Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 99, No. 4, July 1990, pp. 345–352.

Accumulation, distribution and depuration of mercury in the green mussel *Perna viridis* (Linnaeus)

P K KRISHNAKUMAR*, R DAMODARAN and P N K NAMBISAN
School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin 682 016, India
*Karwar Research Centre of Central Marine Fisheries Research Institute, Karwar 581 301, India

MS received 3 August 1989; revised 28 December 1989

Abstract. Accumulation, distribution and depuration of inorganic mercury in the gills, viscera, mantle and adductor muscle + foot of green mussel *Perna viridis* were investigated. Mussels were exposed to 37·5 and 75 $\mu g\ l^{-1}$ mercury as $HgCl_2$ in a static system for 4 days. The rate of accumulation was found to be the maximum in gills followed by viscera, mantle and muscle + foot. Exposed mussels were then transferred to clean seawater in a flow-through system and maintained for 25 days to study the depuration and biological halflives (TB/2) of mercury in the different body parts. After 25 days, mercury loss from the external tissues like gills and mantle was faster than from the internal tissues like viscera and muscle. Gills and mantle showed comparatively shorter TB/2 than the viscera and muscle + foot.

Keywords. *Perna viridis*; mercury; accumulation; biological halflife.

1. Introduction

Accumulation of highly toxic metals by commercial marine species may become harmful to men and other animals and it is a matter of great concern today. Marine mussels are well known for their ability to accumulate pollutants from their environment and have become the subject of various monitoring programmes of the 'mussel watch' type (Goldberg *et al* 1978; Davies and Pirie 1980).

The uptake system in bivalve molluscs act as if they have first order kinetics, so that the rate at which the metal can enter the organism is proportional to the level in the ambient seawater (Phillips 1977; Lakshmanan and Nambisan 1979; Martincic *et al* 1984; Simkiss and Mason 1984; Amiard *et al* 1986; King and Davies 1987). Once the metal has entered the cells they undoubtedly become bound to a variety of ligands and it is the metabolism of these complexes that determines the subsequent fate of the metal and the final body load (Simkiss and Mason 1984). Based upon the rate of loss of a metal, its biological halflife can be determined. The biological halflife may serve as a warning about the persistence and potential for cumulative biological effects of a chemical (Buikema *et al* 1982).

Mercury is probably the most important pollutant both with regards to its effects on marine organisms and its potential hazard to man, and its compounds are included in the 'black list' of all the international conventions (Bryan 1984).

The green mussel *Perna viridis* is having wide spread distribution all along both east and west coasts of India. This is an ecologically and economically important tropical species with outstanding potential as sentinal organism (Krishnakumar 1987). Compared to temperate area mussels, little information is available on the

accumulation, distribution and depuration of mercury in *P. viridis* (Lakshmanan and Nambisan 1979). Our toxicity studies have shown that mercury is highly toxic to *P. viridis* (Krishnakumar *et al* 1987a,b). In this paper an attempt has been made to study the accumulation, distribution, depuration and biological halflives of mercury in the different body parts of *P. viridis*. Although mussels can obtain mercury from both seawater and food, only accumulation from seawater is considered here.

2. Materials and methods

Adult specimens (35–45 mm) were collected from an unpolluted coastal area, which is remote from human activity, near Narakkal (Cochin) in March 1985. Animals were cleaned and acclimated in the laboratory for 4 days under optimal conditions (salinity $35 \pm 0{\cdot}5 \times 10^{-3}$; temperature $29 \pm 0{\cdot}5°C$; pH $7{\cdot}9 \pm 0{\cdot}1$ and dissolved oxygen >4 ml l^{-1}). Mussels were fed with *Synechocystis* sp. and feeding was stopped during and 2 days before the accumulation study.

