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Concentrations of Selected Elements in Oysters (*Crassostrea angulata*) from the Spanish Coast

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Anthropogenic pollutants have contaminated many ecosystems (Evans et al. 1983; Campbell and Evans 1991). Heavy metals are now recognized to be among the most relevant contaminants of the marine environment (Martincic et al. 1984; Rainbow 198.5; Harding and Goyette 1989), and their concentrations are elevated locally in coastal waters (Ober et al. 1987; Schuhmacher et al. 1991, 1992). Since marine organisms living in polluted ecosystems often accumulate metals into their bodies, it has been argued that these organisms can be used as monitors of changes in pollutant concentrations (Luten et al. 1986; López-Artiguez et al. 1989; Peerzada and Kozlik 1992; Schuhmacher et al. 1992).

Oysters have been widely used to determine the levels of contamination by metals in coastal ecosystems (Lytle and Lytle 1982, 1990; López-Artiguez et al. 1989; Sadig and Alam 1989; Vázquez et al. 1990; Peerzada and Kozlik 1992; Páez-Osuna et al. 1990, 1993). They are sedentary, abundant, of relative longevity and easily collected (Hartley and Johnston 1983; López-Artiguez et al. 1989). Moreover, oysters are ecologically important and they are also of economic significance.

Spanish coastal waters are biologically productive and physically diverse marine ecosystems with very important commercial fishing and shellfish industries. A remarkable number of rivers which flow from Spain into the Atlantic Ocean and the Mediterranean Sea are subjected to large loads of toxic industrial residues, including heavy metals (Medina et al. 1986; López-Artiguez et al. 1989; Schuhmacher et al. 1991, 1992). Levels of metals in oysters from the Spanish coast may be of profound interest not only because they document those geographic areas where metal pollution levels may be problematic, but because they may disclose possible health hazards to consumers.

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The purpose of the present study was to determine the concentrations of a number of elements in the oyster *Crassostrea angulata* in different commercially important locations of the Spanish coast. The elements chosen for analysis were arsenic, cadmium, copper, chromium, mercury and lead. Four of them (arsenic, cadmium, mercury and lead) have no known metabolic function and are among the most dangerous toxic trace elements. On the other hand, although copper and chromium are known to play a role in metabolism, they may also be potential pollutants which accumulate in marine organisms and through the food chain may reach to humans. Additionally, the observed concentrations of these elements were compared with those of previously reported surveys.

MATERIALS AND METHODS

The oysters (*Crassostrea angulata*) were randomly purchased from January to March 1992, from seven different locations along the Spanish coast (Figure 1). At each of seven sites, 30 oysters (4-6 cm in length) were collected, placed in labelled polyethylene plastic bags and kept frozen at -20° C until analyzed. Oysters were opened using a plastic knife and soft tissues were removed and homogenized. Composite samples of 4-6 individuals (6 samples for each location) were dried at 40° C until constant weight was attained (Schuhmacher et al. 1991, 1992).

To determine the concentrations of arsenic, cadmium, copper, chromium, mercury and lead in oysters, approximately 1 g of homogenized dry sample was digested in Teflon tubes with 4 ml of 65% HNO_3 (Suprapur, E. Merck, Darmstadt, Germany) and 2 ml of $HClO_4$ (E. Merck, Darmstadt) for 12 hr at room temperature, heating subsequently at 100° C for 5 hr. After adequate cooling, solutions were then made up to 25 ml with double distilled water. Mercury concentrations were determined by the cold vapor atomic absorption method in a cold vapor atomic absorption spectrophotometer (Philips PU 9200X). Stannous chloride solutions were used to reduce mercury to the elemental form (Hg^0). Lead and arsenic concentrations were determined using a Perkin-Elmer 5100 Zeeman spectrophotometer and Spectra A-30 graphite furnace. Cadmium, chromium, and copper concentrations were measured in a computer-controlled sequential inductively coupled plasma spectrometer (Jobin Yvon JY 38 VHR). Together with each series of six samples, one blank was included and analyzed for metal concentrations. To avoid metal contamination, all plastic and glassware was cleaned with HNO_3 and rinsed with deionized water. Samples were kept in acid-washed bottles until metal analyses. As a quality control measure, standard reference materials (NBS-bovine liver, No. 1577a) were included in this study. Triplicate analyses gave the results shown in Table 1.

