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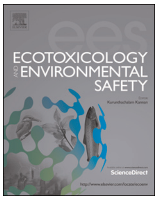

Isopods of the genus *Ligia* as potential biomonitors of trace metals from the gulf of California and pacific coast of the Baja California peninsula.

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ABSTRACT

Supralittoral and high intertidal coastal zones are exposed to pollution from both marine and terrestrial sources and undergo higher deposition rates than the subtidal zone. It is therefore important to identify organisms for this section of the coastal area that can be tolerant to contaminants. The aim of this study was to determine if supralittoral isopods of the genus *Ligia* can be used as biomonitors, since they are abundant and widely distributed. For this purpose, concentrations of trace elements were determined in *Ligia* isopods *in toto* from 26 locations across the Gulf of California and Pacific coast of the Baja California peninsula, which were collected during the summers of 2009 and 2010. The concentrations of trace elements followed the order of; $Zn \geq Cu > As > Cd > Pb > Hg$. Elevated concentrations of copper (up to 1010 μg/g) were detected in *Ligia* from Santa Rosalía (SRo), a locality where industrial mining of copper has historically occurred. Industrial and municipal sewage discharges appear to have contributed to the high concentrations of zinc (326 μg/g) and lead (144 μg/g) found in organisms from Guaymas location. The high mercury concentration in organisms from Mazatlán (M) (2.01 μg/g) was associated with a thermoelectric plant. Natural sources of metals were also detected; coastal upwelling appears to be associated with high cadmium concentrations in *Ligia* from Punta Baja (PB) (256 μg/g) in the Pacific coast, whereas hydrothermal vents may have contributed to high concentrations of arsenic at Ensenada (E) (61 μg/g). Our results suggest that *Ligia* isopods reflect the natural and anthropogenic inputs of trace metals in the environment and could potentially be used as biomonitor organisms of the intertidal rocky shores of the Gulf of California and Pacific coast.

1. Introduction

The supralittoral and high intertidal zones occupy a narrow stretch of the shoreline that is characterized by high organismal diversity. These zones are exposed to pollution from both marine and terrestrial sources and undergo higher deposition rates than the subtidal zone (Li et al., 2008; Ruiz-Fernández et al., 2009; Seung-Kyo et al., 2010; Yap et al., 2009). The aim of this study is to determine if supralittoral *Ligia* isopods can be used as biomonitor organisms.

Crustaceans that spend their entire lives in this narrow margin have proven to be highly informative for monitoring trace element pollution. This is the case for amphipods such as *Talorchestia* and *Talitrus saltator* (Ugolini et al., 2004; Ungherese et al., 2010), *Caprella* (Guerra-García et al., 2010) and isopods, such as *Ligia oceanica* (Hopkin et al., 1985) and *L. italica* (Longo et al., 2013) which have been used as biomonitor organisms of trace metal contamination in the Mediterranean and North Sea. The mechanisms of metal bioaccumulation have been studied in terrestrial isopods

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(i.e., *Porcellio scaber, Oniscus asellus*), and those organisms have an extraordinary capacity to bioaccumulate toxic metals from the environment, especially copper which becomes concentrated in the hepatopancreas (Wieser et al., 1977). Other heavy metals such as zinc, lead and cadmium accumulate in vesicles, such as lysosomes (Paoletti and Hassall, 1999; Prosi and Dallinger, 1988). These characteristics make isopods, both marine and terrestrial, extremely useful as indicators of pollution.

Isopods of the genus *Ligia* have a set of characteristics that make them potentially useful for monitoring pollution in the supralittoral and high intertidal region. First, they are widely distributed along the coast of the Gulf of California, its islands, and the Pacific coast of the Baja California peninsula. Hurtado et al. (2010) report the presence of these isopods in >100 coastal localities in this region, which are subject to varying levels of anthropogenic activity, and they are likely to be found at many yet unexplored localities. Second, they occur exclusively at beaches with rocky supralittoral zones, where they are restricted to the narrow range between the splash zone and the supralittoral. They perform an important ecological function as these isopods are scavengers that primarily feed on algae left by the tides, and in some locations, they are the primary prey for endemic lizards (Grismer, 1994). Crabs, fish, marine birds, and even small mammals can also consume these isopods (Brusca, 1980; Hurtado personal observations; Pennings et al., 2000). Third, *Ligia* isopods have very low dispersal potential, so they are constrained to complete their entire life cycle within the same rocky beach (Hurtado et al., 2010). Thus, individuals must truly reflect the conditions of the beaches where they are found. Fourth, *Ligia* isopods bioaccumulate trace elements from the environment within their tissues. This has been reported in specimens from Guaymas, located on the northern mainland coast of the Gulf of California, where trace element concentrations in *Ligia* tissues reflected local sources of pollution (Güido-Moreno, 2012).

Consistent with their low dispersal potential, long-standing isolation is evident among *Ligia* populations in the Gulf of California and Pacific coast of the Baja California peninsula (Hurtado et al., 2010). Most of the populations represent highly genetically differentiated lineages, which suggests that *Ligia occidentalis* Dana 1853, the only species recognized in this region, is part of a complex of cryptic species (*Ligia occidentalis sensu lato*; Eberl et al., 2013; Hurtado et al., 2010; Markow and Pfeiler, 2010), which we will hereafter refer to as *Ligia*.

