e.g., V, Pb, Cd, Se). Metal levels on this site could be influenced by the proximity to the outlet of the Limón River (Figure 2), which receives waste discharges from coal-mining activities (Ávila et al. 2010), a potential metal pollution source to *P. solida*. *Polymesoda solida* from Punta de Palmas (I and II) showed lower average levels of metals such as Cd (0.26-0.43 µg/g dw), Pb (0.80-0.87 µg/g dw), and V (0.99-1.21 µg/g dw) (Table 1). A more recent study reported elevated average Pb concentrations (about 3 µg/g dw) in *P. solida* collected in the area of Punta de Palmas (identified as Las Palmas by the authors) in Bahía El Tablazo (Marín-Leal et al. 2014). Marín-Leal et al. (2014) described the LMS as a highly complex aquatic system due to its diverse anthropogenic activities occurring within and around its watershed. These diverse sources might contribute to a marked variation (spatially and temporally) in the concentrations of metals in this aquatic system with some metals reaching levels considered unsafe for human consumption.

Table 3. Spearman's correlation "rho" of metals (µg/g dw) in *P. solida*.

	As	Cd	Cu	Pb	Se	V
Cd	-0.149					
	(0.556)					
Cu	0.192	0.317				
	(0.445)	(0.200)				
Pb	-0.280	0.539	0.129			
	(0.261)	(**0.021**)	(0.609)			
Se	0.110	0.311	-0.364	0.209		
	(0.665)	(0.209)	(0.138)	(0.405)		
V	-0.035	0.595	-0.262	0.554	0.601	
	(0.890)	(**0.009**)	(0.293)	(**0.017**)	(**0.008**)	
Zn	0.281	-0.384	0.349	-0.074	-0.366	-0.593
	(0.259)	(0.116)	(0.156)	(0.769)	(0.136)	(**0.009**)

p-values in parenthesis; bold p-values in parenthesis < 0.05

In order to compare to maximum permissible levels (MPL) of metals in seafood set by different regulatory agencies worldwide (Table 4), metal concentrations at each site were converted to wet weight (ww) basis by multiplying the average percent solid (12%) to the dw concentrations shown on Table 1. No MPL was found for Se and V in seafood products. The range of individual concentrations converted to µg/g wet weight (ww) were 0.70 - 1.23 for As, 0.03 - 0.19 for Cd, 1.0 - 3.0 for Cu, 0.07 - 0.30 for Pb, and 2.2 - 4.4 for Zn. Except for As, the rest of the metals were lower than the MPL (Table 4). Although As exceeded the FSANZ (2016) and BFL decree Nº 685 MPL (Brasil, 1998) of 1 µg/g ww, this standard is for inorganic As and not total As (Whyte et al. 2009) (Table 4). Our study only calculated total As, where a significant fraction is considered to be non-toxic organic As (e.g., arsenobetaine) (Sirot et al. 2009). Based on MPL values, concentrations of metals obtained in the clam *P. solida* in this study did not represent a human health hazard. However, other toxic chemicals (e.g. mercury), not included in our study, need to be considered in future studies. In addition, Marín-Leal et al. (2014) reported higher concentrations of Pb in *P. solida* (Table 2) in some sites in the LMS that represented a health risk for human consumption.

Table 4. Maximum permissible metal levels (μg/g ww) in seafood.

	As	Cd	Cu	Pb	Se	V	Zn	References
FAO/WHO	-	1	-	2	-	-	-	FAO/WHO (2000)
BFL	1	1	30	2	-	-	50	Brasil (1965, 1998)
FSANZ	1	2	-	2	-	-	-	FSANZ (2016)
CEC	-	1	-	1	-	-	-	Byrne (2001)

FAO = Food and Agriculture Organization/World Health Organization of the United Nations; BFL = Brazilian Federal Legislation; FSANZ = Food Standards Australia New Zealand (specific for molluscs); CEC = Commission European Community (specific for bivalve molluscs).

Although levels were below maximum permissible values for seafood consumption, the clam *P. solida* from LMS showed the potential to accumulate metals, thus becoming a useful indicator of metal pollution. Overall average metal levels in *P. solida* exhibited the following general pattern: Zn > Cu > As > V > Se > Pb > Cd. The diversity of metal-pollution sources (point and nonpoint sources) throughout the LMS possibly explained the high spatial variability of metal concentrations observed in *P. solida* in this study. Concentrations of some metals like Cd, Pb, and V were generally comparable to metal levels detected in other bivalve species from metal-contaminated aquatic systems such as *M. domingensis*, *T. mactroidea*, *P. caroliniana*, *R. philippinarum*, and *S. cucullata* (Table 2). It is known that metal accumulation in bivalves is species-dependent (Rainbow, 1995; Gupta and Singh, 2011). Physiological properties such as respiration, growth, and reproduction characteristics in bivalves are known to affect metal bioaccumulation (Apeti et al. 2005; Narváez et al. 2005). For instance, Apeti et al. (2005) attributed a rapid increase of Cd and Zn in the juvenile oyster *Crassostrea virginica* (Gmelin, 1791; Ostreidae) to higher growth rates. Therefore, further studies are necessary to understand how the life history (e.g., sex, growth, reproductive cycle), temporal variation in environmental physico-chemical properties, and metal kinetics may influence metal accumulation in *P. solida* from water and sediments, as it has been observed for other bivalve biomonitors of metals (Rainbow, 1995; Gupta and Singh, 2011). In conclusion, this study provided useful information on metal levels in *P. solida* that can be used for future comparisons and/or monitoring studies on metal pollution in the LMS, as well as in other biogeographic regions where this bivalve species occurs.

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