Because mercury concentrations in the reference material were very low (0.004 µg/g) and close to the detection limit, known amounts of mercuric chloride were

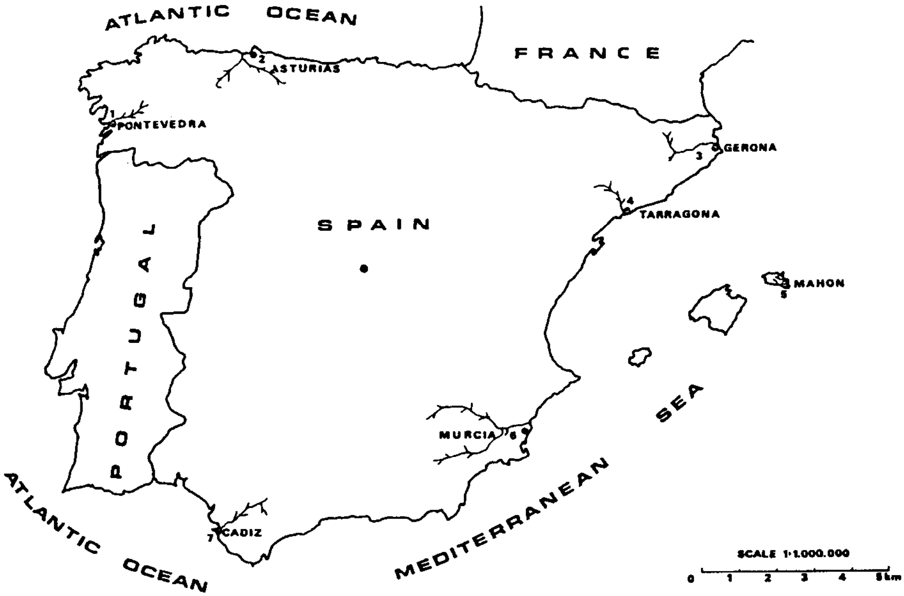

Figure 1. Sites along the Spanish coast from which oysters (*Crassostrea angulata*) were purchased.

Table 1. Concentrations of arsenic, cadmium, copper and lead in reference material.

Sample	As	Cd	Cu	Pb
NBS certified values (μg/g)	0.05±0.01	0.44±0.06	158.01±7.12	0.14±0.02
Concentrations found (μg/g)	0.04±0.01	0.42±0.07	151.96±10.02	0.14±0.02

added to the samples of reference. The mean recovery rate obtained was 96 % . No. reference values were available for chromium. Statistical differences were evaluated by one-way analysis of variance (ANOVA). The method of Scheffé was subsequently used to differentiate between groups. Pearson correlation coefficients between metals were also studied. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Metal concentrations in *Crassostrea angulata* from the Spanish marine coastal waters are summarized in Table 2. All the elements occurred at detectable levels in the oysters. Metal concentrations (dry weight) were increased in this order: mercury < chromium < cadmium < lead < arsenic < copper. The highest mean values for each element were the following: arsenic, 10.03 µg/g (Asturias); cadmium, 3.07 µg/g (Gerona); copper, 305.36 µg/g (Mahón); chromium, 0.79 µg/g (Pontevedra); mercury, 0.23 µg/g (Tarragona) and lead, 34.12 µg/g (Murcia). The highest mean values of metals accumulated in oysters were different for each element according to the sampling site where the oysters were obtained. These differences are mainly due to the anthropogenic contribution derived from the industrial activities which are quite different depending on the geographical situation. The most remarkable feature is the significant difference between lead concentrations found in oysters from different locations (Table 2).

It is important to determine the concentrations of heavy metals in marine organisms which are of commercial value to learn whether these concentrations may constitute a health hazard for consumers. The current food standard regulations in Spain (Boletín Oficial del Estado, 1991) specify the maximum allowable limits of cadmium (1 µg/g wet weight), copper (20 µg/g wet weight), mercury (1 µg/g wet weight), and lead (5 µg/g wet weight). No maximum values for arsenic and chromium have been specified. The results (µg/g wet weight; dry weight/wet weight: 0.171) of the present study for arsenic (0.923), cadmium (0.228), copper (51.85), chromium (0.09), lead (3.14), and mercury (0.023) show that with the exception of copper in some locations, the present levels of metals in oysters of Spanish marine coastal waters are lower than the allowable maximum values.

Correlation coefficients calculated for each pair of metals showed a significant positive correlation ($r = 0.4097$, $P < 0.01$) of copper and cadmium, whereas a significant negative correlation ($r = -0.5011$, $P < 0.01$) of lead and mercury was also found ($N = 42$). No other statistically significant differences were observed. In previous studies, no significant correlations between the concentrations of cadmium, chromium, copper, iron, manganese, lead and zinc were detected in bivalve molluscs collected from non-polluted areas. In contrast, significant correlations were found, with the exception of manganese, in polluted waters (Talbot and Chegwidden 1982).

