Metals in coastal mollusks of Costa Rica

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Abstract: The input of contaminants to coastal ecosystems is a global problem on the rise. Data on metal pollution from tropical sites is often lacking and pilot surveys are urgently needed to provide a general framework to estimate the relative impact of these and other pollutants. The objective of this study was to make accessible data on trace metals collected during pilot surveys (2000-2006) at four coastal embayments of Costa Rica. Cu, Fe, Mn and Zn were analyzed by Flame Atomic Absorption Spectrometry (FAAS) and Al, Cd, Ni, Pb and Sn by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Concentrations of Fe, Pb, Zn, Mn, and Ni were determined by FAAS or GFAAS, in tissues of the rock snail *Acanthais brevidentata*, the infaunal bivalves *Anadara tuberculosa* and *Tagelus affinis* (from three Pacific sites: Culebra Bay, Gulf of Nicoya, and Golfito Bay) and in the clam *Polymesoda arctata* from the Caribbean coast (Moín Bay). In addition, Sn, Cd and Cu were evaluated in tissues of *T. affinis*. A group of specimens of *T. affinis* was depurated for 72 hours in filtered seawater from the collection site. Concentrations varied between and within sites and also between parts of the same organisms. Maximum concentrations (μg/g dry weight) were: Fe 2230 (*P. arctata*-tissues, Moín Bay), Pb 195 (*P. arctata*-tissues, Moín Bay), Zn 961 (*A. brevidentata*-tissues, Golfito Bay), Mn 921 (*P. arctata*-tissues, Moín Bay), and Ni 10.5 (*A. tuberculosa*-shells, Golfito Bay). Minimum concentrations (μg/g dry weight) were: Fe 5.36 (*P. arctata*-tissues, Moín Bay), Pb < 0.20 (*P. arctata*-foot, Moín Bay), Zn 2.75 (*P. arctata*-shell, Moín Bay), Mn 5.5 (*A. tuberculosa*-foot, Gulf of Nicoya) and Ni 0.83 (*A. tuberculosa*-foot, Golfito Bay). Tissues of non-depurated *T. affinis* had maximum-minimum concentrations (μg/g dry weight) of Sn (3.74-2.73), Cd (0.69-0.43) and Cu (21.6-14.8). The concentrations (except for Pb and Mn) were within values reported in recent literature. The relatively high concentration of Pb was probably related to the use at the time of sampling of leaded fuel in small boat operations at the site, while no evident cause was found for the high Mn values. Depuration was partially effective in lowering the metal burden in *T. affinis*. Data gathered during the pilot survey were indicative of relatively clean conditions of Culebra Bay, while the other three sites have important concentrations of certain contaminants, including metals. In spite of the fact that this data was collected more than a decade ago, it remains as the most recent available on trace metals from coastal mollusks of Costa Rica. Rev. Biol. Trop. 63 (4): 1007-1019. Epub 2015 December 01.

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There are numerous scientific reports focusing on spatial and temporal variability of trace metal concentrations in organisms from temperate latitudes, particularly for clams and snails, groups commonly used in pollution monitoring (Bayen, 2012; Menzel, 1979). The situation is different for many tropical sites, where data on metal pollution is often lacking and pilot surveys are urgently needed to provide a general framework to estimate the relative impact of these and other pollutants. These surveys are the first step towards the design of studies aimed at identifying spatial and temporal variability of concentrations. Data generated during pilot surveys are often scattered in space and time, difficult to analyze statistically and usually only available in reports of limited circulation. However, due to the high cost of performing trace metal analyses, more elaborated studies do not often follow up pilot surveys and thus scattered data become the only available information for future reference. Thus, present and future easy access to this type of information might be critical in spite of its inherent limitations.

On the Pacific coast of Costa Rica, Dean, Maurer, Vargas, and Tinsman (1986) published the first report on trace metals in marine organisms from the Gulf of Nicoya estuary. The concentrations found were those expected for non-industrialized estuaries. Fuller et al. (1990) studied metal concentrations at the mouth and upstream the Tárcoles River which discharges untreated waters into this Gulf and chromiun was found to be an important metal contaminant. Guzmán and Jiménez (1992) evaluated the presence of twelve metals in the stony coral *Siderastrea siderea* on the Caribbean coasts of Costa Rica and Panama. They found that metal levels were generally higher than the ranges reported from other coral reefs worldwide. Also on the Caribbean coast of Costa Rica, Rojas, Acuña, and Rodríguez (1998) studied the concentrations of metals in tissues of the sea cucumber *Holothuria mexicana* and reported maximum values (mg/kg dw) of Fe (1 044), Zn (174), Cu (69), Mn (46), and Cd (2.5).

Between the years 2000 and 2006 data on pollutants were collected as part of pilot surveys at four coastal embayments, three located on the Pacific coast and one on the Caribbean coast of Costa Rica. Data on the ecology of the organisms was published by Rojas-Figueroa and Vargas-Zamora (2008), Sibaja-Cordero and Vargas-Zamora (2006), and Tarrant et al. (2008). The surveys also evaluated the use of polychaete worms as indicators of pollution in tropical sites (Dean, 2008), and updated the lists of polychaete, sipunculan, and echiuran worms (Dean, 2009; Vargas & Dean, 2009).

