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Sediment Analysis Does Not Provide a Good Measure of Heavy Metal Bioavailability to *Cerastoderma glaucum* (Mollusca: Bivalvia) in Confined Coastal Ecosystems

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Sediments have traditionally been considered as a sink for metals entering the marine environment, especially in coastal areas. Once in the sediment layer, metals are distributed amongst all different phases of the sediment. Their distribution is governed by physicochemical conditions and, consequently, one fraction is immobilized due to its incorporation into weakly reactive phases of the sediment (bound in interlayer positions of clay minerals or in mineral crystalline lattices). Another fraction may remain weakly bound to organic or mineral phases as sorbed, precipitated, or co-precipitated and complexed forms (Brannon *et al.* 1977) and can be assimilated by detritivorous and suspension-feeding benthic organisms.

Many selective procedures have been suggested for metal extraction from sediments in order to estimate concentrations of fractions which are directly or indirectly available to the biota. The absence of a chemical treatment adequate for accurate quantification of metal bioavailability is well-known (e.g., Förstner and Wittmann 1979; Nirel *et al.* 1986; Nirel and Morel 1990). Nevertheless, a good correlation between metal content in some organisms and in the sediment after a specific extraction treatment has sometimes been found. This has allowed sediments to be frequently used as indicators in pollution studies (e.g., Luoma and Bryan 1982; Tessier *et al.* 1984).

In this paper, concentrations of heavy metals (Fe, Mn, Cu, Pb and Cd) in the cockle *Cerastoderma glaucum*, and in sediments at the same sampling locations are compared. *C. glaucum* is a suspension and deposit feeder, inhabiting a wide range of salinities (4->100°/$_{\infty}$)(Barnes 1980). Heavy metal concentrations in sediments were measured after extraction with seven methods of increasing intensity, up to total dissolution of the samples. These methods were selected from those most frequently used in the literature.

For sampling sites, the study used 8 saltponds in the south of Cádiz Bay, located along a gradient of contamination produced by urban and industrial sewage effluents. The principal aim of the study was to identify areas

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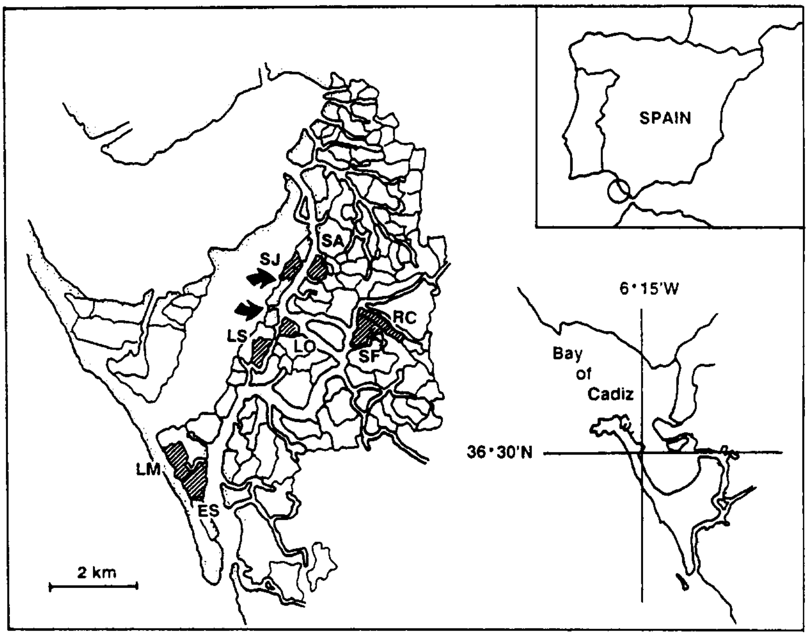

Figure 1. Map of study area with sampling sites. Locations of sewage effluents are marqued by arrows.

with different relative risk from metal pollution, in terms of biological effects and effects on water quality due to natural resuspension of sediments or to human relocation of sediments. The latter is a frequent activity in the area. *C. glaucum* was selected because of its wide distribution in the Bay, and also because it has no commercial value. This second fact means that its distribution and growth is not directly affected by man.

MATERIALS AND METHODS

The study area is a coastal saltmarsh of area 5000 ha, located in the south of Cadiz Bay and subject to a semidiurnal tidal regime of medium amplitude. By the end of the past century, 110 saltworks had been constructed on the marshes. The saltworks are fed with seawater flowing naturally through a complex system of channels, deriving from a main channel that connects the south of the Bay with the Atlantic Ocean (Fig. 1). The tide penetrates the main channel from both ends, the two currents meeting at an intermediate point where sewage from an urban population (close to 130,000 people) and from naval industry is discharged. This sewage is mostly untreated.

