Filtration in Some Tropical Intertidal Bivalves Exposed to Mercury and Cadmium Mixtures

C. V. MOHAN, N. R. MENON* and T.R.C. GUPTA

College of Fisheries, University of Agricultural Sciences, Mangalore - 575 002

Three species of intertidal filter feeding bivalves; *Modiolus carvalhoi, Modiolus* sp. and *Donax spiculum,* exposed to mercury and cadmium filtered significantly less volume of water under individual metal and metal mixture stress. Mercury and cadmium in mixtures interacted additively and more than additively (Synergism) in depressing the filtration rate of the bivalves.

Information on the lethal and sub-lethal effects of metals on aquatic organisms is voluminous (Eisler, 1979). However, the recent trend in heavy metal pollution studies is towards mixture toxicity analysis for determining safe and better water quality criteria (Sprague, 1970, Wong *et al.,* 1978; Weis, 1978; Spehar *et al.* 1978, Christensen *et al.* 1979).

Though the deleterious effects of mercury and cadmium individually on aquatic organisms at lethal and sublethal levels are amply documented (Waltichuk, 1974; Johnston, 1976; Nelson *et al.* 1977; Calabrese *et al.* 1977; Reish & Carr, 1978; Eknath & Menon, 1979; Mohan *et al.* 1983, 1984), studies on the effects of their mixtures to aquatic organisms and their interactions at lethal and sublethal levels are meagre.

Filtration rate test is used as a reliable tool to assess the sub-lethal effects of metals on filter feeding bivalves (Abel, 1976). Since, bivalves depend on the water they filter for both food and oxygen, the results of filtration rate test would have ecological significance. Effects of heavy metals singly on clearance rate of marine mussels have been worked out by Abel (1976), Eknath & Menon (1979) and Mathew & Menon (1984). As the information on effects of heavy metal mixtures on the filtration rate of bivalve molluscs is scanty, the present investigation, was carried out to determine the effects of mercury and cadmium individually and in combinations on the filtration efficiency of three intertidal filter feeding bivalves, *Modiolus carvalhoi, Modiolus* sp. and *Donax spiculum.*

*Present address: School of Marine Sciences, Cochin University of Science and Technology, Cochin-682016

Materials and Methods

Test animals

Sedentary intertidal bivalves, *Modiolus carvalhoi* (12–14 mm, shell length) and *M.* sp. (12–14 mm, shell length) were collected from Someswar rocky shore (12° 47′ N; 74° 51′E) and *Donax spiculum* (15–20 mm, shell length) a burrowing bivalve from the sandy shore of Panambur (12° 57′ N; 74°48′E). The animals were washed in clean seawater, transported to the laboratory and maintained in aerated seawater holding tanks.

Seawater

The seawater collected from the habitat of test animals was stabilized and filtered before use. The salinity and pH of the water used for the experiments ranged between 33.5 and 34.8‰ and 8.15 and 8.30 respectively. All experiments were conducted at room temperature of 30 ± 1°C.

Test solution

The test solutions were prepared by appropriate dilution of stock solutions of mercuric chloride and cadmium chloride. The required mixture concentrations were arrived at by the addition by separately prepared solutions of mercuric chloride and cadmium chloride to seawater.

Filtration rate measurements

Dye clearance technique was employed to assess the rate of filtration (Cole & Hepper, 1954; Badman, 1975). Ten healthy animals of equal shell length were placed in 500 ml test medium under each concentration. The reduction in the dye concentration at fixed intervals (2 h) was estimated colorimetrically with the help of Klett-Sumerson photo electric colorimeter using a blue filter (420–480 nm). The filtration rate was estimated using Quayle's equation (1948):

$$m = \frac{M}{n.t} \log_e \frac{Co}{Ct}$$

where m = filtration rate
M = volume of suspension
n = number of animals
t = duration of experiment
Co=initial concentration of the suspension
Ct=final concentration of the suspension

The bi-hourly readings obtained for each test concentration over a period of 12 h were averaged and standard deviation obtained. The results are expressed as volume of water filtered per hour per animal.