High levels of trace metals, from natural and anthropogenic sources, have been found in intertidal sediments and organisms from different localities inhabited by *Ligia* in the Gulf of California and the Pacific coast of Baja California (Cadena-Cárdenas et al., 2009; Forrest and Ledesma-Vázquez, 2009; Martin and Broenkow, 1975; Méndez et al., 2004; Páez-Osuna et al., 2002; Shumilin et al., 2000).

In this study, we report the concentrations of five metals (cadmium, copper, mercury, lead and zinc) and a metalloid (arsenic) found in the whole tissue of *Ligia* isopods collected in localities with different degrees of contamination across the Gulf of California and the Pacific coast of the Baja California peninsula.

2. Materials and methods

2.1. Sampling and classification of sites

Our field observations identified the summer months as the time when larger isopod individuals are more abundant in the Gulf of California. According to Carefoot (1973), the growth of the supralittoral isopod *L. pallasii* is slow over the winter but accelerates in the spring, so by mid-summer, organisms attain a much larger size. Because larger individuals may accumulate higher concentrations of metals, we directed our sampling efforts to the two consecutive summers. *Ligia* isopods were manually collected from 26 locations across the Gulf of California and the Pacific coast of the Baja California peninsula (Fig. 1). A total of 22 stations were surveyed from July 17 to August 25, 2009, and 17 locations were surveyed from August 16 to 22, 2010 for a total of 13 sampling locations each year. A sample unit consisted of 20 to 30 *Ligia* individuals from a particular station. Whole organisms were pooled from each station, placed in sealed plastic jars and kept on ice during the collection trip, and then frozen upon arrival at the laboratory. One sample unit was collected per location.

2.2. Analytical procedures

Each sample unit was thawed, rinsed with distilled water and dried with a clean paper towel. Organisms were then homogenized *in toto* at room temperature with a mortar and pestle until a paste was formed. The water content of each sample unit was calculated by weighing a portion of the paste before and after drying the sample in a convection oven at 60 °C for 48 h. The digestion of samples was performed using a CEM Corp. Mars$_x$ microwave system (Matthews, NC, U.S.A.), and 1 g of the fresh homogenate paste was placed in a HP-500 vessel with 5 ml of 50% nitric acid. The vessels were mounted on a turntable and digested for 20 min as follows: a 5 min ramp at 100 °C and 65 PSI, a 5 min ramp at 120 °C and 100 PSI, and a 15 min hold at 140 °C and 140 PSI. A second digestion was performed by adding 3 ml of 30% hydrogen peroxide to the sample and digesting it as described above. Distilled water was added to each sample to achieve a total volume of 50 ml. The above procedures were based on the US EPA 3052 method for the digestion of tissue. The following analyses were performed in a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 1100B. Copper and zinc were analyzed by direct aspiration (US EPA methods 7210 and 7950); arsenic, cadmium and lead were analyzed by graphite furnace (US EPA methods 7060A, 7131A and 7421, respectively); mercury was analyzed by hydride generation with an MHS-20 (US EPA method 7471A). Data are expressed as μg/g dry weight.

For quality control/quality assurance purposes, blanks, duplicates and references (DOLT-4 dogfish liver reference materials for trace elements from the National Research Council, Canada) were analyzed for each batch of 10 samples. The percent relative difference ranged from 0% to 10% for all samples and elements, and the percent recovery was 80% for lead, 95% for mercury, 97% for copper, 105% for arsenic, 113% for zinc, and 90% for cadmium. The method detection limits (MDL) were as follows: 0.2 μg/g for copper and zinc, 0.05 μg/g for lead and cadmium, 0.01 μg/g for arsenic, and 0.0005 μg/g for mercury.

3. Calculations

Each sampled location was classified according to its degree of human pressure using an index of general anthropogenic stress (Guerra-García et al., 2010):$Sa=(\sum Pa/Dt)\ \mathrm{x}A$. Sa is the general anthropogenic stress; Pa is the total area of each of the main population centers within a 15 km radius from the site; Dt is the distance of the centroid of each population from the site, and A is a measure of disturbance at the collection site based on a subjective assessment of ease of access and human activity. Locations that are difficult to access and subject to low levels of human activity were assigned an A value of 1 whereas very accessible places with a high degree of human activity were assigned an A value of 5.

To compare the different trace element concentrations at the different sampling sites and between the different years of

