Marine Biology 64, 311–316 (1981)

MARINE BIOLOGY

Toxicity of Zinc, Cadmium and Copper to the Shrimp *Callianassa australiensis*. III. Accumulation of Metals

M. Ahsanullah*, D. S. Negilski and M. C. Mobley**

Marine Science Laboratories, Ministry for Conservation; Queenscliff, Victoria 3225, Australia

Abstract

Callianassa australiensis (Dana) that survived 14 d acute lethality studies were analysed to determine the concentrations of zinc, cadmium and copper in the whole shrimp and in various parts of the body. Using regression analysis, the influence of each metal upon the uptake of the others was studied. Zinc and cadmium appeared to enhance the uptake of each other. In a mixture of zinc and copper, the uptake of zinc was enhanced and that of copper was inhibited. In a mixture of cadmium and copper, the uptake of copper was inhibited by the presence of cadmium, but cadmium uptake was unaffected in the presence of copper. In a mixture of all three metals, similar effects were observed except that zinc and copper, occurring together, appeared to have no effect upon cadmium uptake. Additional 14 d experiments with cadmium suggested that accumulation of this metal was a function of metal concentration in the water and of duration of exposure. The whole shrimp cadmium concentration also appeared to be a function of the size of the shrimp. The variation in concentration factors is described and the need for further research on the effects of combinations of metals on various organisms is emphasized.

Introduction

Through biological amplification, some aquatic organisms may build-up concentrations of metals present in low concentrations in the environment to levels which are harmful to the organisms and exceed public health standards (Thrower and Eustace, 1973; Eustace, 1974; Phillips, 1976a,b). The objective of the present study was to investigate accumulation by the shrimp *Callianassa australiensis* of zinc, cadmium and copper, applied to the water singly and in combination. Shrimp which survived 14 d acute lethality studies were analysed to determine residue levels of the metals in the various parts of the body (Ahsanullah *et al.*, 1981, Negilski *et al.*, 1981).

The results of an additional experiment were used to determine the relationship between cadmium accumulation and shrimp size, duration of exposure to cadmium and concentration of cadmium to which shrimp were exposed.

Materials and Methods

Collection and Acclimation of *Callianassa australiensis* (Dana)

The collection and acclimation of shrimp have been described previously (Ahsanullah *et al.*, 1981).

Metal Accumulation

Shrimp that survived for 14 d in the acute lethality studies (Ahsanullah *et al.*, 1981; Negilski *et al.*, 1981) were rinsed twice in deionized water. They were dissected into three parts: chela, cephalothorax and abdomen by stainless steel scalpel, and care was taken to prevent contamination of the parts. The exoskeletons of the shrimp were not removed. The different parts of the shrimp were placed in polythene bags, freeze-dried and analysed for metal accumulation. The acute lethality studies were carried out during winter months and, as far as possible, only male shrimp were used. Females were reproductive at this time and showed great

*Present address: Australian Atomic Energy Commission, Research Establishment, Private Mail Bag, Sutherland, New South Wales 2232, Australia
**Present address: Lilly Research Laboratories, P.O. Box 708, Greenfield, Indiana 46140, USA

0025-3162/81/0064/0311/$01.20

variation in the state of the gonad and the presence of eggs.

Cadmium Accumulation

In additional experiments, groups of a dozen shrimp were placed in 10 litre Perspex tanks. The shrimp were graded into small ($\leqslant$ 0.7 g wet wt), medium (0.7 to 1.2 g wet wt) and large ($\geqslant$ 1.2 g wet wt), and each tank contained shrimp of the same size range. There were two tanks for each concentration-size combination, and after 2, 4, 7, 10 and 14 d, three shrimp per tank were removed for cadmium analysis. The cadmium concentrations used were 0.006, 0.051 and 0.26 mg l^{-1}, which approximated 0.01, 0.1 and 0.5 of the 14 d LC_{50} value for *Callianassa australiensis* (Ahsanullah *et al.*, 1981). The temperature of the water in each tank was 16 °C ± 2 C°, pH was 8.1 ± 0.05 and salinity 34.3 ± 0.7‰, and the dissolved oxygen was greater than 78% saturation.

Chemical Analysis

The freeze-dried parts of the shrimp were weighed into 50 ml Pyrex test tubes, which were acid-washed in 10% nitric acid for 24 h and then thoroughly rinsed in deionized water prior to use. The test tubes were capped and the parts of the shrimp digested in 5 ml concentrated nitric acid (AR-grade) at 100 °C (6 to 10 h) until clear solutions were obtained. These solutions were appropriately diluted with distilled water and analysed for zinc, cadmium and copper on a Varian A5 atomic absorption spectrophotometer.