Numerous surveys of metal concentrations in oysters have been undertaken in recent years. Table 3 compares the results of the present study with those of earlier studies. Arsenic and copper concentrations were comparable to those previously reported by a number of authors, although they were higher than those reported by Lytle and Lytle (1982). Cadmium, chromium, and lead concentrations were also comparable to the results of previous investigations, whereas, in contrast, mercury concentrations were higher than some values found for different surveys (Lytle and Lytle 1982; López-Artiguez et al. 1989). However, comparisons of levels should be made with caution because of variability in the quality of analytical data. In addition, it should be taken into account that differences in the sampling season, size (age) of oysters, genetic differences, individual variability in metal uptake ability, gonadal maturation of organisms, and induction of metal-binding proteins may also influence the results

Table 2. Metal concentrations (µg/g dry weight) in tissues of *Crassostrea angulata* collected from seven stations along the Spanish coast.[1]

Location of sampling areas	Arsenic	Cadmium	Copper	Chromium	Mercury	Lead
Atlantic Ocean						
Asturias	10.03±0.92[a]	0.64±0.03[a]	55.20±1.88[a]	0.41±0.01[bc]	0.19±0.04[b]	0.41±0.19[b]
Pontevedra	4.45±0.52[b]	1.45±0.18[b]	55.22±9.13[a]	0.79±0.17[acde]	0.16±0.08[bc]	0.63±0.15[b]
Cádiz	9.00±0.91[c]	0.51±0.08[a]	195.99±21.67[c]	0.38±0.08[b]	0.17±0.02[b]	0.04±0.03[b]
Mediterranean Sea						
Murcia	7.23±0.55[cb]	1.30±0.12[b]	28.49±3.23[a]	0.39±0.09[b]	0.07±0.01[b]	34.12±6.31[illegible]
Mahón	5.55±0.20[bd]	1.22±0.11[b]	305.36±31.38[e]	0.71±0.05[acf]	0.13±0.06[bc]	6.64±0.78[c]
Tarragona	4.38±0.30[b]	1.29±0.23[b]	142.43±14.66[b]	0.48±0.16[bd]	0.23±0.06[ac]	0.59±0.13[b]
Gerona	8.30±0.99[c]	3.07±0.14[c]	298.28±15.12[de]	0.62±0.04[bef]	0.13±0.03[bc]	5.01±1.90[bc]

‘Results are expressed as arithmetic means of six samples ± SD. Values in the same row not showing a common superscript (a,b,c,d,e,f) are significantly different at $P < 0.05$.

Table 3. Metal concentrations (µg/g) in oysters from different areas ([1]wet weight; [2]dry weight).

Species	As	Cd	Cu	Cr	Hg	Pb	Location	Reference
Crassostrea angulata							Spain	This study
(wet weight)	0.923	0.228	51.85	0.09	0.023	3.14	Atlantic ocean and	
(dry weight)	4.0-11.2	0.4-3.2	24-345	0.2-1.0	0.04-0.34	0.02-41.4	Mediterranean sea	
Crassostrea virginica[1]	0.122	1.61	31.5	<0.1	0.075	<0.5	St. Louis Bay, MI USA	Lytle and Lytle (1982)
Crassostrea virginica[1]	----	0.44-3.5	5.9-55	ND-1.2	ND-6.6	ND-7.9	Mississippi coast USA	Lytle and Lytle (1990)
Crassostrea angulata[1]	1.18-2.07	0.69-4.07	26.4-535	0.35-0.44	0.018-0.90	0.08-1.24	Huelva estuary, Spain	López-Artiguez et al. (1989)
Saccostrea iridescens[2]	----	3.6	20.4	----	----	----	S. gulf of CA Mexico	Páez-Osuna and Marmolejo (1990)
Crassostrea palmula[1]	----	8.2	150	----	----	----	E.P. Lagoon Mexico	Páez-Osuna et al. (1993)
Saccostrea sp[2]	----	ND-1.32	22-74	----	----	ND-83.3	Darwin Harbor, Australia	Peerzada and Kozlik (1992)
Pinctada radiata[1]	----	0.24-5.57	0.34-3.21	----	----	----	Arabian gulf (Arabian Saudi coast)	Sadig and Alam (1989)
Crassostrea virginica[2]	----	1.5-7.5	3.5-135.7	----	----	3.3-66.6	San Andrés Lagoon, Mexico	Vázquez et al. (1990)

(Lytle and Lytle 1990; Páez-Osuna and Marmolejo-Rivas 1990).

In conclusion, heavy metal concentrations in oysters from Spanish marine coastal waters were mostly comparable to values obtained in previous studies performed in a number of different geographic areas. The present results indicate that metal pollution levels in along the Spanish coast would not be especially problematic. Moreover, according to the standards internationally accepted (Nauen 1983), the human consumption of these oysters should not constitute a health hazard.

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