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Effects of Temperature and Chelating Agents on Cadmium Uptake in the American Oyster

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Estuarine and coastal waters have increasingly become repositories for the effluents from both industrial and agricultural activities. Cadmium concentration in coastal marine organisms reflect increased concentrations of cadmium in seawater (MCINTYRE & MILLS 1975; PHILLIPS 1977; ZAROOGIAN 1976). Although several authors have documented cadmium accululation in oyster (BROOKS & RUMSKY 1967; PRINGLE et al. 1968; SCRUDATO & ESTES 1976; FRAZIER 1976), many questions remain concerning the effects of specific environmental parameters on uptake of cadmium in oyster tissues.

The objective of this research was to evaluate the effect of temperature on cadmium accumulations in the tissue of the American oyster, Crassostrea virginica, under controlled laboratory conditions. Oysters have been reported to accumulate cadmium from seawater containing added cadmium chloride. However, the chemical form of cadmium in seawater has not been defined. This may profoundly influence the mechanism of uptake. Therefore, the present report is also concerned with the effect of chelating agents on the uptake of cadmium.

MATERIALS AND METHODS

Oyster Collection- Single source, adult oysters of similar size (about 3-4 year old) collected from commercial fisherman in Deal Island area of Maryland were kept in clean seawater for two days to allow defecation and then brought back to the laboratory. During the two-day period, several gentle cleanings and a final sorting were performed to insure a vigorous group of animals for the experiments. After two weeks of acclimation to the controlled environmental conditions, oysters were used in exposure experiments.

Design for Exposure System- Cadmium exposure tests were conducted in the laboratory aquaria containing about 50 gal of synthetic seawater which were continuously aerated. The aquaria were equipped with efficient biological filters containing calcite and dolomite. Flow rate in each chamber was well controlled. The pH was monitored daily and maintained at 8.1. Toxicant concentrations for cadmium were selected in the range of 40 to 60 ppb. The photoperiod regimen was set according to natural sunlight. The effects of temperature dependence on cadmium uptake were conducted in the range of 5 to 20 ^{o}C. Salinity was fixed at 15 $^{0}/_{00}$.

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The effects of chelating agents on cadmium uptake were investigated at room temperature. Chelating agents used in this study were nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and humic acid. Cadmium complexes were prepared by addition of $CdCl_2$ to an experimental medium with an excess of a specificied complexing agent.

Toxicant Solution and Water Analysis- Stock solution were prepared by dissolving reagent grade $CdCl_2$ in deionized water. All glassware were cleaned by detergent first, rinsed by tap-water, deionized water, and 8M HNO_3, and finally rinsed with glass deionized-distilled water prior to use.

Samples for water analysis were taken once a week from the middle of each glass aquarium and acidifield with 0.1% nitric acid. After suitable dilution, the acidified water samples were analyzed by an atomic absorption spectrophotometer equipped with a flameless graphite furnace. To verify the accuracy of the method of analysis, known amounts of cadmium compound were added to control water sample to obtain percentage recovery.

Biological Procedures and Residue Analysis- Each experimental group contained about 80 oysters and the cadmium exposure tests lasted for 40 days. During the accumulation period, several oysters from each experimental group were sampled every 10 days. The analytical method used for residue analysis were acid-digestion of the soft parts from oyster. Sampled oysters were first rinsed in cadmium-free seawater, removed from the valves, blended, and then dried in the oven. Drying was conducted at 110°C for 24 h. The dry weighed tissues were digested in concentrated nitric acid and then 30% hydrogen peroxide (2:1, HNO_3:H_2O_2) (FRAZIER 1975). After suitable dilution, the absorbed cadmium content of oysters were determined by reference to standard salt solutions and use of standard addition methods on an atomic absorption spectrophotometer operated in the flame mode. Final concentration of metal was calculated on a dry weight basis

At the end of the 40-day exposure to cadmium, 15 oysters were randomly selected from each tank, and each was dissected into 5 fractions, namely gills, mantle, adductor muscle, hepatopancreas and the remainder. The cadmium content of each fraction was analyzed by the same method as described above.

RESULTS

Levels of cadmium in the aquarium changed slightly during the exposure period. In all experiments, less than 5% of the cadmium originally in each aquarium was transferred to the oysters.

The patterns of cadmium accumulation in the oysters at different temperature during the 40-day experiments are shown in Table 1 and 2.

The results shown in Table 1 represent the average cadmium content expressed in ug per g dry weight tissue for the ten oysters

sampled at the end of each time interval. The distribution of cadmium within oyster tissue fractions after the 40-day exposure are shown on Table 2. The cadmium contents of different tissue fractions were collected by averaging those from the same fraction of each oyster. Although the adductor muscle accumulated substantially less cadmium than other fractions, cadmium uptake by the gill, mantle, hepatopancreas were not significantly different from each other.

To investigate the effects of complex formation on cadmium accumulation by oyster, three different complexes were studied: two with relatively lower molecular weight complexing agents, EDTA and NTA, and the other higher molecular weight, humic acid.

Table 1. Mean Cadmium Content (ppm, dry wt.) in Whole Soft Parts of Oyster at Four Different Temperatures.

