## Bioaccumulation of Cadmium in Marine Organisms

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A general review of cadmium concentrations in marine organisms and studies of cadmium bioaccumulation is presented. Factors which influence cadmium concentrations, such as regional differences, seasonal fluctuations and salinity, are discussed and species which are likely to accumulate cadmium identified. Experimental studies designed to investigate the influence of some of these factors on cadmium bioaccumulation in a filter feeding bivalve mollusk, the American oyster (*Crassostrea virginica*), are presented. Field studies of seasonal dynamics of cadmium in oysters indicate patterns which may be correlated with seasonal physiological activity. The bioaccumulation of cadmium following input to estuarine systems by natural phenomena is observed. Cadmium concentrations in oysters collected from regions of different salinity suggest an inverse relationship between cadmium concentration and salinity. Laboratory experiments designed to investigate mechanisms of cadmium accumulation demonstrate that an inducible cadmium binding protein, similar to metallothionein, is present in the American oyster.

### Introduction

The modification of marine ecosystems as a result of the alteration of natural budgets and cycles of toxic metals during anthropogenic activities is of serious concern to resource managers and public health officials. These concerns are based on the following observations: (1) metals are nondegradable and hence have long half-lives in marine ecosystems; (2) physical, chemical and biological processes may combine under certain circumstances to concentrate metals rather than dilute them; (3) the societal impact of a contaminated system is substantial. From a public health point of view, the contamination of marine food resources with cadmium has particular importance due to the implication of cadmium in chronic health problems. For this reason, an understanding of the environmental and biological factors which control the bioaccumulation of cadmium in marine organisms must be developed. These factors include the physiological and biochemical processes which control cellular cadmium concentrations and the effects of environmental parameters, such as salinity and temperature, on these processes. Some of these factors are discussed below.

### Cadmium Concentrations in Marine Organisms

The results of field surveys in cadmium concentrations in marine biota are summarized in Table 1. A more detailed review of cadmium concentrations in individual species is given elsewhere (1). Although a large data base exists, the utility of much of this data is somewhat limited due to the lack of standardized procedures for sampling, analytical methods, statistical treatment of data and data reporting. In spite of these limitations, several conclusions can be drawn. In general, cadmium is concentrated to a greater extent in mollusks than any other phylum, with scallops (*Pecten maximus*) and

Table 1.	Cd	concentrations	in	marine	organisms."
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Class	Tissue	Cd concentration, $\mu g/g$ wet weight
Microorganisms	_	1.2 -2.6 <sup>b</sup>
Aquatic plants		0.05-75%
Annelids	Whole organism	0.08-3.60 <sup>b</sup>
Crustacea	Muscle	<0.03-13.0%
Mollusks	Whole soft parts	0.01-140
Fish	Muscle	<0.01–2.4
	Liver	0.14-54.0
	Kidney	0.19-9.8
Mammals	Liver	0.5 -4.6
	Kidney	0.1 -15.6

" Data from Frazier (1).

<sup>b</sup> Dry weight basis.

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mussels (Mytilus edulis) exhibiting highest concentrations, followed by the American oyster (Crassostrea virginica). A comparative laboratory study of cadmium accumulation (2) by a crustacea, the American lobster (Homarus americanus), two species of mollusks, the American oyster and the bay scallop (Aquipectens irradians) and a teleostomii fish, the mummichog (Fundulus hetero*clitus*), suggested the following ranking as cadmium bioaccumulators: mollusks > crustacea > teleostomii fish. This ranking is consistent with the observation of field studies. In most organisms, including mollusks, the highest concentrations are found in the visceral mass. Upon further dissection, it is usually the liver or kidney tissues with maximum levels (Table 1).

The data in Table 1 are based on samples taken from many geographical regions. This makes it difficult to compare concentrations between species since the level of cadmium exposure in the environment is not usually quantitated in these field surveys. In order to compare the level of cadmium bioaccumulation in several mollusk species collected from the same area, field sampling was conducted at several stations in the mid-Chesapeake Bay region. Sufficient organisms were obtained for four species to give the data in Table 2. The results indicate that under conditions of equivalent environmental cadmium exposure the American oyster attains the highest tissue concentrations.

