Bivalves such as oysters often accumulate heavy metals, and therefore can be used to monitor changes of pollutant concentrations in the environment. Cultivated oysters from the northwest coast of Mexico are widely used for human consumption and thus have an important commercial value. Information was gathered on the concentration of these elements in oysters (*Crassostrea gigas*) cultivated on the coast of Sonora. Oysters were randomly collected from April to October 1997, from 6 different locations (65 individuals per site) in 4 different months. Metals were determined by microwave digestion followed by atomic absorption spectroscopy. The mean values (µg/g fresh weight) for each metal were: Cd, 0.76; Cu, 3.64; Zn, 17.71; Pb, 0.50; As, 0.05; Hg, 0.03; and Se, 0.21. The results show that, except for Cd, concentrations of regulated metals were under the maximum permitted values specified by regulatory agencies of Mexico and were comparable to those reported from other areas.

Heavy metals are a part of complex biogeochemical cycles whose final destinations are at the bottom of oceans and lakes. After entering the aquatic environment, metals are distributed among water, biotic, and sediment compartments; the latter serves as a final sink for metal pollutants (1, 2). Several organisms, like oysters (*Crassostrea gigas*), bioaccumulate metals that reach very high concentrations. Therefore, oysters have been widely used to determine the levels of metal contamination in coastal ecosystems (3–5). These organisms are sedentary and abundant, with a relatively long life span, and can be easily collected. Thus, they are good ecological bioindicators. Moreover, oysters are considered an appreciated fancy food, with important commercial value (6).

The state of Sonora is located in the northwest of Mexico, with a surface area of 184,934 km² and a coastline of 1200 km. Sonora has a great diversity of industrial activities like mining, agriculture, livestock, and important tourist resorts. Sonoran coastal waters are also characterized by an exceptionally high capture of commercial fish and shellfish and are among the first producers of cultivated oysters in Mexico (7). The aim of this work was to provide information on the concentration of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), arsenic (As), mercury (Hg), and selenium (Se) in the cultivated oyster grown on the coast of Sonora and to identify possible sources of pollution in the Gulf of California.

**Experimental**

**Apparatus**

(a) *Atomic absorption spectrophotometer.*—Varian (Victoria, Australia) Spect AA-20 spectrophotometer equipped with hydride generation atomic system Varian VGA-76, burner head and hollow-cathode lamps. Wavelength and slit were set at (nm): Cd (228.8; 0.5), Pb (217; 1.0), Cu (324.7; 0.5), Zn (213.9; 1.0), As (197.2; 1.0), Hg (253.7; 0.5), Se (196; 1.0).

(b) *Microwave oven.*—Microwave digestion system MDS-81D (CEM Corp., Matthews, NC) equipped with a 600 W magnetron adjustable in 1% increments, a rotating turntable, and a variable-speed exhaust fan to direct any corrosive fumes to a hood. Time, pressure, and power setting were controlled by a programmable microprocessor. Digestions were performed in 100 mL lined digestion vessels (CEM Corp.) equipped with safety relief valves.

(c) *Volumetric flasks, 50 and 100 mL.*—Class A (Kimax, Vineland, NJ), used as containers for digestion.

(d) *Glassware.*—Washed with Pierce solution 20% (v/v); Pierce, Rockford, IL), rinsed with cold tap water followed by 20% (v/v) nitric acid and then rinsed with double-distilled water.

**Reagents**

(a) *Concentrated acid (HNO₃), 70% (v/v); concentrated hydrochloric acid (HCl), 36% (v/v).*—AR SELECT, Mallinckrodt, Phillipsburg, NJ.
Table 1. Microwave digestion program for oyster samples.

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent</th>
<th>Volume, mL</th>
<th>Power, %</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HNO₃</td>
<td>5</td>
<td>95</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>H₂O₂</td>
<td>3</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>—</td>
<td>90</td>
<td>20</td>
</tr>
</tbody>
</table>

* Program for 12 digestion vessels.

