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Elimination of Laboratory-Acquired Cadmium by the Oyster *Crassostrea virginica* in the Natural Environment

Don E. Mowdy

U.S. Food and Drug Administration, Gulf Coast Technical Services Unit, Dauphin Island, AL 36528

Many productive shellfish-growing areas along the coast of the United States are closed because of pollution from domestic and industrial wastes. Expanding industrialization and population in the areas bordering these waters have increased the pollution potential despite federal and state regulations designed to control waste disposition. Pollutants released into the environment are carried from land to the estuarine environment by direct dumping, storm run-off, and polluted streams. Shellfish growing in these contaminated estuaries create a direct health hazard by biologically accumulating these pollutants to concentrations greater than those found in the water.

Many estuarine areas that are closed to shellfish harvesting because of bacterial pollution contain productive shellfish beds which represent a potentially valuable commercial food resource. These shellfish can be used as commercial food products after they have been relayed to approved growing waters and allowed to eliminate the polluting organisms. Such relaying is done under the direction of the state shellfish program, according to the provisions of the National Shellfish Sanitation Program (HOUSER 1965). Relaying is not presently applied to shellfish contaminated with toxic metals for several reasons: There are no standards to control metal pollution (other than mercury) in seafood; few public health problems in this country involve toxic levels of trace metals in oysters; and not enough information is available to indicate the feasibility of such a project.

PRINGEL et al. (1968) reported a laboratory study on uptake and elimination of heavy metals in oysters and clams in which an elaborate system was used to produce a simulated natural environment. Their results indicated that trace metal elimination by shellfish could be a slow process. KOPFLER (1970) transferred oysters containing over 2000 parts per million (ppm) zinc from the waters of Mobile Bay to laboratory tanks to study zinc elimination. Approximately 25% of the zinc was eliminated during a 6-month period.

FRAZIER (1975, 1976) reported seasonal and environmental effects on the dynamics of metals in a population of genetically similar, hatchery-reared American oysters, which were transferred in plastic trays to a metal-contaminated natural environment. Cadmium behavior was found to be similar to that of zinc and copper, with a 50% reduction in body burden between July and October. The 1976 study

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showed that uptake of metals by oyster soft tissue was seasonally dependent, with rapid uptake occurring in summer and fall and delayed uptake in early spring. Frazier's studies indicated that oysters in a given population respond similarly to seasonal and environmental conditions.

GREIG & WENZLOFF (1978) used naturally contaminated water, sediment, and oysters to observe uptake and depuration of trace metals in relayed oysters. Noting an uptake of cadmium from filtered water as well as from water containing sediment, they determined that the oysters did not obtain cadmium directly from sediment. Their depuration experiments showed no substantial decrease in cadmium concentration in naturally polluted oysters relayed from polluted to relatively unpolluted water in Connecticut and in oysters relayed from polluted water in Connecticut to relatively unpolluted water in North Carolina. ZAROOGIAN (1979), attempting to determine if oysters would depurate cadmium acquired from low-dose concentrations over a long period of time, suggested that cadmium depuration was associated with soft tissue weight loss under the conditions of his experiment.

This study demonstrates the feasibility of relaying cadmium-contaminated oysters to observe cadmium elimination. Because oysters containing naturally acquired cadmium were not readily available, oysters containing laboratory-incurred cadmium were studied.

MATERIALS AND METHODS

Oysters: On January 4, 1978, 400 oysters, Crassostrea virginica, were harvested by tonging from a public reef near Cedar Point, Alabama. These oysters were immediately brought to the Gulf Coast Technical Services Unit laboratory on Dauphin Island, Alabama, where they were cleaned by brushing and divided into 4 groups of 100. Each group was placed in a separate fiberglass flume designed to overflow at a volume of about 350 L. Each flume was supplied with flowing estuarine water pumped from Dauphin Island Bay. At the time the oysters were brought to the laboratory, 10 of them were shucked and the meats were composited for cadmium analysis. This sample was found to contain 0.4 ppm cadmium (wet wt.). While the oysters were acclimating in the flumes, feeding activity was observed by visually noting the quantity of fecal and pseudofecal material present from day to day. The dead and injured oysters were removed each day.

Dosing: Two groups of oysters were dosed morning and afternoon with 35 mL of a solution of cadmium nitrate ($Cd(NO_3)_2$) in distilled water containing 0.1 mg/mL cadmium. This calculates to be 0.01 ppm added cadmium. Before each dose was added, the flumes were drained, fecal material was flushed away, and the flumes were refilled with fresh estuarine water. The dose was added while the flumes were being refilled. During dosing, the water in the flumes was circulated with submersible pumps. Undosed oysters were treated in the same way, except that the cadmium dose was not added to their water.