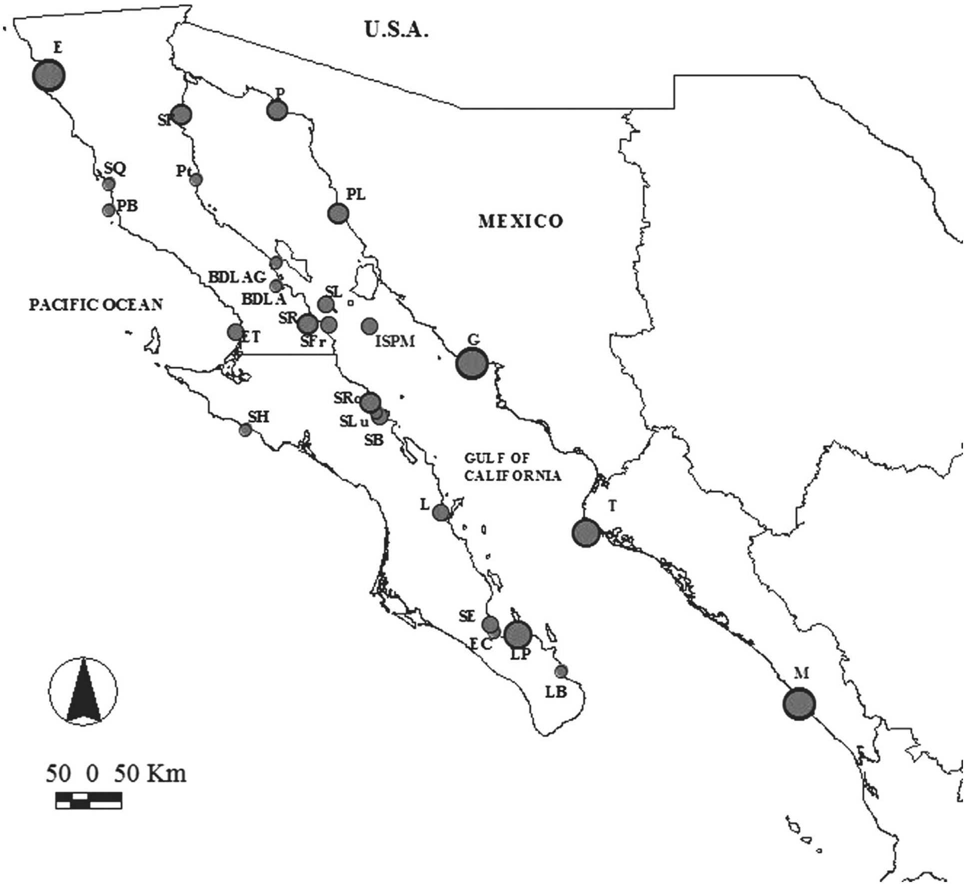

Fig. 1. Locations of the sampling stations during the 2009 and 2010 surveys. The size of the circle on the map corresponds to the anthropogenic stress index (*Sa*) value. Small circles had *Sa* values ranging from 0.1 to 5, medium circles from 5.1 to 10, and large circles had values ranging from10.1 to 41. In this caption, specific *Sa* values are in parentheses, and italic letters indicate the sites that were visited both years. *BDLA*, Bahía de los Angeles (0.70); BDLAG, Bahía de los Angeles la Gringa (0.13); *E*, Ensenada (32.7); EC, El Cajete (1.03); *ET*, El Tomatal (2.63); G, Guaymas (36.3); ISPM, Isla San Pedro Mártir (2); *L*, Loreto (1.86); LB, Los Barriles (1.01); *LP*, La Paz (17.4); M, Mazatlán (40.87); *P*, Puerto Peñasco (5.4); PB, Punta Baja (0.29); PL, Puerto Libertad (4.56); *Pt*, Puertecitos (0.61); *SB*, San Bruno (1.80); *SE*, San Evaristo (1.74); *SF*, San Felipe (8.46); SFr, San Francisquito (1.78); SH, San Hipólito (1.09); SL, San Lorenzo (1.42); SLu, San Lucas (0.72); SQ, San Quintín (0.38); *SR*, San Rafael (4.0); *SRo*, Santa Rosalía (6.0) and *T*, Topolobampo (22.0).

collection, a metal pollution index (*MPI*), defined by Usero et al. (2005), was used. It was obtained with the equation: $MPI=(Cf_1 \text{ x } Cf_2 \ldots Cf_n)^{1/n}$ where Cf_1=the concentration of the first metal, Cf_2=the concentration of second metal, and Cf_n=the concentration of the *n*th metal for each location.

3.1. Statistical analysis

Locations were classified into three groups according to their *Sa*: low ($Sa < 5$), medium (*Sa* between 5.1 and 10), and high ($Sa > 10$). The metal pollution index (*MPI*) value among these three *Sa* groups at each location was compared with a nonparametric test (Kruskal–Wallis). Least squares linear regression analysis was performed between the *Sa* and *MPI* values, and the correlations between *Sa* and the individual element concentrations were also examined with the least squares method. All of the statistical analyses were performed using JMP software version 9.0.2 from the SAS Institute (Sall and Lehman, 1996).

4. Results and discussion

4.1. Anthropogenic stress

Values of *Sa* in the study region varied from 0 to 41 (Fig. 1). The majority of the sampling locations (70%) exhibited a low *Sa* (<5), and they corresponded to the isolated, rocky coastal shores of Baja California and the Sonora coasts, which exhibit little or no obvious human impacts. Three sites (11%) exhibited a medium *Sa* (5.1–10). Five locations (19%) exhibited a high *Sa* (10–41), and they corresponded to sites within, or in the vicinity of cities with more than 100,000 inhabitants, including La Paz, Topolobampo, Ensenada, Guaymas, and Mazatlán (Fig. 1). However, this index was not indicative of mining activities, and some locations, such as the historical copper mining district (since 1896) of Santa Rosalía (SRo), had a medium *Sa* despite the high anthropogenic impacts from this activity. The inclusion of mining impacts in this or another index is recommended for future studies.