Published results obtained during the surveys emphasized the usefulness of the multiparameter approach in the identification of species, sites, and concentrations of concern. For instance, García-Céspedes, Vargas-Zamora and Acuña-González (2004) collected sediment samples between 2000 and 2002 at various locations within these four embayments. They found maximun concentrations of Fe (73 000 μg/g, Culebra Bay), Zn (127 μg/g, Moin Bay), Cu (128 μg/g, Golfito Bay), and Pb (8.2 μg/g, Gulf of Nicoya and Golfito). Lower concentrations of metals were found in sands from Culebra Bay and they considered this site as relatively unpolluted, while Golfito Bay appeared as the most impacted of the four sites. The evaluation of polychlorinated biphenyls (PCBs) in sediments from the four sites was conducted by Spongberg (2004 a, b, c), who found that concentrations were relatively low but highest near the port of Golfito. The presence of PCBs in intertidal sipunculan worms was also evaluated by Spongberg (2006) who found that concentrations ranged from as low as 0.01 ng/g dw in *Antillesona antillarum* from Culebra Bay to a maximum of 67 ng/g dw in *Phascolosoma perlucens* from near the Rincón river mouth close to the entrance of Golfito Bay. Moreover, the cosmpolitan peanut worm *Sipunculus nudus* from the Cocorocas sand flat (Gulf of Nicoya) had relatively high concentrations of PCBs ranging from 17.7 to 41.3 ng/g dw, whether the worms were depurated or not. Spongberg & Witter (2006) reviewed published reports on PCBs from tropical sites including those collected during the pilot study. They concluded that these values are low when compared to data from temperate latitudes. García, Acuña-González, Vargas-Zamora and García-Céspedes (2006) conducted an evaluation of coliform bacteria and beach litter at the four sites. The least polluted location was Culebra Bay with counts for total and fecal coliform bacteria of around 2 MPN (Most Probable Number)/100 mL. The port city of Golfito was the most impacted, with total and fecal coliform bacteria over 50 000 MPN / 100 mL. Plastic material was the most common item found on all beaches at the four sites. Acuña-González Vargas-Zamora, Gómez-Ramírez, and García-Céspedes (2004) studied by molecular florescence the concentrations of dissolved/dispersed petroleum hydrocarbons at the four sites. No

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fluorescence signal was detected in samples from Culebra Bay indicating no oil contamination at the time. Concentrations at the other three sites were below 2 μg/L and considered as indicative of very low contamination with oil.

The importance of low concentrations of other contaminants was not disregarded and the pilot studies also addressed this topic. For instance, no evidence of endocrine disruption was detected in marine turtles (*Lepidochelys olivacea*) nesting near Culebra Bay (Valverde, Selcer, Lara, & Sibaja-Cordero, 2008). Tin compounds like tributyltin oxide (TBT) from antifouling paints, found in extremely low concentrations in seawater, have been linked worldwide to the development of imposex in gastropods. Imposex was detected in the snail *Acanthais brevidentata* from the port of Caldera in the Gulf of Nicoya, but it was absent in Culebra Bay (Gravel, Johanning, McLachlan, Vargas, & Oberdörster, 2006). The presence of other organic residues is also important in this context as emphasized by Cheek (2006). As a follow up of the surveys Spongberg et al., (2011) have reported on the presence of Pharmaceutical and Personal Care Products (PPCPs) in 86 sampling locations in rivers draining into Pacific and Caribbean coastal areas of Costa Rica. The identification of 34 compounds including many antibiotics and caffeine, is noteworthy.

In addition to *A. brevidentata*, three other species of coastal mollusks, including two commercially harvested clams, were also chosen during the pilot studies to evaluate the presence of metals in tissues with the expectation of identifying concentrations of concern. Thus, the objective of this report is to make available these latter data on metals in tissues from mollusks, and to compare the maximum concentrations found to selected examples from the literature.

MATERIALS AND METHODS

Study sites and organisms: Three of the chosen sites (Culebra Bay, Gulf of Nicoya and Golfito Bay) are on the Pacific coast of Costa Rica, where mean tidal range is approximately 3 m. The fourth site, Moín Bay, is on the mid Caribbean coast (Fig. 1), where mean tidal range is less than 0.5 m. The presence of the four species of mollusks collected in this study (Fig. 2) was confirmed for Costa Rica by Wehrtmann and Cortés (2009) who listed them as: *Acanthais brevidentata* (Wood, 1828), *Anadara tuberculosa* (Sowerby, 1833), *Tagelus affinis* (C.B. Adams, 1852) and *Polymesoda arctata* (Deshayes, 1854).

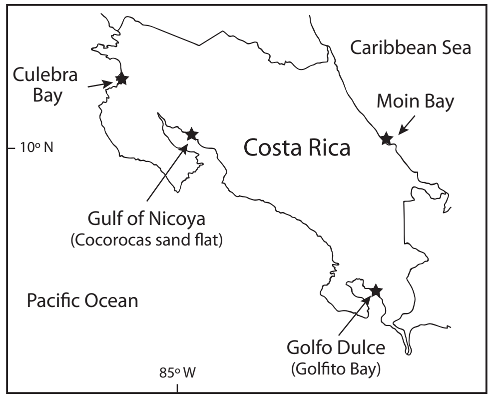

Fig. 1. Study sites on the Pacific and Caribbean coasts of Costa Rica.

Culebra Bay is an embayment on the upper Pacific coast and its mouth opens into the Gulf of Papagayo, an upwelling region. The bay is surrounded by tourism facilities that make use of the numerous white sand beaches. The published records on the marine biodiversity of Culebra Bay have been compiled by Cortés, Vargas-Castillo, and Nivia-Ruiz (2012) but no information on trace metals in organisms was located. The spatial variability of the rocky intertidal fauna, including the snail *A. brevidentata*, has been described by Sibaja-Cordero and García-Méndez (2014).

