Combined toxicity of mercury and cadmium to the tropical green mussel *Perna viridis*

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ABSTRACT: Effects were studied of mercury and cadmium individually and in combination on survival, byssus thread production, oxygen consumption and filtration rate of the intertidal bivalve *Perna viridis* (L.). Mercury and cadmium in mixtures interacted more-than-additively (synergism) in producing mortality in 96 h. Reduction in byssus-thread production, oxygen consumption and filtration rate recorded at sublethal levels of mercury-cadmium mixtures, however, was either less than or more than additive. Less than additive responses probably indicate ion antagonism.

INTRODUCTION

Pollution of the aquatic environment by heavy metals has received considerable attention in recent years (for reviews see Davies 1978, Eisler 1979, Bryan 1984) owing to their toxicity at very low levels, persistence in the environment, and their ability to accumulate in animal tissues. It is well known that metal toxicants seldom occur individually in the aquatic environment. Industrial effluents and agricultural run-offs into aquatic systems almost certainly burden the ecosystem with mixtures of toxic, or potentially toxic, metals. Despite the considerable amount of information available on the effects of individual heavy metals on aquatic organisms, reports on adverse effects of heavy-metal mixtures to aquatic organisms and their interactions at lethal and sublethal levels are quite limited. The importance of complex mixtures of toxicants in determining water-quality criteria and setting guidelines in effluent discharge has been emphasized by Sprague (1970), Marking & Dawson (1975), Spehar et al. (1978), Weis (1978), Weis & Weis (1978), and Wong et al. (1978).

Several studies have stressed possible deleterious effects of mercury or cadmium on aquatic organisms at lethal and sublethal levels (Eisler 1971, Calabrese & Nelson 1974, Waldichuk 1974, Johnston 1976, Nelson et al. 1976, 1977, Reish & Carr 1978, Briggs 1979,

Eknath & Menon 1979, George & Frazier 1982, George 1983, Mohan et al. 1984).

The results presented in this paper report effects of mercury and cadmium individually and in combination on survival and metabolism of the tropical intertidal bivalve *Perna viridis*, which could be an important candidate in the 'Mussel Watch' programme (Goldberg et al. 1978).

MATERIALS AND METHODS

Perna viridis (L.) of 20 to 25 mm shell length were collected from the Someshwar rocky shore (12° 47′ N, 74° 51′ E), along the coast of Mangalore (India) from low tide level and kept in raw seawater (35 ‰) at room temperature (28 °C ± 1 C°) for 72 h prior to the experiments. Raw seawater was collected from the unpolluted area off Someshwar beach, the habitat of the test animal. The seawater was stablised, sanitised and filtered by passing through a biofilter prior to use. Salinity ranged between 34.0 and 35.5 ‰, pH between 8.15 and 8.30.

Ten mussels of 20 to 25 mm shell length were used for each test concentration. They were exposed to either mercury, cadmium or a combination of Hg and Cd for toxicity evaluation. Analar grade mercuric chloride and cadmium chloride formed the source of the metals. Mussels were inspected every 12 h, and were considered dead if they gaped beyond 5 mm and

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showed no mantle retraction or foot movements on mechanical stimulation. Sublethal effects were followed by assessing the performance of the mussel in byssogenesis, oxygen uptake and filtration rate.

Byssus threads, secreted by the pedal gland, help in mooring the mussel to a hard substratum. The rate of thread formation gives an idea of the scope for activity. Estimates of the energy budget of *Mytilus edulis* have shown that 44 % of carbon and 21 % of nitrogen of the total energy intake are utilised by a healthy specimen towards byssus production (Hawkins 1985, Hawkins & Bayne 1985).

The total number of byssus threads produced by 10 mussels during 96 h (12 h light and 12 h dark alternatively for 4 d), under different metal concentrations, were counted and averaged to obtain the rate of byssogenesis.