The metal pollution index (*MPI*) varied from 2.31 to 48.8 in 2009 (Table 1). The highest *MPI* corresponded to Punta Baja (PB) where the *Sa* was low (0.3). In 2010, *MPI* values ranged from 5.14 to 36.4 (Table 2), and the highest *MPI* corresponded to Guaymas (G), a location with high *Sa* (36). No differences were found between the *MPI*s of sites with low, medium and high *Sa* values for both years (Kruskal–Wallis test, *p-value*=0.57), and no correlation was found between the *Sa* and *MPI* values ($R^2=0.02$) for 2009. In 2010, a positive correlation between these two parameters was found ($R^2=0.4$, *p-value*=0.003). However, if the Guaymas site (G) was excluded, the R^2 value decreased to 0.08 and was not significant. *MPI* values from locations with low *Sa* showed greater variation than those with medium or high *Sa* values (Tables 1 and 2).

The lack of a correlation between the *MPI* and the *Sa* indices shows that factors other than direct anthropogenic impacts are

Table 1
Concentrations of trace elements in *Ligia* sample units collected in 2009 and the values of the metal pollution index (*MPI*) for each location (see Fig. 1 for abbreviations).

Sampling location	Metal and metalloid concentration (μg/g dry wt.)						MPI
	Cu	Zn	As	Cd	Pb	Hg	
Low *Sa*							
BDLAG	< DL	53.1	56.4	11.7	30.6	0.918	7.63
PB	258	106	29.0	256	43.8	1.52	48.8
SQ	NS	NS	NS	NS	NS	NS	NS
Pt	< DL	53.2	34.9	35.1	23.3	< DL	2.31
BDLA	13.2	52.7	8.17	66.5	37.7	< DL	4.38
SLu	140	82.3	14.8	26.1	34.1	0.921	22.8
LB	99.7	ND	33.4	182	34.5	0.622	26.5
EC	13.8	111	12.9	17.8	15.6	1.47	14.2
SH	34.8	107	15.2	49.6	18.5	0.842	18.8
SL	NS	NS	NS	NS	NS	NS	NS
SE	5.78	110	12.6	32.4	15.3	0.508	11.2
SFr	76.5	164	11.4	232	13.9	1.73	30.5
SB	36.5	112	36.9	63.0	45.3	< DL	7.75
L	137	161	20.8	232	33.4	1.11	39.8
ISPM	NS	NS	NS	NS	NS	NS	NS
ET	74.8	187	30.5	167	37.0	0.217	28.8
SR	29.1	66.9	41.5	64.7	33.9	1.58	25.6
PL	< DL	107	22.8	140	34.4	0.413	9.95
mean	61.3	105	25.4	105	30.1	0.790	19.9
Std. Dev	72.6	42.5	13.6	87.4	10.3	0.600	21.1
Medium *Sa*							
P	56.4	93.9	31.8	27.5	27.7	0.203	17.2
SRo	1005	163	9.91	36.0	46.3	0.474	33.0
SF	140	137	21.9	8.03	26.7	0.384	18.1
mean	401	131	21.2	23.8	33.6	0.350	22.7
Std. Dev	525	34.9	11.0	14.3	11.1	0.140	8.86
High *Sa*							
LP	76.0	163	14.3	28.2	41.3	1.14	24.8
T	93.8	104	17.3	6.33	21.1	0.853	16.4
E	58.3	167	61.0	36.2	53.8	0.235	25.4
G	NS	NS	NS	NS	NS	NS	NS
M	98.2	109	30.0	23.5	41.5	2.01	29.3
mean	81.6	136	30.7	23.6	39.4	1.06	24.0
Std. Dev	18.3	33.6	21.3	12.6	13.5	0.740	5.44

NS = locations not sampled.
< DL = concentrations under the detection limit.
ND = no data.

playing an important role in the trace element concentrations in supralittoral populations of *Ligia* isopods in the study area. These factors are discussed for each metal and metalloid in the following sections.

In general, the concentrations of trace elements were ordered as follows: Zn ≥ Cu > As > Cd > Pb > Hg for both years (Tables 1 and 2 and Fig. 2).

4.2. Copper

Copper concentrations in *Ligia* isopods were detected in 82% of the sample units in 2009 and in 94% of the sample units in 2010. Concentrations varied from non-detectable to 1013 μg/g (Fig. 2). Low or undetectable concentrations of copper were found at Puerto Libertad (PL), Bahía de los Angeles (BDLAG), Puertecitos (Pt), and El Tomatal (ET); medium concentrations were detected at Punta Baja (PB) and Guaymas (G), and the highest concentration of copper occurred at Santa Rosalía (SRo) in both years (Tables 1 and 2). A study of the trace element composition of Santa Rosalía coastal sediments showed that the location where the *Ligia* isopods were collected is a "hot spot" for copper, zinc, lead, and cobalt (Shumilin et al., 2000). According to these authors, the concentration of copper in the beach sediment was 1906 mg/kg, which was higher than the concentrations found in arroyo bed samples, solid wastes and coastal sediments from the Santa Rosalía mining district and higher than most contaminated locations around the world.

