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Barnacles as Biomonitors of Heavy Metal Pollution in the Coastal Waters of Mazatlán Harbor (Mexico)

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Mazatlán harbor is located in the southern portion of the state of Sinaloa, NW coast of Mexico (Fig. 1). The area accounts for ca. 1200 ha, and according to the Mexican coastal lagoon classification (Lankford 1977) the water body is considered as a III B (III A) type lagoon with an inner barrier shelf. The climate of the region is tropical and subtropical, with a rainy season during summer (Garcia 1964). The main anthropogenic sources of heavy metals in Mazatlán coastal waters are those related to fish and shrimp processing industry, canning and flouring, power plant cooling systems, sandblasting of boats and domestic effluents from adjacent areas. The residence time of the water is relatively short (Páez-Osuna *et al.* 1991) so the industrial and domestic effluents can be expelled thoroughly: however, calm areas can accumulate significant amounts of contaminants and represent a potential hazard for the system.

Recently, biomonitoring of heavy metals has become an important tool for assessing the degree of pollution in coastal waters, specially in temperate latitudes. Organisms used as biomonitors should fulfill several requirements in order to reflect the conditions of the surroundings (Phillips 1980): sedentary, abundant, resistant and tolerating high rates of contaminant concentration and salinity variation, and net accumulators of the metal of interest. Various organisms potentially useful as biomonitors occur in Mazatlán harbor, e.g. oysters, mussels, clams and barnacles. However, no single species is present throughout the water body to be used as a unique cosmopolitan biomonitor. Barnacles are present in different locations where contrasting activities exist, thus, this group of organisms can be considered as a serious candidate for biomonitoring. This paper presents data on the levels and the seasonal changes of Cd, Cu, Cr, Fe, Mn, Ni, Pb and Zn in the soft tissues of the barnacles *Balanus eburneus, Fistulobalanus dentivarians* and *Megabalanus coccopoma* from the waters of Mazatlán harbor, Mexico.

MATERIALS AND METHODS

Barnacles were collected from the intertidal zone at four selected sites considering the different activities developed in Mazatlán harbor; (1) site A,

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in the upstream section, where *F. dentivarians* is associated with mangroves and no discharges are present in the surroundings. (2) Site B, located in the shrimp piers and tuna-fish fleet, nearby the shrimp and fish processing industry (canning and flouring), here *B. eburneus* lives on the base of the piers. (3) Site C, in the mouth of the Estero Infiernillo, which receives principally untreated domestic effluents, here *B. eburneus* lives on the bridge structure. (4) Site D, situated in the pier of the commercial and transportation fleet near to the entrance of the harbor (Fig. 1), where *M. coccopoma* lives adhered on the base of the piers.

Barnacle populations were sampled at regular intervals of 45 days between October 1995 and August 1996. Samples were taken directly from the field detaching the specimens from the substratum with a stainless steel knife. Pre-cleaned plastic containers (Moody and Lindstrom 1977) were used to transport and manipulate the samples, and once in the laboratory, individuals were measured (rostro-carinal axis) and weighed (total fresh weight) prior to the extraction of the soft tissue. About sixty of the largest organisms pressumably adults (*B. eburneus,* mean size 25 mm, mean weight 5.39 g; M. coccopoma 25 mm, 11.57 g; *F. dentivarians 17* mm, 2.54 g) of each location were taken in order to provide enough tissue for the analysis and represent the population, thus pooled samples of soft tissues were obtained for each site during each sampling.

Each tissue subsample of 52-60 individuals was weighed and then dried to constant weight at 60°C. Homogenization and pulverization were achieved by grinding in a teflon mortar. Triplicate tissue aliquots of 0.5 to 1.0 g, were digested with concentrated and distilled nitric acid in silica beakers. The digests and blanks were slowly evaporated to dryness (100 °C) and the remainder dissolved in 1 M HNO_3. After centrifugation (3500 rpm), the solutions were diluted to a final volume of 10-20 ml and placed in acid-washed polyethylene bottles (Moody and Lindstrom 1977). They were then aspirated into an atomic absorption spectrophotometer Shimadzu Model AA-630 using standard flame conditions for each metal. The amount of each metal was determined quantitatively by the internal standard additions method (Páez-Osuna and Tron-Mayen 1995). The samples were spiked with about 1, 2 and 4 times the expected amount of metal in an aliquot of the tissue sample; thus, the average intensities of the added standard additions were used to calculate the concentrations of the tissue samples processed altogether. The accuracy and precision of the employed method was estimated by means of a shrimp homogenate material MA-A3/TM (IAEA 1987). The concentrations of Cu, Cd, Cr, Ni, Mn, Fe and Zn for the standard homogenate, within the acceptable range (twice the given standard deviation) reported for this sample (IAEA 1987). No standard homogenate data were available for Pb. All metal concentrations were expressed as µg g^{-1} dry weight. Differences in average concentrations were