2.1 *Accumulation study*

Two exposure concentrations of 37·5 and 75 $\mu g l^{-1}$ were selected after the acute toxicity (96 h LC_{50} for $HgCl_2$; 155 $\mu g l^{-1}$) experiments (Krishnakumar *et al* 1987b). Twenty mussels each were placed in two separate plastic tubs, each containing 15 l of seawater for each concentration. Control and treatment tubs were kept in duplicate. Optimal conditions were maintained throughout the experiment (salinity $35 \pm 0{\cdot}5 \times 10^{-3}$; temperature $29 \pm 0{\cdot}5°C$; pH $7{\cdot}8 \pm 0{\cdot}1$ and dissolved oxygen >4 ml l^{-1}). The test medium was changed every day and 6–8 animals were removed from both the control and treatment tubs at 1, 2, 3 and 4 days of exposure for mercury analysis. Mercury concentration in the treatment tubs were determined daily and the mean concentrations were 37·51 and 75·05 $\mu g l^{-1}$ for 37·5 and 75 $\mu g l^{-1}$ treatments, respectively. Seawater in the control tubs contained a mean mercury concentration of 0·026 $\mu g l^{-1}$. Before the experiment few mussels were sacrificed to find out their background mercury concentrations (0 day).

2.2 *Depuration study*

After 4 days, mussels from the accumulation study were gently rinsed with seawater and transferred to a seawater flow-through system for depuration study. Filtered (Whatman 1) and aerated seawater is allowed to flow through the system at a rate of 150 ml min^{-1} (salinity $35 \pm 0{\cdot}5 \times 10^{-3}$; temperature $28{\cdot}5 \pm 0{\cdot}5°C$; pH $7{\cdot}9 \pm 0{\cdot}1$ and dissolved oxygen >4 ml l^{-1}). *Synechocystis* sp. cultured in metal free medium was added to the system and maintained at a concentration of 3×10^3 cells ml^{-1}. Mussels were sampled on every 5, 10, 15, 20 and 25 days of the depuration study.

2.3 *Mercury analysis*

Mussels were opened and soft tissues were dissected out using clean stainless steel instruments into gills, mantle, adductor muscle + foot and the remaining body parts

as viscera. Animals (6–8) were dissected out to pool the different body parts. Soft tissues were gently washed with distilled water, blotted dry, weighed and digested using H_2SO_4 and HNO_3 mixture in Bethge's apparatus following the methods of BITC (1976). Seawater mercury levels were estimated following the method of Gardner and Riley (1974). All the analyses were done in triplicate. Total mercury was analysed by using cold vapour atomic absorption technique in a mercury analyser (ECIL, MA-77).

2.4 *Data analysis*

To test the linearity of mercury uptake, regression analysis was carried out using microcomputer. The linearity between the exposure period and mercury concentration in the tissue can be represented by the equation:

$$Yt = a + bt,$$

where Yt is the mercury concentration in the tissue at time t, b the slope of the line which is equal to rate of uptake of mercury and a the natural mercury concentration in the tissue. The bioconcentration factor (BCF) is calculated using the formula:

$$\text{BCF} = \frac{\text{4 day tissue concentration} - \text{0 day tissue concentration}}{\text{Mercury concentration in the seawater}}.$$

Mercury concentration in different body parts after 4 days of exposure period was taken as the initial metal concentration (0 day) for the depuration study. Percentage of the initial concentration of mercury with the progress of depuration time was calculated. Least-square line was fitted through the semilogarithmic plots of the points. The rate of loss of mercury from the body parts of mussels is characterized by the equation:

$$\text{Log } Y = a - bx,$$

where x is the time in days, Y the percent initial concentration of mercury, a the Y intercept and b the slope of the least-square line. The biological halflives (TB/2) were determined by substituting the slope of the least-square line (b) in the equation (Renfro 1973)

$$\text{TB/2} = \frac{\text{Log } 2}{b}.$$

Average TB/2 was calculated for different body parts from two different experiments.

