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Filtration rates and heavy metal toxicity in *Donax incarnatus*

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Abstract

Sub-lethal effects of mercury, copper and cadmium, in dual combination on the filtration rates of the common inter-tidal bivalve *Donax incarnatus* Gmelin were investigated. The results indicated that when dual combinations were present in the medium, conspicuous variations in filtration performance occurred, mostly in the median concentrations tested. The presence of heavy metals in combination at lower and unrealistic higher concentrations elicited comparable filtration performance in the test species Simple additivity observed in majority of cases indicated at least two reactions as 1) independent action of the metal components involved, where the most toxic one influences filtration performance or 2) a joint action of the two in the combination. These results showed an, hitherto, unrecorded reaction by a bivalve to the presence of heavy metals and may be an indication of positive and negative feed backs, both ultimately leading to breakdown. The study also indicated that filtration rate is not a suitable index of stress at least in the case of clams.

Key words: Heavy metal toxity - bivalves

Introduction

In marine molluscs, the filtration rate is a reliable parameter to assess the effects of pollutants and could be carried out with ease. Unlike many functions of potential value as indicators of sub-lethal stress, the filtration rate is based on water quality, as the bivalves depend on the water they filter for both food and oxygen (Abel, 1976). Information on the filtration rates of marine bivalves under stress is available from the works of Cole and Hepper, 1953; Eknath and Menon, 1978; Palmer, 1980; Mathew and Menon, 1984; Prabhudeva and Menon, 1985, 1988; Mohan *et al.*, 1986; Miron, 1994; Stuijifzand *et al.*, 1995 and Knoph, 1996. The filtration rate has been used as a parameter as it has a direct ecological significance, since alterations in feed rate cause variations in growth rate in marine molluscs (Widdows *et al.*, 1982). Relatively protracted biological half-life, inherent toxic nature at sub-lethal concentrations and the capacity to undergo bioaccumulation make heavy metals serious contaminants of the marine ecosystem. The present study was aimed at delineating the sub-lethal effects of mercury, copper and cadmium, in dual combination on the filtration rates of the common inter-tidal bivalve *Donax incarnatus* Gmelin.

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Material and methods

D. incarnatus (size: 20-25mm) was sampled from the sandy beaches of Shakthikulangara (76°21′2″ Long; 9°23′20 ″ Lat.) in southern Kerala along the south-west coast of India. The specimens were transported to the laboratory and maintained under laboratory conditions for 36 hrs in aerated seawater of experimental salinity prior to experimentation (room temperature: 29 ± 1°C; salinity: 32 ppt ; *p*H: 8.4). The concentrations of mercury (as $HgCl_2$), copper (as $CuSO_4.5H_2O$) and cadmium (as $CdCl_2.H_2O$) employed for the study ranged between 1.0 and 5.0 ppb, 0.5 and 6.0 ppb, and 5.0 and 40 ppb respectively and were based on the individual 96 hr LC_{50} values (Hg: 59.1 ppb; Cu: 25.2 ppb and Cd: 320.6 ppb). The concentration of one of the components in the diad was kept constant while the other was made to vary. Stock solution of the toxicants was prepared in double glass-distilled water and added to the experimental media to achieve the desired concentration of metal mixture. The addition did not produce any appreciable variation in *p*H of the medium. The animals were not fed before or during the course of sub-lethal heavy metal exposure. The dye clearance technique was employed to assess the filtration rate. Five animals pre-exposed to the respective toxicant combination (Sprague, 1970) for 24hrs were placed in glass beakers containing two litres of filtered seawater (salinity: 32 ppt) with 2.0 ppm neutral red along with control in duplicate and were allowed to clear the dye. The experiments lasted for 30 minutes and the frequency of sampling was 10 minutes. The amount of water filtered was determined after having found out the reduction in the dye concentration with the help of a Spectrophotometer (Hitachi: model 220-20) at 435 nm. No significant reduction in dye concentration was noted when the experiment was conducted without the animals at room temperature. After the experiments the animals were dissected, soft tissues removed, cleaned in distilled water, dried at 80°C for 48 hrs and dry weights taken to constancy and the results expressed as ml $h^{-1}mg^{-1}$ (dry wt.). Filtration rate was calculated using Abel's equation (Abel, 1976). Student's "t" test was employed to assess whether there was any significant variations in the sub-lethal response registered in respect of the test animals. The additive indices were calculated using the toxicity unit concept (Marking and Dawson, 1975) and EC_{50} employing Probit analysis (Finney, 1971).

