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# Sandy Beach Molluscs as Possible Bioindicators of Metal Pollution 2. Laboratory Studies

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Donax serra and Bullia rhodostoma have been selected as possible indicators of metal pollution (CLOETE and WATLING 1981) and the results of a survey of the metal concentrations in these two molluscs have been summarised (part 1 of this investigation). The present study is concerned with the ability of these molluscs to accumulate metals, without being killed by levels encountered, in such a way that the rates of accumulation can be related to the average metal concentration in the surrounding water.

## MATERIALS AND METHODS

D. serra and B. rhodostoma were collected from an unpolluted beach, transferred immediately to the laboratory and allowed to acclimatise in tanks containing sand and seawater, the latter being renewed each day.

Comparative accumulation studies were carried out in polythene tanks containing 5 l of washed sand and 40 l seawater. The water was aerated continuously throughout each 3-week experiment. Aliquots of 5000 μg/ml stock solutions of the metal chlorides were added to achieve the appropriate concentrations in each tank. The experimental solutions were renewed daily, at which time the molluscs were dislodged from the sand and placed on its surface so that burrowing behaviour could be observed.

Possible metal loss from these solutions was tested under the conditions of the experiment, but in the absence of the molluscs. No losses were observed for cadmium, nickel, cobalt or chromium in the 24-h period. However, zinc concentrations decreased by 5% at 50 μg/l and 10% at 10 μg/l; copper concentrations decreased by about 30% at both 50 and 10 μg/l levels and lead losses of 30% at 60 μg/l and 50% at 10 μg/l were observed in the 24-h period.

At the end of each experiment the wet tissues of individuals were removed from their shells and frozen prior to chemical analysis. The oxidative dissolution and trace-metal analysis of these samples has been described (WATLING and WATLING 1979) and the results are expressed as μg/g in wet tissue.

## RESULTS AND DISCUSSION

Specimens (20 per treatment) were exposed to cadmium in the range 0-100 µg/l; the water temperature was between 14-16°C throughout the 3-week experiment. All individuals survived the experiment. D. serra individuals were dissected and the separate tissues analysed; the overall mean cadmium concentrations were then calculated from the tissue analyses (Table 1). The smaller B. rhodostoma individuals were analysed whole and the mean concentration and standard deviation for each suite of results was calculated (Table 1).

Table 1. Cadmium accumulation by D. serra and B. rhodostoma

| Treatment (µg/l) | | Donax serra Wet mass (g) | Donax serra Dry mass (g) | Donax serra µg/g in wet tissue Zn | Cd | Cu | Bullia rhodostoma Wet mass (g) | Bullia rhodostoma Dry mass (g) | Bullia rhodostoma µg/g in wet tissue Zn | Cd | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | x̄ | 18.2 | 3.1 | 11.1 | 0.10 | 0.60 | 2.21 | 0.43 | 39 | 5.5 | 2.6 |
| | s | | | | | | 0.72 | 0.15 | 9 | 0.9 | 1.2 |
| 25 | x̄ | 16.1 | 3.2 | 11.5 | 0.54 | 0.64 | 2.60 | 0.49 | 38 | 5.7 | 2.4 |
| | s | | | | | | 0.83 | 0.18 | 8 | 1.6 | 0.5 |
| 50 | x̄ | 15.1 | 2.4 | 11.0 | 1.05 | 0.64 | 2.70 | 0.51 | 39 | 6.5 | 2.3 |
| | s | | | | | | 0.97 | 0.17 | 11 | 1.2 | 0.5 |
| 100 | x̄ | 16.4 | 2.6 | 11.5 | 2.19 | 0.67 | 2.15 | 0.41 | 40 | 11.4 | 2.8 |
| | | | | | | | 0.79 | 0.14 | 10 | 2.6 | 0.7 |

Tissue cadmium concentrations increased with increasing concentration in the test solution for both species, but particularly in D. serra where a 20-fold increase is observed. This was probably a reflection of the initial low cadmium concentration of this species as compared with that of B. rhodostoma. If the rate of cadmium accumulation (µg/g/day) is calculated for the 100 µg/l treatment, the mean rate of accumulation in B. rhodostoma (0.3 µg/g/day) was somewhat greater than that of D. serra (0.1 µg/g/day) for this 3-week experiment. For comparison, zinc and copper concentrations were also determined in each sample but these remain relatively constant irrespective of the treatment.

Cadmium accumulation by the various tissues of D. serra is shown in Table 2. Concentrations in each tissue increased relative to the solution cadmium concentration, the greatest accumulation occurring in the gill tissue. In terms of accumulation factors (the ratio of the mean concentration of the study element in the tissues of the treated individuals to the mean concentration in tissues of the control individuals), the data may be summarised as: gill>>>digestive gland>muscle>mantle>gonad>foot>syphon.

Table 2. Cadmium accumulation in D. serra tissues (μg/g wet tissue)

| Tissue | Control | 25 μg/l | 50 μg/l | 100 μg/l |
|---|---|---|---|---|
| Mantle | 0.08 | 0.30 | 0.70 | 1.18 |
| Gill | 0.27 | 3.38 | 8.08 | 19.67 |
| Digestive gland | 0.08 | 0.42 | 1.02 | 1.82 |
| Syphon | 0.15 | 0.30 | 0.46 | 0.73 |
| Muscle | 0.14 | 0.65 | 1.31 | 2.97 |
| Gonad | 0.11 | 0.49 | 0.75 | 1.11 |
| Foot | 0.07 | 0.18 | 0.24 | 0.47 |

Specimens were exposed to 20 μg/l of one of the elements zinc, cadmium, copper, lead, nickel, cobalt and chromium for three weeks. There were 20 individuals in each tank and the water temperature varied between 21-23°C during the experimental period. All individuals survived the experiment. The whole tissues were analysed and the mean concentration and standard deviation for each suite of results were calculated (Table 3). Accumulation factors and rates of accumulation are also listed.