3. Results

3.1 *Accumulation*

Significant linear mercury accumulation was found in all the body parts of mussels with progress of exposure time (figure 1). The rate of accumulation in the gills were

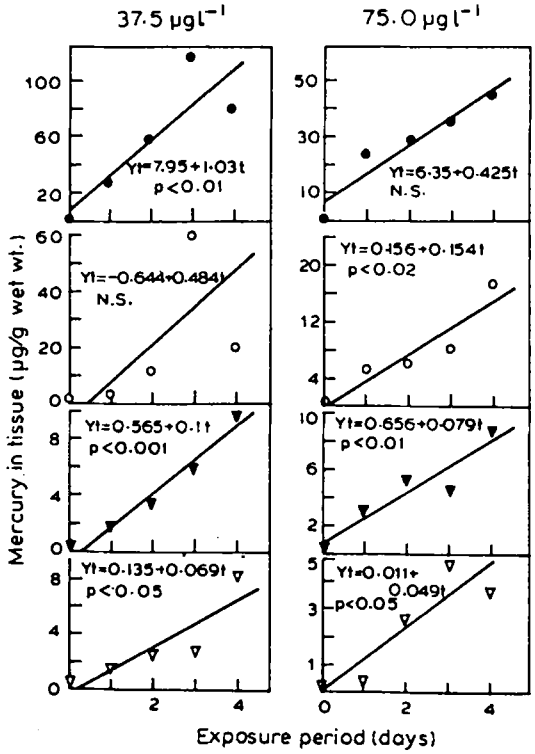

Figure 1. Accumulation of mercury in gills (●), viscera (○), mantle (▼) and muscle + foot (▽) of *P. viridis* exposed to 37·5 and 75 $\mu g l^{-1}$ of $HgCl_2$.

maximum, followed by viscera, mantle and muscle + foot. However, the rate of accumulation showed inverse relationship with metal concentration in all the body parts (table 1). BCF was found to be maximum in gills, followed by viscera, mantle and adductor muscle + foot. In all the body parts BCF decreased with increase in concentration of the exposure medium (table 1).

3.2 *Distribution*

Mussels exposed to 75 $\mu g l^{-1}$ showed frequent valve closure after second day of the exposure. Gills of mussels exposed to 37·5 and 75 $\mu g l^{-1}$ (will be referred hereafter in the text as the 37·5- and 75-group respectively) attained the maximum concentration of 79·92 and 45·48 $\mu g g^{-1}$ wet weight respectively. In both experiments, tissue mercury levels followed the order, gills > viscera > mantle > muscle + foot.

3.3 *Depuration*

Mercury concentration in the different body parts of mussels after 25 days of depuration study is shown in table 1. The linearity of mercury loss and TB/2 in the

Table 1. Tissue concentration ($\mu g\,g^{-1}$ wet wt.), rate of accumulation, BCF and TB/2 of mercury in different body parts of *P. viridis*.

	Gills		Viscera		Mantle		Muscle + foot	
	a	b	a	b	a	b	a	b
Background (0 day)	3·319		0·320		0·126		0·309	
After 4 days of accumulation	79·22	45·48	20·10	17·57	9·92	8·97	8·34	3·72
Rate of accumulation	1·03	0·425	0·485	0·154	0·10	0·07	0·069	0·049
BCF	2177	604	531	227	262	117	207	48
After 25 days of depuration	40·76	25·51	16·22	12·28	4·17	4·38	4·73	2·66
TB/2(days)	31·68	21·5	81·36	64·05	19·94	24·48	38·11	47·78
Mean TB/2 (days)	26·37		72·71		22·11		42·95	

a, 37·5 $\mu g\,l^{-1}$; b, 75 $\mu g\,l^{-1}$.

different body parts are shown in figure 2. After 25 days 37·5-group showed only 19% reduction in viscera. Mantle showed maximum reduction of 53%. In 75-group maximum reduction (51%) attained in mantle, while in muscle and viscera mercury reduction was slow (30%).