**Oyster Sampling**

Cultivated oysters were collected from 6 different locations along the Sonoran coast between April and October 1997. At each of the 6 sites, 65 oysters (6–9 cm in length) were collected. Sampling was repeated 4 times at low tide. Samples were rinsed with sea water and transported on ice in polyethylene plastic bags to the laboratory within 12 h of collection. Water temperature ranged between 25 and 30°C, and salinity was 38–43%.

**Sample Preparation**

At each of the 6 sites, oysters were cleaned with distilled water and were not deputated, so that the soft tissue included the digestive gland and sediment particles. In choosing oysters for preparation of composite samples (40 oysters) at each site, emphasis was placed on organisms of nearly equal length to limit as far as possible differences attributable to size and age. They were then opened with a knife, removed from their shells, homogenized, and stored frozen until analyzed.

**Microwave Digestion**

Homogenized samples (1.25 ± 0.003 g) were weighed in a microwave digestion lineal vessel and 5 mL nitric acid (50%, v/v) was added. Twelve vessels were digested at a time in 2 consecutive procedures (Table 1). First, they were digested for 2 min at 95% power, 5 min at 90% power, and 15 min at 85% power. Then 3 mL (30%, v/v) H₂O₂ was slowly added to the vessels, which were kept in a hood until all bubbling ceased. The vessels were returned to the microwave oven and a second digestion of 4 min at 100% power and 20 min at 90% power was applied. During the 2 procedures, the pressure was set at 90 psi. After microwave digestion, the samples were cooled, vented, and diluted with double-distilled water (8). Samples of Cd, Cu, Pb, Zn, and Hg were diluted to 50 mL. Digested solutions for Hg, As, and Se were run under the same conditions previously described under Preparation of Standards. Samples of Cd, Cu, Pb, Zn, and Hg were diluted to 50 mL; Se and As were diluted to 100 mL.

**AAS Measurements**

Cd, Pb, Cu, and Zn were analyzed directly by flame AAS.
Table 3. Metal concentrations (µg/g fresh wt) in tissue of Crassostrea giga collected along the Sonoran coast

<table>
<thead>
<tr>
<th>Element</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.40–1.43</td>
<td>0.76</td>
</tr>
<tr>
<td>Copper</td>
<td>1.40–7.87</td>
<td>3.64</td>
</tr>
<tr>
<td>Zinc</td>
<td>8.00–30.83</td>
<td>17.71</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;0.40–0.60</td>
<td>0.50</td>
</tr>
<tr>
<td>Arsenic</td>
<td>&lt;0.02–0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.02–0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.17–0.28</td>
<td>0.21</td>
</tr>
</tbody>
</table>

* n = 24.

(a) Hg determination.—For Hg, the cold vapor technique was used after reduction with SnCl₂ (20%, w/v) in HCl (20%, v/v).

(b) As and Se determination.—As and Se were determined with the hydride generation atomic system. The measuring cell was heated and the solution was reduced with 0.35% NaBH₄ prepared with 5 mL (10%, w/v) NaOH and 10M HCl. For all metals, nonatomic species are reported for Varian, except Hg at 253.7 nm; however, the background absorption was low, and no correction was made.

Quality Control

Standard Reference Material (SRM), NBS 1566a, reagent blanks, and duplicate samples were run with each digestion series. Relative standard deviation (RSD) for flame AAS standards were <0.5% and for VGA methods were <1%. All instrumental measured values in samples with <20% RSD were considered negatives. Method precision was estimated from the average coefficient of variation (% CV) calculated from replicate analyses of 5 samples of SRM on different days. All CV values were <10% except for As. The proficiency testing was based on interlaboratory comparisons organized by EMA (Entidad Mexicana de Laboratorios de Prueba), a Mexican organization dedicated to accreditation and quality assurance, and by the Mercury Quality Assurance Program (Canadian Food Inspection Agency). For both programs, accuracy was good.

