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# Distribution of Trace Metals in Different Tissues in the Rock Oyster *Crassostrea iridescens:* Seasonal Variation

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In the Pacific region, the rock oyster, *Crassostrea iridescens* Hanley 1954, is present on the coast, and is associated with subtidal rocky substrates (Páez-Osuna et al. 1995). The species is geographically distributed from La Paz, Gulf of California, to Northern Peru (Keen 1971). This species and other oysters, are extensively used in monitoring programs in the marine/estuarine environment due to their ability to concentrate pollutants (i.e. trace metals) to several orders of magnitude above surrounding ambient levels (Páez-Osuna et al. 1995; Schuhmacher and Domingo 1996; Szefer et al. 1997; Al-Madfa et al. 1998). Besides, *C. iridescens* is widely utilized for human consumption and has an important commercial value.

The aim of this study was to determine, during a year, the concentrations of nine trace metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in soft tissues (Gonad, mantle, gills, adductor muscle and digestive gland) of the rock oyster *Crassostrea iridescens* inhabiting the Northwest coastal region of Mexico.

## MATERIALS AND METHODS

From January 1994 to December 1994, a population of *Crassostrea iridescens* was collected by scuba diving from Punta Cerritos, Sinaloa, Northwest coast of Mexico situated between 23° 10' and 23° 29' N and 106° 24' and 106° 40' W (Fig. 1); a zone characterized by the absence of significant anthropogenic activities. Samplings were carried out every 45 days to allow the analysis of organisms in different physiological phases (Latouche and Mix 1981); collecting from 58 to 64 specimens of similar size (10.5±0.9 cm) to minimize the effect of body weight (NAS 1980). Daskalakis (1996) examined the variability of metal concentrations in *Crassostrea virginica* and found that the sampling error decreases significantly with increasing numbers of individuals per pool. The confidence interval of the mean decreased by a factor of 0.6 when 50 oysters were used per pooled sample. Such results indicate that an increasing size of pooled samples is an option to detects changes in environmental contaminants.

In the laboratory, prior to analysis, the oysters were depurated for 48 hr. After that, bivalves were measured and freed of their shells to separate the tissues. In

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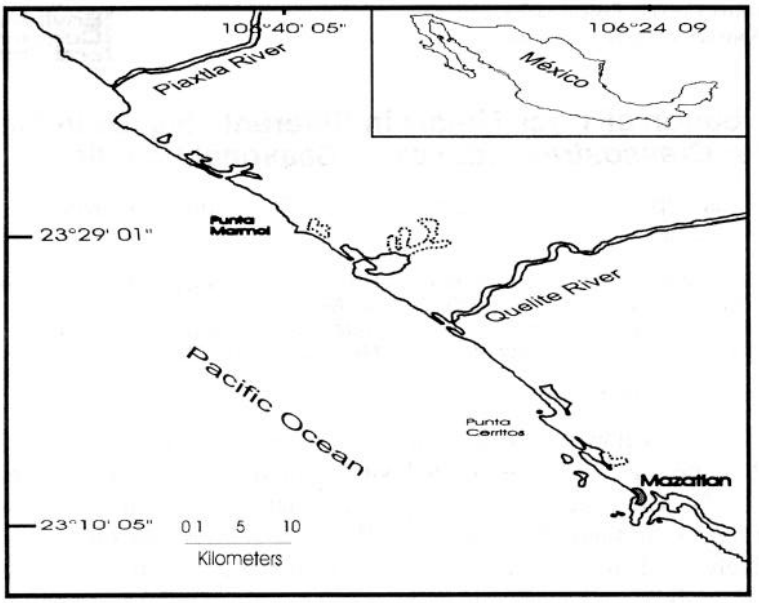


**Figure 1.** Location of sampling site ( • )

*Crassostrea iridescens* (Páez-Osuna et al. 1995) as the mussel *Mytilus edulis* (Lobel and Wright 1982) the gonad develops associated to the crystalline style sac, therefore, the gonad and some portions of style sac were taken to represent the gonadal tissue, while the remaining tissues were considered as somatic or nongonadal.