In 37·5-group, viscera showed longest TB/2 (81·36 days), while mantle showed shortest TB/2 (19·94 days). In 75-group also viscera showed longest TB/2 (64·05 days), while gills showed shortest TB/2 (21·5 days). The order of mean TB/2 in the different body parts are: viscera > muscle + foot > gills > mantle.

4. Discussion

The mercury accumulation pattern observed in different body parts was found to be different from each other (figure 1). This may be due to the differences in the physiological functions these tissues perform (Eganhouse and Young 1978). In the present study maximum mercury accumulation was observed in the gills followed by viscera, while minimum in the mantle and muscle + foot. Similarly higher accumulation of inorganic mercury was reported in gills, kidney and digestive glands and lower in the adductor muscle and foot of *M. edulis* (King and Davies 1987). High mercury concentrations were reported in the gills of bivalves by several authors (Unlu *et al* 1972; Dillon and Neff 1978; Lakshmanan and Nambisan 1979; Denton and Burdon-Jones 1981). These type of high metal uptake in the gills may be due to their functional activity and position relative to incoming ambient water (Dillon and Neff 1978). Moreover absorption of the metal ions by the mucous sheets may also add to the greater concentration in the gill tissues as reported by Smith *et al* (1975).

After 4 days of exposure, mercury distribution in the different body parts of

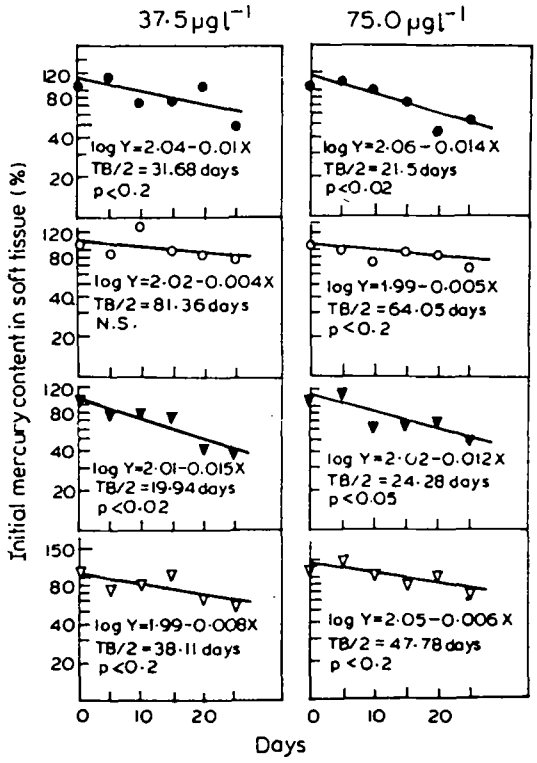

Figure 2. Depuration of mercury from gills (●), viscera (○), mantle (▼) and muscle + foot (▽) of *P. viridis*.

P. viridis indicates that the ability of the animal to accumulate mercury vary from organ to organ as reported in *Mytilus edulis* (Amiard *et al* 1986). In the present study mussels showed a time integrated linear accumulation of mercury (figure 1). Smith *et al* (1975) reported an increase in rate of accumulation of mercury by freshwater clams, with increase in concentration in the medium. However, the reverse was observed in the present study. This may be due to the higher concentration of mercury used in the exposure study (75 $\mu g l^{-1}$). Valve closure and reduction in filtration rate was observed in bivalves exposed to higher concentration of metals (Akberali and Trueman 1985). Mercury concentration of 74 $\mu g l^{-1}$ was found to be inhibiting 50% of the filtration rate (EC_{50}) in *P. viridis* (Krishnakumar 1987).

