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Bioaccumulation and Depuration of Some Trace Metals in the Mussel, *Perna viridis* (Linnaeus)

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Bivalves are well known for their ability to concentrate heavy metals in their tissue from environmental water. Substantial enrichment of heavy metals in bivalve molluscs have been reported (Eustace 1974; Ratkowsky et al. 1974; Phillips 1976; Talbot and Cheguwidden 1982; Eisenberg and Topping 1984; Pfeiffer et al.1985). Experimental studies on the accumulation of these pollutants by molluscs have been extensively conducted (Brookes and Rumsby 1965; Pringle et al. 1968; Ayling 1974; Aoyamma et al. 1978). The depuration of accumulated metals in a toxicant free medium has also been studied (Cunningham and Tripp 1973 & 1975; Schulz-Baldes 1974; Simpson 1979; Riisgaord 1984). Bivalve molluscs may form useful tools in monitoring heavy metal pollution. However, such studies are scant in tropical species (D'silva and Kureishy 1978; Lakshmanan and Nambisan 1979 & 1985). This paper reports the bioaccumulation and depuration of Hg, Cu, Zn and Pb by the mussel Perna viridis (Linnaeus) from seawater and explores its suitability as an indicator organism for metal pollution.

MATERIALS AND METHODS

The mussels were collected from the sea near Cochin and acclimatized in the laboratory for 3 to 4 days (water characteristics: salinity = 25×10^{-3}, temperature = $30 \pm 2^{0}C$, pH = 7.4 ± 0.2, dissolved O_2 = $95 \pm 5\%$ saturation). Animals of uniform shell length (average 61.5mm) were taken for the study.

Uptake and release experiments were conducted in static replenishing seawater systems following standard methods. Ten animals were exposed in each glass aquarium of 10L capacity containing 5L test medium. The metals studied (viz., Hg, Cu, Zn & Pb) were introduced as their aqueous salt solutions prepared from $HgCl_2$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ and $Pb(NO_3)_2$ (BDH Analar grade), respectively. Separate experiments were conducted for each metal. Controls were maintained through out. The test medium was aerated

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and renewed once every 24 h. The animals were not fed during the experiment. Bioconcentration was measured in the exposed mussels at intervals of 2, 4 and 6 days. Three animals were taken from each tank and the total metal content in the soft tissue were determined by Flame Atomic Absorption spectrophotometer (Varian Techtron 1100 or Perkin Elmer 2380) following the AOAC (1975) method. Mercury was estimated using a Mercury Analyser (ECIL Model MA 5800) after wet digesting the tissue in Bethge's apparatus using con. HNO_3 and con. H_2SO_4 (4:1 v/v). The distribution pattern of the metals in the various body components was determined after dissecting the treated animals into mantle (mantle + gonad), muscle (adductor + foot), gills and the remaining soft parts were taken as visceral mass, at definite intervals of time (Table 2). Replicates were run in all cases.

Depuration of trace metals in P. viridis was studied by removing 3 animals each after a 4 day continuous exposure to specified concentrations of the metal ions (0.10 ppm Hg, 0.10 ppm Cu, 0.50 ppm Zn and 1.0 ppm Pb) and transferring them to toxicant free seawater medium. They were sampled periodically to determine the time required to eliminate the accumulated metal. The data were statistically analysed by student t-test according to Snedecor and Cochran (1967) and significant difference between control and experimental groups were found ($p \leq 0.05$ to $p \leq 0.001$).

RESULTS AND DISCUSSION

The results are presented in Tables 1 and 2 and Figure 1. Mercury content in the soft parts of the mussels exposed to different concentrations of Hg^{2+} (0.05, 0.10 & 0.20 ppm) increased significantly and reached a value of 9.756 ug/g (wet weight) in animals exposed for 2 days to 0.2 ppm Hg against a background level of 0.08 ug/g. The corresponding values in animals exposed to 0.05 and 0.10 ppm Hg were 2.501 and 6.004 ug/g respectively. The amount of Hg in the tissue was very much dependent on the concentration in the medium and length of exposure (Table 1). At higher concentrations of Hg there was rapid initial uptake. The rate decreased subsequently. Bio-concentration factor (C.F.) was the highest in 0.05 ppm Hg (404 at 6 days exposure) and there was gradation in C.F. with increasing metal concentration (Table 1).