Tests of chronic exposure and acute toxicity were performed on the coastal isopod *Idotea baltica*, which is distributed in the Baltic and Mediterranean seas (de Nicola Giudici and Guarino, 1989). Organisms were exposed to solutions of different concentrations of copper sulfate for 120 days, and the results showed that the survival of *Idotea* is inversely related to the concentration of copper; the mean LT_{50} with 1 mg/l of copper sulfate was between 12 and 30 days. Tolerance was higher in females followed by males with the lowest tolerance observed in juveniles. Sublethal effects were observed at concentrations as low as 0.005 mg/l, and survival was strongly reduced in the embryonic and juvenile stages. Long-term exposure at 0.5 mg/l caused reduced growth and survival to 10% by the 120th day with females being markedly more resistant than the males. Therefore, the authors conclude that *Idotea* populations are unable to survive long-term copper exposure.

Copper concentrations in coastal water samples were measured at the Guaymas location (G) by Güido-Moreno (2012), and values ranged from < ND to 0.22 mg/l with a mean of 0.10 mg/l. These values are high compared with background Cu concentrations in seawater, which are commonly 0.0001 mg/l (Förstner, 1983). Therefore, it is likely that isopods from (G) are affected by

Table 2
Concentrations of trace elements in *Ligia* sample units collected in 2010 and the values of the metal pollution index (*MPI*) for each location (see Fig. 1 for abbreviations).

Sampling location	Metal and metalloid concentration (μg/g dry wt.)						MPI
	Cu	Zn	As	Cd	Pb	Hg	
Low*Sa*							
BDLAG	NS	NS	NS	NS	NS	NS	NS
PB	NS	NS	NS	NS	NS	NS	NS
SQ	75.6	113	18.3	111	21.8	0.775	25.8
Pt	18.9	56.7	< DL	42.0	27.9	0.294	5.14
BDLA	18.9	94.3	13.4	85.4	25.2	0.226	15.0
SLu	NS	NS	NS	NS	NS	NS	NS
LB	NS	NS	NS	NS	NS	NS	NS
EC	NS	NS	NS	NS	NS	NS	NS
SH	NS	NS	NS	NS	NS	NS	NS
SL	18.6	93.2	25.6	43.7	24.1	0.503	16.9
SE	57.9	154	7.05	55.8	22.3	0.309	17.0
SFr	NS	NS	NS	NS	NS	NS	NS
SB	18.8	75.2	9.05	3.72	20.8	0.207	7.68
L	56.3	75.0	3.92	25.7	59.0	0.657	16.0
ISPM	55.0	91.7	32.5	43.9	26.2	0.953	23.8
ET	< DL	91.7	3.98	140	25.4	0.898	7.84
SR	18.6	74.4	17.8	27.9	26.6	0.372	13.8
PL	NS	NS	NS	NS	NS	NS	NS
mean	33.9	92.0	13.2	57.9	27.9	0.520	14.9
Std. Dev	24.8	26.9	10.4	42.0	11.2	0.280	6.71
Medium*Sa*							
P	18.9	75.5	8.67	33.3	24.4	0.434	12.8
SRo	1013	188	21.1	17.5	26.8	< DL	9.90
SF	54.2	72.3	17.9	5.48	21.7	0.416	12.3
mean	362	112	15.9	18.8	24.3	0.280	11.7
Std. Dev	564	65.7	6.44	14.0	2.58	0.250	1.54
High*Sa*							
LP	38.8	136	6.98	6.33	25.0	0.233	10.5
T	38.4	76.9	17.1	5.32	24.8	0.730	13.0
E	92.0	73.6	6.85	45.3	24.6	0.294	15.7
G	325	326	14.4	16.5	144	0.647	36.4
M	NS	NS	NS	NS	NS	NS	NS
mean	123	153	11.3	18.4	54.5	0.480	18.9
Std. Dev	136	119	5.24	18.7	59.4	0.250	11.9

NS=locations not sampled
< DL=concentrations under detection limit.

this metal because the mean copper concentration in seawater from (G) was twice as high as the threshold for effects on survival and growth in *Idotea* (de Nicola Giudici and Guarino, 1989).

However, *Ligia* isopods were still present under these conditions, and there might be several explanations for this. One is that free copper, which is believed to be the form toxic to aquatic organisms, is removed by precipitation (malachite formation). Reeve et al. (1977) found that soon after addition, measurable copper was only 50–75% of that added. Second, copper toxicity is influenced by environmental factors, such as dissolved oxygen, temperature, turbidity, carbon dioxide, phosphate and magnesium salts, organic compounds, etc. (Bender et al., 1970). Third, studies of terrestrial isopods (*Porcellio scaber*) show that the bioavailability of metals in the laboratory is often higher than under field conditions due to environmental heterogeneity, which provides food choice for animals that is absent from many laboratory test systems (Van Wensem et al., 1992). Fourth, organisms living in contaminated locations show higher tolerances to contaminants. According to Moraitou-Apostolopoulou (1978), the 48 h LC_{50} of copper sulfate for the marine copepod *Acartia clausi* was 0.034 mg/l for populations from an uncontaminated area and 0.082 mg/l for populations from a polluted area. Brown (1977) performed a histochemical hepatopancreas analysis of a copper-tolerant*Asellus meridianus* population, in which particularly large (5–10 μm diameter) spherical inclusions were present in the cecae. The author concludes that the hepatopancreas is an important storage organ for this metal. Recent studies of acclimation and fitness costs in the intertidal copepod *Tigriopus japonicus* confirmed enhanced Cu resistance after just one generation. However, the acquired Cu resistance had a fitness cost as the intrinsic population growth rate of this Cu-resistant lineage was significantly lower than the control (Kwok et al., 2009).