To study oxygen uptake, mussels were pre-exposed to selected toxicant concentrations for 24 h. Ten individuals were kept in glass containers with 2 l of test solution and the containers were sealed with inert liquid paraffin in order to avoid exchange of gases with the atmosphere. The experiment was run for 10 to 12 h; water samples were drawn every 2 h and analysed for oxygen. The values represented are therefore averages of 5 or 6 readings, recorded from each test concentration. Results were expressed as μg oxygen consumed mg^{-1} dry weight h^{-1}.

To assess the rate of filtration, a dye clearance technique was employed involving addition of a known concentration of Neutral red to the test solution so as to achieve 2.0 ppm and allowing the mussels to clear the dye. The reduction in dye concentration at fixed intervals was estimated colorimetrically (420 to 480 nm). Filtration rate was estimated using Quayle's (1948) equation.

To determine the toxicity of metal mixtures, the additive toxicity index developed by Marking & Dawson (1975) was used. The toxicity unit, or sum of the biological effects of metal mixtures, is calculated based on the formula

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = S \qquad (1)$$

where A and B = metals; subscripts i and m = toxicities (the 96 h LC_{50} values) of the individual metal and metal mixtures respectively; S = sum of biological effects. The following relation is used to arrive at an appropriate index:

$$\frac{1}{S} - 1 \text{ for } S \leq 1 \text{ (greater-than-additive toxicity)}$$

$$S(-1) + 1 \text{ for } S \geq 1 \text{ (less-than-additive toxicity)}$$

When an index is negative, it indicates less-than-additive toxicity, zero indicates additive toxicity and a positive value, more-than-additive. If the 95 % confidence limits overlap zero, it is considered as simple additivity.

Lethal and sublethal toxicites of Hg and Cd were assessed first, and then their combined toxicity. Metal-metal combinations are shown as effects of different Hg concentrations in combination with a constant Cd concentration, and *vice versa*. Combinations are represented as (Hg + Cd) for Hg varying and Cd constant, and (Cd + Hg) for Cd varying and Hg constant.

RESULTS

Table 1 gives LC_{50} values, and Table 2 ET_{50} values, for Hg and Cd individually and in combination. The 96h LC_{50} values for Hg and Cd were 0.23 and 2.5 ppm respectively (Table 1). The Hg + Cd mixture, in which concentrations ranged from 0.02 to 0.18 ppm Hg with a constant 0.8 ppm of Cd, produced LC_{50} values (as toxic

Table 1. *Perna viridis*. LC_{50} (ppm) upon exposure to Hg, Cd, (Hg + Cd) and (Cd + Hg) salts over periods up to 96 h with respective additive indices

Time (h)	Hg	Cd	Hg + Cd (0.8 ppm)	Cd + Hg (0.12 ppm)
48	1.00	–	0.13	0.12
60	0.52	7.0	0.085	0.72
72	0.25	5.0	0.07	0.60
84	0.23	3.1	0.05	0.42
96	0.23	2.5	0.045	0.35
	(0.21–0.25)*	(2.4–2.55)*	(0.042–0.046)*	(0.33–0.36)*
	Additive indices			
Hg + Cd	+0.92**	(0.89–0.95)*		
Cd + Hg	+0.52**	(0.49–0.54)*		

* 95 % confidence limits
** More than additive

Table 2. *Perna viridis*. $ET_{50}(h)$ (effective time to kill 50 % of test population) when exposed to Hg, Cd, (Hg + Cd) and (Cd + Hg) as a function of concentration