Copper content in the mussel, after exposure to various concentrations (0.025, 0.05 & 0.1 ppm), ranged from 11.38 to 22.44 ug/g wet wt. in 6 days period. The C.F. for Cu varied from 224 (in 0.10 ppm) to 455 (in .025 ppm).

Zinc content in mussels exposed to Zn^{2+} increased at all concentrations, the highest being in 2.0 ppm solution. At this concentration, the tissue Zn level registered a four-fold increase at the end of 6 days. The highest body burden of Zn was 53.63 ug/g wet wt. (control : 13.61 ug/g). The C.F. varied from 27 to 41, the highest being at 0.5 ppm and the lowest at 2.0 ppm Zn. The efficiency of Zn uptake by the mussel is rather low.

Table 1. Accumulation and Bioconcentration factors of trace metals in soft tissue by the mussel P. viridis exposed to metal solution in sea water of habitat salinity

Metal ion	Concentration in exposure water (ppm)	Tissue concentration of metals, ug/g wet wt. ($\bar{x}$ ± S.D) after different periods			Bioconcentration factor at 6 days
		2 days	4 days	6 days	
	Control	0.09±0.01	-	-	-
2+	0.05	2.50±0.24	10.56±0.81	20.18±0.97	404
Hg	0.10	6.00±0.32	15.88±0.89	21.29±1.05	213
	0.20	9.76±0.72	21.63±1.22	-	126 (at 5 days)
	Control	3.29±0.17	-	-	-
2+	0.025	4.24±0.39	8.14±0.46	11.38±0.53	455
Cu	0.050	5.33±0.46	10.83±0.30	14.93±0.53	299
	0.100	6.91±0.59	15.10±0.93	22.44±0.75	224
	Control	13.61±0.43	-	-	-
2+	0.50	15.99±0.45	18.19±0.48	20.41±0.59	41
Zn	1.00	22.94±0.68	30.15±0.86	39.24±0.91	39
	2.00	28.88±0.88	42.17±1.04	53.63±1.46	27
	Control	1.86±0.08	-	-	-
2+	0.25	35.08±1.22	55.10±1.0	79.77±1.3	319
Pb	0.50	61.27±1.67	104.33±3.8	140.74±4.2	281
	1.00	158.18±2.80	279.85±4.0	398.08±4.8	398
	2.00	357.12±4.40	663.79±6.4	917.88±7.1	459

Table 2. The accumulation pattern of trace metals in the various body components and concentration factor in P. viridis exposed to metal ions in seawater of habitat salinity

Metal ion Conc. ppm	Organ/tissue % of whole soft parts	Metal, ug/g control	Component residue metals, (ug/g wet. $\bar{x}$ ± S.D.) after different periods			Bioconc. factor at 6 days
			2 days	4 days	6 days	
2+ Hg (0.1 ppm)	Muscle (22.8)	0.08±0.00	2.80±0.10	6.93±0.20	10.49±0.19	105
	Mantle (37.7)	0.08±0.01	2.76±0.11	6.73±0.11	10.10±0.37	101
	Gills (13.7)	0.20±0.01	23.05±1.05	32.31±1.81	45.70±0.85	457
	Viscera (25.6)	0.08±0.00	2.64±0.08	7.22±0.21	12.53±0.33	125
2+ Cu (0.1 ppm)	Muscle	4.97±0.20	6.88±0.33	7.303±0.30	10.85±0.20	109
	Mantle	3.95±0.18	6.06±0.33	10.88±0.43	14.29±0.50	143
	Gills	8.48±0.30	14.67±0.51	22.22±0.63	30.89±0.80	309
	Viscera	5.05±0.20	10.46±0.35	16.39±0.65	22.22±0.74	222
2+ Zn (1.0 ppm)	Muscle	18.35±0.46	28.13±1.04	33.90±0.78	36.12±1.04	36
	Mantle	18.08±0.46	20.22±0.54	24.04±0.89	26.54±0.86	27
	Gills	28.67±0.72	33.94±0.87	42.04±1.10	46.00±1.73	46
	Viscera	25.03±0.52	31.45±0.74	37.26±1.19	41.57±1.15	42
2+ Pb (2.0 ppm)	Muscle	1.81±0.04	163.9±4.7	284.4±6.6	416.4±8.3	208
	Mantle	1.18±0.04	81.9±5.3	155.3±5.4	201.4±5.0	101
	Gills	2.50±0.09	369.7±4.2	706.6±8.8	990.8±12.8	495
	Viscera	1.64±0.04	194.5±2.3	393.9±6.2	556.8±6.4	278