Research on the genetic effects of copper on subsequent generations using the harpacticoid copepod *Amphiascoides atopus* (Puello-Cruz et al., 2014) is now in progress to better understand copper toxicity and resistance in *Ligia* isopods.

4.3. Zinc

Zinc was present in 100% of the sample units in both years, and concentrations ranged from 52.7 μg/g at BDLA up to 326 μg/g at G (Tables 1 and 2 and Fig. 2). Zinc concentrations at Guaymas Bay (G) have been associated with municipal wastewater discharge and industrial activities with reported sediment concentrations of between 366 and 476 mg/kg (Méndez et al., 2004). *Ligia* from other industrial ports in our study area, such as Topolobampo and Mazatlán, did not exhibit high zinc concentrations, and no correlation was found with *Sa*. Factors such as the different types of industrial activities at these ports may have influenced the results.

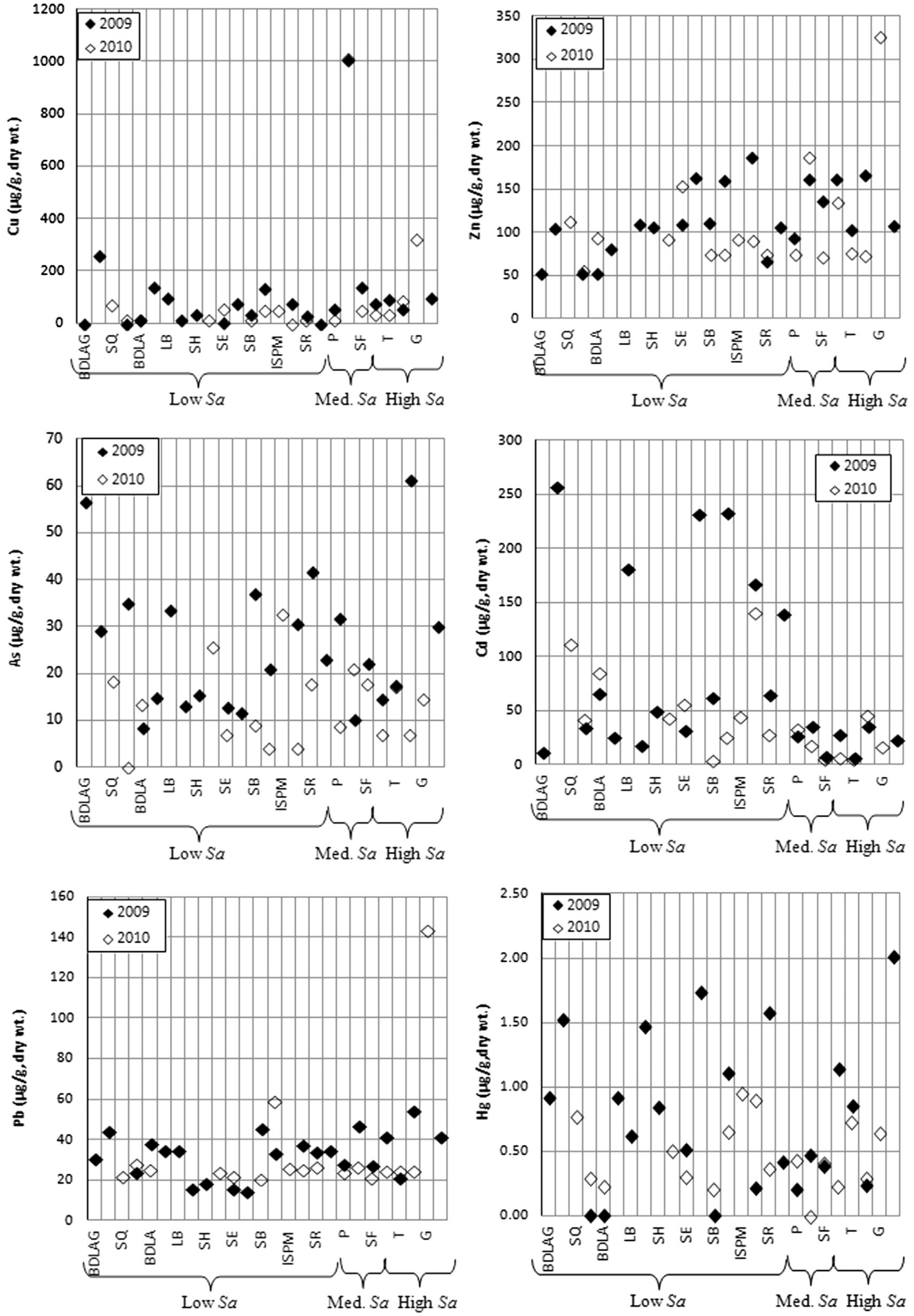

Fig. 2. Concentrations of Cu, Zn, As, Cd, Pb and Hg in *Ligia* sample units from the different locations (see Fig. 1 for location abbreviations) collected in the summers of 2009 and 2010.