Metal concentration (ppm)	Hg	Cd	Hg + Cd (0.8 ppm)	Cd + Hg (0.12 ppm)
0.04			110.0	
0.06			73.3	
0.08			65.0	
0.12			48.3	
0.15			45.0	
0.30	68.33			
0.40				83.3
0.50	63.33			
0.60				73.3
0.75	55.00			
0.80				60.0
1.00	51.66			53.3
1.20				48.3
4.0		76.66		
5.0		65.0		
10.0		48.33		

units) as follows: 48h, 0.89; 60 h, 0.69; 84 h, 0.54; 96 h, 0.52. The additive index calculated for the 96h LC_{50} (Hg + Cd) mixture was + 0.92, indicating more-than-additive interaction (Table 1). In the (Cd + Hg) combination, concentrations ranged from 0.4 to 1.2 ppm of Cd along with 0.12 ppm of Hg. LC_{50} values (in toxic units) were 48 h, 1.0; 60 h, 0.81; 72 h, 0.76; 96 h, 0.66. The additive index was + 0.52 for 96 h LC_{50} indicating a more-than-additive interaction (Table 1).

There was a decrease in the number of byssus threads produced with increasing concentration of Hg, Cd, (Hg + Cd) and (Cd + Hg) (Fig. 1a to d). Hg concentrations of 0.038 ppm and 0.029 ppm caused a 50 % reduction in the number of byssus threads produced during the dark and light phases of the experiments, respectively, whereas for Cd it was 0.73 and 0.87 ppm. In the (Hg + Cd) mixture, the effective concentrations to cause 50 % reduction (EC_{50} values) were 0.04 ppm +0.3 ppm (dark) and 0.008 ppm + 0.3 ppm (light). In the (Cd + Hg) combination a concentration of 0.569 ppm + 0.03 ppm and 0.97 ppm + 0.03
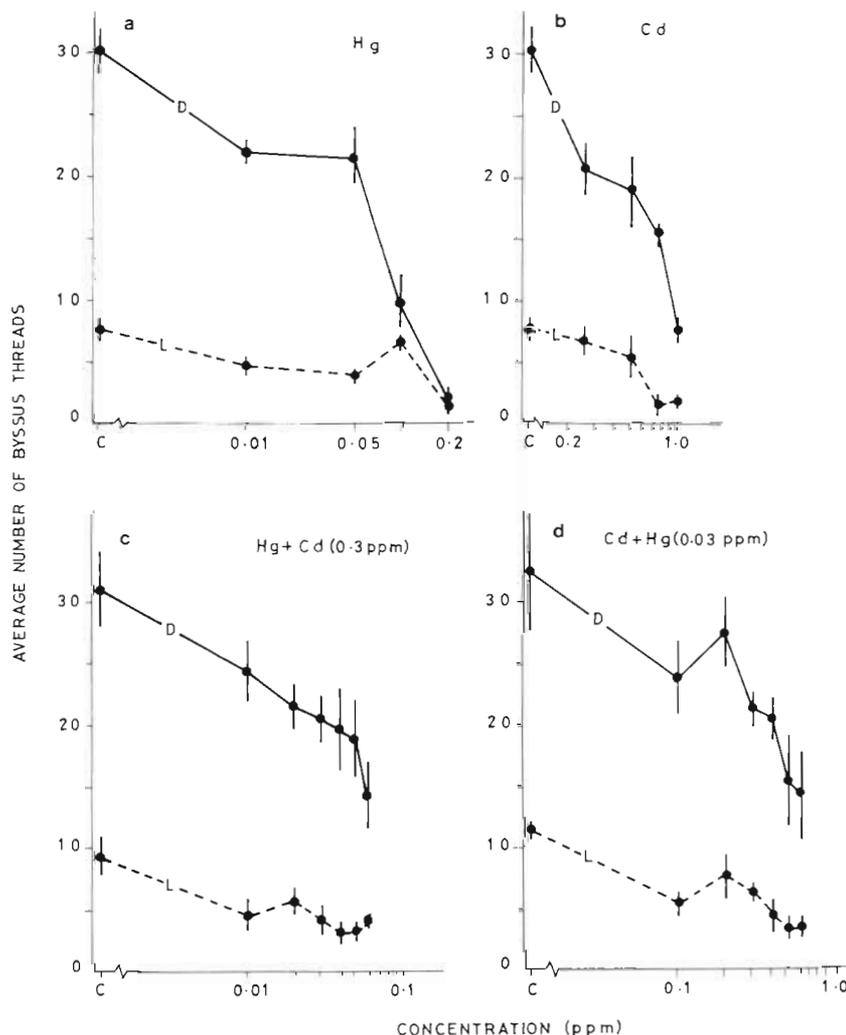