At present, nearly all the saltworks in the area are dedicated exclusively to semi-intensive marine fish culture. Water exchange with the sea is controlled by means of gates. The extent of exchange depends on the area of the saltpond water reservoir, the number of gates available and, principally, the height of the bottom of the pond with respect to average sea level.

Differences among these factors mean that environmental conditions, especially salinity, vary considerably from one saltpond to another.

The sampling locations used in the study are depicted in Fig. 1. The saltponds were chosen according to their degree of water pollution. Thus, using pollution indices (heavy metal, organic matter and detergent concentrations in water and sediments) previously established for the area (Sales *et al.* 1983; Quiroga *et al.* 1992), saltponds SJ, LO, SA and LS are in highly polluted locations, saltponds SF and RC are affected by moderate levels of pollution, and saltponds ES and LM are considered to be little affected by pollution.

Surface sediments (approximately 0-3 cm depth) were sampled over an area of 1 m^2 in each saltpond. The sediments, which were mainly clays, were homogenized "in situ" and a subsample ($\approx$ 1 kg) was transported to the laboratory within 3 hours. Sediments were then dried at 105°C and ground to a particle size finer than 100 μm. Different aliquots were treated according to the following procedures for chemical extraction: Method A (Cosma *et al.* 1982); A sample of around 5 g of sediment is treated with 15 mL of a 25% acetic acid solution and 50 mL 1*N* of hydroxylammonia chloride for 19 hr, stirring from time to time. Method B (Berry Lyons and Gaudette 1979); A 1-g sediment sample is treated for 15 min with 25 mL of 10% HCl in a boiling water bath. The sample is kept in suspension by means of mechanical agitation. Method C (Forstner and Wittmann 1979); A 3-g sample is treated at room temperature with concentrated HCl and HNO_3 (3:1 in volume) for 30 min. Method D (Jackson 1978); A 2-g sample is extracted with 25 mL of 6*M* HCl in a boiling water bath for 30 min. Method E (Cosma *et al.* 1978); A 2-g sediment sample is extracted with 8*M* HNO_3 in a steam bath for 30 min. Method F (Adapted from Katz *et al.* 1977); A sample weighing 2 to 4 g (depending on the metal to be analyzed) is extracted 3 times with 8-10 mL of concentrate HCl and HNO_3 (10:1 in volume) for 30 min in a boiling water bath. The treatment is performed in 100-mL centrifuge tubes which are kept agitated. After each extraction, the sediment is separated from the acid extract by centrifugation and it is washed twice with 3-4 mL of 1*M* HCl. Washes are added to the extracts. Method G (Buckley and Cranston 1970); Around 0.75 g of sediment is digested with 9 mL of HF and 1.5 mL of concentrated HCl and HNO_3 (10:1 in volume). The treatment is performed in teflon vesels at 80°C for 19 hr. After cooling, 20 mL of water and 5.4 mL of HBO_3 are added in order to enhance metal fluoride dissolution, and the mixture is stirred. In the course of all the methods tested, acid extracts were filtered before final dissolution. Organic carbon in sediments was determined according to the method of El Rayis (1985).

C. glaucum individuals of the dominant size range found in each saltpond were collected close to the locations where sediment samples were taken, with the exception of saltponds LO and RC where no bivalves were found.

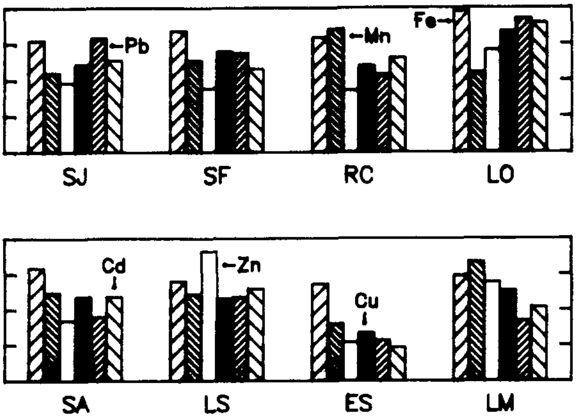

Figure 2. Sediment concentrations of 6 heavy metals at the 8 sampling sites, after extraction by method G (see text for details). Results are normalized to organic carbon (%). One interval on the y-axis represents: 0.5 % dry wt (Fe); 75 ppm (Mn); 40 ppm (Zn); 6 ppm (Cu); 15 ppm (Pb); 0.4 ppm (Cd).