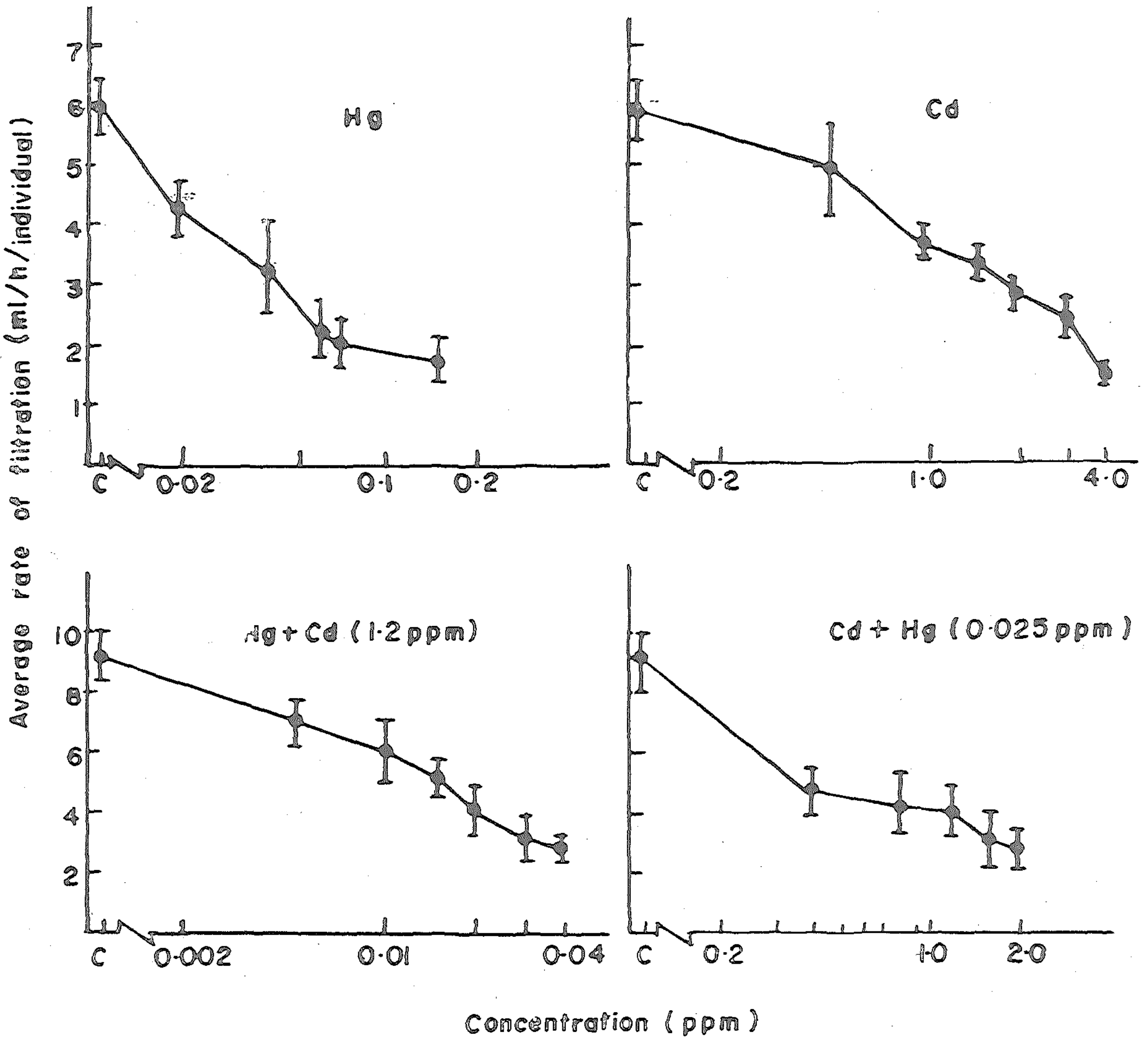

Fig. 1. Mean filtration rate (ml/h/individual) of *Modiolus carvalhoi* exposed to sub-lethal concentrations of Hg, Cd, Hg + Cd and Cd + Hg. Vertical bars represent standard deviation.