Fig. 1. *Perna viridis*. Mean number of byssus threads secreted under sublethal concentrations of (a) Hg, (b) Cd, (c) (Hg+Cd), and (d) (Cd+Hg) during dark (D) and light (L) regimes (48 h each). Vertical bars: standard deviation

Table 3. *Perna viridis*. Effective concentrations (EC_{50} ppm) which caused a 50 % reduction in the number of byssus threads produced, filtration rate, and oxygen consumption. (Values based on regression analyses)

Metal	Byssogenesis		Filtration rate	Oxygen consumption
	Dark phase	Light phase		
Hg	0.038	0.029	0.02	0.059
Cd	0.73	0.87	0.82	0.76
Hg + Cd	0.04 + 0.3	0.008 + 0.3	0.014 + 0.35	Data insufficient
Cd + Hg	0.569 + 0.03	0.97 + 0.03	0.36 + 0.016	Data insufficient

ppm produced 50 % reduction in the dark and light phases respectively (Table 3). In both (Hg + Cd) and (Cd + Hg) combinations the metals produced either a less-than-additive or a more-than-additive response in the rate of byssogenesis.

Fig. 2a to d gives the mean oxygen consumption rate of *Perna viridis* exposed to different concentrations of Hg, Cd, (Hg + Cd) and (Cd + Hg). Hg at 0.059 ppm caused 50 % reduction in oxygen uptake. Cd also individually depressed oxygen consumption; uptake was reduced by 50 % at 0.76 ppm. *P. viridis* exhibited the following pattern of oxygen uptake when exposed to a combination of (Hg + Cd): immediate reduction at lower concentrations, temporary elevation at medium concentrations and a subsequent decline reaching very low levels in the highest concentration. A combined concentration of 0.02 ppm Hg with 0.55 ppm Cd

resulted in more than 50 % reduction in oxygen consumption. In all the combined concentrations employed, the rate of consumption was less than that recorded in controls. In both combinations metals in mixtures reduced oxygen consumption to a greater extent than either of the metals did singly at lower individual concentrations. However, this trend was not maintained in higher concentrations, at least in the case of (Cd + Hg) mixture (Fig. 2c, d).

Mean filtration rates of *Perna viridis* exposed to different concentrations of Hg, Cd, (Hg + Cd) and (Cd + Hg) are presented in Fig. 3a to d. Hg at a level of 0.02 ppm resulted in 50 % reduction in the quantity of water filtered, and a concentration of 0.2 ppm caused 91 % reduction in filtration rate. At 0.82 ppm Cd caused 50 % reduction in filtration rate. Combinations of the 2 metals in (Cd + Hg) reduced filtration rates to
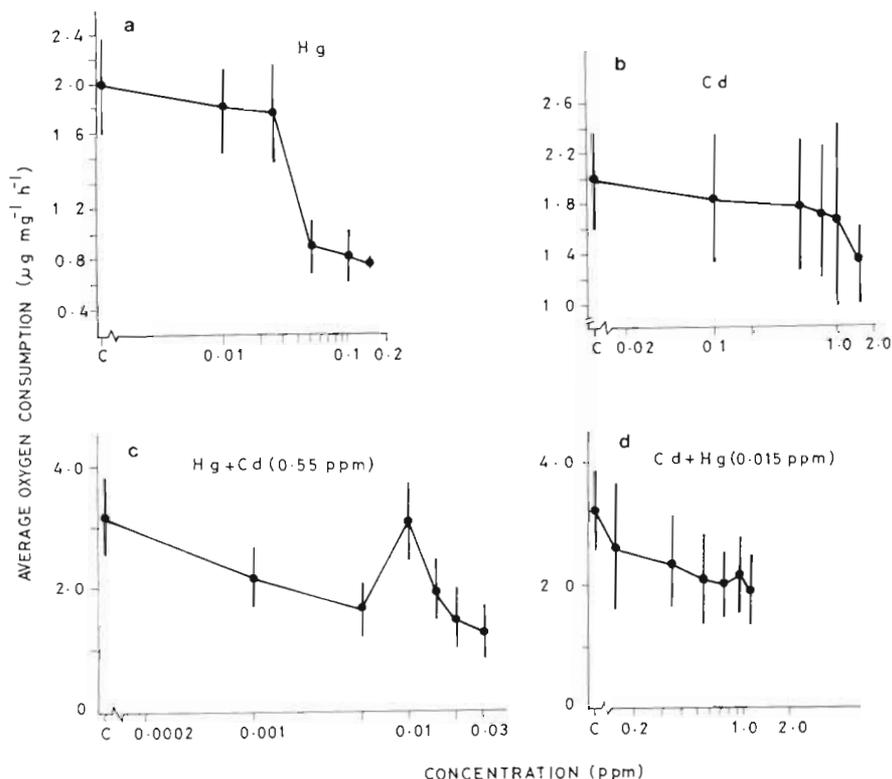