The Gulf of Nicoya is an estuary on the central Pacific coast of Costa Rica. Since the Mid-20th Century the Gulf has been the main fishing ground of the country for finfish and shellfish. Research on the Gulf conducted mainly after 1980 makes it one of the best known tropical estuaries worldwide (Vargas, 1995, Vargas & Mata, 2004). The

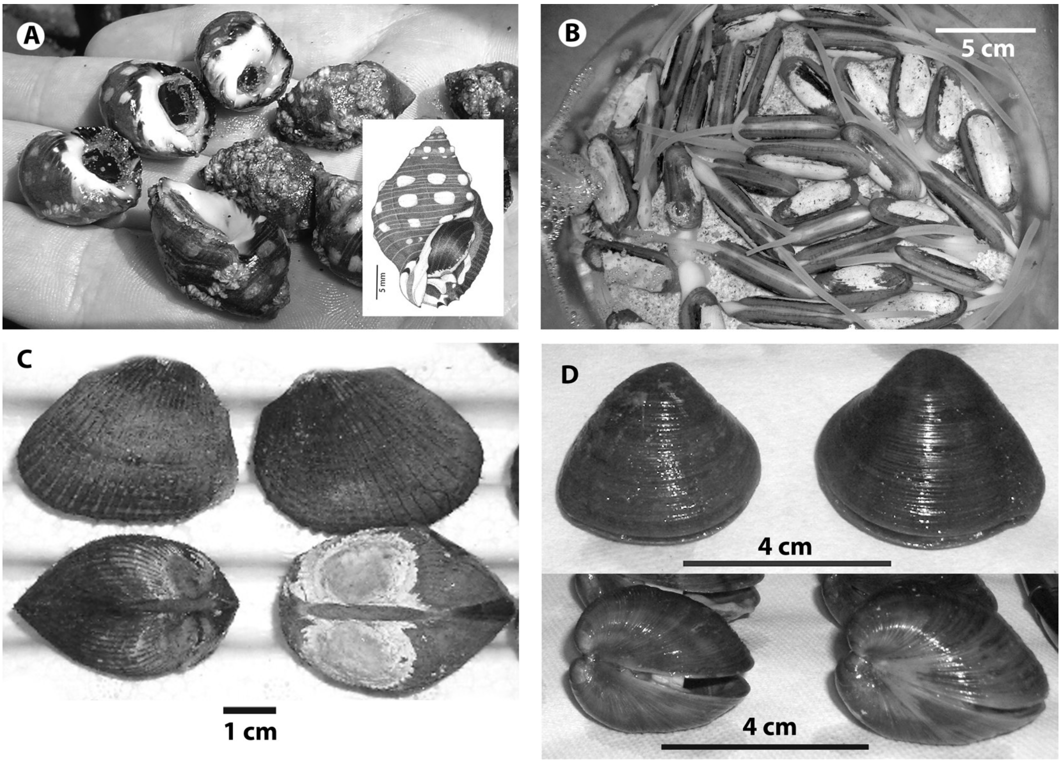

Fig. 2. A. *Acanthais brevidentata* snails from the rocky intertidal zone at the port of Caldera, Gulf of Nicoya (Reproduced from Gravel et al. 2006). **B.** *Tagelus affinis* clams during the depuration period in clean sea water (Note long siphons), Cocorocas sand flat, Gulf of Nicoya. **C**. Shell of *Anadara tuberculosa* from the edge of the Purruja mangrove forest, inner Golfito Bay, Golfo Dulce. Botton right: eroded shell. **D**. *Polymesoda arctata* clams from the Moin River mouth, Moin Bay,Caribbean coast.

ark clam *Anadara tuberculosa* (known locally as "piangua") continues to be extracted for human consumption after the decline of the bigger *Grandiarca grandis* (known locally as "chucheca") due to overfishing (Stern-Pirlot & Wolff, 2006). This overfishing prompted the harvesting of smaller bivalves, like the white razor clam *Tagelus affinis*. The snail *A. brevidentata* is also a common gastropod on the rocky shores of the Gulf, particularly near the port of Caldera (Sibaja-Cordero & Vargas-Zamora, 2006).

Golfo Dulce is an estuarine embayment on the South Pacific coast of Costa Rica. Its deep bathymetry, similar to a fjord, has encouraged comparative research leading to significant discoveries, such as the scarcity of infaunal invertebrates below 50 m (León-Morales & Vargas, 1998), the anammox reaction in the anoxic water column, and the presence of bacterially-derived bacteriohopanepolyols (Dalsgaard, Canfield, Petersen, Thamdrup, and Acuña-González 2003; Rush et al., 2014). Golfito Bay is a small and shallow embayment on the Northern shore of the gulf. It houses the main port for the region and the city of Golfito is located along its shores. The Purruja mangrove forest is on the South-Eastern shore of the bay. There, *A. tuberculosa* is extracted for local consumption (Silva-Benavides & Bonilla, 2001). So far the only report on pollutants from Golfo Dulce is by Spongberg and Davies (1998) who studied the concentrations of organochlorinated pesticides in sediments.

Moín Bay is on the central Caribbean coast of Costa Rica. It houses the main sea port facilities for container ships and is under the process of further development. Part of the Moín River runs parallel to the shore and is bordered by mangroves. An oil refinery is located several kilometers upstream. Brackish waters are found at the river mouth and a port facility for tourism river boats operates there. Here the clam *Polymesoda arctata* was found to be abundant and is occasionally harvested.

The four species of molluks were chosen for several reasons: the snail *A. brevidentata* is a top predator in the rocky intertidal zone, where specimens are abundant and easy to identify in the field. The snails cluster together at low tide in shaded spaces which facilitates obtaining enough biomass for study. The three filter feeding infaunal bivalves are also easy to identify and to collect from intertidal sediments, and are haversted for local consumption. Moreover, the four species are easy to transport and to maintain in the laboratory. These characteristics are among those cited by Cunningham (1979) for mollusks to be used as bioindicator organisms. *A. brevidentata* snails were hand collected from rocks exposed at low tide at Culebra Bay (in July 2003 and October 2004), the port of Caldera (Gulf of Nicoya, in October 2004) and at the Golfito bay mouth (in October 2004). Specimens of *T. affinis* were purchased on site from a fisherwoman working at the Cocorocas sand flat in February 2006. Ark clams (*A. tuberculosa*) were hand collected from muddy sediments at the edges of mangrove stands in the Gulf of Nicoya (Cocorocas mangrove, in October 2004) and Golfito Bay (Purruja mangrove, in April 2006). *P. arctata* was also hand collected from muddy sediments between the roots of mangrove trees at the Moín river mouth, in July and September 2006.