Data Analysis

Initially, tables of data were compiled and inspected for trends in metal accumulation (Tables 1-3). Then regression analysis was used to identify the way in which each metal influenced the uptake of the other metals. The regression models were fitted in three stages for each metal. Taking zinc, for example (Table 1):

(1) Linear and quadratic trends were fitted to the data from the application of zinc alone.

(2) The effect of adding cadmium (or copper) and its interaction with zinc were examined by constructing a model for the zinc and zinc plus cadmium (or copper) data.

(3) The interaction of all three metals was examined by fitting a model to all the data in Table 1.

Similar approaches were used for the other metals (Tables 2 and 3). Each analysis of metal accumulation required the lumping together of three or four shrimp. When a sufficient number of individuals was not available from one tank, shrimp from different tanks containing almost identical metal concentrations were lumped together, in which case the weighted averages of the water concentrations were entered in Tables 1-3.

Results

Zinc Accumulation

Zinc Alone. The zinc content was higher for those *Callianassa australiensis* kept in dosed sea water than in the control, and the concentration factors decreased with increasing zinc concentration (Table 1).

Zinc Accumulation in Presence of Cadmium. Where zinc concentrations were comparable, zinc accumulation was higher in those shrimp exposed to zinc-cadmium mixtures than in those exposed to zinc alone. Concentration factors were also higher.

Zinc Accumulation in Presence of Copper. For comparable zinc concentrations, zinc accumulation was higher in those shrimp exposed to zinc-copper mixtures than in those exposed to zinc alone. This increase was most apparent at low copper concentrations. Concentration factors were also higher. As in the case with zinc-cadmium mixtures, most marked increases occurred in the cephalothorax and abdomen.

Zinc Accumulation in Presence of Cadmium and Copper. Zinc accumulation was higher in those shrimp exposed to all three metals than in those exposed to zinc alone. Increases were most marked in the cephalothorax and abdomen. Concentration factors were also higher.

Regression analysis of the data showed:

(1) With increasing zinc concentration in the water, the zinc concentration in whole *Callianassa australiensis* increased in quadratic fashion (for the zinc alone data).

(2) For the zinc alone and zinc-copper mixture data, the addition of each of the following terms to the regression model significantly increased the amount of variation in zinc whole-body concentration accounted for: zinc, copper (linear and quadratic terms) and zinc-copper interaction ($P<0.05$). For the zinc alone and zinc-cadmium mixture data, the zinc, cadmium and zinc-cadmium terms were significant.

(3) The addition of both cadmium and copper to the water gave a significant negative three-way interaction term. The following model was selected as the most plausible, given (1) and (2), with 78% of the variation of the dependent variable explained:

$$\begin{aligned} ZN_{body} &= 44 + 58\ Zn + 41\ 834\ Cu + 10\ 452\ Cu^2 \\ &- 34\ 621\ Zn\ Cu - 1\ 751\ Cd + 2\ 008\ Zn\ Cd \\ &- 8\ 209\ Zn\ Cd\ Cu, \end{aligned}$$

where Zn_{body} = concentration of zinc in the whole shrimp, Zn = concentration of zinc in sea water, Cd = concentration of cadmium in sea water, and Cu = concentration of copper in sea water.

Table 1. *Callianassa australiensis.* Accumulation of zinc in presence of zinc, cadmium and copper, over 14 d. CF: conncention factor

Treatment ($mg\,l^{-1}$)	Accumulation (μg Zn g^{-1} dry wt) Chela	Cephalothorax	Abdomen	Whole shrimp	CF (whole shrimp)
Zn					
0 (Control)	23.1	42.2	40.2	35.4	
0.44	84.7	63.5	72.4	74.7	170
0.88	72.7	98.5	52.4	74.5	85
0.98	34.7	67.7	55.0	50.4	51
1.22	36.2	87.0	72.8	61.3	50
Zn+Cd					
0.96 + 0.34	82.2	104.4	180.5	119.0	125
1.07 + 0.33	119.0	644.5	422.0	408.6	382
1.15 + 0.39	105.0	580.7	494.6	357.2	308
1.22 + 0.51	89.6	694.7	422.5	393.4	322
Zn+Cu					
1.00 + 0.025	97.5	582.0	497.5	369.1	369
1.07 + 0.042	113.3	567.0	578.7	385.4	360
1.10 + 0.051	103.5	538.0	615.5	362.9	330
1.14 + 0.063	79.5	310.5	235.5	204.5	179
1.23 + 0.125	87.7	320.3	255.7	187.9	153
Zn+Cd+Cu					
0.95 + 0.35 + 0.034	102.0	477.0	418.5	334.2	344
1.04 + 0.35 + 0.047	82.0	403.3	371.8	291.9	280
1.09 + 0.33 + 0.041	147.0	525.0	530.0	377.7	360
1.11 + 0.47 + 0.099	104.0	512.7	448.7	368.1	332