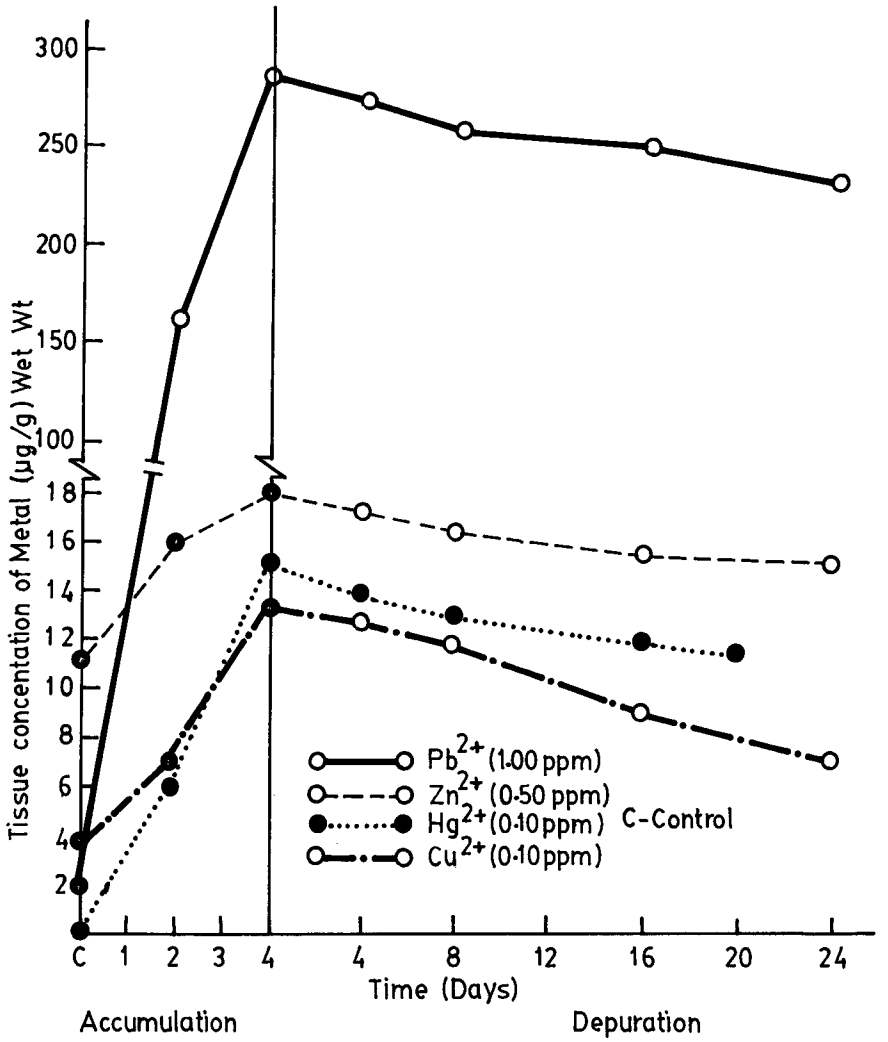

Figure.1. Uptake and release of Cu, Hg, Zn and Pb by P. viridis as a function of time.