The size ranges of specimens analyzed, expressed in terms of shell length (mm), were 20.0±5.1, 20.0±3.5, 19.8±1.9, 23.2±2.2, 26.1±3.7, and 22.8±2.1 for saltponds SJ, SF, SA, LS, ES and LM respectively. Animals were kept alive for 48 hr in seawater filtered to 0.45 μm, and then drained and freeze-dried. After, whole animals (without shell) from each saltpond were pooled into several 4-g samples that were ashed at 500°C for 24 hr, the ash being dissolved with 0.1*M* HCl (Gutiérrez *et al.* 1978). Metals were analyzed with a Perkin Elmer model 373 atomic absorption spectrophotometer, using external standards. All reagents were from Merck, using Milli-Q water for dilutions. The final volume reached by acid extracts of sediments and organisms depended on the concentration of the metal to be analyzed. All analyses were replicated. Analytical methodology used for bivalves was tested using a reference standard (Mussel Tissue, BCR material No 278). The results obtained ranged from 61.4% for Mn to 94.1% for Cu, in relation to the certified values.

RESULTS AND DISCUSSION

Figure 2 shows metal concentrations in sediments after the total dissolution of samples (method G). Since the sediments were not screened, metal contents are normalized to organic carbon. In general, the spatial distribution of the metals analyzed in sediments does not correspond to the degree of water pollution. Two exceptions can be cited: i) except for Fe, the lowest concentrations for all metals occurred in saltpond ES. Here, there is little pollution, and the sediment is slightly coarser than elsewhere. ii) The highest concentrations of Cu, Pb and Cd, which are common pollutants, were found

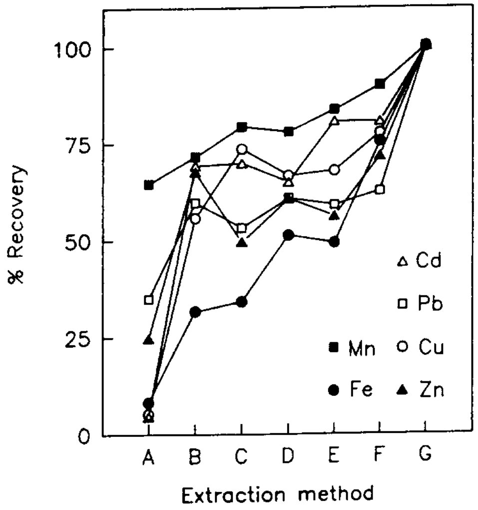

Figure 3. Percentage recovery of 6 heavy metals from sediment samples after extraction by 6 different digestion methods (A-F).

in the saltpond closest to the discharge points of sewage to the area (saltpond LO). For other extraction methods (A to F), there was no good correlation between metal content and proximity to the source of pollution.

Figure 3 shows the average percentage recovery of the six metals after extraction by the different methods. Comparing metals amongst themselves, it can be seen that the highest values for each method were achieved by Mn, with the lowest being Fe in all but one case. The latter is related to the high abundance of Fe in the sediment. Taking the mean percentage recovery of all metals and its coefficients of variation, the extraction efficiency of the different methods can be compared as follows:

$$A < B = C \leq D \leq E < F$$

In addition, method F gave the least variable results when different aliquots of a single sample were analyzed. The Coefficients of Variation (CV) for this method, with n=17, ranged from 4% for Mn to 7.5% for Pb (data not presented). CVs were as much as twice these values for the other methods tested.

Figure 4 shows the concentrations of six heavy metals in *C. glaucum* at the different sampling sites. Correlation coefficients between the concentration of each metal in *C. glaucum*, and its concentration in the sediment after extraction by each method are shown in Table 1. Only two correlations were statistically significant (Fe with methods C and D, for $P<0.05$). In all other instances correlations were not significant. At the same time, no significant

