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Accumulation of Seven Metals by *Crassostrea gigas*, *Crassostrea margaritacea*, *Perna perna*, and *Choromytilus meridionalis*

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The molluscs Crassostrea margaritacea, Perna perna and Choromytilus meridionalis are potentially useful biological indicators of metal pollution in the South African coastal marine environment (DARRACOTT and WATLING 1975) and metal concentrations in these species are being determined as part of the South African marine pollution monitoring programme (WATLING and WATLING 1976a, 1979, 1982; ORREN et al. 1980). However, few data on comparative rates of metal accumulation in these species are available.

The Pacific oyster Crassostrea gigas is a hardy, fast-growing oyster which has been introduced into Knysna estuary on the south coast of South Africa to be cultivated and grown on a commercial basis. Metal concentrations in C. gigas from Knysna have been found to be very low in comparison to many of the values reported for this species growing in other parts of the world (WATLING and WATLING 1976b). However, C. gigas has been shown to accumulate metals to very high levels (THROWER and EUSTACE 1973) and can be used for both field and laboratory experiments (e.g. PRINGLE et al. 1968; THORNTON et al. 1975). In a number of cases it has been possible to relate the accumulation of selected metals by this species to metal pollution from industrial or other sources (BOYDEN 1975; BOYDEN and ROMERIL 1974; RATKOWSKY et al. 1974).

In this preliminary study the accumulation of seven elements by C. margaritacea, P. perna and C. meridionalis was investigated. At the same time the opportunity was taken to compare these rates of accumulation with those achieved by C. gigas under the same controlled experimental conditions.

MATERIALS AND METHODS

C. gigas and C. margaritacea were taken from the rack systems in the estuary and P. perna were collected from the rocky shore at Knysna Heads (WATLING and WATLING 1976b) and C. meridionalis were collected at Bloubergstrand near Cape Town (ORREN et al. 1980). All the experimental animals were maintained on floating trays in a large flowing-water pond at Knysna.

Experimental solutions containing 100 µg/L of one of the elements were prepared using stock solutions of the metal chlorides of zinc, cadmium, copper, lead, nickel and cobalt, and of sodium dichromate. Solutions were renewed daily and aerated continuously

during the three-week experiment. The stabilities of sea water solutions containing low metal concentrations were investigated for the conditions of this experiment. Losses of up to 25% for lead were recorded at the end of the 24-h period but it was also found that the greatest losses occurred during the final 6 h. No losses were detected for the other elements.

Comparative accumulation studies were carried out using 10 of each species (40 individuals) in each tank containing 40 L sea water. The experimental animals were suspended in the tanks on plastic nets in order to facilitate their removal and examination. At the end of the experiment the wet tissues of individuals were removed from their shells and frozen preparatory to chemical analysis.

The frozen specimens were thawed, weighed into clean dry flasks and oven-dried at 90°C for 24 h. The dried samples were reweighed, dissolved in 25 mL of redistilled analar-grade nitric acid and the sample solution boiled and evaporated to near dryness. The residue was dissolved in 25 mL of a 4:1 nitric-perchloric acid mixture. This solution was fumed to dryness at about 120°C. The white residue was redissolved in 10 mL of 10% v/v nitric acid and the metal concentrations in this solution determined by atomic absorption spectrometry.

Composite standards containing zinc, cadmium, copper, lead, iron, manganese, nickel, cobalt and chromium in the range 0.1-20 µg/mL, in the presence of sodium, potassium, calcium and magnesium in the range 100-5000 µg/mL, were prepared in 10% v/v nitric acid. A Varian-Techtron AA5 with AA6 readout module and BC6 background corrector was used for all measurements. Background correction was applied to the determination of zinc, cadmium, lead, nickel and cobalt and the slotted tube (WATLING 1978) was used to increase the sensitivity of the lead determination. The instrument was calibrated using the composite standards and the results are expressed as µg/g metal in wet tissue.

RESULTS AND DISCUSSION

Mean tissue metal concentrations of *C. gigas*, *C. margaritacea*, *P. perna* and *C. meridionalis* exposed to seven elements are summarised in Table 1. The results indicate that all seven elements are accumulated to a greater or lesser extent by each of the four species.

It is also possible to calculate the rate of accumulation (µg/g/week) of each element by each species for a given set of experimental conditions (Table 2) and these figures can be used to compare the uptake rates of different metals by a given species or the uptake rates of a single element by different species. When these data are examined, some striking differences between oysters and mussels become apparent.

TABLE 1

Metal concentrations in mollusc tissues following 3-week exposure to 100 µg/L of one of seven elements (results as µg/g wet tissue)

Species		Zn	Cd	Cu	Pb	Ni	Co	Cr
Crassostrea gigas								
Control	$\bar{x}$	120	0.57	11.2	<0.05	0.04	0.01	0.19
	s	48	0.12	3.3	-	0.02	0.01	0.06
Treatment	$\bar{x}$	132	6.3	23.3	1.34	0.47	0.41	0.65
	s	50	2.3	3.7	1.24	0.24	0.11	0.23
Crassostrea margaritacea								
Control	$\bar{x}$	115	1.13	1.42	0.08	0.03	<0.02	0.21
	s	20	0.25	0.38	0.04	0.02	-	0.09
Treatment	$\bar{x}$	128	2.70	7.36	1.78	0.28	0.07	0.61
	s	33	0.90	3.64	0.79	0.09	0.03	0.13
Perna perna								
Control	$\bar{x}$	12,0	0.57	0.74	0.22	0.38	<0.02	0.28
	s	3.1	0.19	0.14	0.05	0.11	-	0.06
Treatment	$\bar{x}$	12.7	2.05	1.79	2.93	0.82	0.46	0.81
	s	4.3	0.54	0.90	0.64	0.35	0.12	0.29
Choromytilus meridionalis								
Control	$\bar{x}$	14.3	0.27	1.16	0.15	0.09	0.02	0.24
	s	2.5	0.06	0.12	0.06	0.06	0.01	0.09
Treatment	$\bar{x}$	15.8	1.40	1.44	3.32	0.65	0.38	0.52
	s	2.6	0.16	0.11	0.72	0.13	0.10	0.18