Table 1. *Percentage reduction in filtration rate of Modiolus carvalhoi*

Hg		Cd		Hg+Cd (1.2 p.p.m.)		Cd+Hg (0.025 p.p.m.)	
Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction
0.02	28.07	0.50	17.82	0.005	23.57	0.4	48.87
0.04	46.05	1.00	38.82	0.01	33.91	0.8	53.39
0.06	62.86	1.50	43.87	0.015	42.95	1.2	56.51
0.08	66.22	2.00	53.28	0.020	55.11	1.6	66.85
0.14	70.76	3.00	59.16	0.030	63.94	2.0	70.08
		4.00	77.31	0.040	67.81		

The metal mixture experiments were done in such a way as to embrace the various concentrations of one metal (mercury) with a constant concentration of the other metal (cadmium) and *vice-versa*. For all purposes they will be designated as Hg + Cd (Hg (varying) + Cd (constant) and Cd + Hg Cd (varying) + Hg (constant) from hereafter.

Results

Fig. 1 and Table 1, respectively summarise the mean filtration rate and percentage reduction in filtration under Hg, Cd, Hg + Cd and Cd + Hg stress. Sub-lethal levels of Hg and Cd individually reduced the volume of water filtered significantly. The EC–50 values (concentration where filtration rate is reduced by 50%) for Hg and Cd individually were 0.05 p.p.m. and 2.40 p.p.m. respectively. In mixtures, Hg and Cd interacted more than additively in depressing the filtration rate (Table 4). Under Hg+Cd combination, the filtration rate was reduced by 55% in a concentration of 0.02 p.p.m. Hg with 1.2 p.p.m. Cd. The combination EC–50 value expressed in terms of empherical toxic unit (Sprague, 1970) is 0.9. In Cd+Hg combination the filtration rate was reduced by 53% in a mixture of 0.83 toxic units (0.8 p.p.m. Cd + 0.025 p.p.m. Hg). In both the combinations the mixture EC–50 expressed in terms of toxic units is less than 1 and according to the emperical toxic unit method of mixture toxicity analysis of Sprague (1970), it clearly indicates the involvement of a more than additive interaction between Hg and Cd in depressing the filtration rate.

Modiolus sp.

Hg and Cd singly and in mixtures depressed the filtering activity considerably (Fig. 2, Table 2). Under individual metal stress Hg and Cd recorded EC 50 values of 0.005 p.p.m. and 3.4 p.p.m. respectively. These values were well below their respective 96h–LC50 values. In both the combinations, Hg and Cd interacted more than additively at sub-lethal levels (Table 4). Under Hg+Cd combination, 0.013 p.p.m. Hg with 1.7 p.p.m. Cd brought about more than 50% reduction in the volume of water filtered, the EC 50 value being 0.74 toxic units. The two metals interacted more than additively in Cd+Hg combination in reducing the filtration rate. A mixture concentration of 0.60 toxic units reduced the filtration rate by 51%.

Donax spiculum

The mean filtration rate and the percentage reduction in the volume of water filtered are presented in Fig. 3 and Table 3 respectively. Sub-lethal levels of these two metals singly and in mixtures reduced the filtration rate significantly. Hg levels of 0.05 to 0.06 p.p.m. registered 50% reduction in the volume of water pumped, over the gills, while Cd concentrations of 0.5 p.p.m. brought about 50% reduction. These two metals interacted less than additively and more than additively under Hg + Cd and

Table 2. *Percentage reduction in filtration rate of Modiolus sp.*

Hg		Cd		Hg + Cd (1.7 p.p.m.)		Cd + Mg (0.025 p.p.m)	
Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction
0.02	12.19	0.50	9.94	0.005	36.67	0.5	51.12
0.04	43.46	1.00	19.86	0.01	48.45	1.0	55.26
0.06	59.15	1.50	18.74	0.015	61.04	1.5	61.92
0.08	56.21	2.00	25.59	0.020	61.78	2.0	68.15
0.12	64.42	3.00	38.15	0.03	68.60	2.5	71.56
		4.00	61.74	0.50	82.38		

