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Biological Availability of Sediment-Bound Cadmium to the Edible Cockle, *Cerastoderma edule*

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Essential metals in biological systems are characterised by their ability to bind reversibly with important biochemical species. The toxicity of heavy metals stems, in part, from their ability to co-ordinate more strongly with the same biochemical species. The higher affinity of heavy metals, or, more correctly, of heavy metal ions, for organic ligands arises from their possession of filled d-orbitals and vacant bonding orbitals promoting synergic bonding with organic species. A consequence of this high affinity is that heavy metals tend to accumulate in man because of a lack of an efficient excretion mechanism. However, the co-ordination of heavy metals to biological systems is not irreversible as various marine creatures will decrease their body burdens of heavy metals when transferred to a clean environment (PRINGLE et al. 1968).

Several toxic mechanisms for heavy metals have been identified (LUCKEY et al. 1975). The reaction of mercury or cadmium with thiol groups in proteins may inhibit enzyme action. Enzyme activation by metals is often associated with stabilisation of the secondary or tertiary structure of the protein. Displacement of a lighter essential metal such as zinc by a chemically similar heavier metal such as cadmium may modify this structure and alter the action of the enzyme. Metals such as gold, cadmium, mercury and lead are capable of combining with the cell membrane and thereby altering its permeability (PRINGLE et al. 1968). Other toxic mechanisms include the chelation of essential metabolites and the catalytic decomposition of these compounds. The observed effects of heavy metal toxicity in living organisms include lack of vigour, inhibition of growth and metabolism, poor fertility and high infant mortality.

The mechanisms of trace metal uptake by marine organisms are not well understood. Possible pathways include ingestion of particulate suspended matter, uptake via pre-concentration in the food chain, or ion-exchange (BROOKS and RUMSBY 1965).

The low concentrations of heavy metals found in the marine environment are measured in nanograms per gram where-as levels in biota are of the order of micrograms per gram, illustrating the ability of marine creatures to concentrate or accumulate such elements. Bio-accumulation of metals is especially high in the invertebrates (WALDICHUK 1974). The action of filter feeding is thought to give rise to this effect. It is not surprising therefore

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to find that heavy metals often concentrate in the gills (VERNBERG et al. 1974) (TOPPING 1973).

Of all the marine organisms, molluscs appear to have the greatest ability to concentrate heavy metals (PRESTON 1973). This property has resulted in the use, or suggestion, of molluscs as indicators of metal pollution in marine environments (GOLDBERG et al. 1978). However, it has been reported, (PRESTON 1973), that oysters exhibit the highest cadmium levels in areas of industrial outfall whereas crabs in such areas have relatively low cadmium concentrations. Conversely where crabs have high cadmium levels oysters contain significantly less of the metal than in polluted areas. Such a result may be interpreted as indicating a variation in physico-chemical form of the element i.e. it was available to one species but not to the other.

A number of laboratory studies of the bio-availability of sediment-bound metals have been reported. For example, cadmium bound to hydrous iron oxide was available to clams but the presence of organic material on the sediment inhibited cadmium uptake (LUOMA and JENNE 1976). Similarly the bio-availability of silver, zinc and cobalt bound to a variety of sediments has been studied (LUOMA and JENNE 1977). Silver was most readily assimilated by clams when the metal was associated with calcium carbonate or manganese oxide. When the metal was bound to biogenic calcium carbonate (crushed clam shells), iron oxides or organic sediments, uptake by the clams was much lower.

We now report the bio-availability of cadmium (present as Cd^{2+}) to the edible cockle, Cerastoderma edule, from the four sediments:- iron oxide, manganese oxide, calcium carbonate and biogenic calcium carbonate.

MATERIALS AND METHODS

All of the sediments were prepared using double distilled water and analytical grade reagents. Iron oxide sediment. 400 ml of approximately 0.3 M solution of ferric chloride was prepared and 1 M sodium hydroxide added slowly, with stirring, until precipitation was complete. The sediment produced was filtered on a Buchner funnel and washed with 3 x 100 ml portions of sea-water. The precise chemical construction of the iron oxide produced by such a precipitation reaction is not known but is believed to be a hydrated ferric oxide, $Fe_2O_3 \cdot nH_2O$. For the subsequent step of doping the sediment with cadmium ions the sediment was retained in the wet form obtained after filtration. A small sample of the wet sediment was weighed and then heated to constant weight on a boiling water bath. From the weighings, the solid content of the wet sediment was obtained. Using this data, sufficient wet sediment was taken to provide about 10 g of dry sediment. The wet sediment was placed in a conical flask and about 100 ml of double distilled water added. An aqueous solution (about 200 ml) containing 500 µg cadmium (as cadmium chloride) was then added slowly, with swirling, to the sediment slurry in the flask. When the addition was complete the

flask was stoppered and shaken for 24 hours. The sediment slurry was then filtered and washed with three 100 ml portions of sea-water.