Results of the depuration study clearly indicate that the ability of different body parts to eliminate mercury vary from each other. Comparatively rapid mercury loss was found in the external tissues like gills and mantle (figure 2). Most probably the excretion of mercury might have occurred across the body surface of gills and mantle, since these tissues are always in direct contact with the ambient medium. Similar rapid decline of mercury was noticed in gills together with an increase in foot and muscle of bivalves (Cunningham and Tripp 1975; Smith *et al* 1975; Fowler *et al* 1978; Denton and Burdon-Jones 1981).

Comparatively less mercury was lost from viscera and muscle + foot of mussels in

the present study. Similarly, high amount of mercury was found to be retained in the visceral organs of *M. galloprovincialis* and *Tapes decussatus* (Miettinen *et al* 1972). In *Rangia cuneata* large amount of mercury was retained in viscera after 8 days of depuration (Dillon and Neff 1978). These types of high mercury retention in viscera may be due to slower release of metal or due to a net internal flow towards viscera as noticed by Dillon and Neff (1978).

The dosage, duration of exposure to a specific metal, duration of depuration and physiological conditions of the animals may affect the TB/2 of the heavy metals (Unlu *et al* 1972; Cunningham and Trip 1973). Metallothioneins were found to be playing a fundamental role in accumulation and elimination of heavy metals in mussels (Roesijadi 1982; Viarengo *et al* 1985). They suggested that the different TB/2 of metals are related to the different capacity of cells to eliminate the metals bound to thioneins or metallothioneins themselves. The slow depuration of mercury from viscera and muscles could be related to the chemical and physical difficulty in removing mercury or mercury containing substances (like metalloprotein) to the exterior (Dillon and Neff 1978; Viarengo 1985).

Cunningham and Tripp (1975) have recognised following categories of heavy metal release: (i) increase in TB/2 with increase in body burden of heavy metals, (ii) stable TB/2, when an equilibrium is maintained with a proportionate increase in the rate of heavy metal loss as its body burden increase, and (iii) decrease in TB/2 with increase in body burden. In the present study, release of mercury from the viscera apparently followed the first category of metal release and from the gills followed the third category.

Acknowledgement

Financial assistance from the Indian Council of Agricultural Research, New Delhi is gratefully acknowledged.

References

Akberali H B and Trueman E K 1985 Effects of environmental stress on marine bivalve molluscs: Review; *Adv. Mar. Biol.* **22** 101–198

Amiard T C, Berthat B, Metayer C and Amiard J L 1986 Contribution to ecotoxicological study of cadmium, copper and zinc in the mussel *Mytilus edulis*. II. Experimental study; *Mar. Biol.* **92** 7–13

Bryan G W 1984 Pollution due to heavy metals and their compounds; in *Marine ecology* Part-3 (ed.) O Kinne (New York: John Willey) vol. 5, p 1229

Buikema A L Jr, Niederlehner B R and Cairns J Jr 1982 Biological monitoring, part. IV. Toxicity testing; *Water Res.* **16** 239–262

The Bureau International Technique du chlor (BITC) 1976 Standardisation of methods for the determination of traces of mercury, Part II Determination of total mercury in materials containing organic matter; *Anal. Chim. Acta* **84** 231–257

Cunningham P A and Tripp M R 1973 Accumulation and depuration of mercury in the American oyster *Crassostrea virginica*: *Mar. Biol.* **20** 14–19

Cunningham P A and Tripp M R 1975 Accumulation, tissue distribution and elimination of $^{203}HgCl_2$ and $CH_3\ ^{203}HgCl_2$ in the tissues of the American oyster *C. virginica*; *Mar. Biol.* **31** 321–324

Davies I M and Pirie J M 1980 Evaluation of a mussel watch project for heavy metals in Scottish coastal waters; *Mar. Biol.* **57** 87–94

Denton G R W and Burdon-Jones C 1981 Influence of temperature and salinity on the uptake, distribution and depuration of Hg, Cd and Pb by the black lip oyster *Saccostrea echinata*; *Mar. Biol.* **64** 317–326