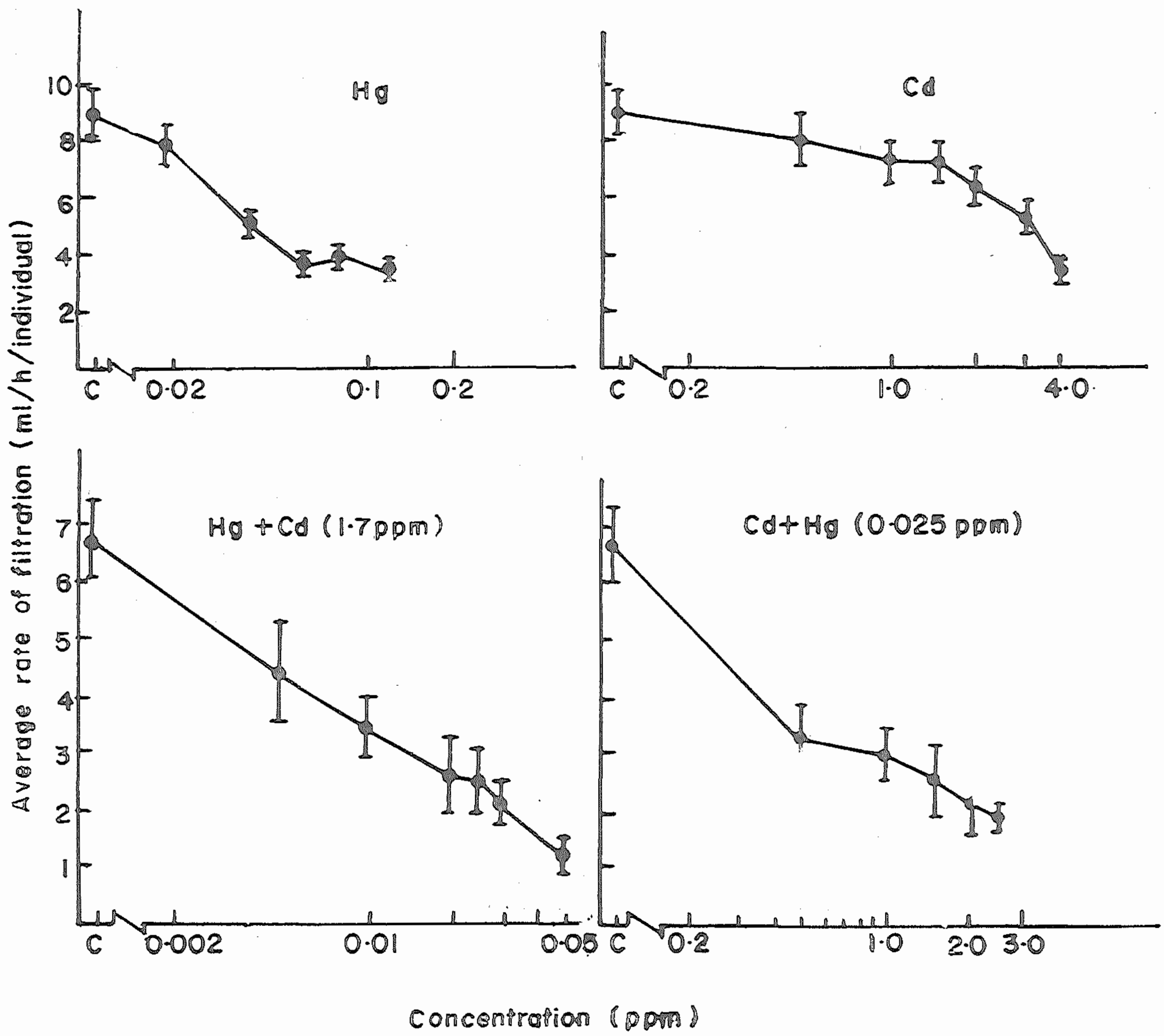

Fig. 2. Mean filtration rate (ml/h/individual) of *Modiolus* sp. exposed to sub-lethal concentrations of Hg, Cd, Hg + Cd and Cd + Hg. Vertical bars represent standard deviation.

