Bull. Environ. Contam. Toxicol. (1992) 48:185–193

Environmental Contamination and Toxicology

Toxic Responses of Bivalves to Metal Mixtures

Philip Mathew and N. R. Menon

School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin-682 016, India

Toxicity is a biological response which, when quantified in terms of concentration of the toxicant, can constitute the basis for a bioassay procedure. Bioassay (toxicity) tests are defined as estimation of the amount of biologically active substances by the level of their effect on test organisms (Chapman and Long 1983). Bryan (1976) is of the view that factors like form of the metal, environmental characteristics, physiological status of the animal, acclimation and acclimatization to the metal, etc., can considerably influence the toxicity of a metal to marine animal. Although there is a growing body of information on the toxicity of individual heavy metals to economically important species of bivalves, (Portmann 1970; Scott and Major 1972; Ahsanullah 1976; Eisler 1977) literature on the lethal toxicity of metal mixtures to bivalves under controlled conditions is rather limited. Mohan et al. (1986) investigating into the combined toxicity of mercury and cadmium to Perna viridis, observed that mercury and cadmium in mixture interacted more than additively in producing mortality in 96 hr. Mac Innes and Calabrese (1977) found out that interaction of metals could be temperature dependent. When Perna indica was exposed to a combination of Cu and Ag, the toxicity of these metals increased (Prabhudeva and Menon 1988). The concentration of metals in the mixture can also influence metal interaction (Prabhudeva and Menon 1990). In the present investigation the toxic effects of combinations of copper - mercury and copper - cadmium at lethal levels to two marine bivalve species, Perna indica and Donax incarnatus, have been delineated.

MATERIALS AND METHODS

Perna indica and Donax incarnatus (size range : 20 - 25 mm) were collected from the rocky beaches of Sakthikulangara and sandy beaches of Ambalapuzha, respectively, and transported to the laboratory in large polythene tubs in seawater collected from the sampling site. The animals were laboratory conditioned in aerated seawater (Salinity : ca 32‰) at 30±1°C for 36 hr prior to experimentation.

Send reprint requests to (N.R. Menon) at the above address.

The animals were not fed during the experiments. The seawater employed for the experimental purpose was collected from the Arabian Sea off Cochin, transported to the laboratory, allowed to settle for 2 hr, filtered using a biofilter and well aerated before use (Salinity : ca 32‰ , pH : 8.2-8.4).

Stock solutions of the toxicants were prepared in double glass distilled water ($CuSO_4.5H_2O$ for Cu; $HgCl_2$ for Hg and $CdCl_2.H_2O$ for Cd being the source of metals) and added to the test medium to get the desired concentration.

Lethal toxicity tests were conducted over 96 hr. Inability to close the valves upon mechanical stimulus and valve gaping beyond 5 mm, were the criteria used to indicate death. Dead individuals were removed from the experimental media at 12 hr intervals. The experimental vessels were fibre glass troughs of 5 L capacity containing 4 L filtered seawater. Ten animals were exposed to each concentration along with control in duplicate. Cumulative mortality were taken at 12 hr intervals. Twenty five (5 x 5) combinations were tested to complete one set of experiments on combined toxicity of two metals. The concentrations of the toxicants used in combination studies were derived from the respective individual 96 hr LC_{50} values delineated before hand. The 96 hr LC_{50} values and their 95% confidence limits were calculated using Probit analysis (Finney 1971). The toxicity unit method as suggested by Marking and Dawson (1975) was employed for the determination of additive index.

RESULTS AND DISCUSSION

Perna indica and Donax incarnatus were subjected to comprehensive tests to delineate lethal effects of heavy metals. The results are given in Table 1a to 4b. The toxicity of copper to Perna indica did not vary appreciably when the animals were exposed to a constant concentration of mercury along with varying copper concentrations (Table 1a). The additive index was simple additive. In the reciprocal experiment (copper constant and mercury varying), it was observed that significant mortality occurred only in the highest concentration (Table 1b).

Comparable results of mortality of Perna indica were obtained when they were exposed to copper (constant) and cadmium (varying) (Table 2a). Significant mortality was found to occur only in higher concentrations. However, cadmium was found to become more toxic in the presence of copper even though the confidence limits recorded showed high flexibility. The additive index was simple additive. In the reciprocal combination (Table 2b), it was found that the combination was more toxic with a relatively high copper concentration and low cadmium. Thus, with 300 ppb of cadmium, the 96 h LC_{50} occurred in a medium which contained 24.7 ppb of copper, while with 1100 ppb of cadmium, a copper concentration of 18.3 ppb produced 50% mortality of the test population; the additive index showing a gradation from simple additivity to less than additivity.