Fig. 2. *Perna viridis*. Average oxygen consumption (light) (μO_2 mg^{-1}h^{-1}) under sublethal concentrations of (a) Hg, (b) Cd, (c) (Hg+Cd) and (d) (Cd+Hg). Vertical bars: standard deviation
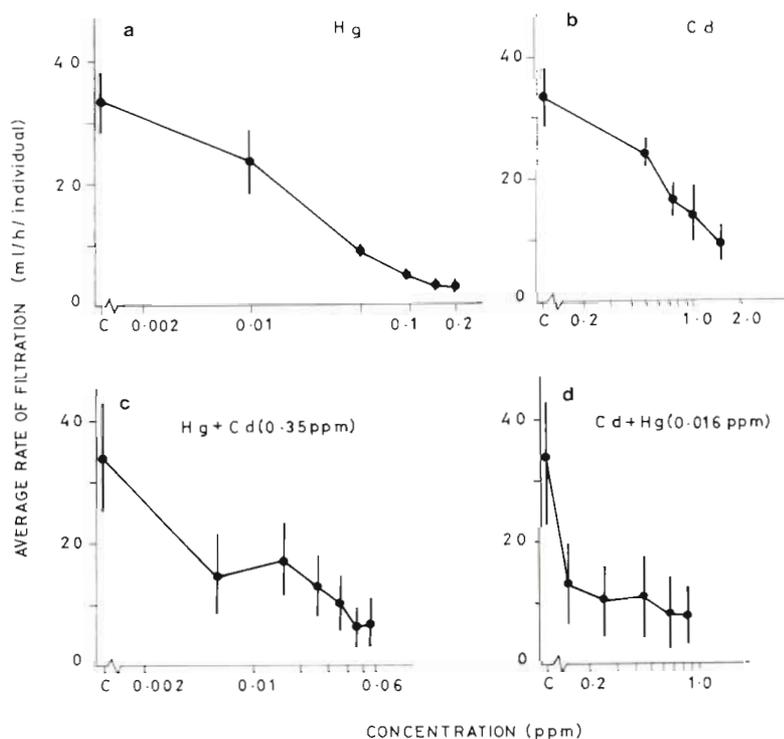
Fig. 3. *Perna viridis*. Average rate of filtration (light) (ml ind^{-1} h^{-1}) under sublethal concentrations of (a) Hg, (b) (Cd), (c) (Hg+Cd), and (d) (Cd+Hg). Vertical bars: standard deviation

a greater extent than either of the metals individually, indicating more-than-additive interaction as observed with lethal toxicity (Table 1).