In a similar manner an iron oxide sediment containing, nominally, 5 μg/g cadmium was prepared. A control sediment was also prepared (nominally zero cadmium) the sediment being shaken only with double distilled water. Manganese oxide sediment. The sediment was prepared by precipitation - an approximately 0.5 M solution of manganese(II) sulphate being reacted with a 1 M solution of sodium hydroxide. As with the iron oxide sediment, the precise nature of the manganese oxide sediment is not known. N.B. The manganese oxide sediment, was brown in colour. Manganese oxide sediments doped with cadmium at the nominal level of 50, 5 and 0 μg/g were prepared, as for the iron oxide sediments. Calcium carbonate sediment. An approximately 2 M solution of calcium chloride was prepared (400 ml) and sodium carbonate solution (∿1.5 M) was slowly added until precipitation was complete. The precipitate was washed and then doped with cadmium, as above. Biogenic calcium carbonate. Biogenic calcium carbonate sediment was obtained by crushing washed cockle shells to powder. The sediment so obtained was then treated as above to provide cadmium-doped and control sediments. Preparation of aquaria. The sea-water was prepared by dissolving 38 g of synthetic sea-salt in 1 ℓ of double distilled water. Each tank of water was well aerated using diffuser stones. The air supply for filtration and aeration was provided by electrically operated air pumps. The aquaria were placed in a large tank which was supplied by a water flow from a refrigerated water bath. By maintaining the refrigerated water at 4 °C and adjusting the flow of water entering the tank by means of a screw-clip on a rubber tube, the temperature of the aquaria could be held at 10 °C ± 2 °C. Each of the aquaria was supplied with a similar quantity of fish-food. The food (10 drops) was dissolved in 100 ml of double distilled water and 2 ml of this solution was pipetted into each tank. The edible cockles used in this work were obtained from Bioserv Ltd., Worthing. Prior to placing the cockles in the sediment-containing tanks, they were kept for 5 days in sediment-free tanks to allow time for acclimatization. Only active animals were selected at the end of the acclimatization period for subsequent exposure to the synthetic sediments. For each synthetic sediment three aquaria were prepared to contain the 50 μg/g, 5 μg/g and control (0 μg/g) sediments. Into each aquarium six cockles were placed, to allow sampling of two cockles after 24 hours, 48 hours and 96 hours.

ANALYTICAL PROCEDURES

Sediments were analysed (i) immediately after preparation and (ii) after 8 days in the aquaria. Analysis was performed using a Varian Techtron A.A.5. atomic absorption spectrophotometer with background correction. Determinations were by calibration curve, the standards being "matrix adjusted" to contain the relevant quantities of acid and matrix metal element. In selected instances, the magnitude of any error introduced by incorrect "matrix matching"

of standards was assessed either by comparison of the calibration curves obtained from adjusted standards containing various amounts of matrix element, or by the method of multiple additions. None was observed.

Cockles were prepared for analysis by opening the shell and well-rinsing the interior of the animal with flowing tap water, followed by rinsing with double distilled water. The sample was weighed wet, freeze dried and re-weighed. Digestion with 10 ml of concentrated nitric acid followed by reduction of volume to 3 ml and then addition of double distilled water to 10 ml gave a suitable solution for analysis. Determinations of cadmium were carried out by atomic absorption spectrophotometry by calibration curve and by multiple addition using background correction. No non-specific absorption was observed. Cockles exposed to the biogenic calcium carbonate sediment (nominally 50 µg/g cadmium) were also analysed for zinc. Samples of artificial sea-water and double distilled water were analysed for cadmium by electrothermal rather than flame atomization using an Instrumentation Laboratory Inc. IL151 spectrophotometer equipped with an IL555 flameless atomizer.

RESULTS AND DISCUSSION

Although sediments were doped with sufficient metal to provide for concentration of 50 µg/g and 5 µg/g, concentrations of only 24.4 µg/g and 2.0 µg/g were attained for the biogenic calcium carbonate sediment. By contrast the other sediments showed total uptake of the cadmium initially added. A lower adsorptive capacity of the biogenic calcium carbonate was thus suggested. This lower binding strength of cadmium to the biogenic calcium carbonate may result from the manner of preparation of this sediment. This sediment was prepared by mechanical crushing rather than precipitation resulting in a larger particle size and consequent lower surface area.