TABLE 2

Rates of metal accumulation (µg/g/week)
for a 3-week exposure to 100 µg/L of one of seven elements

Species	Zn	Cd	Cu	Pb	Ni	Co	Cr
Crassostrea gigas	4.0	1.92	4.0	0.44	0.14	0.13	0.15
Crassostrea margaritacea	4.3	0.52	2.0	0.57	0.08	0.02	0.13
Perna perna	0.23	0.49	0.35	0.90	0.15	0.15	0.18
Choromytilus meridionalis	0.50	0.38	0.09	1.06	0.19	0.12	0.09

The four species were exposed to 100 µg/L of each of the elements for a period of three weeks. During this period the oysters have accumulated zinc and copper at the fastest rates,

lead and cadmium at intermediate rates and the remaining elements are accumulated more slowly. Lead is accumulated at the fastest rate by the mussels, in spite of the possible 25% loss from solution during each 24-h period; zinc and cadmium in both mussels and copper in P. perna are accumulated at intermediate rates and, as was found in the oysters, nickel, cobalt and chromium are accumulated very slowly.

The zinc levels which are being tested here are already substantially more than those found in many so-called polluted areas. However, the zinc concentrations determined for these experimental animals, particularly the oysters, are very much lower than some which have been reported for individuals growing in polluted areas (e.g. THROWER and EUSTACE 1973). It must be concluded that these concentrations have been achieved during very long exposure periods or, alternatively, that the chemical or physical form of the zinc in solution plays an important role in the uptake mechanism. Further experiments on the accumulation of zinc associated with food or sediment particles or complexed by naturally occurring organic compounds, complemented by biochemical studies such as those described by COOMBS (1972, 1974), could clarify the mechanism by which zinc is normally accumulated.

Data concerning the accumulation of individual elements by selected species of oysters and mussels under controlled experimental conditions have been reported but very few of these studies give comparative rates of accumulation for more than one element. SHUSTER and PRINGLE (1969) showed that zinc, copper, cadmium and chromium were accumulated by Crassostrea virginica. The rates of accumulation estimated for the first three weeks of their long-term experiment are 122, 46, 10.3 and 1.3 µg/g/week of zinc, copper, cadmium and chromium, the solution concentrations being 0.1, 0.05, 0.1 and 0.1 respectively. These accumulation rates are all much greater than those determined for C. gigas and C. margaritacea but their relative order is the same. The accumulation of copper and zinc by Mytilus viridis has been investigated (D'SILVA and KUREISHY 1978) but due to the extreme sensitivity of this species to copper, the concentrations of this element in the prepared solutions were an order of magnitude lower than those of zinc. The rate of copper uptake from a solution containing 0.01 µg/mL copper during a five-week experiment was estimated to be 0.22 µg/g/week. This is comparable with the rate achieved by P. perna and C. meridionalis in the presence of 0.1 µg/mL copper. The estimated rate of zinc uptake by M. viridis from a solution containing 0.1 µg/mL zinc is 1.16 µg/g/week which is considerably greater than that achieved by either P. perna or C. meridionalis from a solution of the same concentration.

The toxic effect of copper in possibly causing a lower accumulation rate cannot be discounted. The upper concentration

which can be tolerated is well defined. Both P. perna and C. meridionalis survived a three week exposure to 0.2 µg/mL copper with no apparent effects, yet concentrations of 0.25 and 0.23 µg/mL copper respectively are sufficient to kill 50 per cent of the individuals after only four days (WATLING 1981). Even lower concentrations can be detected by these mussels, for which 0.16 and 0.12 µg/mL copper cause a 50 per cent reduction in P. perna and C. meridionalis filtering rates respectively. The results of equivalent experiments on the effects of zinc, cadmium and lead show that these elements are much less toxic than copper (WATLING 1981).

From the point of view of indicating metal pollution in the marine environment, the tissue-metal concentrations which are determined for individuals from a polluted environment must be significantly greater than those determined for individuals from an unpolluted area. With this in mind "accumulation factors" for each species and element have been calculated from the summarized data (Table 3). The accumulation factor is defined as the ratio of the mean concentration of the study element in the tissues of treated individuals to the mean concentration in the tissues of "control" individuals which have not been exposed to that element.

TABLE 3

Accumulation factors

Species	Zn	Cd	Cu	Pb	Ni	Co	Cr
Crassostrea gigas	1.1	11.1	2.1	45	11.8	41	3.4
Crassostrea margaritacea	1.1	2.4	5.2	22	9.3	7	2.9
Perna perna	1.1	3.6	2.4	13	2.2	46	2.9
Choromytilus meridionalis	1.1	5.2	1.2	22	7.2	19	2.2

On the basis of these accumulation factors, it appears that zinc has not been accumulated under these experimental conditions by either oysters or mussels. Moderate accumulation factors have been determined for cadmium, copper, nickel and chromium and the very high accumulation factors estimated for lead and cobalt suggest that these elements are accumulated very rapidly. The results indicate that C. gigas, C. margaritacea, P. perna and C. meridionalis could be used to indicate the presence of all these elements in the marine environment, with the exception of zinc.

The question remains whether the comparative lack of accumulation of these elements is due to a chemical mechanism in the solution whereby the element is rendered unavailable to the mollusc,

(e.g. precipitation or complexation) or to some form of descrimination, whether passive or active, on the part of the mollusc.

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