Metal analyses: After collection the mollusks were washed with sea water from the sites to remove dirt from the shells and transported to the laboratory in acid-washed polyester bags. At the laboratory the organisms were counted, rinsed with filtered sea water, blotted dry on a paper towel, weighed, separated each into equal-numbered groups of individuals, and stored frozen (-18 ºC) in a heat sealable, acid-washed polyester bags until analyses. Prior to analyses individuals of *A. brevidentata* were placed in acid-washed polyester bags and the shells crushed on a vise. Shell fragments were removed by hand and tissues saved for analyses. Bivalve shells were opened with a plastic knife and tissues (gills, digestive tract, muscles, and mantle) removed from the shell. In specimens of *A. tuberculosa* and *P. arctata* the foot was cut off and analyzed separately and shell fragments for analyses included the periostracum. A group of *T. affinis* was kept in a plastic container filled with filtered sea water from the site (Fig. 2) and aerated with plastic aquarium pumps to explore if organisms were able to survive and remove sediments and other matter from their digestive tracts. Sea water, feces, and dead organisms, were discarded every 12 hours and the containers filled with fresh filtered sea water from the site. The procedure was repeated six times for a total of 72 hours. Surviving organisms (more than 90 %) were then stored frozen as above until trace metal analyses.

All metal determinations were performed at the Centro de Contaminación Ambiental (CICA) at the University of Costa Rica, under validated and accredited protocols by the Costa Rican Accrediting Agency (ECA). Groups of 10-30 specimens were randomly subsampled from each batch and weighed after drying in an oven at 65 ºC for 48 hours. Dried tissues were finely ground to a homogeneous powder and samples of 1.00 g were placed in Teflon containers with 6 mL of high purity nitric acid, 2 mL of high purity hydrochloric acid, and pressure-digested in a microwave oven (*CEM® MARSx*). After digestion, the containers were cooled at room temperature and the liquid was transferred to 100 mL volumetric flasks and diluted with deionized water. Certified reference materials and blank tests were also run. Cu, Fe, Mn and Zn were analyzed by Flame Atomic Absorption Spectrometry (Perkin Elmer® 3300) and Al, Cd, Ni, Pb and Sn by Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer® HGA 600). Calibration curves were produced against certified standards for each element, according to the CICA accredited protocols. All the results were expressed in µg/g dry weight (dw). Concentrations of Fe, Pb, Zn, Mn, and Ni were determined in mollusks collected at the four sites (Culebra, Gulf of Nicoya, Golfito, Moín Bay), while Cd, Cu, and Sn were determined only in non-depurated *T. affinis* clams from the Gulf of Nicoya. Detection limits typical values were 0.40 µg/g for Al, 0.0020 µg/g for Cd, 1.6 µg/g for Cu, 0.10 µg/g for Sn, 1.9 µg/g for Fe, 0.91 µg/g for Mn, 0.022 µg/g for Ni, 0.027 µg/g for Pb, and 18 µg/g for Zn.

RESULTS

The concentrations of metals found in tissues of the snail *A. brevidentata* from Culebra Bay are shown in Table 1, and those found in *A. brevidentata*, the clams *T. affinis* and *A. tuberculosa* from the Gulf of Nicoya are listed in Table 2. The metal concentrations in *A. brevidentata* and *A. tuberculosa* from Golfito Bay are included in Table 3 and those for the clam *P. arctata* from Moín Bay are listed in Table 4. The maximum and minimum concentrations are summarized in Table 5.

TABLE 1

Trace metals (Fe, Pb, Zn, Mn, Ni) concentrations (µg/g dw) in tissues (n = 3) of the rocky intertidal snail *Acanthais brevidentata*. Culebra Bay, Gulf of Papagayo, Pacific, Costa Rica, July 2003 - October 2004.

Date	Statistics	Iron	Lead	Zinc	Manganese	Nickel
Jul.-03	Max.	208.5 ± 3.8	8.8 ± 1.0	601 ± 21	9.41 ± 0.97	2.99 ± 0.11
	Median	137.8 ± 5.1	4.57 ± 0.20	519 ± 16	9.2 ± 1.3	2.57 ± 0.08
	Min.	134.1 ± 4.0	2.36 ± 0.10	382 ± 21	5.93 ± 0.74	1.18 ± 0.08
Oct.-04	Max.	179.7 ± 4.6	22.9 ± 1.2	289.7 ± 6.2	9.1 ± 1.1	2.18 ± 0.09
	Median	108.9 ± 5.9	22.8 ± 1.2	155.0 ± 3.4	8.8 ± 1.4	2.03 ± 0.14
	Min.	87.8 ± 7.5	21.3 ± 1.0	148.7 ± 4.0	<5.2	1.44 ± 0.12

The uncertainty (±) corresponds to the expanded uncertainty using a coverage factor k = 2.

TABLE 2

Trace metals (Fe, Pb, Zn, Mn, Ni, Cd, Sn, Cd, Cu) concentrations (µg/g dw) in tissues of the intertidal snail *Acanthais brevidentata* (Caldera Bay), the razor clam *Tagelus affinis* (Cocorocas sand flat), and the ark clam *Anadara tuberculosa* (Cocorocas muddy mangrove edge), middle and upper Gulf of Nicoya estuary, Pacific coast, Costa Rica. October 2004 - February 2006.