Dillon T M and Neff J M 1978 Mercury and the estuarine clam, *Rangia cuneata* Gray. II. Uptake, tissue distribution and depuration; *Mar. Environ. Res.* **1** 67–76

Eganhouse R P and Young D R 1978 *In situ* uptake of mercury by the intertidal mussel *Mytilus californianus*; *Mar. Pollut. Bull.* **9** 214–217

Fowler S W, Heyrand J and La Rosa J 1978 Factors affecting methyl and inorganic mercury dynamics in mussels and shrimps; *Mar. Biol.* **46** 267–276

Gardner R and Riley J P 1974 Mercury in the Atlantic around Iceland; *J. Cons. Inst. Explor. Mer.* **35** 202–204

Goldberg E D, Bowen V T, Farrington J W, Harvey G, Martin J H, Parker P L, Riseborough R W, Robertson W, Schneider E and Gamble E 1978 The mussel watch: *Environ. Conserv.* **5** 101–125

King D G and Davies I M 1987 Laboratory and field studies of the accumulation of inorganic mercury by the mussel *M. edulis* (L): *Mar. Pollut. Bull.* **18** 40–45

Krishnakumar P K 1987 *Physiological effects of some heavy metals on Perna viridis (Linnaeus)*, Ph.D. thesis, Cochin University of Science and Technology, Cochin

Krishnakumar P K, Damodaran R and Nambisan P N K 1987a Carotenoid content and tolerance of green mussel *Perna viridis* to acute mercury and zinc toxicity: *Indian J. Mar. Sci.* **16** 155–157

Krishnakumar P K, Damodaran R and Nambisan P N K 1987b Acute toxicity of selected heavy metals to green mussel *Perna viridis*; *Indian J. Mar. Sci.* **16** 263–264

Lakshmanan P T and Nambisan P N K 1979 Accumulation of mercury by the mussel *P. viridis*; *Curr. Sci.* **48** 672–674

Martincic D, Nurenberg H W, Stoeppler M and Bremica M 1984 Bioaccumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Seas); *Mar. Biol.* **81** 177–188

Miettinen J K, Heyraud M and Keckes S 1972 Mercury as a hydrospheric pollutants. II. Biological half time of methyl mercury in four Mediterranean species, a fish, a crab, and two molluscs; in *Marine pollution and sea life* (ed.) M Ruivo (London: Fishing News Books Ltd) pp 295–298

Phillips D J H 1977 The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environment—a review; *Environ. Pollut.* **13** 281–317

Renfro W C 1973 Transfer of ^{65}zinc from sediment by marine polychaete worms; *Mar. Biol.* **21** 305–316

Roesijadi G 1982 Uptake and incorporation of mercury into mercury binding proteins of gills of *M. edulis* as a function of time; *Mar. Biol.* **66** 151–157

Simkiss K and Mason A Z 1984 Cellular responses of molluscan tissues to environmental metals; *Mar. Environ. Res.* **14** 1–4

Smith A L, Green R H and Lutz A 1975 Uptake of mercury by fresh water clams (family Unionidae); *J. Fish. Res. Board Can.* **32** 1297–1303

Unlu M Y, Heyrand M and Keckes S 1972 Mercury as a hydrospheric pollutants. I Accumulation and excretion of $HgCl_2$ in *Tapes decussatus* L.; in *Marine pollution and sea life* (ed.) M Ruivo (London: Fishing News Books Ltd.) pp 292–295

Viarengo A 1985 Biochemical effects of trace metals; *Mar. Pollut. Bull.* **16** 153–158

Viarengo A, Palmero S, Zanicchi G, Capelli R, Vaissiere R and Orunesu M 1985 Role of metallothionein in Cu and Cd accumulation and elimination in the gills and digestive glands of *M. galloprovincialis*; *Mar. Environ. Res.* **16** 23–26