In contrast to Zn, a very high biomagnification potential was exhibited for Pb by the mussel. The whole soft-tissue content of Pb in the animal varied from 80 (in 0.25 ppm) to 918 ug/g wet wt. (in 2.0 ppm) in 6 days period (control = 1.86 ug/g). It is significant to note that, unlike the other metals, the C.F. continuously increased with increasing concentration of Pb, showing greater uptake efficiency. The C.F. varied from 281 to 459.

The distribution pattern of the four metal ions in the various body components along with the C.Fs are presented in Table 2. Gills of the mussel form the major site of accumulation for all the metals. This was followed by viscera. Accumulation in the muscle/mantle region was relatively low. The C.Fs attained by the gills for the four metals were 457 (Hg), 309 (Cu), 46 (Zn) and 495 (Pb). The order of preference of metals by the body components (based on C.F.) was as follows:

Hg	:	Gills	>>	Viscera	> Muscle >	Mantle
Cu	:	Gills	>	Viscera	> Mantle >	Muscle
Zn	:	Gills	>>	Viscera	>> Muscle >	Mantle
Pb	:	Gills	>>	Viscera	>> Muscle >	Mantle

The results of depuration studies are presented in Fig 1. The depuration of accumulated Hg by the mussel was a slow process in a metal free medium. Only 23.3% of the accumulated metal was lost during a 20 day period. Mercury content declined from 15.22 to 11.67 ug/g (control = 0.086 ug/g). The rate of release of copper was faster in the mussel in a pollutant free medium. Thus, Cu content declined from an exposure value of 13.34 ug/g to 6.96 ug/g during a clearance period of 24 days (control = 3.71 ug/g). In all, 63.3% of Cu was eliminated in the process. The rate of depuration of Zn also was slow. About 40% of the accumulated metal was lost during a depuration period of 24 days. The metal content reached to a value of 15.27 from 17.93 ug/g during the experimental period (control Zn = 11.25 ug/g). In 1.0 ppm Pb-exposed animals the metal content reached to a value of 286.64 ug/g in 4 days period (control = 1.91 ug/g). Although the rate of uptake of Pb was a rapid process, release of accumulated Pb was rather slow. Thus, as much as 81.5% of Pb was retained in the tissue at the end of 24 days depuration. The final tissue burden of Pb was 237.05 ug/g. Total purification of metals could not be achieved in the mussel during 20/24 days.

The results of the experiment clearly indicate the ability of the mussel P. viridis to accumulate appreciable quantities of trace metals from the environmental water. The amounts of metals in the tissue were very much dependent on the exposure concentration and length of time. The decreasing CFs with increasing metal concentration may be due to the deactivating effect of the metal ions on the animal at higher concentrations. Sequestering of the metals and subsequent release of additional metals could also lead to lower CFs. The low bioavailability of the metals may be another reason. The rapid and continuous accumulation of Pb even at higher concentrations may be attributed to its non-lethality to the animal (non-lethal up to 10 ppm).

The high concentrations of trace metals found in the gill tissues of the mussel is explained as due to the filter feeding of the mollusc whereby uptake occurs across this organ. Brookes and Rumsby (1965) had observed rapid accumulation and large concentration of Hg and other trace metals in gill tissues of some of the New Zealand bivalves (scallops, mussels and oysters).

Similar observations have also been made by Smith et al. (1975) and Cunningham and Tripp (1975) in certain molluscs. The relatively high content of Cu found in the viscera of the mussel may be due to the high rate of uptake and subsequent loss of Cu in the fecal matter. Brookes and Rumsby (1965) also observed a similar phenomenon.

The slow rate of metal release in P. viridis indicates the possibility of forming strong metal complexes with the tissue components. However, there was a steady release of Pb by the animal throughout the experiment. Pringle et al. (1968) found that in Crassostrea virginica lead loss was characterised by an increase in the $B_{\frac{1}{2}}$ value with an increase in the body burden of Pb. When tissue Pb was very high little Pb was lost and they suggested a permanent deposition of the metals. Schulz-Baldes (1974) reported that the rate of loss of Pb in the mussel Mytilus edulis was very much dependent on the internal Pb concentration in the animal. The slow rate of release of Pb observed in the present study may be explained similarly.