Table 2. *Callianassa australiensis.* Accumulation of cadmium in presence of cadmium, zinc and copper, over 14 d. CF: concentration factor; bd: below detection limits (0.1 $\mu g\,g^{-1}$); nd: no data

Treatment ($mg\,l^{-1}$)	Accumulation (μg Cd g^{-1} dry wt) Chela	Cephalothorax	Abdomen	Whole shrimp	CF (whole shrimp)
Cd					
0 (Control)	bd	0.1	1.3	0.43	
0.006[a]	nd	nd	nd	1.4	233
0.051[a]	nd	nd	nd	5.0	98
0.260[a]	nd	nd	nd	34.0	130
0.325	1.35	29.0	51.0	23.5	72
0.570	3.33	39.0	60.0	28.7	50
Cd+Zn					
Control		0.84	2.5	0.98	
0.30 + 0.93	3.5	44.0	33.0	26.5	88
0.33 + 1.07	1.7	55.5	58.0	39.6	120
0.36 + 1.04	4.0	63.5	158.3	63.9	183
0.39 + 1.15	3.4	56.0	69.0	40.7	104
0.53 + 1.18	5.0	100.0	148.0	97.9	185
Cd+Cu					
0.29 + 0.032	2.1	16.9	51.6	23.1	80
0.32 + 0.044	2.0	36.6	76.8	35.5	111
0.39 + 0.059	1.9	53.3	88.7	45.3	116
Cd+Zn+Cu					
0.35 + 0.95 + 0.034	4.0	19.0	53.0	26.7	89
0.35 + 1.04 + 0.047	5.0	48.5	84.0	46.0	128
0.43 + 1.05 + 0.069	3.0	29.7	69.3	31.5	73
0.47 + 1.11 + 0.099	5.0	39.5	56.6	29.9	59

[a]Results of additional Cd experiments

Table 3. *Callianassa australiensis.* Accumulation of copper in presence of copper, zince and cadmium, over 14 d. CF: concentration factor

Treatment ($mg\ l^{-1}$)	Accumulation (μg Cu g^{-1} dry wt)				
	Chela	Cephalothorax	Abdomen	Whole shrimp	CF (whole shrimp)
Cu					
0 (Control)	42.0	230.0	728.0	301.8	
0.058	53.0	510.0	1 247.0	510.3	8 798
0.098	53.0	556.0	1 156.0	497.7	5 079
0.195	68.0	865.0	1 436.0	729.6	3 742
0.533	88.0	948.0	1 097.0	663.9	1 246
Cu+ Zn					
0.025 + 1.00	66.0	204.0	282.0	174.2	6 700
0.042 + 1.07	40.0	306.0	484.0	251.9	5 360
0.063 + 1.14	63.0	210.0	272.0	178.8	2 838
0.110 + 1.18	55.0	361.0	464.0	255.1	2 319
0.140 + 1.24	66.0	329.0	409.0	265.5	1 967
0.250 + 1.25	57.0	349.0	644.0	401.8	1 576
Cu+Cd					
0.032 + 0.29	50.0	214.0	426.0	202.2	6 972
0.044 + 0.32	47.0	255.0	571.0	278.1	6 953
0.059 + 0.39	43.0	388.0	521.0	286.7	5 030
Cu+Zn+Cd					
0.034 + 0.95 + 0.35	55.0	275.0	385.0	244.5	11 643
0.047 + 1.04 + 0.35	82.0	452.0	768.0	450.7	9 589
0.069 + 1.05 + 0.43	49.0	193.0	335.0	180.6	2 617
0.099 + 1.11 + 0.47	52.0	235.0	361.0	193.4	1 465

Cadmium Accumulation

Cadmium Alone. The experimental results showed that as the concentration of cadmium in the water increased, the cadmium concentration of the shrimp also increased and the concentration factors decreased (Table 2). The whole shrimp concentrations from the additional experiments increased with time and gave no indication of tending towards an asymptote during the 14 d exposure. Small shrimp accumulated higher concentrations of cadmium than medium or large-sized shrimp at all the test concentrations. For example, small medium and large shrimp reached concentrations of 12, 7 and 3 $\mu g\ g^{-1}$ Cd after 2 d exposure in 0.26 mg Cd l^{-1}. Similar results were obtained for the other exposure concentrations for various periods of time. After 14 d exposure to 0.26 mg l^{-1}, the cadmium levels in small shrimp were two and three times greater than those in medium and large shrimp, respectively.