Results

D. incarnatus exposed to constant concentrations of 1.0 and 3.0 ppb of mercury and varying levels of copper, showed clear cut increase in filtration rates only in the combination containing 0.5 ppb of copper. However, an increase in copper concentration up to 4.0 ppb with constant mercury load produced a reduction in filtration rate (Table 1a & b). In the presence of 1.0 to 5.0 ppb of mercury along with 0.5 ppb of copper there was significant elevation in the filtration rates of the test animals. (Table 1c). Contrary to the above observation, the presence of 1.0 to 4.0 ppb of mercury along with 2.0 ppb of copper resulted in a reduction in filtration rate (Table 1d). The experimental animals registered significant reduction in filtration, when exposed to 5.0 ppb of cadmium along with 1.0 ppb of mercury. Exposure to higher concentrations of cadmium with constant mercury load evoked little impact on the filtration performance as evidenced by the amount of water filtered in unit time (Table 2a). Filtering less quantity of water than the control even in the lowest cadmium concentration of 5.0 ppb along with 3.0 ppb of mercury, the animals tended to filter less and less as the cadmium concentration increased to 20 ppb. Curiously enough, an increase in cadmium concentration to 30 ppb and 40 ppb with constant load of mercury produced a significant increase in the filtration rate of the exposed animals (Table 2b). Depicting statistically significant reduction in filtration rate at the minimal mercury concentration tested, the performance was found to increase with increasing mercury load at constant cadmium concentration [5.0 ppb] (Table 2c). A four-fold increase in the cadmium concentration in the test medium along with 1.0 to 5.0 ppb of mercury evoked reduced rates of filtration in two combinations (Table 2d). The filtration rate of experimental animals increased with increasing copper concentration in the presence of constant cadmium load. More or less comparable results were obtained when *D. incarnatus* was exposed to 0.5 ppb and 2.0 ppb of copper along with 5.0 to 40 ppb of cadmium in both lower and higher combinations (Table 3a-d).

Discussion

The concept of assessing filtration rate to explain stress arose out of the knowledge that metabolism and activity are interrelated. When bivalves are exposed to a toxicant, reduction of contact with the medium can be brought about by the secretion of mucous and/or closure of shells. Unlike many systems of potential value as indicators of sub-lethal stress, filtration rate is an important one, since, this activity gives a direct idea on the quantity of water propelled through the gills and the quantum of water used for life supporting activities (Mohan *et al.*, 1986). Studies have gone to indicate a close agreement between scope for activity and filtration rate (Bayne *et al.*, 1973). Observations on the filtering efficiency of *Perna perna* and *P. indica* have shown that physiological flexibility accompanying food availability is reflected in the filtration rate, oxygen consumption and hence scope for growth (Bayne, 1985). The results indicated that when dual combinations were present in the medium, conspicuous variations in filtration performance occurred, mostly in the median concentrations tested. For example, the presence of mer-

Table 1. *Average filtration rate (ml hr^{-1} mg^{-1} dry wt.) in* D. incarnatus *under sub- lethal concentrations of Hg (constant) and Cu (varying) [1a&b]; Cu (constant) and Hg (varying) [1c&d], along with the respective standard deviations, percentage performance, EC_{50} level, additive index and control*

Table 1a **1. 0 ppb Hg + Cu**

Copper (ppb)	Filtration rate (ml hr^{-1} mg^{-1}dry wt.) Mean	SD	Performance (% of control)	EC_{50} (ppb)	Additive index
0.5	0.297*	0.074	176.8		
1.0	0.048	0.032	28.6		
2.0	0.152	0.071	90.1	12	-1.09
4.0	0.163	0.033	97.0		(SA)
6.0	0.181	0.051	107.8		
Table 1b **3. 0 ppb Hg + Cu**					
0.5	0.232	0.063	138.1		
1.1	0.089	0.055	52.9		
2.0	0.106	0.029	63.1	1.1	-1.58
4.0	0.161	0.073	95.8		(SA)
6.0	0.197	0.083	117.3		
Control	0.168	0.059	100.0		

Table 1c **0. 5 ppb Cu + Hg**

Mercury (ppb)	Filtration rate (ml hr^{-1} mg^{-1}dry wt.) Mean	SD	Performance (% of control)	EC_{50} (ppb)	Additive index
1.0	0.297*	0.074	176.8		
2.0	0.191	0.054	113.7		
3.0	0.232	0.063	138.1	ND	ND
4.0	0.242	0.061	144.0		
5.0	0.242	0.101	144.0		
Table 1d **2. 0 ppb Cu + Hg**					
1.1	0.152	0.071	90.1		
2.0	0.132	0.055	78.5		
3.0	0.106	0.029	63.1	4.6	-3.43
4.0	0.075	0.029	44.6		
5.0	0.182	0.053	108.3		
Control	0.168	0.059	100.0		

* $p<0.05$

Table 2. *Average filtration rate (ml hr^{-1} mg^{-1} dry wt.) in* D.incarnatus. *under sub- lethal concentrations of Hg (constant) and Cd (varying) [2a&b]; Cd (constant) and Hg (varying) [2c&d], along with the respective standard deviations, percentage performance, EC_{50} level, additive index and control*