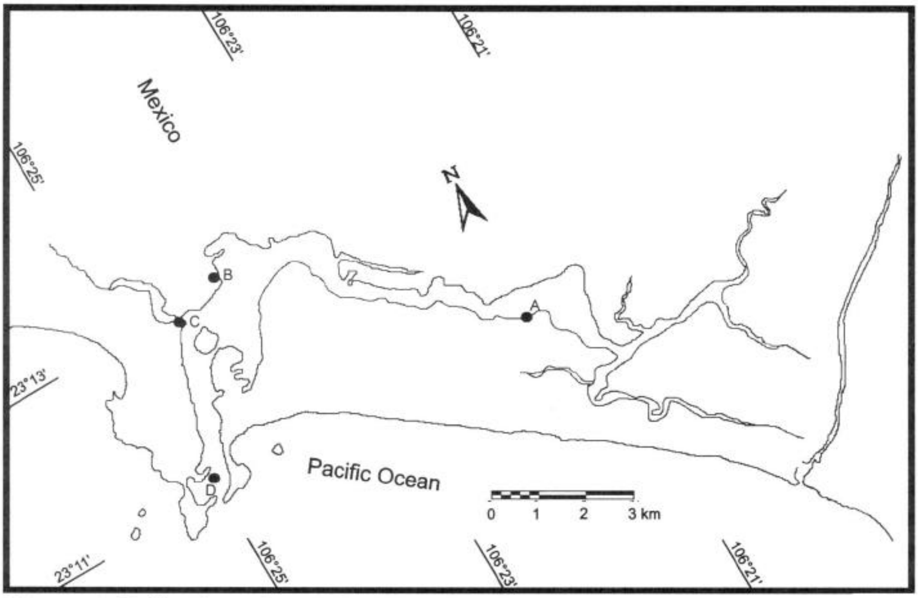

Figure 1. Collection sites (•) in Mazatlan harbor on the NW coast of Mexico.

assessed by a one-way analysis of variance and the Bonferroni's multiple comparison test using the GraphPad Prism 2.0 for Windows 97 (GraphPad Software Inc. San Diego CA).

RESULTS AND DISCUSSION

The results of the analysis of the eight metals studied in the three species of barnacles from all sites are summarized in Table 1. Certain elements were not measured due to the insufficient material available, and *F. dentivarians* was collected only eventually since it was absent in some months in site A. Zn and Fe were the most abundant elements in the four populations of barnacles, followed by Cu. Mn preceded Pb, Cd, Ni and Cr. The concentrations of most of the metals studied in *Balanus eburneus* varied seasonally at both sites, with a similar pattern for Cr, Cu, Pb and Zn and a different pattern for Cd, Ni and Mn. *M. coccopoma* from site D exhibited seasonal variation in the concentrations of the metals with a similar pattern for Cr and Mn, and a different pattern for Cd, Zn, Ni and Pb.

Seasonal fluctuations of metals have been observed in several organisms and the causes have been the source of different proposals (Fowler and Oregioni 1976, Walker and Foster 1979, Phillips 1980), such as the food supply, changes in feeding rates, changes in body weight (as energy reserves are accumulated or depleted), changes in run-off of particulate metal to the coastal waters as a consequence of high precipitations and

Table 1. Average metal concentration (µg/g dry weight) over the whole study and standard deviation (±SD) in barnacles from Mazatlán harbor.

Metal	Site A *F. dentivarians*	Site B *B. eburneuss*	Site C *B. eburneus*	Site D *M. coccopoma*
Cd	1.8±0.3 (n=4)	4.4±3.6 (n=5)	4.1±3.9 (n=7)	25.8±8.3 (n=7)
Cu	26.1±3.6 (n=4)	124±89 (n=5)	148±220 (n=7)	25.0±4.4 (n=7)
Cr	2.8±1.3 (n=4)	2.5±2.0 (n=5)	4.7±4.2 (n=7)	2.2±1.0 (n=7)
Fe	-	265±326 (n=2)	-	622±681 (n=6)
Mn	37.0±8.0 (n=3)	28.1±17.6 (n=5)	53.0±17.4 (n=5)	51.7±19.9 (n=7)
Ni	10.5±0.1 (n=2)	8.4±6.4 (n=5)	8.4±4.4 (n=6)	7.7±1.8 (n=7)
Pb	15.5±3.0 (n=2)	12.4±5.2 (n=5)	14.8±6.2 (n=8)	12.1±7.8 (n=7)
Zn	1676±225 (n=4)	30030±46040 (n=5)	5589±3440 (n=5)	1848±810 (n=7)