Each tissue (adductor muscle, gills, digestive gland, gonad and mantle) was weighed separately and then lyophilized for 48 hr. Pulverization and homogenization were achieved by grinding the tissues in a teflon mortar. Samples and blanks for analysis were prepared by digesting aliquots of dry material with concentrated quartz-distilled nitric acid using the multiple standard addition method and the metal concentrations were determined by flame atomic absorption spectrophotometry (Páez-Osuna et al. 1995), and the confidence intervals (P=0.05) were calculated using the method described by Miller and Miller (1988). All glassware and plastic devices used in the manipulation of the samples were completely acid-washed (Moody and Lindstrom 1977). The performance of the method was evaluated by analyzing a mussel homogenate as a reference material MA-M-2/T (IAEA 1985). Details of the analysis have been previously mentioned by Páez-Osuna et al. (1995). All metals were in the acceptable range, only Fe and Mn were underestimated. The percentage of recovery of reference material for each metal was calculated in relation to the interval reported (IAEA 1985). The values for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were 82.4, 94.4, 79, 79.8, 63.8, 71.6, 101.5, 65.2, 115.3 %, respectively.

## RESULTS AND DISCUSSION

Table 1 shows heavy metal concentrations in different tissues during the study period.

Correlation coefficients were calculated for each pair of total metal concentrations. Only Pb-Zn (r = -0.79) and Cd-Cu (r = -0.79) showed a significant correlation at P = 0.05 level of confidence (T-students); and according to Szefer et al. (1994) this is due to the similar physical/chemical properties of the elements involved. Besides, correlations between each total metal concentrations and total length were carried out, and no correlation coefficients were significant at P = 0.05 level of confidence.

Besides, correlations between metals per each tissue were carried out. Only Cd-Cr in gonad (r= 0.84), Co-Zn in adductor muscle (r= 0.83), Cr-Fe in mantle (r= 0.76) and Ni-Pb in gills (r= 0.77) were statistically significant (T-students test, P= 0.05). A similar work was carried out by Sarkar et al. (1994) in the bivalves *Anadara granosa* and *Crassostrea cucullata*. They found significant correlations only between Fe and Cu in the digestive gland for *A. granosa*, and Cu-Zn and Mn-Zn in gills of *C. cucullata.*

More correlations between tissues and between metals were computed. Only the coefficients (in parenthesis) were statistically significant for gills-adductor muscle Cd-Cd (0.93), Cd-Cu (-0.87), Fe-Fe (0.78) and Mn-Zn (-0.85); gills-mantle Cd-Cd (0.85), Cd-Cu (-0.82), Co-Cr (-0.78), Cu-Cu (0.76), and Fe-Fe (0.80); gills-gonad Cu-Cu (0.81); gills-digestive gland Cr-Mn (-0.84); adductor muscle-mantle Cd-Cu (-0.82); adductor muscle-gonad Cd-Ni (085), and Fe-Ni (0.82); mantle-gonad Co-Co (081), Cu-Zn (087), Fe-Fe (0.98), and Zn-Zn (0.85); mantle-digestive gland Co-Mn (-0.79), and Cu-Cu (0.83); gonad-digestive gland Cr-Cu (0.82), Fe-Fe (0.83), and Fe-Pb (0.85). According to Rainbow (1990) metals as Fe, Zn, Cu, Cr, Co, Mn and Ni play a variety of roles in the biochemistry as enzyme cofactors; and significant correlations between metals and tissues could be due to some biochemical requirements and/or passive sequestration of the metals to intracellular metal-binding ligands (Rainbow 1990) according to the stability constants of the metal and their relative concentrations (Mason et al. 1988). This distribution may be changed due to several physiological processes.

The highest concentration of total heavy metal in the soft tissue of *C. iridescens* for Cd, Co, Cr, Cu, Fe and Zn was registered in Winter-Spring Months, while for Mn, Ni and Pb the peak was in Summer-Fall months. The main factors that regulate heavy metals concentrations in bivalves tissues are the quantity in the water column (dissolved and/or particulate) and some biological parameters (i.e. reproductive cycle).