Detection limits (DL).—For each element, the DL was estimated as proposed by VARIAN and multiplied by volume over weight (µg/g fresh wt): Cd, 0.06; Cu, 0.12; Zn, 0.03; for the rest of the metals (As, 0.024; Hg, 0.01; and Se, 0.016). DL was determined by analyzing samples with known concentrations of analyte and establishing the minimum level at which metals can be reliably detected.

Method accuracy and precision.—These values were evaluated by comparing the results obtained for the reference material, Oyster 1566a, with its certified values (Table 2). Experimental values for all metals (mean recovery) were in good agreement with the certified values (96–102%) except for As (71%) and Hg (125%). The hydride generation technique has been widely applied for determination of low levels of As however, numerous factors influence its accuracy. Causes of errors include improper sample preparation, volatilization losses during digestion, or inappropriate oxidation. In this study, HNO₃ and H₂O₂ were used as oxidizing agents. HNO₃ is a good oxidizing agent but produces NO₂ fumes that may interfere in the hydride generation by reoxidation of the analyte. In this study, the presence of NO₂ fumes were reduced by adding H₂O₂ as a second digestion step and diluting the digestion solutions with water before the KI prereduction step. It was observed that the lowest recoveries occurred in winter when more time was needed to stabilize the solution. The use of urea, pH controls for each of the solutions, and the use of SRMs need further investigation.

For Hg the cold vapor generation is a stable technique; however, results indicate problems in detection at low concentration and/or the need to optimize the sample size. It was also used for Dogfish muscle and liver Certified Reference Materials (DORM-2 and DOLT-2) purposed by the National Research Council, Canada. Recoveries and % CV were 100.05% and 1.72 for DORM-2 and 101.72% and 3.17 for DOLT-2.

Because Pb concentration in the reference material was low (0.371 ± 0.014 µg/g) and close to the detection limit (0.4 µg/g), a known amount of standard (3 µg/mL) was added to oyster samples before microwave digestion. The mean recovery rate obtained was 95%. McCarthy et al. reported 0.24 µg/g as estimated DL. They found minimum levels of 0.86 µg/g using the flame AAS. From spiked shellfish, they recovered 91.5%.

Calculations

Standard concentrations and samples were measured 3 times, and results were averaged. Metals concentration was calculated by multiplying the measured value by its dilution (50 or 100) and dividing by weighed portion.

Results and Discussion

Metal concentrations found in C. gigas from the Sonoran coastal waters are summarized in Table 3. Zn was the most abundant element in the oyster (17.71 µg/g), followed by Cu, Cd, Pb, Se, and Hg. Metal concentrations showed a wide variation, ranging from 1.4 to 7.87 µg/g for Cu and 8 to 30.83 µg/g for Zn. These metal concentrations were lower than those reported previously by Páez-Osuna (9, 10) in Saccostrea iridiscens and C. corteziensis; Schumacher and Domingo (3) in C. angulata; Páez-Osuna et al. (11) in

Table 4. Correlations between elements in oysters (Crassostrea gigas)

<table>
<thead>
<tr>
<th>Element</th>
<th>Cadmium</th>
<th>Zinc</th>
<th>Arsenic</th>
<th>Selenium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.376</td>
<td>-0.448</td>
<td>0.354</td>
<td>-0.500</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.404</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.531</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Selenium</td>
<td>-0.403</td>
<td>—</td>
<td>0.389</td>
<td>—</td>
</tr>
</tbody>
</table>

* Significant correlation = 90%.

b Significant correlation = 95%.
C. palmula but similar to those for Pinctada radiata (5). Zn and Cu are known to vary widely between crustacean species; therefore, these differences were expected. Findings suggest that Cu interacts in diverse physiological responses with other elements like Zn, Cd, Ag, and Hg. These metals are chemically similar to Cu and may be displaced, causing toxicological effects. It has been reported that Cu is one of the most toxic metals to C. virginica and C. gigas, affecting their embryonic development (12, 13).