Since the oyster has one of the highest potentials for cadmium bioaccumulation in the marine ecosystem, the remainder of this paper will concentrate on studies involving this species. Several laboratory studies of cadmium uptake by oysters have been performed. The experiments of Schuster and Pringle (3) with the American oyster resulted in cadmium concentration factors (ratio of tissue concentration to seawater concentration) in excess of 1000 after 16 weeks of exposure to cadmium. These experiments were cut short by oyster mortality and thus the concentration factor never reached the natural levels reported in the literature of greater than 100,000 (4). Zaroogian and Cheer (5) investigated the uptake of cadmium by the American oyster over a 10-month period. When exposed to 0.005

 
 Table 2. Cd concentration in four species of mollusks collected from the same region of Chesapeake Bay.

Species	Cd concentration, $\mu g/g$ wet weight"
Rangia cuneata	<0.1-2.0
Macoma balthica	0.3-0.8
Mya arenaria	0.5-2.1
Crassostrea virginica	2.1-3.8

" Range of concentrations in total soft tissues.

ppm cadmium in seawater the final concentration in whole oyster tissue was 13.6 ppm (wet weight basis) or a concentration factor of approximately 3000. Since seawater cadmium concentrations in excess of 0.005 ppm have been reported in the literature, the concern for contamination of oysters with this toxic metal is well founded. Brooks and Rumsby (6) studied the uptake of cadmium in the oyster, *Ostrea sinuata* L., and found that the concentration factor was dose-dependent, i.e., the concentration factor decreased as the exposure level increased. This indicates that the accumulation capacity of the oyster is limited and can be saturated.

# Factors Affecting Cadmium Accumulation in Oysters

Correlations of metal concentrations in oysters with field observations of environmental conditions have suggested four interesting relationships which affect bioaccumulation of cadmium. First, there appears to be a general regional difference in soft tissue cadmium concentrations in the American oyster between the Atlantic coast of the United States and the Gulf coast (Table 3). Whether this trend is due to differences in cadmium concentrations in seawater between these two regions or due to biological differences in oyster populations is not known.

The second observation is the significant seasonal alterations in cadmium concentrations in whole soft tissues of the American oyster under natural conditions of exposure (10). Figure 1 presents the concentration of cadmium in American oysters for the years 1972–1975. The oysters in these studies were genetically similar populations (i.e., progeny from a single parent pair) which were maintained in plastic trays in the Rhode River, a natural tributary of the Chesapeake Bay. The general seasonal trend for cadmium concentrations is illustrated by the 1973–1975 data, where a maximum is reached in April or

 Table 3. Regional comparison of Cd concentrations in the American oyster, Crassostrea virginica."

	Cd concentration,		
Region	$\mu$ g/g wet weight	Reference	
Atlantic Coast			
Atlantic Coast, U.S.	0.38-2.39	(7)	
Atlantic Coast, U.S.	0.01-7.80	(3)	
Connecticut, U.S.	3.1 -5.6 <sup>b</sup>	(8)	
Chesapeake Bay, U.S.	<0.6 -2.5	(9)	
Chesapeake Bay, U.S.	0.6 -3.2	(10, 11)	
Gulf Coast			
Florida West Coast, U.S.	0.3 -1.1	(12)	
Mobile Bay, U.S.	<0.05-1.6	(13)	

" Summarized from review of Frazier (1).

<sup>b</sup> Corrected from dry weight basis to wet weight basis.



FIGURE 1. Seasonal dynamics of cadmium concentrations in American oyster whole soft tissues during the years 1972-1975. Oysters sampled in these studies were genetically similar populations maintained in plastic trays in an uncontaminated region of the Chesapeake Bay.

May followed by a decline throughout the summer. This pattern of cadmium concentrations is probably due to the depletion of glycogen stores in the spring followed by the growth of the oyster during the summer which merely dilutes the tissue cadmium concentrations. An interesting variation in the seasonal pattern is observed in the year 1972. The large increase in cadmium concentrations during the summer (June-September) is probably related to a pulse loading of the entire Chesapeake Bay region as a result of the massive influx of metals and other contaminants attendant to the tropical storm Agnes. This storm caused severe flooding in the Susquehanna, Potomac, and James river watersheds which feed into the Chesapeake Bay. These data clearly demonstrate the impact of natural phenomena on metal contamination of estuarine biota.