According to Roesijadi y Robinson (1994) all aquatic animals contain a wide variety of membrane-bound intracellular deposits, many of which bind metals.

**Table 1.** Heavy metal concentrations (µg $g^{-1}$, dry weight) in different tissues in the rock oyster *Crassostrea iridescens* (means and confidence intervals).

| Tissue | Cd | Co | Cr | Cu | Fe | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| JANUARY | | | | | | | | | |
| Gill | 1.1±0.02 | 0.7±0.16 | 2.1±0.4 | 59±0.9 | 31±3.4 | 29±0.3 | 2.5±0.4 | 4.6±0.3 | 836±11 |
| Ad. Muscle | 0.6±0.06 | 1.7±0.04 | 2.4±0.3 | 6±1.7 | 20±2.4 | 3.5±0.4 | 1.1±0.1 | 4.6±1 | 168±10 |
| Mantle | 1.4±0.01 | 1.0±0.1 | 2.3±0.2 | 40±0.1 | 29±3.6 | 8.7±0.2 | 1.7±0.1 | 2±0.8 | 492±101 |
| Gonad | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| D. Gland | 1.6±0.1 | 1.6±0.1 | 1.9±0.3 | 78±1.6 | 121±4 | 11.5±1 | 2.3±0.3 | <L.D. | 787±33 |
| MARCH | | | | | | | | | |
| Gill | 1.1±0.1 | 1.5±0.2 | 3.5±0.1 | 57±0.3 | 23±3.3 | 22±0.3 | 2.5±0.5 | 2.6±0.4 | 1242±14 |
| Ad. Muscle | 0.6±0.5 | 1.9±0.1 | <L.D. | 7.3±1 | 5.7±3.4 | 4±0.3 | 2.1±0.4 | 4.1±0.4 | 231±6 |
| Mantle | 1.1±0.1 | 2.0±0.1 | <L.D. | 29±0.9 | 17.5±6 | 6.9±0.3 | 2±0.4 | 3.1±0.6 | 543±29 |
| Gonad | 1.3±0.1 | 1.6±0.1 | <L.D. | 23±0.1 | 25±0.6 | 5.3±0.1 | 0.8±0.3 | <L.D. | 386±23 |
| D. Gland | 5.9±0.2 | 1.8±0.1 | 1.9±0.1 | 48±1 | 97±0.6 | 10±0.2 | 3±0.6 | <L.D. | 685±115 |
| APRIL | | | | | | | | | |
| Gill | 1.8±0.1 | 2.1±0.1 | 0.8±0.1 | 37±0.3 | 13±1 | 26±0.4 | 1.6±0.7 | <L.D. | 824±65 |
| Ad. Muscle | 0.7±0.1 | 0.6±0.1 | 1.4±0.1 | 5.5±0.1 | 0.5±0.4 | 4.2±0.1 | 1±0.2 | 3.7±0.7 | 156±11 |
| Mantle | 1.5±0.1 | 0.6±0.3 | 1±0.1 | 18±0.4 | 15±3 | 9.8±0.1 | 1.8±0.1 | 5.8±0.6 | 387±29 |
| Gonad | 2.2±0.1 | 0.6±0.4 | 1.4±0.1 | 14±0.3 | 24±4 | 7.7±0.1 | 1.7±0.1 | 2.1±0.3 | 224±5 |
| D. Gland | 14±0.5 | 0.9±0.2 | 1.3±0.2 | 37±0.8 | 70±3 | 14±0.2 | <L.D. | 3.9±1 | 572±40 |
| JULY | | | | | | | | | |
| Gill | 3.7±0.1 | 1.4±0.1 | 1.3±0.6 | 42±0.3 | 27±2 | 31±2 | 4±0.2 | 5.9±0.3 | 1294±27 |