Table 1a. *Perna indica*. 96 hr LC_{50} (ppb), when exposed to constant concentration of mercury salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Mercury (ppb)	Copper 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
30	20.3	10.1 - 40.6	-0.32 (SA)
25	ND	ND	
20	23.2	17.7 - 30.4	-0.32 (SA)
15	20.2	10.2 - 40.0	-0.12 (SA)
10	20.2	18.0 - 22.9	-0.06 (SA)

SA : Simple additive

ND : Not determined

Table 1b. *Perna indica*. 96 hr LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.

Copper (ppb)	Mercury 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
20	11.4	10.7 - 12.1	-0.06 (SA)
15	*		
10	*		
5	*		
1	*		

SA : Simple additive

* : No mortality

Table 2a. *Perna indica*. 96 hr LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.

Copper (ppb)	Cadmium 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
20	767.2	460.2 - 1279.1	-0.19 (SA)
15	1677.7	704.2 - 3996.7	-0.28 (SA)
10	*		
5	*		
1	*		

SA : Simple additive

* : No mortality

Table 2b. *Perna indica*. 96 hr LC_{50} (ppb), when exposed to constant concentration of cadmium salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Cadmium (ppb)	Copper 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
1100	18.3	17.0 - 19.7	-0.22 (LA)
900	19.9	18.5 - 21.5	-0.23 (LA)
700	21.3	17.2 - 26.3	-0.22 (SA)
500	21.6	20.6 - 22.6	-0.17 (SA)
300	24.7	23.3 - 26.2	-0.24 (SA)

SA : Simple additive

LA : Less than additive

Donax incarnatus was exposed to a combination of copper and mercury, the former maintained at a constant concentration and the latter varying. With increasing copper concentration, the toxic effect of mercury was found to decrease (Table 3a). The toxicity of mercury was reduced when applied in combination with copper. Further, the toxicity of copper was considerably reduced in the presence of low levels of mercury (Table 3b). Similarly, copper became less toxic in the presence of low levels of cadmium. However, there was oscillation of additivity from simple to less than additive (Tables 4a and b).

Probably the most exciting and potentially useful development in pollution biology has been the method of predicting toxicity of mixtures of toxicants (Sprague 1970). The methodology developed helps to measure the simultaneous effects of more than one metal that could be expressed numerically. The majority of techniques for evaluating the toxicity of mixtures of chemicals follow mathematical models for joint toxicity that yields their harmonic mean of the LC_{50}'s of the components (Finney 1971). The simple additive reaction when *Perna indica* was exposed to combinations of copper, mercury and cadmium indicates that the comparatively low concentrations of the metals employed react together and that the presence of one did not in any way express or suppress the effect of the other. This brings us to the important question as to whether the combined toxicity of metal combinations is also mainly concentration dependent and not metal dependent. The present series of experiments were conducted using an essential and a non-essential metal in combination with the contention that the animals will detoxify the non-essential metal and would handle the essential metal later. However, the results do not support this assumption. Further, when copper and cadmium were applied in concert, the 96 hr LC_{50} values showed variations, not only from the individual metal stand point but also in dual combination. In the presence of copper, the LC_{50} of cadmium varied considerably and the variation was not copper - concentration dependent when the metals were administered in dual combination. This forces us to assume that it is not possible to ignore the amount administered versus the amount reaching the site of action, so as to cause mortality.

The toxicity unit concept (Marking and Dawson 1975) seems to be a mere redefinition of the simple similar action model and that it has been employed to characterize the toxicity of mixtures of pollutants without regard for dose-dependent mortality brought about by individual metals. This can be easily seen in some of the two metal combinations conducted in the present instance. The shifting of simple additivity to less than additivity or more than additivity was found to be controlled by the concentration of one of the metals rather than both. Less than additive reaction recorded in the case of *Donax incarnatus* probably speaks for independent-similar action of metals.

Examination of the data obtained on two-metal combinations, especially with reference to cadmium and copper, shows that shifting from less than additivity to more than additivity and

Table 3a. *Donax incarnatus*. 96 hr LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of mercury salt, along with the respective 95% confidence limits and additive indices.

Copper (ppb)	Mercury 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
20	90.5	ND	-1.33
15	51.1	ND	-0.46
10	*		
5	*		
1	*		

SA : Simple additive

ND : Not Determined

* : No mortality

Table 3b. *Donax incarnatus*. 96 hr LC_{50} (ppb), when exposed to constant concentration of mercury salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Mercury (ppb)	Copper 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
30	20.2	15.7 - 26.0	-0.31 (LA)
20	33.6	22.2 - 50.8	-0.67 (LA)
15	47.4	34.2 - 65.6	-1.13 (LA)
10	*		
5	*		

LA : Less than additive

* : No mortality

Table 4a. *Donax incarnatus*. 96 hr LC_{50} (ppb), when exposed to constant concentration of copper salt with varying concentrations of cadmium salt, along with the respective 95% confidence limits and additive indices.