variations related to the reproductive cycle. In the context of Mazatlán harbor, the stratification of waters and the intermittent flow of industrial effluents as additional factors may produce seasonal changes in heavy metal levels in the harbor waters, and therefore also elicit changes in the levels of the bioavailable metal fractions. For instance, the shrimp industry operates only during September-April, and more strongly in October, November and December, when the product is more intensively processed and commercialized.

Seasonal variation in Cd concentration was not systematic in the three species and the four selected sites (Fig. 2). The highest concentrations of Cd (range 17.4-42.1 µg/g) in the soft tissue of barnacles were found at site D at the entrance of the harbor, where *M. coccopoma* was collected. These levels indicate that this species is a good accumulator of the metal and the site has a high bioavailability of Cd, which is related probably with the natural enrichment of Cd in the Baja California waters associated with upwellings (Segovia-Zavala *et al.* 1998). The lowest concentrations of Cd were found at site A which is relatively clean and where *F. dentivarians* was present. The concentrations detected here are in the same order of magnitude as the levels found for *Balanus amphitrite, Balanus uliginosus* and *Tetraclita squamosa* in Fujian, China (Rainbow et. al. 1993), and below the levels reported for *Balanus amphitrite* in Hong Kong (Phillips and Rainbow 1988, Rainbow, 1993).