After the oysters had acclimated 12 days in the flumes, dosing was begun. It was discontinued after 3 1/2 days because apparent oyster activity had stopped. The water temperature was below 10°C and the salinity had fallen below 6 parts per thousand (ppt). The poor water conditions continued for 55 days, during which time activity of both dosed and undosed oysters appeared to be minimal. Dosing was resumed on March 15 when oyster activity was good. After 3 1/2 days, activity slowed and dosing was again discontinued for 8 days. Dosing was then resumed for another 2 days, ending on March 29. The oysters were sampled and analyzed for cadmium. The dosed oysters were found to contain 10.4 ppm cadmium and the undosed oysters, 0.4 ppm. Total dosing time during the 76 days that the oysters were in the flumes was 9 days. The oysters were held in the flumes with running estuarine water for 2 additional days while the analysis was being performed.

Elimination: On March 31, 100 oysters from the experimental (dosed) flumes and 100 oysters from the control (undosed) flumes were transferred to perforated plastic trays (56 cm X 56 cm X 6 cm) in single layers. Twenty oysters were placed in each tray, and the trays, tied in stacks of 2 and 3, were suspended in the water from a pier in Dauphin Island Bay. The waters of Dauphin Island Bay are classified as "prohibited" to shellfish harvesting because of sewage pollution; however, the area is remote from sources of industrial pollution. Dense populations of oysters are found in many areas within the bay, including the area around the pier. The cadmium concentration in these waters is low, as indicated by the failure of the control oysters to increase their cadmium content during the dosing period. The flumes in which the oysters were held were supplied with water from this same area. Analysis of the water from this area was performed at a later date and found to contain 0.03 ppm cadmium.

Environmental parameters: While the oysters were being dosed and while they were in the bay, water temperatures and salinity readings were recorded 3 times each work day. The temperatures were read on a hand-held thermometer and the salinity readings were taken with an American Optical salinity refractometer.

Sampling: The first sample on the day the oysters were transferred to the bay consisted of 5 oysters from each group. After the transfer, 10 oysters were taken from each group during the first 5 sampling intervals. Because some of the oysters died in the trays, it became necessary to reduce the number of oysters per sample to 8 per group. This provided 10 samples over 10 sampling intervals, varying from 7 to 19 days.

Analytical procedure: After collection, the oysters were brought immediately to the laboratory, where they were promptly cleaned, shucked and blended. Analytical portions of approximately 10 g were weighed, and the ashing procedure was begun during the day of collection. Cadmium analyses were performed by the dry-ash procedure described by CAPAR (1977) with the following modifications: (1) 4% instead of 15% sulfuric acid was mixed with

the weighed sample. (2) The ashed sample was dissolved in 20% perchloric acid instead of 1 mL concentrated nitric acid and 10 mL water. (3) The standard dilutions were prepared with 4% perchloric acid instead of 1% nitric acid. The levels of cadmium present in the samples and standard solutions were sufficient for direct aspiration into the atomic absorption spectrophotometer for cadmium determination.

Each sample was analyzed in triplicate, along with a reagent blank. A fourth portion of each sample was fortified with a known amount of cadmium for a recovery check. Each fortified control portion contained 1 ppm added cadmium, and each fortified experimental portion contained 10 ppm added cadmium. The 1 ppm recoveries ranged from 93.2% to 103.1%, with an average recovery of 98.6%. The 10 ppm recoveries ranged from 93.3% to 103.2%, with an average recovery of 98.6%.