Examination of the concentration of cadmium in the sea-water and the sediment eight days after the sediments had been placed in the tanks showed that a marked desorption of cadmium occurred only in the case of the biogenic calcium carbonate where the cadmium level had fallen from 24.4 µg/g to 15.2 µg/g. No significant desorption of cadmium from the other sediments was observed.

Analysis of cockles kept in tanks with the various sediments showed a significant increase in cadmium levels only for those specimens exposed to the biogenic calcium carbonate sediment. LUOMA and JENNE (1977) have reported that cobalt and zinc were most readily available to clams when bound to biogenic calcium carbonate but uptake was slow. Silver bound to biogenic calcium carbonate exhibited similar availability to zinc, but when bound to precipitated calcium carbonate availability was much higher. Thus the reverse situation appears applicable to cadmium when compared with silver.

Of the other sediments studied, there was some evidence to suggest that cadmium was available to C. edule from precipitated

calcium carbonate but this was much lower than for the biogenic calcium carbonate. No uptake of cadmium from either the iron oxide or manganese oxide sediments was observed. In agreement with this finding it was observed that the cadmium concentrations of these sediments had not decreased after eight days in sea-water. The iron oxide sediment used in these experiments had been prepared several weeks prior to the introduction of the cockles. The zero availability of cadmium may have resulted therefore from the aged nature of the sediment and it has been reported (LUOMA and JENNE 1977) that silver was more readily available to clams when bound to freshly precipitated iron oxide than when bound to aged iron oxide.

The zero availability of cadmium bound to manganese oxide is of interest as silver bound to manganese oxide is readily available to clams, although cobalt and zinc, when bound to the same sediment, were of much lower availability. HALCROW et al. (1973) have reported an apparent higher availability of cadmium when associated with manganese rich sediments. The zero availability of cadmium bound to manganese oxide in this study may therefore be due to either the strong adsorptive properties of the sediment or the adverse effects (small particle size causing blocking of the mantle) of the physical nature of the sediment on the vigour of the cockles.

Concentration factors calculated from our results give values ranging from 2.6×10^2 to 7×10^3. These values may be considered low when compared with the values of 10^3 to 10^4 generally reported for molluscs (PRESTON, 1973). It would seem that lower concentration factors (i.e. the ratio of metal concentration in the environment to the concentration in the sample) occur when the

TABLE

Comparison of cadmium and zinc concentrations in C. Edule

Sample No.	Cd concentration µg/g	Zn concentration µg/g	Zn:Cd Ratio	Sample Description
C95	0.24	77	321	"As Received" cockles held in sea-water only for 72 h.
C96	0.69	88	128	
C97	0.73	76	105	
C98	2.4	60	25	After 24 h, in aquarium containing 24.4 µg/g Cd/Biogenic $CaCO_3$
C99	2.3	70	30	
C104	3.7	58	15	48 h, as above
C105	3.4	65	19	
C110	7.2	117	16	96 h, as above
C111	7.4	89	12	

ambient cadmium concentration is high. This implies that a bio-available metal is not necessarily completely taken up by the organism or that uptake is at a rate which may be longer than the duration of these studies (5 days maximum exposure).

The chemical similarity of cadmium and zinc and the ability of cadmium to form similar complexes to zinc with proteins raises the question of competitive effects between the two metals in organisms. The table compares zinc and cadmium concentrations for cockles at various stages of exposure to biogenic calcium carbonate containing 24.4 µg/g cadmium. Initially a mean cadmium level of 0.55 µg/g was accompanied by a mean zinc concentration of 80.9 µg/g. After exposure to the sediment for 24 h. the cadmium concentration rose to 2.4 µg/g and the zinc level fell to 65.4 µg/g. Further exposure led to another reduction in the zinc/cadmium ratio. Clearly an inverse relationship exists between the cadmium and zinc concentrations in cockles exposed to biologically available cadmium. These results correlate with those of STENNER and NICKLESS (1975) and BRYAN and HUMMERSTONE (1977).

In conclusion, cadmium bound to biogenic calcium carbonate is readily available to C. edule but cadium bound to precipitated calcium carbonate is of considerably lower bio-availability. Cadmium bound to iron oxide and manganese oxide is unavailable, at least in short term exposures. Availability appears to be determined by the ability of the metal to desorb from the sediment.

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