Cadmium Accumulation in Presence of Zinc. The addition of zinc to the water increased the accumulation of cadmium by the shrimp, most markedly in the cephalothorax and abdomen. Concentration factors were also raised.

Cadmium Accumulation in Presence of Copper. Over the range of cadmium concentrations studied, the metal uptake by the shrimp appeared unaffected by the presence of copper in the water. In contrast to the concentration factors in cadmium alone, concentration factors for the mixture increased with increasing cadmium.

Cadmium Accumulation in Presence of Zinc and Copper. Cadmium accumulation in the presence of all three metals was similar to that in the presence of cadmium alone, as were the concentration factors.

As a result of the regression analyses several features of these data can be noted:

(1) Cadmium whole-shrimp concentrations increased with cadmium concentrations in the water in a linear fashion.

(2) The addition of zinc to the water increased the accumulation of cadmium, the interaction between zinc and cadmium being significant and positive. The addition of copper to the water (not containing zinc) did not produce either a significant copper term or a significant cadmium and copper interaction term.

(3) The addition of both zinc and copper to the water gave a significant negative zinc-copper interaction term. The selected model, with 82% of the variation explained was:

$$Cd_{body} = 4 + 71\ Cd - 36\ Zn + 149\ Cd\ Zn - 382\ Zn\ Cu.$$

Copper Accumulation

Copper Alone. The copper content was higher for those shrimp kept in dosed sea water than in the control, and

Table 4. *Callianassa australiensis.* Effect of presence of other metals in the water upon accumulation of a metal by shrimp. The first metal is the one accumulated. Upward- and downward-pointing arrows indicate increased and decreased uptake, respectively; u: unaffected; i: inconsistent. CF: Concentration factor

Metals	Chela	Cephalothorax	Abdomen	Whole shrimp	CF
Zn+Cd	↑	↑	↑	↑	↑
Zn+Cu	↑	↑	↑	↑	↑
Zn+Cd+Cu	↑	↑	↑	↑	↑
Cd+Zn	u	↑	↑	↑	↑
Cd+Cu	u	u	u	u	i
Cd+Zn+Cu	u	u	u	u	u
Cu+Zn	u	↓	↓	↓	↓
Cu+Cd	u	↓	↓	↓	↓
Cu+Zn+Cd	u	↓	↓	↓	↓

the concentration factors decreased markedly with increasing copper concentration (Table 3).

Copper Accumulation in Presence of Zinc. Where copper concentrations were comparable, copper accumulation was lower in those shrimp exposed to copper-zinc mixtures than in those exposed to copper alone. Concentration factors were also lower.

Copper Accumulation in Presence of Cadmium. For comparable concentrations, copper accumulation was lower in those shrimp exposed to copper-cadmium mixtures than in those exposed to copper alone, as were the concentration factors.

Copper Accumulation in Presence of Zinc and Cadmium. In the presence of all three metals, copper concentrations in whole shrimp and all body sections, except the chela, were lower than those in individuals exposed to copper only at comparable concentrations. The concentration factors were also depressed at the higher copper levels.

As a result of the regression analyses the following features can be noted:

(1) Copper concentrations in whole shrimp increased with copper concentrations in the water in a quadratic fashion.

(2) The addition of zinc to the water reduced the uptake of copper, as did the addition of cadmium.

(3) The addition of both zinc and cadmium produced a significant positive interaction term between zinc and cadmium. The model selected, with 78% of variation explained, was:

$$\begin{aligned} Cu_{body} &= 198 + 3\,659\ Cu - 5\,164\ Cu^2 + 322\ Cd\ Zn \\ &\quad - 1\,584\ Cu\ Zn - 6\,487\ Cu\ Cd. \end{aligned}$$

Discussion

In an ideal study of the accumulation in animals (shrimps, invertebrates) of metals from water containing mixtures of metals, the combinations of concentrations used would be selected so as to cover a range which may be expected to occur in the environment, and all animals would be analysed for all metals. In the present study on *Callianassa australiensis*, this was not the case, as the experiments were designed for acute lethality studies (Ahsanullah *et al.*, 1981; Negilski *et al.*, 1981). Nevertheless, because of the paucity of data on the effects of interactions of heavy metals, it was thought that these results should be reported.