The above observations on seasonal dynamics of

metals are related to uptake under natural conditions. The third environmental factor which affects cadmium bioaccumulation in oysters relates to the seasonal effect of temperature on accumulation of cadmium from contaminated environments. Zaroogian and Cheer (5) reported that cadmium accumulation from an experimentally contaminated environment did not occur until the water temperature was greater than 15°C. Similar observations have been reported for zinc and copper uptake from contaminated environments (11). These data indicate that cadmium contamination may be a more serious problem during the summer months.

The last observation is that there is a definite inverse relationship between salinity and tissue cadmium concentrations in mollusks, i.e., the higher the salinity, the lower the tissue concentration (14). In order to further investigate this relationship, American oysters were collected from several regions of the Rappahanock River, a tributary of the



FIGURE 2. Salinity dependence of cadmium, zinc, calcium, and magnesium in (●) mantle and (■) digestive gland of the American oyster. Samples were collected from a tributary of the Chesapeake Bay. The dashed line in the calcium and magnesium graphs indicate theoretical concentrations of these ions.

Chesapeake Bay, at various salinities. The oysters were dissected into mantle tissue (the tissue involved in shell formation) and digestive gland, including the stomach and digestive diverticula. The concentration of cadmium in these two tissues is given in Figure 2 as a function of salinity, along with similar data for zinc, calcium, and magnesium.

Several observations can be made from these data. Cadmium concentrations are higher in digestive gland than mantle tissue which is consistent with other field data. Zinc and calcium concentrations are higher in mantle than digestive gland, and magnesium concentrations are essentially the same in both tissues Cadmium concentrations in both tissues decline significantly between 13 and 18% salinity. A similar effect is seen in the zinc data. Calcium concentrations increase with salinity up to 13%, then level off, indicating the presence of a homeostatic mechanism. Magnesium concentrations increase linearly with salinity, although apparently not in equilibrium with estuarine waters.

These observations suggest that oyster tissues have some control over concentrations of several metals. The fact that magnesium concentrations in both tissues are the same and increase linearly indicates that a major element, not actively regulated by tissues, is uniformly distributed in the oyster in relation to the environmental concentrations. The salinity dependence of cadmium and zinc concentrations must then be related to the effects of salinity on the cellular control mechanisms and/or the effects of salinity on the physical chemistry of the metal in seawater. One mechanism which has been suggested as a possible bioaccumulation pathway is related to calcium uptake. The suggestion is that at low salinities, the mechanisms which are involved in calcium transport for shell deposition must operate at an elevated rate due to the depletion of calcium in fresher water. Cadmium bioaccumulation is enhanced as a result of nondiscriminant uptake of this ion along with calcium. The data presented here do not contradict this hypothesis, but at 13 % it appears that the oyster has adequate calcium levels in the tissues and yet the effect of salinity on cadmium concentration does not become significant until 18 % salinity. The question of the mechanisms of cadmium bioaccumulation is still unresolved. One last point is that the reversal of the cadmium and zinc relationship in the mantle and digestive gland indicates that cadmium is not merely mimicking zinc in these tissues, but that metal specific concentration effects are involved.

### **Cadmium Uptake in Oysters**

The uptake of cadmium by the American oyster

was investigated in this laboratory. Three year old, genetically similar oysters were randomly divided into a control and treatment group. Oyster samples were taken after one and two weeks of exposure to 160  $\mu$ g Cd/l. (ppb). Tissues were dissected into mantle and digestive gland. The concentration of cadmium and zinc in these tissues are given in Table 4. Cadmium rapidly accumulated in the tissues studied. Higher levels were attained in digestive gland than mantle. The concentrations of cadmium were still increasing at the end of two weeks. Exposure to cadmium significantly increased the concentration of zinc in mantle tissue.