Species & Date	Statistics	Iron	Lead	Zinc	Manganese	Nickel	Tin	Cadmium	Copper
T. brevidentata tissues. Oct. 04	Max.	523 ± 14	22.3 ± 1.0	544 ± 16	14.4 ± 1.0	3.17 ± 0.11			
	Median	472 ± 18	16.8 ± 1.1	442 ± 19	14.0 ± 1.6	3.04 ± 0.07			
	Min.	420 ± 11	11.38 ± 0.52	340 ± 11	13.7 ± 1.3	2.90 ± 0.09			
T. affinis 72 h depuration Feb. 06	Max.	290.3 ± 6.5	5.04 ± 0.26	153.5 ± 3.1	24.97 ± 0.90	1.60 ± 0.06			
	Median	171.1 ± 4.0	3.29 ± 0.38	147.6 ± 3.2	20.22 ± 0.91	1.27 ± 0.06			
	Min.	148.1 ± 4.2	2.16 ± 0.18	127.8 ± 2.8	14.7 ± 1.0	1.00 ± 0.07			
T. affinis non depurated. Feb. 06	Max.	2160 ± 55	1.37 ± 0.17	206.7 ± 4.1	255.2 ± 3.6	4.13 ± 0.09	3.74 ± 0.52	0.69 ± 0.06	21.6 ± 2.6
	Median	1524 ± 40	1.17 ± 0.17	162.9 ± 3.1	176.4 ± 2.3	2.80 ± 0.08	3.01 ± 0.48	0.66 ± 0.06	18.6 ± 2.4
	Min.	1151 ± 30	0.93 ± 0.16	146.7 ± 3.0	104.0 ± 1.1	2.00 ± 0.08	2.73 ± 0.51	0.43 ± 0.08	14.8 ± 2.4
A. tuberculosa foot. Oct. 04	Max.	256.1 ± 5.3	5.57 ± 0.39	61.12 ± 0.9	8.9 ± 1.6	1.87 ± 0.10			
	Median	180.0 ± 5.1	5.53 ± 0.52	57.2 ± 1.1	8.7 ± 1.2	1.40 ± 0.08			
	Min.	140.4 ± 6.4	4.23 ± 0.33	52.88 ± 0.9	5.5 ± 1.2	0.88 ± 0.08			
A. tuberculosa tissues. Oct. 04	Max.	661 ± 20	17.1 ± 1.2	65.1 ± 1.8	27.59 ± 0.49	1.52 ± 0.05			
	Median	559 ± 20	6.03 ± 0.49	58.3 ± 1.2	12.67 ± 0.66	1.30 ± 0.05			
	Min.	472 ± 14	5.75 ± 0.42	55.25 ± 0.7	9.89 ± 0.70	1.00 ± 0.05			

The uncertainty (±) corresponds to the expanded uncertainty using a coverage factor k = 2.

TABLE 3
Trace metals (Fe, Pb, Zn, Mn, Ni) concentrations (µg/g dw) in tissues (n = 3) of the rocky intertidal snail *Acanthais brevidentata*, (Golfito Bay, mouth), and in tissues and shells of the ark clam *Anadara tuberculosa* (inner Golfito Bay, Purruja mangrove edge). Golfo Dulce, Pacific, Costa Rica, October 2004 - April 2006.

Species & Date	Statistics	Iron	Lead	Zinc	Manganese	Nickel
T. brevidentata	Max.	341.9 ± 7.4	24.6 ± 1.7	961 ± 32	11.8 ± 1.7	2.73 ± 0.14
tissues. Oct. 04	Median	274.8 ± 6.7	7.08 ± 0.73	894 ± 30	10.9 ± 1.6	2.24 ± 0.13
	Min.	153.1 ± 5.2	15.2 ± 1.0	574 ± 21	6.8 ± 1.3	1.94 ± 0.10
A. tuberculosa	Max.	218.8 ± 4.5	1.80 ± 0.14	64.14 ± 0.84	18.25 ± 0.99	1.29 ± 0.07
foot. Apr. 06	Median	209.9 ± 4.3	1.73 ± 0.13	56.94 ± 0.76	10.6 ± 1.0	1.00 ± 0.07
	Min.	188.7 ± 4.3	1.66 ± 0.13	60.72 ± 0.81	6.86 ± 0.95	0.83 ± 0.06
A. tuberculosa	Max.	1333 ± 38	23.4 ± 1.6	100.2 ± 2.8	38.97 ± 0.81	1.46 ± 0.07
tissues. Apr. 06	Median	944 ± 30	23.0 ± 2.1	76.6 ± 1.6	33.90 ± 0.35	1.42 ± 0.13
	Min.	809 ± 24	11.05 ± 0.92	81.9 ± 2.0	32.76 ± 0.54	0.96 ± 0.09
A. tuberculosa	Max.	241.2 ± 6.7	4.71 ± 0.18	3.87 ± 0.37	51.07 ± 0.49	10.50 ± 0.51
shell. Apr. 06	Median	189.7 ± 5.4	3.87 ± 0.15	3.39 ± 0.30	44.06 ± 0.41	6.71 ± 0.28
	Min.	158.6 ± 4.2	2.76 ± 0.11	2.85 ± 0.28	27.31 ± 0.37	3.96 ± 0.21

The uncertainty (±) corresponds to the expanded uncertainty using a coverage factor k = 2.

TABLE 4
Trace metals (Fe, Pb, Zn, Mn, Ni) concentrations (µg/g dw) in tissues and shells of the clam *Polymesoda arctata* from the Moin river mouth, Caribbean, Costa Rica. July and September 2006.