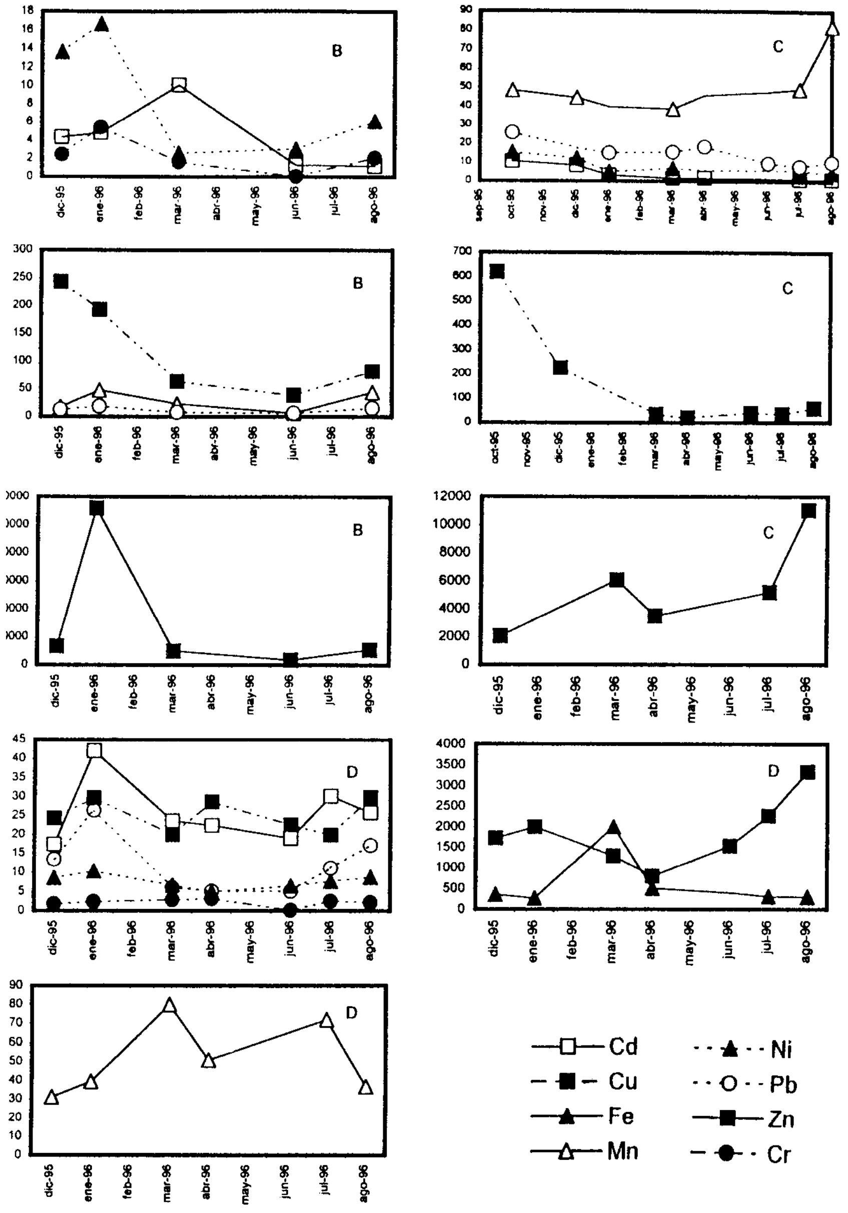

Figure 2. Seasonal variations of heavy metal concentrations in *B. eburneus* from the piers (Site B), from the mouth of Estero Infiernillo (Site C), and *M. coccopoma* from piers near to the entrance of Mazatlán harbor (Site D).

Seasonal variations are evident for Cr concentrations, in *B. eburneus* the maximum (12.9 µg/g) is present in the winter and the minimum (<0.15 µg/g) in June. In the case of *M. coccopoma* the pattern is different, the maximum is evident in April. The highest level of Cr was detected in *B. eburneus* from site C on December 1995, this site is located in the mouth of the Estero Infiernillo and near to the shipyards. In the remaining sites and sampling times the levels were below 5.5 µg/g, such values reveal natural concentrations or low contamination. Rainbow *et al.* (1993) reported for *B. amphitrite* 95% confidence limits from 1.9-20.1 to 4.4-32.3 µg/g depending on the contamination gradient in Fujian, China, while in Hong Kong coastal waters, Phillips and Rainbow (1988) and Rainbow (1993) reported up to 28 and 7.2 µg/g, respectively.

Seasonal fluctuations of Cu show a maximum (621 µg/g) in October and a minimum (20.8 µg/g) in April in *B. eburneus* in two sites, while in the other species a seasonal pattern is not evidenced clearly (Fig. 2). The low levels of spring and summer found here for *B. eburneus* are very similar to the reported range (11-64 µg/g) for the Indian River lagoon (Florida coast) in the same species (Barber and Trefry 1981). In general the levels of Cu found here for *B. eburneus* in sites C and D are comparable with those in *B. amphitrite* from contaminated regions (Rainbow *et al.* 1993; Rainbow 1993). Seasonal variation of Fe and Mn were noted only in *M. coccopoma* with a maximum (2000 and 79.9 µg/g, respectively) on March 1996 and a minimum (267 and 31.3 µg/g, respectively) in December. The comparison of the mean Fe and Mn values of *B. eburneus* between sites B and C, show that the site adjacent to the shipyards, having the highest levels, is influenced by the domestic effluents which is due to a higher degree of bioavailability of the two metals in the site. Similarly, regarding the Pb concentrations, the highest values (26.0 and 26.4) were detected in *B. eburneus* from site C on October 1995, and *M. coccopoma* from site D on January 1996, while the lowest level (5.1 µg/g) was measured in *M. coccopoma* from site D on May 1996, these levels indicate Pb contamination in the area, and particularly in site C. Rainbow and Smith (1992) reported comparable levels in the polluted waters of Hong Kong.

Several authors (Walker *et al.* 1975, Barber and Trefry 1981, Anil and Wagh 1988, Rainbow 1995) have considered barnacles as good indicators of zinc in coastal waters. Differences among the four sites and the three species are evident, with the lowest Zn values (1474-1894 µg/g) in *F. dentivarians* from site A and the highest levels (3546-112121 µg/g) in *B. eburneus* from site B, the maximum is about 18 times higher respect to the minimum value. Even though these barnacles are not closely related it is possible to conclude that the waters from site B are contaminated by Zn. The probable source of this element is related to the several docking activities: sandblasting of boats, bilge waters, effluent discharges from shrimp and fish industry.

Considering that even at a species level the individuals show differences in the uptake and detoxification of metals, Phillips and Rainbow (1988) stated that it is valid to compare metal accumulation among different species in rank orders but not directly. In general (Table 1) and in decreasing order, higher concentrations of metals were detected in the barnacles *B. eburneus* collected in the intermediate waters where the principal port activities are located, *M. coccopoma* from the site with marine influence and *F. dentivarians* from the upstream waters, a null or poorly contaminated site which does not receive wastes directly. The multiple comparison of mean metal concentrations in the soft tissues of the barnacles over the whole study revealed significant differences only in the case that involved the Cd from site D, i.e. *M. coccopoma* showed higher Cd content than the obtained levels in site A for *F. dentivarians* ($P<0.001$), site B for *B. eburneus* ($P<0.001$) and site C for *B. eburneus* ($P<0.001$).

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