| Ad. Muscle | 1.2±0.1 | 0.8±0.1 | 2.4±0.2 | 3.3±0.1 | 12±6 | 3.6±0.1 | 2.1±0.3 | 1.3±0.4 | 149±8 |
| Mantle | 2.8±0.3 | 1.1±0.1 | 1.9±0.1 | 10±0.4 | 25±0.6 | 7.5±0.8 | 0.6±0.2 | 1.8±0.3 | 474±22 |
| Gonad | 2.4±0.1 | 1.0±0.1 | 1.9±0.2 | 7.9±0.4 | 33±0.8 | 25±0.7 | 3.8±0.1 | <L.D. | 213±15 |
| D. Gland | 11.1±0.3 | 1.4±0.1 | 2.2±0.1 | 37±1.8 | 92±7.8 | 13±0.5 | 2.7±0.1 | 1.9±0.4 | 821±89 |
| AUGUST | | | | | | | | | |
| Gill | 2.8±1.1 | 1.2±0.1 | 2.35±0.1 | 38±1 | 31±0.3 | 37±0.3 | 3.5±0.5 | 8.7±0.2 | 1274±10 |
| Ad. Muscle | 0.9±0.1 | 1.3±0.2 | 1.5±0.3 | 2.4±0.3 | 11±2.1 | 3.4±0.3 | 1.2±0.2 | 2.5±0.4 | 129±22 |
| Mantle | 2.6±0.7 | 1.8±0.2 | 2.5±0.7 | 15±0.6 | 43±3.4 | 5.6±0.4 | 2.5±0.3 | 2.9±0.8 | 336±52 |
| Gonad | 1.8±0.1 | 0.8±0.2 | 1.0±0.2 | 10±0.6 | 54±1.4 | 35±0.1 | 2.9±0.1 | 6.7±0.2 | 177±0.7 |
| D. Gland | 6.5±0.2 | 0.6±0.3 | 1.5±0.1 | 46±0.5 | 141±2 | 9.8±0.4 | 2.1±0.2 | 7.9±0.5 | 668±24 |
| OCTOBER | | | | | | | | | |
| Gill | 2.1±0.1 | 1.8±0.1 | 2.5±0.5 | 52±0.5 | 30±2.1 | 31±0.4 | 2.2±0.6 | 3.8±0.4 | 906±14 |
| Ad. Muscle | 0.8±0.1 | 0.7±0.1 | 2.5±0.5 | 4.9±0.2 | 11±0.9 | 3.1±0.2 | 1.5±0.1 | <L.D. | 110±15 |
| Mantle | 2.5±0.2 | 0.4±0.1 | 1.4±0.3 | 27±0.7 | 35±1.8 | 8.9±0.3 | 3.0±0.2 | 5.6±0.7 | 415±6 |
| Gonad | 3.1±0.9 | 0.5±0.1 | 4.6±0.2 | 24±0.6 | 40±6 | 5.7±0.3 | 3.0±0.3 | <L.D. | 365±3 |
| D. Gland | 4.5±0.3 | 1.0±0.1 | 2.3±0.3 | 72±2 | 85±1.8 | 13±0.1 | 3.7±0.3 | 5.6±0.4 | 647±13 |
| DECEMBER | | | | | | | | | |
| Gill | 2.5±0.1 | 0.6±0.2 | 2.8±0.2 | 40±0.3 | 27±0.1 | 37±0.7 | 1.8±0.3 | 4.2±0.2 | 1044±32 |
| Ad. Muscle | 0.7±0.1 | 0.1±0.1 | 2.9±0.3 | 3.6±0.1 | 2.8±0.9 | 2.4±0.4 | 0.6±0.2 | 1.5±0.9 | 97±75 |
| Mantle | 2.8±0.7 | 1.0±0.1 | 2.7±0.3 | 29±0.5 | 33±5.5 | 10±0.1 | 1.8±0.2 | 3.6±0.3 | 614±8 |
| Gonad | 3.0±0.1 | 0.9±0.5 | 2.5±0.5 | 2.9±0.2 | 40±1.7 | 6.8±0.3 | <L.D. | 1.6±0.8 | 541±2 |
| D. Gland | 2.9±0.1 | 0.8±0.3 | 2.6±0.5 | 56±0.5 | 104±6 | 11±0.8 | 1.2±0.2 | 4.9±0.4 | 337±2 |