Of the nonessential metals, Cd presented the highest mean values (0.76 μg/g). The highest Cd concentration (1.43 μg/g) was detected at site 5, which is located close (25 km) to an important modern agricultural zone where the use of agrochemicals is a normal practice. The soil and water of this area also have high salinity levels that may modify redox potential, affecting Cd concentrations in oysters. Sites 3 and 6 also showed high values (0.66 and 0.60 μg/g, respectively). Cd was often found at levels similar to those reported for C. corteziensis (14) and P. radiata (5) but higher than those reported for C. angulata (3). The mean value (0.76 μg/g) was also very similar to that reported for shrimp Penaeus vannamei and P. stylirostris from the northwest coast of Mexico (9, 10). Different authors reported high Cd concentrations in plankton and sediment along the Gulf of California (15, 16). According to these authors, the accumulations of Cd in the trophic chain can probably be explained by the atmospheric route or by marine current transport. The rest of the elements detected in C. gigas (Pb, As, and Hg) were lower than some values reported for C. angulata and C. virginica (3). Heavy metals in coastal areas may come from freshwater runoff and the atmosphere. The Sonora state has a great diversity of mineral deposits like silver, lead, zinc, gold, and copper which could provide a natural source of Cd in this coastal area. The majority of the areas also have tourist activities which may be impacting the zone (7).

Mexican regulations (17) specify a maximum allowable limit (wet weight) for Cd (0.5 μg/g), Pb (1.0 μg/g), and Hg (1.0 μg/g). Limits for the rest of the elements have not been specified. The results of this study show that, except for Cd (0.76 μg/g), concentrations of regulated metals in oysters are lower than the allowable maximum levels. However, this level of Cd concentration in whole oysters included the digestive gland, sediment particles, and water. Because oysters in the region are consumed as a fresh product, they are traditionally not depurated. Therefore, including a depuration system before oyster consumption may reduce this mean value.

Because Pb mean value in oysters and its maximum allowable limit are close to the DL, it may be necessary to make some method modifications. New microwave systems may permit increased sample size, making metal detection by AAS easier, especially at low levels. Monitoring programs require a rapid multi-element analysis of food with good accuracy. Almost all studies of oysters from the northwest coast of Mexico in the past decade used wet digestion after AAS for metals detection, and accuracy was evaluated by the International Atomic Energy Agency (IAEA) intercalibration program (6, 11, 14). Acceptable ranges were reported for metals such as Cd, Cu, and Zn, but in some cases Pb recovery was >150% (6). Hosh (12) reported total Cd, Cu, Zn, and Pb in clams by microwave digestion and AAS. The accuracy of tests for these metals was estimated by using SRM, NBS Oyster 1566a, and indicated good recovery. Differences in digestion and detection methods for metals may affect the accuracy of test results; therefore, a unified scheme for metal analysis in food is necessary.

Correlations between the elements analyzed in oysters are shown in Table 4. Hg and Zn showed the highest correlation followed by Pb:Se. Other correlations, significant at the 95% confidence level for oyster, were Pb:Zn, As:Zn, and Se:Cd. A significant correlation was found at 90% confidence level for Pb:Cd, Pb:As, and Se:As. Different authors (11, 13) have suggested that a consistent association between particular groups of metals such as Zn, Cd, and Pb may indicate particular biochemical pathways or reflect their common source. Zn correlated with almost all metals in this study. A 1:7 relationship for Hg and Se was observed, although a poor correlation was found for this metal pair. This relationship could be used to regulate the methylation of inorganic mercury (18).

Conclusions

Heavy metal concentrations in oysters from the Sonoran coast were mostly similar to those reported from other geographic areas. Cd in cultivated oysters may originate from the natural mineral composition of the zone, or from various activities in the Sonora state.

Acknowledgments

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References