Table 2a **1.0 ppb Hg + Cd**

Cadmium (ppb)	Filtration rate (ml hr^{-1} mg^{-1}dry wt.)		Performance (% of control)	EC_{50} (ppb)	Additive index
	Mean	SD			
5	0.75*	0.028	44.6		
10	0.148	0.1.2	88.1		
20	0.185	0102	110.1	3.0	1.36
30	0.146		0.058	86.9	(SA)
40	0.143	0.068	85.1		

Table 2b **3.0 ppb Hg + Cd**

5	0.166	0.057	98.8		
10	0.1.5	0.056	62.5		
20	0.085*	0.012	50.6	17.1	-1.54
30	0.334*	0.120	198.8		(SA)
40	0.381**	0.071	226.8		
Control	0.168	0.059	100.0		

Table 2c **5 ppb Cd + Hg**

Mercury (ppb)	Filtration rate (ml hr^{-1} mg^{-1}dry wt.)		Performance (% of control)	EC_{50} (ppb)	Additive index
	Mean	SD			
1.0	0.075*	0.028	44.6		
2.0	0.166	0.076	98.8		
3.0	0.166	0.057	98.8	ND	ND
4.0	0.168	0.031	100.0		
5.0	0.209	0.085	124.4		

Table 2d **20 ppb Cd + Hg**

1.1	0.185	0.102	110.1		
2.0	0.175	0.054	104.2		
3.0	0.185*	0.012	50.6	43.1	-0.67
4.0	0.130	0.028	77.4		(LA)
5.0	0.167	0.073	99.4		
Control	0.168	0.059	100.0		

* $P<0.05$ ** $P < 0.01$

Table 3. *Average filtration rate (ml hr^{-1} mg^{-1} dry wt.) in* D. incarnatus. *under sub- lethal concentrations of Cd (constant) and Cu (varying)[3a&b]; Cu (constant) and Cd (varying) [3c&d], along with the respective standard deviations, percentage performance, EC$_{50}$ level, additive index and control.*

Table 3a **5 ppb Cd + Cu**

Cadmium (ppb)	Filtration rate (ml hr^{-1} mg^{-1}dry wt.)		Performance (% of control)	EC$_{50}$ (ppb)	Additive index
	Mean	SD			
0.5	0.244	0.063	99.2		
1.0	0.319	0.069	129.7		
2.0	0.322	0.122	130.9	ND	ND
4.0	0.389	0.123	158.1		
6.0	0.189	0.103	76.8		

Table 3b **20 ppb Cd + Cu**

0.5	0.198	0.069	80.4		
1.0	0.310	0.114	126.0		
2.0	0.295	0.099	119.9	ND	ND
4.0	0.289	0.043	117.5		
6.0	0.191	0.053	77.6		
Control	0.168	0.059	100.0		

Table 3c **0.5 ppb Cu + Cd**

Mercury (ppb)	Filtration rate (ml hr^{-1} mg^{-1}dry wt.)		Performance (% of control)	EC$_{50}$ (ppb)	Additive index
	Mean	SD			
5	0.244	0.063	99.2		
10	0.139*	0.040	56.5		
20	0.198	0.069	80.4	28.1	-0.68
30	0.362	0.109	147.2		(SA)
40	0.419*	0.080	170.3		

Table 3d **2.0 ppb Cu + Cd**

5	0.322	0.122	130.9		
10	0.294	0.032	119.5		
20	0.295	0.099	119.9	ND	ND
30	0.368*	0.061	149.6		
40	0.428*	0.101	173.9		
Control	0.168	0.059	100.0		

*P<0.05

cury and copper reduced filtration rate when the copper concentration was either 1.0 ppb or 2.0 ppb along with 1.0 ppb or 3.0 ppb of mercury. The presence of heavy metals in combination at lower concentration and naturally unrealistic higher concentrations elicited comparable filtration performance in the test species. Simple additivity observed in majority of cases can indicate at least two reactions. Independent action of the metal components involved, where the most toxic one influences filtration performance or a joint action of the two in the combination. These results show a hitherto unrecorded reaction by a bivalve to the presence of heavy metals and may be an indication of positive and negative feed backs, both ultimately leading to breakdown. Further, being an intertidal bivalve, *D. incarnatus* is subjected to wide environmental fluctuations, which could result in varying physiological and behavioural performance. It is also noteworthy that this sand dwelling burrowing bivalve is subjected to spatial confinement. Therefore, the behavioural pattern of this bivalve could be an admixture of both its physical and physiological ability to compensate. The variations noticed in the filtration performance between concentrations and metal combinations could be an index of organismic compensation. The presence of cadmium and copper in the media was found to enhance the animal's capacity to filter more water, in majority of cases. It is known that bivalves, which have got such well-developed siphons as in the case of *D. incarnatus*, can regulate filtration rate by manipulating the internal lumen of the siphon. Probably, the concentrations were not sufficient to result in either a conspicuous impairment of filtration mechanism or an increase in the filtration capacity of the test animals. This is evident from the results obtained during the present study. The study also indicated that filtration rate is not a suitable index of stress at least in the case of clams.

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