L.D.: Limit of detection (Cr: 0.65; Ni: 0.3 and Pb: 1.1 µg $g^{-1}$); -- Individuals with gonad absent

These structures are generally associated with the digestive or excretory tissues of invertebrates (e.g. midgut, digestive glad, hepatopancreas, etc). In the present study, the highest concentrations of Cd and Fe were determined, for each sampling, in the digestive gland. Simkiss y Mason (1983) reported that Fe is commonly accumulated in the hepatopancreas of molluscs. Regarding the highest concentrations of the other metals, Co, Cu, Ni and Pb; these were determined in some tissues (included digestive gland), but in Cr, digestive gland was not

included; and Mn and Zn, were always observed in the gills.

Recently, the reproductive cycle of *C. iridescens* was described by Frías-Espericueta et al. (1997): resting phase from November to January; gametogenesis from January to March, maturation phase from April to May; and spawning phase from July to October. Latouche and Mix (1981) considered Zn as a key element regarding possible relationships with the reproductive cycle, and these authors concluded that Zn, in *Mytilus edulis,* was transferred from somatic to gonadal tissue to perform some biochemical reactions. However, in our study this phenomenon was not observed; clearly (Table 1) Zn-gonad burdens not increased during gametogenesis and maturation phases and always Zn-gonad burdens were lower than those values determined in gills mantle and digestive gland (except digestive gland for December sampling). Lobel and Wright (1982) found that Zn concentrations in *Mytilus edulis,* were significantly lower in gonadal tissue than in somatic tissue, and concluded that gonadal state had little effect on zinc variability in these bivalves.

The Mn behaviour in *C. iridescens* was noticeable . This element was increasing its concentration in the gonad during gametogenesis and maturation phases until August (highest value, 35 µg $g^{-1}$), after that Mn-gonad burdens decreased. A correlation between gonadal index (average gonadal dry weight / average somatic dry weight) (Latouche and Mix 1981) and Mn-gonad burdens was carried out. However, the correlation coefficient (r = 0.70) was not statistically significant at P=0.05% confidence level (T-students test). Páez-Osuna et al. (1995) with the same species, found a maximum Mn-gonad burdens that was correlated positively with the maturation of the organism. They suggested that Mn may perform some biochemical function during maturation or prespawning phases.

Table 2 shows a comparison between trace metals concentrations of this study

**Table 2.** Trace metal concentrations (µg $g^{-1}$, dry weight) in whole oysters from different areas.

| Species | Area | Cd | Cu | Fe | Mn | Pb | Zn | Ref. |
|---|---|---|---|---|---|---|---|---|
| *Pinctada radiata* | Halul (Arabian Gulf) | 0.4 | 3.7 | | | 5.6 | | A |
| *Crassostrea angulata* | Gerona (Spain) | 3.07 | 298 | | | 5.0 | | B |
| *Crassostrea gigas* | Langebaan (South-Africa) | 9.0 | 33 | | 12 | 1 | 424 | C |
| *Crassostrea gigas* | Saganoseki (Japan) | 19.6 | 5110 | 146 | | 14.5 | | D |
| *Crassostrea iridescens* | Northwest coast (Mexico) | 2.4 | 24 | 34 | 10.8 | 3.1 | 443 | E |

(A) Al-Madfa et al. (1998), (B) Schuhmacher and Domingo (1996), (C) Watling and Watling (1976), (D) Szefer et al. (1997) (E) this study.

with oysters from different areas. These values were similar with those determined by Watling and Watling (1976) in oysters from Langebaan South-Africa, a site considered unpolluted; and lower than those determined by Szefer et al. (1997) in *Crassostrea gigas* from Saganoseki, Japan, a site considered as one of the most metal-contaminated in the world.

A general conclusion is that seasonal differences are due to metal concentrations in the water column and/or the mechanisms of uptake, storage and release of metals involved in these processes, which determine the relation and concentrations of metals between tissues (Simkiss and Mason 1983; Roesijadi and Robinson 1994). Besides, According to Rainbow (1990) it could help to any understanding and appreciation of the significance and biology of heavy metal concentrations in marine organisms.

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