Body part. Site #, date	Statistics	Iron	Lead	Zinc	Manganese	Nickel
Foot. Site 1.	Max.	486 ± 11	1.44 ± 0.34	126.4 ± 2.0	53.0 ± 2.4	2.90 ± 0.17
Sept. 06	Median	198 ± 13	0.92 ± 0.37	161.8 ± 2.1	37.2 ± 3.1	1.67 ± 0.19
	Min.	150 ± 11	<0.25	122.7 ± 2.2	31.8 ± 2.7	1.90 + 0.22
Foot. Site 2.	Max.	773 ± 15	<0.30	99.9 ± 2.6	28.1 ± 2.9	1.70 ± 0.20
Jul. 06	Median	261 ± 16	<0.23	84.2 ± 2.0	19.9 ± 3.9	1.15 ± 0.27
	Min.	213 ± 11	<0.20	77.5 ± 1.8	15.2 + 2.6	0.90 ± 0.18
Tissues. Site 1.	Max.	2230 ± 68	27.7 ± 3.4	485 ± 13	921 ± 14	3.80 ± 0.17
Sept. 06	Median	1573 ± 37	26.2 ± 2.3	481.0 ± 9.0	499.5 ± 8.7	3.15 ± 0.19
	Min.	1401 ± 41	21.9 ± 2.8	183.3 ± 5.2	410.5 ± 9.3	3.06 ± 0.21
Tissues. Site 2.	Max.	1585 ± 50	195 ± 14	181.3 ± 3.8	404.5 ± 6.6	3.76 ± 0.16
Jul. 06	Median	1249 ± 44	88.0 ± 7.0	164.0 ± 4.0	401.8 ± 6.2	3.48 ± 0.16
	Min.	5.36 ± 0.37	0.98 ± 0.07	157.5 ± 3.4	260.2 ± 4.7	2.47 ± 0.13
Shell. Site 1.	Max.	81.7 ± 2.1	1.68 ± 0.06	2.85 ± 0.40	14.91 ± 0.51	6.55 ± 0.44
Sept. 06	Median	47.8 ± 3.0	1.09 ± 0.07	2.77 ± 0.44	13.96 ± 0.68	4.87 ± 0.48
	Min.	39.4 ± 2.4	0.75 ± 0.04	2.75 ± 0.41	10.23 ± 0.56	4.04 ± 0.20
Shell. Site 2.	Max.	88.2 ± 2.3	105.5 ± 6.7	4.63 ± 0.35	13.08 ± 0.54	8.82 ± 0.58
Jul. 06	Median	48.7 ± 2.8	44.4 ± 6.7	3.17 ± 0.47	12.36 ± 0.59	5.80 ± 0.46
	Min.	39.0 ± 1.6	1.18 ± 0.05	2.97 ± 0.43	11.81 ± 0.45	1.73 ± 0.40

The uncertainty (±) corresponds to the expanded uncertainty using a coverage factor k = 2.

Among the five determined metals at the four sites (Fe, Pb, Zn, Mn, and Ni), concentrations of iron above 1400 µg/g-dw were found in tissues of non-depurated *T. affinis* from the Gulf of Nicoya (Cocorocas flat, Table 2). On the Caribbean coast, iron was found to be greater than 1400 µg/g-dw in tissues of *P. arctata* (Table 5). Concentrations of lead in *T. brevidentata* were around 22 µg/g-dw in Culebra, Gulf of Nicoya and Golfito (Table 1,

TABLE 5
Maximum (**A**) and minimum (**B**) concentrations (µg/g dry wt) of iron, lead, zinc, manganese and nickel found in mollusks at four coastal embayments of Costa Rica.

Site/	Culebra Bay		Gulf of Nicoya		Golfito Bay		Moín Bay	
Metal	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Fe	208	87.8	2 160	140.4	1 333	153.1	2 230[a]	5.36[f]
Pb	22.9	2.36	22.3	0.93	24.6	1.66	195[b]	<0.20[g]
Zn	601	148.7	544	52.8	961[c]	2.85	485	2.75[h]
Mn	9.4	<5.2[i]	255	5.5	51.7	6.8	921[d]	10.2
Ni	2.99	1.18	4.13	0.87	10.5[e]	0.83[j]	8.8	0.90

[a] Fe: *Polymesoda arctata*-tissues. [b] Pb: *P. arctata*-tissues, [c] Zn: *Acanthais brevidentata*-tissues. [d] Mn: *P. arctata*-tissues. [e] Ni: *Anadara tuberculosa*-shells, [f] Fe: *P. arctata*-tissues, [g] Pb: *P. arctata*-foot, [h] Zn: *P. arctata*-shell, [i] Mn: *A. brevidentata*-tissues, [j] Ni: *A. tuberculosa*-foot.

Table 2 and Table 3), while the highest concentration (195 µg/g-dw, Table 5) was found in *P. arctata* at the Caribbean port. Zinc above 300 µg/g-dw was found in the snail *T. brevidentata* at the three Pacific sites, with a maximum of 961 µg/g-dw (Golfito Bay-tissues, Table 5). Maximum concentration for the Caribbean coast was 485 µg/g-dw in tissues of *P. arctata* (Table 4). Manganese above 100 µg/g-dw was found in non-depurated *T. affinis* clams from the Cocorocas sand flat (Table 2), with a maximum of 255 µg/g-dw (Table 5), while much higher values were detected in tissues of *P. arctata*, with a maximum of 921 µg/g-dw (Table 5). Nickel in concentrations above 2 µg/g-dw was present in tissues of the snail *A. brevidentata* from Culebra Bay, Gulf of Nicoya, and Golfito Bay (Tables 1, 2, 3), with the highest concentration (10.5 µg/g-dw) in the shell of the ark clam *A. tuberculosa* from Golfito (Table 5). Concentrations of nickel were above 2 µg/g-dw in tissues and shell of *P. arctata* from the Caribbean coast, with a maximum of 8.8 µg/g-dw (Table 5).

Maximum concentrations of tin, cadmium and copper were respectively 3.74 µg/g-dw, 0.69 µg/g-dw, and 21.6 µg/g-dw, all in non-depurated tissues of the clam *T. affinis* (Table 2). The 72 hour depuration for *T. affinis* yielded interesting results as concentrations of Fe, Zn, Mn, and Ni were lower in depurated as compared with non-depurated tissues (Table 2). However, Pb concentrations were higher in depurated tissues.

Table 6 includes examples of maximum concentrations of metals in other species of molluks from selected locations around the world. When maximum concentrations in Table 5 are compared with those in Table 6, lead (195 µg/g-dw) and manganese (921 µg/g-dw) both in *P. arctata*, were found much higher in this study.