The subcellular distribution of cadmium and zinc following one week of exposure to cadmium was determined (Table 5). In the mantle more cadmium was associated with the cell organelles and debris than cytosol, while in the digestive gland the inverse was true. The possibility that this result was an artifact due to the fibrous nature of the mantle tissue resulting in poor homogenization was considered. However, the fact that zinc distribution was the same in both tissues suggested that the cadmium effect was real. These data again indicate differential metabolism of cadmium and zinc in these two tissues.

The binding of cadmium and zinc to cytosolic macromolecules was also investigated by Sephadex G-75 gel-permeation chromotography. The association of cadmium with a protein was observed; this protein was identified as metallothionein-like on the basis of the position of the peak on the Sephadex column profile (calibrated with rat hepatic metal-

Table 4. Uptake of Cd by the American oyster, Crassostrea virginica, and its influence on tissue Zn concentrations.<sup>a</sup>

	Cadmium concentration, $\mu g/g$ wet weight		Zinc concentration, $\mu g/g$ wet weight	
Length of exposure <sup>b</sup>	Mantle	Digestive gland	Mantle	Digestive gland
0 1 week 2 weeks	$\begin{array}{c} 0.72 \pm 0.19 \\ 25.4 \pm 2.2 \\ 82.6 \pm 7.6 \end{array}$	$   \begin{array}{r}     1.33 \pm 0.35 \\     52.0 \pm 9.9 \\     *c   \end{array} $	$991 \pm 169$ 1030 ± 105 1840 ± 305	$766 \pm 101$ 843 ± 173 *c

<sup>a</sup> Exposure to 160  $\mu$ g Cd/l. in 20% artificial seawater.

<sup>b</sup> Mean  $\pm$  SE (n=4).

<sup>c</sup> Insufficient data.

 

 Table 5. Percentage of total cellular Cd and Zn in cytosol of American oyster (Crassostrea virginica) tissue.<sup>a</sup>

Tissue	Cd, %	Zn, % <sup>b</sup>
Mantle	$37.9 \pm 4.2$	$43.0 \pm 4.6$
Digestive gland	$54.5 \pm 4.2$	$40.1 \pm 3.6$

 $^a$  Following one week of exposure to 160  $\mu g$  Cd/l. in 20% artificial seawater.

<sup>b</sup> Mean  $\pm$  SE (n=4).



FIGURE 3. Binding of cadmium and zinc to cytoplasmic macromolecules of American oyster digestive gland. Oysters were exposed to cadmium (160  $\mu$ g Cd/l.) for one week. Samples of cytosol were fractionated by gel-permeation chromatography on a Sephadex G-75 column. The elution volume of rat hepatic metallothionein is indicated by MT.

lothionein) and the 254/280 nm absorption ratio. (Fig. 3). The binding behavior of zinc to cytoplasmic macromolecules was unexpected. In control oysters, the majority of cytoplasmic zinc was either free or associated with a very low molecular weight compound. This observation suggests the possibility that intracellular zinc accumulation in oysters may involve an active transport mechanism. Secondly, the increase in tissue zinc concentration attendant to cadmium exposure was not a result of binding to metallothionein as would be expected from mammalian studies, but was characterized by an increase in low molecular weight zinc. It appears that cadmium exposure may have stimulated zinc transport into oyster soft tissues. Although these results are preliminary, they suggest that unique mechanisms of metal uptake and accumulation may occur in marine biota and must be understood in order to be able to predict bioaccumulation potentials in these organisms.

#### Summary

The bioaccumulation of cadmium in marine organism is a potential problem from a public health point of view. Particular attention must be focused on mollusks because of their high affinity for cadmium. Significant bioaccumulation of cadmium occurs in the American oyster at concentrations as low as 0.005 ppm. Environmental concentrations in excess of this level have been reported. Several factors affect cadmium concentrations in oyster tissues, including regional effects, seasonal influences and salinity dependence. These factors can be expected to influence the bioaccumulation of cadmium in other species as well. Laboratory studies designed to investigate mechanisms of cadmium accumulation in oysters demonstrated the existence of an inducible cadmium-binding protein similar to metallothionein. Exposure to cadmium also influences zinc metabolism in these organisms but in a manner different that that observed in mammalian species. These results suggest that unique mechanisms of metal uptake may exist in marine organisms.

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