Table 3. *Percentage reduction in filtration rate of Donax spiculum*

Hg		Cd		Hg + Cd (0.4 p.p.m.)		Cd + Hg (0.03 p.p.m.)	
Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction	Concentration (p.p.m.)	Percentage reduction
0.01	2.05	0.10	33.33	0.005	12.73	0.1	46.21
0.02	23.38	0.25	41.54	0.01	45.99	0.2	57.37
0.04	41.51	0.50	51.21	0.02	38.50	0.3	59.82
0.06	57.73	0.75	60.40	0.03	54.69	0.4	70.98
0.08	60.15	1.50	64.46	0.04	70.87	0.6	70.87
0.10	69.60			0.05	81.14		

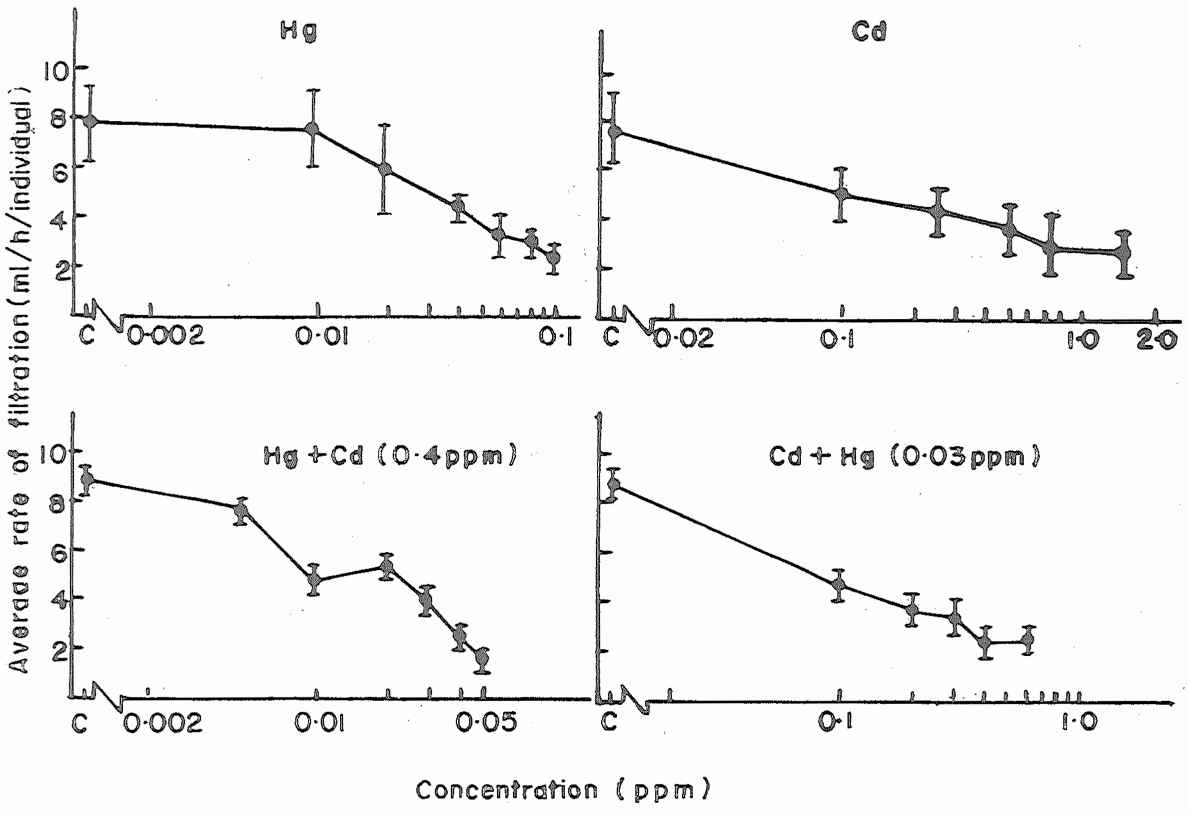

Fig. 3. Mean filtration rate (ml/h/individual) of *Donax spiculum* exposed to sub-lethal concentrations of Hg, Cd, Hg + Cd and Cd + Hg. Vertical bars represent standard deviation.

Cd + Hg combinations respectively (Table 4). In Hg + Cd combination a mixture concentration of 0.03 p.p.m. Hg with 0.4 p.p.m. Cd reduced the filtration rate by 54%. The combined EC 50 value is 1.35 toxic unit. Since the combined toxic unit is < 1, the interaction is less than additive, however, the two metals have exhibited joint interaction.

Under Cd + Hg combination, the metals were found to interact, more than additively. The EC 50 was 0.95 toxic units.

Discussion

The sub-lethal effects of metals are regarded more important than lethal effects themselves and more often sub-lethal effects turn out to be more insidious and injurious than short-term lethal effects (Waldichuck, 1974). Unlike many systems of potential value as indicators of sub-lethal stress, the filtration rate test is based on a response of ecological significance, since bivalves depend on the water they filter for both food and oxygen (Abel, 1976). The first observable response of bivalves of low levels of metal stress is shell closure. This behaviour drastically reduces the volume of water pumped over the gills. The findings of Epifanio & Srna (1975) and Abel (1976) that the filtering efficiency and feeding mechanisms of bivalves is impaired by the presence of pollutants in low concentrations is in conformity with our observations. In the present study, all the 3 bivalves collected from two different ecological niches were found to filter considerably less volume of water under metal stress either singly or in mixtures. The rate of filtration