The present study confirms the ability of the mussel P. viridis to reflect the environmental concentrations of mercury, copper, zinc and lead in their body tissues. The metal levels also declined in the animal in a pollutant-free medium. This suggests the mussel P. viridis as a potential sentinal organism in environmental monitoring of trace metal pollution.

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REFERENCES

AOAC (1975) Association of Official Analytical Chemists, Official Methods of Analysis. 12th edn. Mc Graw-Hill, New York.

Aoyamma I, Yoshinobu I, Yoriteru I (1978) Experimental study of the concentration process of trace element through a food chain from the view point of Nutrition ecology. Water Res 12: 831-836.

Ayling GM (1974) Uptake of cadmium, zinc, copper, lead and chromium in the Pacific Oyster, Crassostrea gigas, grown in the Tamer River Tasmania. Water Res 8: 729-738.

Brookes RR, Rumsby MG (1965) Biogeochemistry of trace element uptake by some New Zealand bivalves. Limnol Oceanogr 10: 521-527.

Cunningham PA, Tripp MR (1973) Accumulation and depuration of mercury in the American Oyster, Crassostrea virginica. Mar Biol 20: 14-19.

Cunningham PA, Tripp MR (1975) Factors affecting the accumulation and removal of mercury from tissues of the American Oyster, Crassostrea virginica. Mar Biol 31: 311-319.

D'silva C, Kureishy TW (1978) Experimental studies on the accumulation of copper and zinc in the Green Mussel. Mar Pollut Bull 9: 187-190.

Eisenberg M, Topping JJ (1984) Trace metal residues in shellfish from Maryland Waters, 1976-1980. J Environ Sci Health 19B: 649-672.
Eustace IJ (1974) Zn, Cd, Cu and Mn in species of finfish and shellfish caught in the Derwent Estuary, Tasmania, Aust J Mar Freshwat Res 25: 209-220.
Lakshmanan PT, Nambisan PNK (1979) Accumulation of mercury by the mussel, Perna viridis (Linnaeus). Curr Sci 48: 672-674.
Lakshmanan PT, Nambisan PNK (1985) Uptake and loss of mercury in three bivalve molluscs, viz. Perna viridis (Linnaeus), Villorita cyprinoides var. cochinensis and Meretrix casta (Chemnitz). Proceedings of the symposium on Harvest and Post harvest Technology of Fish. Society of Fisheries Technologists (India) PP 419-423.
Pfeiffer WC Drude de Lacendra L, Fiszman M, Lima NRW (1985) Heavy metals in seafood of Sepetila bay, Riode Janeiro CIENC CULT 37: 297-302.
Phillips DJH (1976) The common mussel, Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper II. Relation of metals in the mussels to those discharged by the industry. Mar Biol 38: 71-80.
Pringle BH, Hissang DE, Katz EL, Mulacoka ST (1968) Trace metal accumulation by estuarine mollusks. J Sanit Engng Div Am Soc Civ Engrs 94: 455-475.
Ratkowsky DA, Thrower SJ, Eustace IJ, June O (1974) A numerical study of the concentration of some heavy metals in Tasmanian oysters. J Fish Res Bd Can 31: 1165-1171.
Riisgoard Hu (1984) Mercury pollution in the waters around Harbooere Tange and Limfjord Denmark. Mar Pollut Bull 15: 129-133.
Schulz-Baldes M (1974) Lead uptake from seawater and food and lead loss in the common mussel Mytilus edulis. Mar Biol 25: 177-193.
Simpson RD (1979) Uptake and loss of zinc and lead by mussels (Mytilus edulis) and relationship with body weight and reproductive cycle. Mar Pollut Bull 10: 7-78.
Smith AL, Green RH, Lutz A (1975) Uptake of mercury by freshwater clams (Family unionidae). J Fish Res Bd Can 32: 1297-1303.
Snedecor GW, Cochran WG (1967) Statistical methods 6th edn. Oxford and IBH Publishing Co, New Delhi.
Talbot V, Chegwidden A (1982) Cadmium and other heavy metal concentrations in selected biota from Cockburn Sound, Western Australia. Aust J Mar Freshwat Res 33: 779-788.
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