A chronic toxicity experiment with *I. baltica* by Bat et al. (1999) showed that survival decreased with increased zinc, copper and lead concentrations; males were more sensitive than females, and zinc was found to be more toxic than copper or lead. The LT_{50} for 1 mg/l of zinc was 5 days and concentrations as low as 0.01 mg/l caused mortality in males after 20 days and in females after 25 days. The mean zinc concentration in seawater at G was 0.24 mg/l (Güido-Moreno, 2012), and this concentration exceeded the experimental threshold of mortality in *Idotea* after 20 days (Bat et al., 1999). However, as with copper, *Ligia* isopods also appear to have mechanisms that allow them to survive in supralittoral environments with high zinc concentrations.

4.4. Arsenic

Arsenic was found in 97% of the sample units, and concentrations ranged from $<$DL to 61.0 µg/g (Fig. 2). In 2009, the highest concentration of arsenic was found in a sample unit from Ensenada (E), and similar concentrations were detected at Bahía de los Angeles-La Gringa (BDLAG) (56.4 µg/g). In 2010, however, the concentration of arsenic at E decreased to 6.85 µg/g, and the highest concentration was observed in a sample unit from Isla San Pedro Mártir (ISPM) (Tables 1 and 2). Arsenic was the only element found at different concentrations at sites sampled in both 2009 and 2010 (Wilcoxon test, *p-value*=0.0025).

Ensenada (E) is located 15 km north of Punta Banda where shallow-water hydrothermal vents occur at a depth of 40 m, and arsenic enrichment has been reported around these vents (Prol-Ledesma et al., 2004). However, arsenic concentrations decreased considerably at this location in 2010; oceanographic conditions (i.e., currents and wind patterns) could have changed and affected the transport of arsenic to the shore. Active underwater geothermal springs may have also contributed to the high arsenic concentrations detected in *Ligia* from Bahía de los Angeles-La Gringa (BDLAG). Coastal geothermal springs have been reported ~90 km south of this locality, at San Francisquito Bay, and further south in Bahía Concepción (Forrest and Ledesma-Vázquez, 2009).

Compared with other metals, there are few studies of arsenic toxicity in aquatic invertebrates. Canivet et al. (2001) used freshwater macroinvertebrates, including an isopod (*Asellus aquaticus*), to test for arsenic and chromium toxicity. Isopods were relatively resistant to arsenic with an LC_{50} of 2.31 mg/l. In addition, arsenic accumulation in tissues of *A. aquaticus* exposed to 0.1 mg/l over a 10 day period was 26.6 µg/g. This concentration was higher than that in the control animals, but few mortalities occurred during this period (11%). The authors note the possible accumulation of arsenic in the hepatopancreas, where up to 60% of the total body burden of metals, such as copper and cadmium, are accumulated and taken out of circulation (Van Hattum et al., 1996). The mean concentration of arsenic in the water at G was 2.9 mg/l (Güido-Moreno, 2012), and the concentration in *Ligia* from this locality was 14.4 µg/g (Table 2). Therefore, it is likely that isopods accumulate arsenic directly from seawater, but this semi-metal is not as toxic to isopods, as the other metals analyzed here.

4.5. Cadmium

Cadmium concentrations varied considerably among locations (Fig. 2). They were highest at Punta Baja (PB) on the Pacific coast of the Baja California peninsula (256 µg/g) followed by Loreto (L), San Francisquito (SFr), and Los Barriles (LB). Lower cadmium concentrations were found at Topolobampo (T), La Paz (LP), San Felipe (SF), and San Bruno (SB) in both years (Tables 1 and 2). Higher concentrations were found at locations with lower *Sa* (R^2=0.11, *p-value*=0.03), and this correlation was strengthened if only the 2009 data were used (R^2=0.22, *p-value*=0.02).

Coastal upwelling appears to be associated with increased concentrations of cadmium in organisms at some locations. Strong and frequent local upwelling occurs at different localities along the Pacific coast of the Baja California peninsula (Zaytsev et al., 2003), which is a possible explanation for the unusually high levels of cadmium found in organisms. Studies of the mussel *Mytilus californianus* as a biomonitor of cadmium (Segovia-Zavala et al., 2004) have found significantly higher concentrations of cadmium (17 µg/g) at Punta Banda, a location characterized by intense upwelling, compared with lower concentrations (9.8 µg/g) at a site with lower intensity upwellings. Our results support this hypothesis because very high cadmium concentrations ($>$100 µg/g) in *Ligia* were detected at Punta Baja (PB), El Tomatal (ET) and San Quintin (SQ) along the Pacific coast of the Baja California peninsula.

Toxicity experiments with *Idotea baltica* by de Nicola Giudici and Guarino (1989) showed that cadmium was less toxic than copper. The LT_{50} for cadmium at 1 mg/l was 12 days for juveniles and 40 days for adults, i.e., the LT_{50} slopes were significantly different, which supports the idea that the metals have different toxic effects. As with copper, females were more tolerant than males, and sublethal effects were observed in juveniles at 0.5 mg/l. The mean cadmium sea water concentration at G was 0.09 mg/l (Güido-Moreno, 2012), and the concentration of Cd in *Ligia* from G was 16.5 µg/g (Table 2). The cadmium concentration in the water at this location was lower than the experimental toxicity threshold. The highest concentration found in *Ligia* from PB was 15 times greater than this “safe” value.

It has been speculated that regular exposure to cadmium from oceanic upwelling has contributed to the development of defenses against this metal, such as the specific cadmium-binding metallothioneins that have evolved in some marine organisms (Monosson, 2012). More research is needed to confirm this hypothesis.