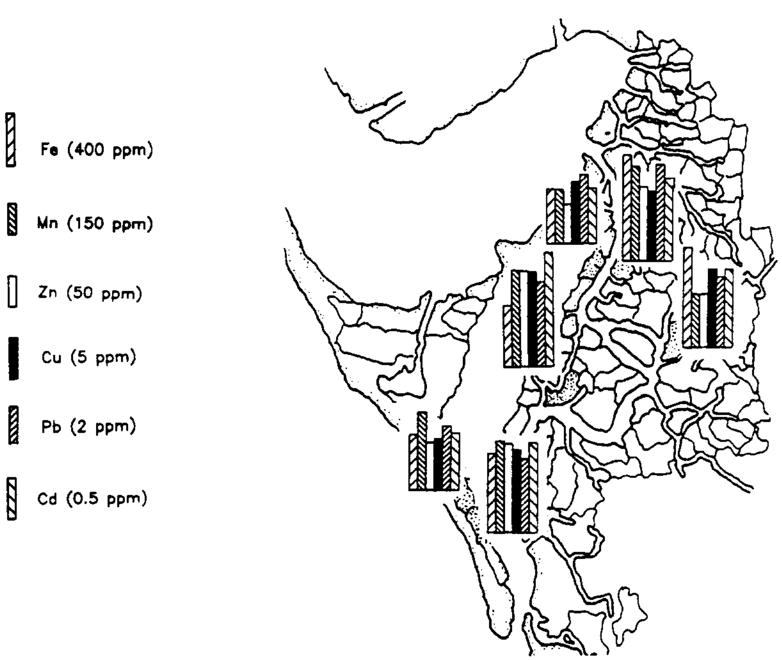

Figure 4. Concentrations of 6 heavy metals (ppm dry wt) in *C. glaucum* at the 6 sampling sites.

correlations were found between metal concentrations in *C. glaucum* and body size or morphometric parameters which can be used as indices of condition in bivalves. The volumetric and gravimetric meat-to-shell ratios tested were: Dry weight of meat·100 / shell-cavity volume (Higgings 1938), dry weight of meat·100 / dry weight of shell (Mann and Ryther 1977), and ash-free dry-meat·1000 / shell lenght^3 (Mann and Ryther 1977).

Concerning metal concentrations in sediments, none of the extraction methods gave results which led to a good description of the pollution gradients existing in the area. These results suggest that heavy metal input to each saltpond is determined primarily by the amount of water exchanged with the exterior. Subsequently, sediment uptake of heavy metals is regulated by environmental conditions within the saltponds. On the other hand, neither did any of the extraction methods allow an estimation of metal bioavailability, judging by data from the bioindicator used. In view of the feeding habits of *C. glaucum*, a relationship between heavy metal concentration in these organisms and in sediments is to be expected. The reason for the lack of concordance between these values may be that methods of acid digestion are not really selective enough for different chemical phases of the sediment. On the other hand, isolated chemical treatment of samples, and also the sequential extraction methods, define "operative phases" in the sediment that may have little relation to the real phases amongst which metal distribution effectively occurrs (Förstner and Wittmann 1979). Bioavailability depends on the real (and unknown)

Table I. Correlation matrix between metal concentrations in *C. glaucum* and in sediment after extraction by 7 different digestion methods (A - G).

Method	Fe	Mn	Zn	Cu	Pb	Cd
A	0.34	0.04	0.61	0.13	0.34	0.46
B	0.61	0.22	0.36	0.11	0.31	<0.01
C	0.78*	0.10	0.10	0.03	0.26	0.39
D	0.76*	0.06	0.68	0.33	0.20	0.44
E	0.70	<0.01	0.04	0.10	0.43	0.21
F	0.30	0.20	0.30	0.08	0.27	0.22
G	0.55	0.01	0.12	0.05	0.36	0.23

* Statistically significant correlations

distribution of metals, which is governed by physicochemical conditions both in the overlying water column and in interstitial water, with Eh and pH values being particularly important. Salinity, temperature and others environmental conditions show large spatial and temporal variations within the studied area, especially in the saltponds, due to a high and variable degree of stagnation. Environmental conditions also affect two processes in which organic matter is involved: i) flocculation, as a preliminary step in sedimentation; ii) the mineralization rate in the sediment. Organic matter plays an important role in heavy metal geochemistry, for which especially complex mechanisms for incorporation of heavy metals into sediments and subsequent accumulation in benthic organisms could be expected in these ecosystems. This could explain the unsatisfactory results in relation to the initial objective, irrespective of the extraction method. It is very likely that the results would have been similar had any other digestion procedure been selected.

These results suggest that an assessment of environmental impact of heavy metals in partitioned and very stagnant areas cannot be undertaken with the use only of the methodology utilized in this work. It can be inferred that in such ecosystems, physicochemical variations have a great influence on the behavior of metals in the environment, including their assimilation by the biota. This influence can be more important than the size of the metal inputs to different locations within the areas.

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