Cadmium Dose (ppb)	Water Temp. (°C)	Days Exposed 10	20	30	40
53±2	5	14.2	--	21.1	25.2
53±2	10	25.8	29.6	39.1	54.6
45±2	15	38.2	45.2	65.3	85.5
45±2	20	--	--	73.4	123

Table 2. Distribution of Cadmium in Oyster Tissue Fractions after Exposure to Cadmium (45±2 ppb) for 40 days.

Tissue Fraction	Cadmiun Content per g Tissue (ppm)[a] 10°C[b]	15°C	20°C
Adductor muscle	13.8	25.2	23.3
Mantle	42.3	83.0	126
Gill	69.5	147	144
Hepatopancreas	58.7	130	145
Remainder	29.6	68.0	72.0

a. Cadmium Contents are based on dry weight tissue
b. Cadmium concentration in this experimental medium was 53±2 ppb.

Both chelating agents like EDTA and NTA are used as components of detergents. Besides, EDTA is used extensively in washing powders to stablize perborate and may be found in sewage effluents in concentrations up to 1 ug/mL (GARDINER 1975). Humic acid occurs in soils and readily forms complexes with Cd^{2+} (SCHNITZER & KHAN 1972; GUY & CHAKRABARTI 1975). Cadmium contents in oysters exposed to 0.05 ug/mL cadmium for 40 days were different from that of those exposed to same level of cadmium with EDTA, NTA, or

humic acid. The concentrations of cadmium for the total soft parts and separate tissue fractions of the oysters sampled from each experimental group are indicated in Table 3.

Table 3. Accumulation of Cadmium in Different Tissue Fractions of Oysters Exposed to 0.05 ug/mL in Seawater as $CdCl_2$ or Complexed Cadmium at Room Temperature.

	Mean Cadmium Content per g dry tissue (ppm)				
Tissue Fraction	Control group	$CdCl_2$	$CdCl_2$ + NTA[a]	$CdCl_2$ + EDTA[a]	$CdCl_2$ + Humic acid[b]
Adductor Muscle	2.8	28	35	23	30
Mantle	10.1	160	142	85	145
Gill	10.6	290	203	154	174
Hepatopancreas	13.8	240	197	103	145
Remainder	11.9	160	96	58	82
Total	7.1	193	97	59	106

a. Concentrations of NTA and EDTA were at 125 ppb
b. Concentration of humic acid was at 250 ppb

Results shown in Table 3 indicate that the added chelators decrease the accumulation of cadmium in all of oyster tissues except the adductor muscle, where the accumulation is lowest, as compared to that without the presence of chelators. Besides, the results also show that the effects of chelator EDTA on the toxicity of cadmium in American oyster differ significantly from that of the other chelators. Although it is difficult to compare the effects of humic matter on uptake to that of the other chelators because of its uncertain molecular weight, the ability of a chelator to reduce the accumulation of heavy metal in oyster has been related to the stability constant of the metal-chelator complex (KNEZOVICH et al. 1981).

DISCUSSION

The concentrations of 40 to 60 ppb cadmium used in these experiments were chosen to correspond to naturally occuring concentrations in heavily polluted estuarine systems.

Although the dry weight data show that Crassostrea virginica accumulates cadmium regardless of the water temperature, that rate of accumulation at high temperature was more than that at the lower temperature, supported greater metabolic rate at warmer seawater, so that more seawater passed over the gills.

Temperature affects many physiological processes in molluscs. GALTSOFF (1928) reported that the maximum pumping rate for C. virginica occurred at 25°C; below this temperature pumping rate

was decreased with the water temperature. Similar decreasing environmental temperatures were reported in Ostrea lurida (HOPKINS 1933). Thus, with high temperature and increased metabolism, more rapid turnover of all tissue constitutents, including Cd or other contaminant would be expected.

The distribution of Cd in the oyster tissues was nearly uniform except the adductor muscle (see Table 2). The relatively low rate of Cd accumulation in the adductor muscle may be resulted from the isolating effect of its thick surrounding membrane. If cadmium were actively involved in a particular physiological process, one might expect its distribution to be localized in areas where this process predominates. Instead, its rather generalized distribution suggests that it is associated with a fundamental component of the oyster tissues. Since cadmium combines readily with certain proteins, it suggested that cadmium may be distributed throughout the oyster tissues as Cd-protein complexes. Besides, other workers even suggested that Cr uptake occurs primarily by passive diffusion. Although the present data do not indicate the mechanism responsible for Cd accumulation it seems consistent with this hypothesis.

The reduction of heavy metal toxicity by organic chelating agents has been reported for several species (LEWIS et al. 1971). MILANOVICH et al. (1976) showed that naturally occurring yellow humic matter, as well as EDTA reduced the toxicity of copper to Escherichia coli. In this study, all of the added organic chelators reduced the toxicity of cadmium to C. virginica. The ability of a chelator to reduce cadmium tocicity in oyster could be explained as its strength of forming a stable cadmium complex. The fact that EDTA forms a relatively strong complex with cadmium and is very effective in reducing toxicity, indicates that the unbound, labile fraction of cadmium is the toxic species.

Although our results from experiments indicated that medium temperature and types of organic ligand in seawater are important in determining cadmium toxicity to oyster, other environmental parameters, such as salinity, pH, cadmium level of seawater, etc., must also be considered when determining the deleterious effects of cadmium released into marine and estuarine ecosystems. Because all parameters affect the biological availability, they in turn affect the uptake of cadmium in oyster.

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