The results presented in Table 3 allow the following generalisations. Metals in combination can bring about more-than-additive or less-than-additive reactions of byssogenesis and filtration. The results obtained for byssogenesis indicate that metal concentrations which produced 50 % reduction in performance can vary drastically depending on time of day (light/dark). Especially in the combination (Hg + Cd), Hg can exert little change in toxicity during the dark phase, while during the light phase, conspicuously low concentrations of Hg created deleterious effects.

DISCUSSION

The recent trend in heavy-metal studies is to analyse and predict lethal and sublethal toxicity levels of metal mixtures. Several studies (Sprague 1970, Spehar et al. 1978, Wong et al. 1978, Christensen et al. 1979, Eisler 1979) have stressed the importance of mixed toxicities in determining water-quality criteria. Interactions between metals may increase or decrease the degree of toxicity, depending upon the nature of individual pollutants. Since metals usually occur as mixtures in the marine environment, rather than alone, information on their interactions provides a more realistic

assessment of their toxicity to marine organisms than observations with individual metals.

In the present study, the concentrations of Hg and Cd employed were considerably higher than the average mercury concentrations in seawater encountered in the northern hemisphere. According to Florence & Batley (1980), this is 0.03 ppb. *Mytilus edulis* is reported to survive and grow at reduced pace in 0.3 ppb (Strömgren 1982). The high concentrations used in the present study were a compromise for the time factor, as the experimental duration was short. Further, laboratory studies showed that *Perna viridis* remained active for more than 1 wk at concentrations as high as 0.1 ppm Hg and 0.8 ppm Cd (own unpubl. data). The Marking & Dawson (1975) additive indices of +0.92 and + 0.52, respectively, for (Hg+Cd) and (Cd+Hg) combinations, reveal joint interaction, i.e. more than additive in bringing about mortality of test specimens. Even though both combinations produced a more-than-additive effect, the additive index was higher in the (Hg + Cd) combination. This suggests that varying concentrations of Hg with a constant concentration of Cd in the test medium were relatively more lethal to *P. viridis*. The higher additive index reflects 2 things: firstly, the involvement of a more-than-additive (synergistic) interaction; secondly, low levels of 2 metals causing a fixed response. It is evident that the concentrations of metals in mixtures also affect the degree to which component interactions are additive or synergistic. A

comparable result was obtained when *P. viridis* was subjected to combinations of zinc and copper (Prabhudeva & Menon 1986).

Literature on the combined toxicities of Hg and Cd to bivalves is limited. The reasons for the increased toxicities of mixtures are still not clearly understood. These may include increase in the rate of uptake, formation of toxic metabolites, reduction in excretion, alteration of distribution, and inhibition of detoxification (Moulder 1980). This could account at least partially for the results obtained in the present study. The presence of 2 metal ions in the test medium may accentuate toxic effects at comparatively low concentrations. Perhaps, depurative processes of the mussel are affected deleteriously when forced to ward off 2 metal ions.

Several workers have studied the toxicity of metal mixtures, and a few have considered possible reasons for increased or decreased toxicities of metal mixtures. Barnes & Stanbury (1948) found that mixtures of cupric sulphate and mercuric chloride were far more toxic to the copepod *Nitocra spinipes* than either of the salts alone. Corner & Sparrow (1956) reported that 50 ppm of mercury (as $HgCl_2$), in 23 h, or 1000 ppm of copper (as copper sodium citrate) caused 50 % mortality of *Artemia salina*, whereas 25 ppm of Hg and 500 ppm of Cu killed 50 % *A. salina* in 23 h indicating a clear-cut additive reaction. Additive effects of Hg and Cu have been documented in developing sea urchin eggs (Marakkami et al. 1976). MacInnes & Calabrese (1977) reported the interaction of metals to be temperature dependent. Antagonism and less-than-additive interactions have also been documented for Hg and Cu mixtures. Moulder (1980) observed the protective action of Cu against the toxic action of Hg in the euryhaline amphipod *Gammarus duebeni*. Her reasons for the resulting less-than-additive interaction were: (1) the possibility of Cu-Hg complex formation, thereby reducing Hg availability; (2) Cu occupying binding sites on the surface of the gammarid that would be otherwise occupied by Hg; (3) Cu detoxifying Hg within the tissue.