could be affected by at least three important factors, namely, frequency of valve closure, rate of ciliary activity and changes in the level of gill irrigation (Reddy & Menon, 1979). It was found in the present investigation that too high concentrations either arrested filtration entirely or caused the animals to close their shells. Valve closure mechanisms and inhibitory effect of ciliary action (Brown & Newell, 1972) can significantly affect the amount of water drawn into the body. The findings that for each individual metal and their mixtures tested the concentration required to reduce filtration rate by 50% was lower than their corresponding 96h–LC 50 is in agreement with the finding of Abel (1976).

Table 4. *The EC 50 (p.p.m.) values for the intertidal bivalves*

Species	Metal			
	Hg	Cd	Hg + Cd	Cd + Hg
M. carvalhoi	0.05	2.40	0.02 + 1.20 (0.9)*	0.80 + 0.025 (0.60)*
M. sp.	0.055	3.40	0.013 + 1.70 (0.74)*	0.5 + 0.025 (0.60)*
D. spiculum	0.055	0.50	0.03 +0.4 (1.35)*	0.20 + 0.03 (0.95)*

*Figures in parenthesis show the emperical toxicity unit

The interesting feature of the present study is the finding that the two metals in mixture at sub-lethal levels interacted more than additively (synergism) in depressing the filtration rate of all the bivalves. Here the mixture toxicities were greater than summed component toxicities. This finding is contrary to the observation of Sprague (1970) that simple additivity may not be involved at low sub-lethal levels. The reasons for increased effects in mixtures are still not clearly understood. These may include increase in the rate of metal uptake, formation of toxic metabolities, reduction in excretion, alteration of distribution and inhibition of detoxication. It may be assumed here that the presence of two metal ions in the test medium accentuate the toxic effect and the commencement of sub-lethal toxicity is at a comparatively lower concentration. To quantify physiological reactions at sub-lethal levels would require definition of toxicity indices based on detailed physiological studies under combined metal stress which would ultimately help in describing more meaningful water quality criteria.

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