Copper (ppb)	Cadmium 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
20	246.0	242.9 - 250.4	-0.55 (LA)
15	236.8	203.1 - 299.2	-0.33 (LA)
10	160.4	153.8 - 167.4	0.11 (MA)
5	116.5	93.3 - 145.1	0.78 (MA)
1	109.4	77.1 - 153.0	-0.05 (SA)

LA : Less than additive

MA : More than additive

SA : Simple additive

Table 4b. *Donax incarnatus*. 96 hr LC_{50} (ppb), when exposed to constant concentration of cadmium salt with varying concentrations of copper salt, along with the respective 95% confidence limits and additive indices.

Cadmium (ppb)	Copper 96 hr LC_{50}(ppb)	95% Confidence limits	Additive Index
300	ND	ND	
250	26.6	15.7 - 45.3	-0.84 (LA)
200	38.1	21.1 - 68.8	-1.13 (LA)
100	*		
50	*		

LA : Less than additive   ND : Not determined

* : No mortality

then to less than additivity occurs with respect to *Donax incarnatus*. Since the seawater used for the experiments came from the same stock, this could not be the effect of complexation alone (Mac Innes 1981). It is safe to assume that copper, an essential and highly toxic metal, when present in higher concentrations was the main offender and cadmium had no effect, death would have been controlled by the toxic reactions of copper alone, indicating independent-dissimilar action. The present study has attempted to demonstrate the relevance of adopting multivariable procedures to estimate toxicity (Alderice 1972).

Acknowledgments. The first author (PM) is thankful to the University Grants Commission, New Delhi for the award of Research Fellowship during the tenure of which the present work was carried out.

REFERENCES

Ahsanullah M (1976) Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port, Victoria. Aust J Mar Freshwat Res 27 : 187-196

Alderice DF (1972) Factor combinations: Responses of marine poikilotherms to environmental factors acting in concert. In: Kinne O (ed) Marine Ecology, vol. I Environmental Factors, Part III, Wiley - Interscience, Chichester, pp 1659-1722

Bryan GW (1976) Heavy metal contamination in the sea. In: Johnston R (ed) Marine Pollution. Academic Press, New York and London, pp 185-302

Chapman PM, Long ER (1983) The use of bioassays as part of a comprehensive approach to marine pollution assessment (view point). Mar Pollut Bull 14(3) : 81-84

Eisler R (1977) Toxicity evaluation of a complex metal mixture to the softshell clam *Mya arenaria*. Mar Biol 43 : 265-276

Finney DJ (1971) *Probit Analysis*. Cambridge University Press, London, p 333

Mac Innes JR (1981) Response of embryos of the American oyster *Crassostrea virginica* to heavy metal mixtures. Mar Environ Res 4(3) : 217-227

Mac Innes JR, Calabrese A (1977) Responses of the embryos of the American oyster, *Crassostrea virginica* to heavy metals at different temperatures. In: McLusky DS, Berry AJ (eds) Physiology and Behaviour of Marine organisms, Pergamon Press, Oxford, pp 195-202

Marking LL, Dawson VK (1975) Method for assessment of toxicity or efficiency of mixtures of chemicals. Investigations in Fish Control, US Department of Interior, Fish and Wild life service, Washington DC, 67 : 1-7

Mohan CV, Gupta TRC, Shetty HPC, Mehon NR (1986) Combined toxicity of mercury and cadmium to the tropical green mussel *Perna viridis*. Dis Aquat Org 2 : 65-72

Portman JE (1970) A discussion of results of acute toxicity tests with marine organisms using a standard method. FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing. FIR : MP/70/E-31, pp 1-13

Prabhudeva KN, Menon NR (1988) Toxicity of copper salts on the brown mussel Perna indica : Individually and in combination. In: Joseph MM (ed) The First Indian Fisheries Forum, Proceedings. Asian Fisheries Society, Indian Branch, Mangalore, pp 277-279

Prabhudeva KN, Menon NR (1990) Metal interaction during accumulation in Perna indica I. Effects of silver on copper accumulation. In: Hirano R, Hanyu I (eds) The Second Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines, pp 951-954

Scott D, Major C (1972) The effect of copper (II) on survival, respiration and heart rate in the common blue mussel Mytilus edulis. Biol Bull 143 : 679-688

Sprague JB (1970) Measurement of pollutant toxicity to fish II. Utilizing and applying bioassay results. Wat Res 4: 3-32

Received May 8, 1991; accepted August 30, 1991.