Table 3. Comparative uptake of seven elements by D. serra and B. rhodostoma (μg/g wet tissue)

| Metal | Control | Treatment 20 μg/l | Accumulation factor | Rate of accumulation (μg/g/day) |
|---|---|---|---|---|
| Donax serra | | | | |
| Zn | 11.1 ± 1.3 | 13.8 ± 3.8 | 1.2 | 0.13 |
| Cd | 0.17 ± 0.04 | 0.32 ± 0.04 | 1.9 | 0.01 |
| Cu | 0.83 ± 0.20 | 1.75 ± 0.61 | 2.1 | 0.04 |
| Pb | 0.04 ± 0.03 | 0.65 ± 0.17 | 16.2 | 0.03 |
| Ni | 0.31 ± 0.11 | 1.18 ± 0.40 | 3.8 | 0.04 |
| Co | <0.02 | 0.66 ± 0.18 | ∿33 | 0.03 |
| Cr | 1.0 ± 0.6 | 2.6 ± 1.4 | 2.6 | 0.08 |
| Bullia rhodostoma | | | | |
| Zn | 33 ± 11 | 33 ± 9 | 1.0 | 0 |
| Cd | 4.3 ± 1.7 | 7.7 ± 4.4 | 1.8 | 0.16 |
| Cu | 1.2 ± 0.4 | 1.9 ± 0.8 | 1.6 | 0.03 |
| Pb | 0.09 ± 0.06 | 1.2 ± 0.4 | 13.3 | 0.05 |
| Ni | 0.18 ± 0.08 | 0.57 ± 0.17 | 3.2 | 0.02 |
| Co | <0.02 | 0.71 ± 0.24 | ∿35 | 0.03 |
| Cr | 0.6 ± 0.2 | 2.7 ± 1.2 | 4.5 | 0.10 |

Clearly the ability to distinguish between contaminated and uncontaminated samples is essential if molluscs can be used as bioindicators, and the accumulation factor can be used to indicate whether measurable metal accumulation has occurred relative to the normal tissue-metal concentration. On the basis of these factors (Table 3) it would seem unwise to use D. serra and B. rhodostoma as indicators of the presence of zinc in the environment. However, the accumulation factors determined for the remaining elements are great enough to give a definite indication that metal accumulation is taking place (from the point of view of a marine pollution survey).

Rates of accumulation of each element by each species under these particular experimental conditions have also been calculated; these values can be used to compare the accumulation rates of a particular metal by different species or the accumulation rates of a number of metals by the same species. It is interesting to note that the rates of accumulation of lead and cobalt, the elements with the highest accumulation factors, are very similar to those of most other elements. The high accumulation factors for these two elements are a relection of the extremely low initial concentration in the tissues. Too few measurements are available to assign a rank order based on accumulation rates.

The accumulation of zinc, cadmium and copper in the range 0-500 μg/l by D. serra was investigated in a one-week experiment. Some mortalities occurred after two weeks and the experiment was terminated. The results are summarised in Table 4. Lead, iron and manganese contents were also determined but the concentrations of these elements were unaffected by the treatments.

Table 4. Uptake of zinc, cadmium and copper by D. serra (μg/g wet tissue)

| Treatment | | Zn | Cd | Cu |
|---|---|---|---|---|
| Control | $\bar{x}$ | 16.3 | 0.08 | 1.1 |
| | s | 4.8 | 0.03 | 0.3 |
| 100 μg/l | $\bar{x}$ | 17.3 | 1.42 | 5.7 |
| | s | 4.8 | 0.36 | 1.1 |
| 250 μg/l | $\bar{x}$ | 19.5 | 2.53 | 7.8 |
| | s | 4.6 | 1.00 | 1.3 |
| 500 μg/l | $\bar{x}$ | 20.5 | 3.19 | 17.0 |
| | s | 3.8 | 1.16 | 3.8 |

An increased tissue concentration is observed with increasing solution concentration for each element. The greatest accumulation factor occurs for cadmium but copper shows the greatest rate of accumulation (2.3 μg/g/day in the 500 μg/l treatment).

It is concluded that D. serra and B. rhodostoma would accumlate metals, were they present as pollutants in the sandy beach environment. With the exception of the final experiment, the metal levels tested were not apparently toxic to these species during the three-week experiments. But they were, nevertheless, greater than the levels which would be expected to occur in a sandy beach environment.

The problems of losses of metals from solution has been noted, in so far as the concentrations to which the experimental animals were subjected is not known but may be lower than intended. However, some preliminary studies indicate the greater quantity of "missing" metal becomes adsorbed on to the surface sand grains. The question remains as to whether the experimental animals are then subjected to greater than expected metal concentrations at the sand/water interface or whether the metal adsorbed on to the sand grains is then less "biologically available". No correlation between measured losses from solution and accumulation rates was discerned.

Only metal chlorides were used in the preparation of the experimental solutions. Almost certainly the form of a metal in the environment will affect the rate at which it is accumulated. As nothing specific is known about the types of metallic pollutants likely to be washed on to East Cape sandy beaches, or on their relative rates of accumulation or toxic effects, it is impractical and premature to suggest that D. serra and B. rhodostoma can be used as monitoring organisms. But in view of the results so far obtained, and bearing in mind that D. serra and B. rhodostoma are being used in the national marine pollution monitoring programme, it is concluded that further work on the accumulation and effects of metals would be justified.

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