4.6. Lead

Lead was detected at all locations with the highest concentrations observed in Guaymas (G) (143 µg/g) (Fig. 2). Low lead concentrations were observed at San Francisquito (SFr), El Cajete (EC), San Hipolito (SH), and San Evaristo (SE) during both years (Tables 1 and 2), which are all near small fishing communities (*Sa*$<$2) along the Baja California peninsula. Lead concentrations increased with higher *Sa* (R^2=0.18, *p-value*=0.006); the correlation was slightly improved by only using the 2009 data (R^2=0.22, *p-value*=0.02) and was significant with or without the Guaymas (G) location.

Anthropogenic activities at the Guaymas Bay include the discharge of municipal and industrial effluent, the presence of shipyards, fishing, the loading and unloading of different products and materials, and regular dredging of the bay. A study of the different geochemical fractions of sediment in oyster farms in Sonora found significantly higher levels of lead in the sediment from Guaymas (46.6 µg/g) compared with other harbors in the region (Garcia-Rico et al., 2004). Higher sediment concentrations of lead (81 µg/g) were also found near the municipal wastewater outflow (Méndez et al., 2004). In 2006, however, the main municipal wastewater outflow was shut down, but other, smaller sources of sewage and contaminated sediments remain in the bay.

According to Brown (1977), freshwater isopods (*Asellus meridianus*) exposed to 0.5 mg/l of lead nitrate experienced 100% mortality after the 9th day in non-lead-tolerant populations; tolerant populations remained alive until day 14. After the experiment, the organisms were dissected and were found to have accumulated up to 20,000 µg/g of lead in their tissues with higher concentrations found in the hepatopancreas and intestines of the lead-tolerant individuals. The author concluded that these organs are a repository of metals in tolerant populations. The mean concentration of lead in seawater at G was 0.6 mg/l (Güido-Moreno, 2012), and this concentration is high enough to bioaccumulate in the food chain and create lead-tolerant populations of isopods that might survive under this condition through hepatopancreas storage of this metal. However, for lead and copper contamination, it is important to evaluate the physiological and energetic costs of this mechanism to the individual organisms and, consequently, the population. Additionally, there is evidence that copper is released during the molting period in terrestrial isopods (Wieser and Klima, 1969), so more research is needed to determine the effects of the molt process on the retention of metals in the hepatopancreas in aquatic isopods.

4.7. Mercury

Mercury concentrations were the lowest of all the trace elements analyzed ($\leq$ 2.0 μg/g) (Fig. 2). The highest concentrations were observed at Mazatlán (M) (2.01 μg/g) followed by San Francisquito (SFr) (1.73 μg/g) and Punta Baja (PB) (1.52 μg/g). The lowest concentrations were found at San Bruno (SB), Bahía de los Angeles (BDLA), and Puertecitos (Pt) in 2009 (Tables 1 and 2). These locations varied in their *Sa* values and exhibit different upwelling patterns; only San Francisquito (SFr) is located near a hydrothermal vent. In Mazatlán, however, the thermoelectric plant has been suggested as the source of mercury enrichment in the coastal sediments around this city (Ruiz-Fernández et al., 2009; Ruiz-Fernández and Hillaire-Marcel, 2009), so the higher mercury concentrations in the *Ligia* isopods from M are probably derived from this power plant.

An acute toxicity test for mercury in the marine isopods *I. baltica* and *Sphaeroma serratum* resulted in lower LC_{50} concentrations for this metal compared with copper and cadmium (Prato et al., 2006). The highest LC_{50} was observed in *S. serratum* (1.38 mg/l of $HgCl_2$) whereas *I. baltica* registered LC_{50} values of 0.20 mg/l of $HgCl_2$. The total mercury concentrations in seawater samples from G were lower ($<$DL-0.01 mg/l) than this experimental toxicity threshold (Güido-Moreno, 2012), and the concentration of mercury in *Ligia* from this location was also low (0.647 μg/g). The concentration of Hg in *Ligia* from M was three times this “safe” concentration. However, to determine the potential mercury toxicity in organisms from Mazatlan, it will be necessary to measure its concentration in the water and compare it with the experimental toxicity thresholds.

5. Conclusions

The concentrations of trace elements in the supralittoral populations of *Ligia* isopods in the Gulf of California and the Pacific side of the Baja California peninsula reflect local anthropogenic and natural conditions. High concentrations of copper in *Ligia* from the Santa Rosalía (SRo) area are consistent with mining activities at this location. Industrial and municipal sewage discharges appear to influence the high concentrations of zinc and lead in *Ligia* observed at Guaymas (G), and the concentrations of mercury at Mazatlán (M) may be associated with its thermoelectric plant. While natural sources, such as upwelling, may have contributed to the high cadmium concentrations in *Ligia* isopods at localities along the Pacific coast of Baja California, hydrothermal vents may have influenced the high concentrations of arsenic at Ensenada (E).

Considering that one-fifth of the total area of the Mexican Republic is now leased to mining companies, with a particularly high number of concessions in the states of Baja California, Sonora and Sinaloa (El Universal, 2014), we suggest the use of *Ligia* isopods as biomonitors of trace metals due to their high tolerance of these contaminants and their wide distribution in the intertidal rocky shores of the Gulf of California.

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