The addition of any one of the three metals to sea water produced an increase in the metal concentration of the shrimp exposed to it (Table 1-3). The additional experiments on cadmium suggested that accumulation was a function of cadmium concentration in the water and duration of exposure. Similar results have been obtained for a number of crustaceans (Fowler and Benayoun, 1974; Renfro *et al.*, 1975; Nimmo *et al.*, 1977) and for phytoplankton, polychaetes, molluscs and fish (e.g. Betzer and Pilson, 1975; McLusky and Phillips, 1975; Saward *et al.*, 1975).

When pairs of metals are present in sea water, the ways in which each influences the uptake and effects of the other may differ according to the particular pair of metals (Table 4), and the particular species exposed to them. For example, in the present study, both cadmium and copper enhanced the accumulation of zinc by *Callianassa australiensis*, whereas both zinc and cadmium decreased the uptake of copper. Braek and Jensen (1976) investigated the combined effects of zinc and copper ions on the growth of four common species of marine phytoplankton and concluded that zinc and copper acted synergistically to all except one species, and with this species an antagonistic effect was observed. D'Agostino and Finney (1974) reported that cadmium and copper inhibited growth and development of the F_2-generation of the copepod *Trigriopus* sp. at 44 and 64 $\mu g\ l^{-1}$ Cd and Cu, respectively. These two metals acted synergistically when tested in combination at concentrations of 4.4 and 6.4 $\mu g\ l^{-1}$ cadmium and copper, respectively (concentrations ten times less than those used individually), and produced an impairment equivalent to that when tested individually.

With triads of metals, as with pairs, the exact influence of each metal on the uptake of the others differs according to the metal being considered. For zinc accumulation, the presence of cadmium and copper enhanced the uptake in the whole shrimp and all three parts of the body. For the whole shrimp, the regression equation can be used as a predictor of body concentration. The negative three-way interaction term occurs because the sum of the enhancements caused by cadmium and copper when applied separately is higher than the enhancement caused by the two metals in combination. For cadmium accumulation, the addition of zinc to water containing cadmium enhanced the uptake of cadmium in the shrimp. The addition of copper to the water containing zinc and cadmium produced a reduction in the zinc enhancement effect. This can be seen from the regression equation for whole-body concentration if appropriate values for each metal are inserted (e.g. 0 and 1 for zinc; 0.3 and 0.5 for cadmium, and 0 and 0.05 for copper). In the case of copper accumulation, the presence of zinc and cadmium produced inhibitory effects upon metal uptake in the whole shrimp, cephalothorax and abdomen and these effects were not additive, producing a third interaction term in the regression model, as in the case of cadmium accumulation.

For all experimental treatments, the chela had the lowest concentrations of metals, whereas the abdomen had higher concentrations than the cephalothorax in the cases of cadmium and copper, but lower in the case of zinc. The accumulation of a metal by the chela was generally not affected by the presence of other metals in the water, whereas the cephalothorax and abdomen followed the same pattern as the whole shrimp. It has been suggested that metals are selectively localized in the tissues of crustaceans (Bryan, 1976; Nimmo *et al.*, 1977), for zinc the highest concentration being reached in hepatopancreas and gills, for copper in the liver and haemocyanin in blood, and for cadmium possibly in liver and gills. Such selectivity could explain the patterns of metal accumulation in the present study, although specific tissues were not analysed.

Although many toxicology studies have investigated the effects of single metals, an industrial effluent may contain combinations of different metals. The present results show that the response of the surviving shrimp to a metal differs according to which other metals are present and, quite apart from this, the response is no doubt also dependent on environmental factors such as temperature, salinity and presence of dissolved oxygen. Hence to establish water quality criteria, it is important to understand the way in which such interactions influence the effects on aquatic organisms of metals individually and in combination.

Acknowledgements. Our sincere thanks are due to Ms P. Sedmach for her assistance in the laboratory; tissue analyses for heavy metals were carried out at Arthur Rylah Institute, Ministry for Conservation. Thanks are due to the staff, especially Mr J. Bacher for the help provided; Ms F. Tawfik for heavy metal analysis of experimental solutions; Drs L. C. Collett and N. Coleman for editorial comments on the manuscript and Ms C. Chrimes for typing the final manuscript.

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Date of final manuscript acceptance: June 26, 1981.
Communicated by G. F. Humphrey, Sydney