Byssogenesis has been employed as a useful tool to assess sublethal pollutant effects in *Mytilus edulis* (Roberts 1975) and *Perna viridis* (Eknath & Menon 1979, Reddy & Menon 1979, Mathew & Menon 1983). Byssus-thread production could be affected by reduced pedal activity, differences in shell closure ability, or direct interference in the synthesis of byssal threads. In the present study Hg concentrations of 0.029 to 0.038 ppm reduced byssal-thread production by 50 % during light and dark phases respectively. This observation is in general accordance with that reported by Martin et al. (1975). Byssal-thread production was inhibited at Cd levels 0.25 ppm and above; thread production

was reduced by 50 % at Cd levels of 0.73 or 0.87 ppm depending on the time of observation. By comparison, Briggs (1979) observed inhibition of byssal-thread production in *M. edulis* at concentrations between 1.0 and 5.0 ppm Cd. Comparison of the results with EC_{50} values of individual metals reveals the possibility of the 2 metals acting less than additively in decreasing byssogenesis. The combination EC_{50} values were well below the 96 h LC_{50} values (Table 3).

Changes in oxygen consumption are valuable indicators of sublethal stress and are frequently used to evaluate changes in metabolism due to environmental alteration (Hawkins et al. 1986). Oxygen uptake in *Perna viridis* decreased with increasing Hg levels; concentrations between 0.04 and 0.05 ppm reduced uptake by 50 to 60 %, about 1/5 of the 96 h LC_{50} value (Fig. 2a to d). A similar observation was recorded for Cd. In *P. viridis* exposed to Hg + Cd combined, oxygen uptake showed a general decreasing trend with increasing concentrations. The combination of Cd+Hg also depressed oxygen uptake in *P. viridis*. Bivalve molluscs can turn to anaerobiosis under unfavourable conditions. Salanki (1965, 1968) observed that bivalves close their valves tightly and stop filtering under unfavourable conditions resulting in decreased oxygen uptake. Since complete valve closure in *P. viridis* is not possible due to interference by the byssal mass, some water may enter. Rhythmic shell movements in bivalves are closely related to respiration and filtration rate (Morton 1970, Reddy & Menon 1979, Mathew & Menon 1983). Oxygen consumption in bivalves represents the product of 2 factors: ventilation volume and efficiency of gas exchange. Hence fluctuations in respiratory levels may be due to variation in the water volume moved through the gills and to interruptions.

Variations in filtration rate are valuable indicators of sublethal stress. The filtration-rate test is based on an organismic response of ecological significance, since bivalves depend on the water they filter for both food and oxygen.

Perna viridis filters considerably less water under Hg stress. According to Epifanio & Srna (1975) filtration rate in bivalves is reduced under pollutional stress. Filtration rate can be affected by at least 3 important factors: frequency of valve closure, rate of ciliary acitivity, and changes in the level of gill irrigation (Reddy & Menon 1979). Under Cd stress, *P. viridis* temporarily elevates filtration rates at lower concentrations; at higher concentrations there is a clear-cut reduction. (Hg+Cd) combinations cause considerable reduction in filtration rate; this indicates that the metals in combination are more harmful than either of them alone.

A conspicuous feature of this study is that lethal and sublethal effects did not reflect the same trend in metal

additive reactions. While concentrations of heavy metal salts employed in combination may produce more-than-additive lethality, such reactions need not be reflected at sublethal levels. The reason may be that the concentrations selected for the sublethal toxicity tests were well above thresholds and hence, individual-metal-induced reactions in the mixture could not be delineated. It is possible that concentrations at ppb levels, if employed, would have given sublethal results comparable to those of lethal effects, as found in the case of *Perna indica* (Menon & Baby unpubl. data).

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