DISCUSSION

The generation and interpretation of data on trace metals in marine molluks are difficult for several reasons. Metal concentrations vary in individuals of the same species depending on their sex, age, and on the physiological and environmental conditions. In addition, parts (foot, tissues, gills, shell) of the same organism may accumulate metals in different concentrations (Cunningham, 1979; Zuykov, Pelletier, & Harper, 2013). The differences in accumulation of trace metals in parts of the same organism, is well known. The early report by Brooks and Rumsby (1965) on the scallop *Pecten novaezelandiae* illustrates this fact: Concentrations (µg/g) of metals in soft tissues were: Fe (6 900), Pb (23), Zn (368), Mn (306), Cd (299) and Cu (14), while for instance the gills had Fe up to 21 600 µg/g, the kidney had 137 µg/g of Pb, 2 630 µg/g of Zn, 2 660 µg/g of Mn, and 106 µg/g of Ni. The intestine had 131 µg/g of Cu, and the visceral mass had 2 000 µg/g of Cd. Samples collected relatively close to each other may also show differences due to point sources

TABLE 6
Examples of concentrations (µg/g dry wt) of metals found in selected mollusks

	A	B	C	D	E	F	G	H
Fe	3400	ND	ND	790 / 1100	ND	ND	ND	361.8
Pb	1.46	30.3	6.8	ND	0.10 - 8.46 / 0.11 - 46.34	2.06	ND	ND
Zn	4380	783	650	36 / 104	2.02 - 3700 / 2.04 - 80,724	ND	755	1.8
Mn	ND	ND	ND	18 / 19	ND	ND	ND	45.9
Ni	7.92	ND	6.5	ND	1.60 - 54 / 0.08 - 45.51	6.70	0.43	ND
Cd	2.57	2.85	0.70	1.1 / 0.30	0.02 - 20.6 / 0.02 - 374.1	0.50	0.67	2.9
Cu	866	229	440	8.2 / 4.6	1.13 - 560 / 1.25 - 166.36	ND	53	1.4

ND = No data. **A**: Sbriz et al. (1998, Table 4), maximum values in clams and oysters. Dominican Republic. **B**: Ming-Shiou et al. (2000, Table 2), maximun in snails, clams and oysters. Taiwan. **C**: Cohen (2001, Table 3), maximum in clam *Tagelus californianus*, California, USA. **D**: Otchere (2003, Table 3), maximum in the ark clam *Anadara senilis*, Ghana, dry season / wet season. **E**. Bayen (2012, Table 4): ranges reported in the literaure review for: mangrove gastropods / mangrove bivalves. **F**. Pérez-Cruz, et al. (2013, Table 3), clam *Polymesoda arctata*, Mexico, Caribbean. **G**. Rojas de Astudillo et al. (2005,Table 2, maximum in oysters),Venezuela. **H**. Ndome et al. (2010, Table 1, mean concentrations), sandy beach clam *Donax rugosa*, Nigeria.

of different magnitudes. Data presented in this study include examples of this variability and point out to the need for more detailed studies in space and time. Some metals are essential in the physiology of molluks. For instance, iron is found in hemoglobin, a respiratory red pigment that gives the name “blood cockles” to *Anadara* ark clams, while other mollusks may contain hemocyanin, a green pigment with copper. Clark (2001) classifies metals in two groups: transitional (like Fe, Cu, and Mn), which are essential at low concentrations, and metalloids (like Pb and Sn) which are not essential but are toxic at low concentrations. Cadmiun is also not essential but bivalve molluks tend to accumulate it. Many enzymes contain zinc and other metals (Lane et al., 2005). Clark (2001) also indicates that in some marine ecosystems the natural amount of a substance might be altered due to anthropogenic actions, and this can be considered as contamination. But at the same time if there is a bigger natural input of such a substance in other areas, its concentration there could be higher than that caused by human intervention.

With the above facts in mind the four coastal sites evaluated for trace metals in this study differ in the nature and magnitude of the inputs from natural sources and human activities. For instance, the Gulf of Nicoya is under the influence of estuarine water mass circulation patterns (Voorhis, Epifanio, Maurer, Dittel, & Vargas, 1983) that may bring contaminants from the Tempisque (agricultural) and Tárcoles (agricultural, industrial, sewage) rivers in contact with mollusks located far apart from the point sources. On the other hand, no major river discharges wastes into Culebra Bay where sewage and runoff inputs are also minimal in this the driest region of Costa Rica. Thus, the expected low concentrations of metals in Culebra Bay were so for Fe, Mn, and Ni, but Pb and Zn were similar to values found in the other Pacific sites (Table 5). Golfito Bay is under the direct influence of runoff (heavy rains are present year-round) and untreated sewage from the city of Golfito settled along its shores. Concentrations of Fe and Pb were similar in the Gulf of Nicoya and in Golfito, but Zn and Ni in Golfito had the highest values of the four embayments (961 and 10.5 µg/g-dw, respectively, Table 5). Concentrations of Pb were around 22 µg/g in tissues of *A. tuberculosa* from the Gulf of Nicoya and Golfito (Table 5).

On the Caribbean coast Moín Bay receives inputs (agricultural, industrial) from the Moín River and from the operation of many small river boats equipped with outboard fuel-leaded engines. The highest concentration of Pb found in *P. arctata* from Moín Bay (195 µg/g-dw) is probably related to this activity. The highest mean concentration of Mn (921 µg/g-dw) also found in *P. arctata* is difficult to identify as to its possible source. Nonetheless, according to Simkiss & Mason (as cited in Páez-Osuna, 2005), it is usual to observe high concentrations of metals in some tissues of mollusks as part of a detoxification process related to proteins, and also it has been found that some female mollusks have higher Mn and Zn concentrations than males (Marina & Enzo, 1983). In our study we did not discriminate between males and females. The nature of the substrate is also important in studies of trace metals. Finer sediments usually contain higher concentrations of contaminants than coarser sediments (Ujevic, Odzak, & Baric, 2000). In this context clams like *A. tuberculosa* (Gulf of Nicoya and Golfito) and *P. arctata* (Moín Bay) are found in fine grained sediments, while *T. affinis* lives in an extensive coarse sand flat at the mouth of the Lagarto River, which drains mostly grass lands for cattle raising. The snail *A. brevidentata* lives on sedimentary and igneous rocks in Culebra Bay, Gulf of Nicoya, and Golfito Bay. Recent evidence also suggests that the nature (type and species of microalgae) of phytoplankton ingested by bivalves influences the assimilation of certain metals, Hédouin et al. (2010).