RESULTS AND DISCUSSION

The oysters were held in the flumes with flowing estuarine water for 2 days after dosing was discontinued, pending analysis. The experimental sample taken on the day of transfer to the bay, contained 5.9 ppm cadmium (Table 1), which was 57% of the amount of cadmium found in the same group of oysters 2 days earlier. The control sample taken that same day showed no change in cadmium concentration. During the next 42 days, the cadmium concentration in the experimental oysters decreased to 3.0 ppm, which represented a 71% loss of cadmium from the day dosing was discontinued. CHOU et al. (1978) reported that about 50% of the total cadmium in oyster tissue is unbound or free. Concentration of free or unbound cadmium where flushing or cleansing occurs during pumping activity could account for the rapid elimination of cadmium during the 2 days before transfer to the bay and during this 42-day period. CASTERLINE & YIP (1975) reported that cadmium in oysters and rat organs was principally bound to large protein molecules with significant amounts associated with smaller molecules. This finding could explain the slower rate of elimination that occurred during the remainder of the present study. The sample taken after 60 days in the bay showed no decline in cadmium concentration compared with the 42-day sample. The temperature range during this period (43-60 days) was not extreme, but the salinity fluctuated widely and dropped to a low level of 2 ppt. As the salinity fluctuated upward during the next sampling interval, 61-73 days, the decline of cadmium concentration resumed, leveling off at 2.1 ppm. No change was noted in the cadmium concentration for the next 31 days (i.e., days 74-104). This is unexplained. The temperature fluctuated betwen 23 and 34°C and the salinity varied between 4 and 22 ppt. These temperature and salinity conditions are not adverse for oysters in this area. The remaining decline of the cadmium concentration in the oysters amounted to 0.8 ppm over the last 42 days of the study. The temperature fluctuated in a narrow range between 25 and 34°C, and the salinity ranged from 12 to 23 ppt. Both conditions were within ranges apparently conducive to good oyster activity.

TABLE 1

Average cadmium concentrations[a] in experimental and control oysters and averages and ranges of water temperature and salinity during cadmium elimination by the oyster, Crassostrea virginica

Sampling Interval[b] Days in Bay	Cadmium in Oysters ppm (wet wt.)		Daytime Water Temperature (°C)		Daytime Salinity (ppt)	
	Experimental	Control	Average	Range	Average	Range
0	5.9	0.4	-	-	-	-
1-19	4.8	0.4	23	19-25	19	13-26
20-36	3.9	0.4	22	18-26	17	13-24
37-42	3.0	0.4	25	23-26	11	9-13
43-60	3.0	0.4	27	22-31	6	2-11
61-73	2.1	0.3	29	27-31	10	6-16
74-88	2.1	0.3	30	27-34	9	4-14
89-104	2.1	0.3	31	29-33	16	12-22
105-118	1.7	0.3	30	26-34	18	12-23
119-133	1.7	0.3	28	25-33	19	16-21
134-147	1.3	0.2	32	28-34	17	14-19

[a]Cadmium concentrations are averages of 3 determinations on each sample.
[b]Samples collected on last day of each sampling interval.

GALTSOFF et al. (1964) described the optimum temperature for maximum ciliary activity in the oyster to be 25 to 26°C, with a decline of activity as the temperature rises above 32°C and falls below 7°C. He also found the favorable salinity range to be 5-30 ppt. Below 5 ppt he found that the oysters were active, but shell movement and water transport were abnormal. The ciliary activity of the gill epithelium decreased immediately on contact with low salinity water. When extreme prolonged salinity changes occurred, respiration and feeding activity ceased until conditions became more favorable.

Reporting on the effects of temperature, salinity, and turbidity on depuration of bacteria by Gulf Coast oysters, Crassostrea virginica, PRESNELL et al. (1967) found the depuration rate to be most affected by salinity changes. The rate of depuration was greatly diminished by salnities below 7 ppt. Gulf Coast oysters were also found to be more tolerant of higher temperatures than those described by GALTSOFF (1964).

Low salinity may have contributed to the lack of elimination between days 43 and 60 of this study, when the salinity dropped rapidly from 10 to 4 ppt and remained low. Although the lack of elimination between days 74 and 104 is unexplained, the rapid fluctuation of salinity over a broad range, 2-22 ppt, could have contributed to a slowing of activity.

This study showed that laboratory-dosed oysters eliminated cadmium in the natural estuarine environment and suggested that the rate of elimination is affected by changing water temperature and salinity. The rapid elimination which ocurred before and during the first 42 days in the bay suggested the presence of free or unbound cadmium as reported by CHOU et al. (1978). The slower elimination which occurred during the later part of this study could be due to the slow elimination of bound cadmium in the absence of free cadmium.

GREIG AND WENZLOFF (1978) reported that relayed naturally contaminated oysters showed no significant elimination of cadmium after 40 weeks. ZAROOGIAN (1979) found no appreciable elimination with declining temperatures after the oysters had been dosed at a low level (0.015 ppm) for 40 weeks and the cadmium concentration monitored for 16 weeks. This is in agreement with PRINGLE et al. (1968), who stated that trace metal depletion follows the biochemical turnover within the animal and is a slower process than the release of cellular material.

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