In Costa Rica the clams *A. tuberculosa*, *T. affinis* and *P. arctata* are under different fishing pressures as food items. However, research has been focused mainly on the heavily harvested *A. tuberculosa* (Stern-Pirlot & Wolff, 2006). Hossen, Hamdan and Rahman (2014) found concentrations of Pb of 2.65 to 4.36 µg/g-dw in *Anadara granosa* in Malaysia. They point out that the maximum permissible limit of Pb by the Malaysian Food Authority is 2.00 µg/g-dw. According to this reference level, the *A. tuberculosa* specimens from Costa Rica analyzed in this study were not suitable for human consumption, nor were specimens of the other bivalves analyzed. The results obtained with the 72 hour depuration experiment of *T. affinis* clams are important in the context of lowering the content of certain metals enough to make clams suitable for human consumption. Median concentrations of Fe, Zn, Mn, and Ni were lower in depurated tissues when compared to non-depurated. However, the increase of Pb in depurated tissues is interesting and deserves further study.

We have also included some reports on trace metal concentrations from tropical molluks. The maximum values (µg/g-dw) reported are: Fe 3400, Pb 46.34, Zn 4 380, Mn 45.9, Ni 54, Cd 374.1, and Cu 866. In this context the relatively high concentrations of Pb (195) and Mn (921) found in this study are noteworthy. Depuration could be applied also to the widely used food item *A. tuberculosa* from the Gulf of Nicoya and Golfo Dulce. However, depuration seems to produce unexpected results. For instance, Yap Muhamad, Cheng and Tan (2011) found no significant difference in the depuration levels between Cu and Zn in soft tissues of *A. granosa* from Malaysia. Their study also evaluated the concentration of metals in the shells and concluded that in the long term shells may be able to accumulate higher concentrations of metals than the soft tissues. The relatively high concentrations of Ni and Mn found in shells of *A. tuberculosa* may be indicative of similar mechanisms in this species. The concentrations of trace metals found in this pilot survey are generally high for mollusks that continue to be harvested for human consumption.

It must be taken into account that the data on metals presented herein was collected more than a decade ago but remains as the most recent reference for trace metal concentrations in coastal mollusks from Costa Rica. Input sources of contaminants to the four embayments are expected to have increased over the past decade due to local coastal development and to water and sediment loads carried by the rivers. A new detailed spatial and temporal evaluation of metals in mollusks and other invertebrates is advised.

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RESUMEN

Metales en moluscos costeros de Costa Rica. La llegada de contaminantes a los ecosistemas costeros es un problema global en aumento. Los datos sobre contaminación por metales en sitios tropicales son escasos y se necesitan con urgencia estudios para proveer un marco de referencia y estimar el impacto relativo de estos y otros contaminantes. El objetivo de este trabajo fue hacer accesible los datos de metales traza recolectados durante estudios piloto (2000-2006) en cuatro bahías de Costa Rica. Cu, Fe, Mn and Zn fueron analizados por Absorción Atómica de llama (AAL) y Al, Cd, Ni, Pb y Sn por Absorción Atómica en Horno de grafito (AAHG). Las concentraciones de Fe, Pb, Zn, Mn, y Ni fueron determinadas en moluscos mediante AAL o en AAHG, en tejidos del caracol de las rocas *Acanthais brevidentata*, los bivalvos infaunales *Anadara tuberculosa* y *Tagelus affinis* de la costa Pacífica (Bahía Culebra, Golfo de Nicoya, y Bahía de Golfito) y en la almeja *Polymesoda arctata* de la costa Caribe (Bahía de Moín). Adicionalmente, Cd, Cu y Sn, fueron evaluados en tejidos de *T. affinis*. Un grupo de *T. affinis* fue depurado por 72 horas en agua de mar del sitio, filtrada. Las concentraciones (μg/g peso seco) variaron entre y dentro de los sitios y también entre las partes de los mismos organismos. Las concentraciones máximas fueron: Fe 2230 (*P. arctata*-tejidos, Bahía de Moín), Pb 195 (*P. arctata*-tejidos, Bahía de Moín), Zn 961 (*A. brevidentata*-tejidos, Bahía de Golfito), Mn 921 (*P. arctata*-tejidos, Bahía de Moín) y Ni 10.5 (*A. tuberculosa*-concha, Bahía de Golfito). Las concentraciones mínimas fueron: Fe 5.36 (*P. arctata*-tejidos, Bahía Moín), Pb <0.20 (*P. arctata*-pie, Bahía de Moín), Zn 2.75 (*P. arctata*-concha, Bahía de Moín), Mn 5.5 (*A. tuberculosa*-pie, Golfo de Nicoya) y Ni 0.83 (*A. tuberculosa*-pie, Bahía de Golfito). Los tejidos de *T. affinis* no depurada mostraron concentraciones máxima-mínima de Sn (3.74-2.73), Cd (0.69-0.43) y Cu (21.6-14.8). Las concentraciones, excepto para Pb y Mn, estuvieron dentro de valores reportados en la literatura reciente. La relativamente alta concentración de Pb probablemente estuvo relacionada con el uso de gasolina adicionada con plomo en la operación de embarcaciones menores en la época de la toma de muestras, mientras que no se encontró causa evidente para los altos valores de Mn. La depuración fue parcialmente efectiva en bajar la carga de metales en *T. affinis*. Los datos obtenidos durante el estudio piloto son indicativos de condiciones relativamente limpias en Bahía Culebra, mientras que los otros tres sitios tienen concentraciones importantes de ciertos contaminantes, incluyendo metales. No obstante que los datos fueron recolectados hace más de una década, estos son los más recientes disponibles para moluscos costeros de Costa Rica.

Palabras clave: *Acanthais, Anadara*, *Polymesoda, Tagelus*, contaminación, estuario, almejas, pesquería.

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