

## Uptake and loss of mercury, cadmium and lead in marine organisms

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Experiments were conducted on *Tilapia mosambica*, *Perna viridis* and *Villorita cyprinoides* on the uptake and loss of Hg, Cd and Pb in various tissues of these organisms. Hg was found to be highly toxic to the clams as compared to the fish and mussels. Pb was found to be the least toxic. Hg was found to accumulate in greater amounts as compared to Cd and Pb. Preferential accumulation and subsequent retention was observed for each metal to a particular tissue, thus Hg showed affinity towards muscles, Cd towards liver and Pb towards gills. Similar trends of distribution were observed in tissues of fishes collected from the Indian coast except that Pb showed affinity towards gills as well as kidney. The rate and degree of depuration varied for different metals. Since, the metals concentrations found in the tissues of these organisms reflect the external concentrations, makes them ideal tools for monitoring environmental levels.

The increasing occurrence of heavy metals through the disposal of complex effluents has stimulated many studies on the toxicity and uptake of these elements by the marine biota<sup>1-4</sup>. It has been observed that through biological amplification, some aquatic organisms may build-up concentrations of metals present in low concentrations in the environment to levels which are harmful to the organisms and exceed public health standards.

Our estuarine waters are being increasingly burdened by metal pollution and if such pollution continuous large populations of fish and shell fish may be damaged. Very low concentrations prevailing at times may not affect the organisms directly, but may render them more susceptible to diseases and poor health condition. This study offers a realistic view on the metal accumulation and depuration by some estuarine organisms of economic importance to the local people. Secondly the distribution of Hg, Cd and Pb in the various tissues of *Perna viridis*, *Villorita cyprinoides* and *Tilapia mosambica* were determined and the accumulation trend was compared with earlier field studies<sup>5-8</sup> done in our laboratory on fishes collected from the Indian coast. Certain sublethal effects which became apparent during the study period were discussed.

### Materials and Methods

The test species *T. mosambica*, *P. viridis* and *V. cyprinoides* were collected from their natural habitats

and acclimatized in 1000L fibre glass tanks for a period of 10 days in running sea water. Fish, mussels and clams were held in separate tanks and exposed to  $0.1 \pm 0.003$  ppm of Hg, Cd and Pb. These concentrations are usually encountered at industrial and municipal discharge sites. Test solutions were prepared from HgCl<sub>2</sub>, CdCl<sub>2</sub> and PbNO<sub>3</sub> salts. Before the start of the experiment samples were taken to check initial tissue levels of these metals. Subsequent samples were taken after every 48 hr period. The animals were fed on fish meal and mixed algal culture. The accumulation period lasted for 12 days at the end of which the organisms were kept in running sea water to determine the rate of depuration of metal. A control was run simultaneously. Metal concentrations were analysed on Hilger and Watts atomic absorption spectrophotometer using methods described earlier<sup>5,9</sup>. Methods were checked by analysing reference material for metals (obtained from Research Council of Canada) and the recovery obtained was above 91%. Coefficient of variation for all these metals ranged from 3-11% in replicate analysis.

### Results

Metal concentrations in various tissues were treated statistically in order to reduce the effects of random errors. The data for uptake and loss of metal by the tissues of the organisms were treated together for this purpose and fed to the computer. A second order polynomial relation could be deduced to arrive at the best fit. These curves for the metal concentration in various tissues are presented in Figs 1-3.

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**Mercury**—Mercury was found to be highly toxic to the clams as compared to the fish and mussels. 100% mortality of clams was observed within 48 hr at 0.1 ppm concentration. Impairment of feeding and sluggish behaviour was observed in fish and reduced byssus activity in mussels. Control organisms were healthy and normal throughout the study period. The reduced behavioural activities gained momentum and came to normal at the end of depuration period. Accumulation and depuration of Hg from gills, muscles and liver of *Tilapia* are presented in Figs. 1(A-C). The gills accumulated higher amounts of Hg with a maximum value of 21.68 ppm on the 14th day which came down to 8.75 ppm at the end of the experiment suggesting accumulation of Hg with time and its release in clean sea water. In muscles the rate of uptake reached a maximum of 1.79 ppm on the 18th day of the experiment and 1.47 ppm after depuration. This delayed maximum and marginal loss after exposure to clean sea water indicating incorporation of Hg with muscle proteins. No accumulation was observed in the liver probably because of its non affinity to lipids. The rate of uptake and losses are calculated and presented in Tables 1 and 2 for each organ.

In mussels (Fig. 1D-F) maximum amount of Hg was taken up by the adductor muscle followed by mantle and byssus gland, the maximum concentrations levels being 37.27, 26.25 and 13.96 ppm Hg respectively. The slow rate of depuration observed in adductor muscles may be due to methylation of Hg with muscle proteins. Rapid accumulation was observed in clams. The initial concentration of Hg in the soft parts of clams was 0.001 ppm which reached a level of 4.11 ppm within 48 hr causing 100% mortality.

**Cadmium**—Cadmium was not found to be toxic to *Tilapia* and mussel. 100% mortality was observed in clams after 5 days of exposure. Slow rate of feeding was observed in fish. Byssal thread formation was normal in mussels. The uptake and loss of Cd by gills, muscles and liver of *Tilapia* are given in Fig. 2A-C. The rate of accumulation of Cd is faster in liver as compared to muscles and gills. However the rate of depuration is slow, causing retention of Cd in the liver. The probable reason for this is the association of Cd with lipids and the metallothionein compounds. Cd metallothionein has been reported in the liver of the sea lion and plaice<sup>10,11</sup>.

Table 1—Accumulation rate ( $\mu\text{g}/\text{days}$ ) of Hg, Cd and Pb in different tissues of *T. mosambica* and *P. viridis*

Days		1	2	4	6	8	10	12
<i>Tilapia</i>								
Hg	Muscles	0.19	0.186	0.16	0.139	0.116	0.09	0.069
	Gills	2.3	2.13	1.78	1.44	1.09	0.75	0.4
	Liver	0.0005	0.0004	0.0004	0.0003	0.0003	0.0002	0.00008
Cd	Muscles	0.103	0.094	0.078	0.062	0.045	0.029	0.013
	Gills	0.125	0.107	0.088	0.070	0.051	0.033	0.015
	Liver	3.28	2.19	1.96	1.72	1.48	1.24	1.00
Pb	Muscles	0.017	0.014	0.01	0.006	0.001	0.003	-0.007
	Gills	0.282	0.264	0.228	0.193	0.157	0.122	0.086
	Liver	0.31	0.28	0.24	0.19	0.15	0.12	0.06
<i>Mussel</i>								
Hg	Byssus	2.042	1.888	1.576	1.264	0.953	0.641	0.328
	Mantle	3.485	3.219	2.687	2.156	1.624	1.093	0.561
	A. Muscle	5.128	4.794	4.124	3.454	2.785	2.115	1.446
Cd	Byssus	1.286	1.183	0.976	0.769	0.562	0.355	0.140
	Mantle	0.914	0.85	0.722	0.593	0.465	0.336	0.200
	A. Muscle	1.288	1.195	1.007	0.819	0.632	0.444	0.257
Pb	Byssus	0.549	0.503	0.412	0.321	0.23	0.138	0.047
	Mantle	1.007	1.683	1.435	1.186	0.938	0.689	0.441
	A. Muscle	1.76	1.639	1.398	1.157	0.916	0.675	0.433

The accumulation of Cd in various tissues of *P. viridis* did not show much variations. Fig. 2(D-F). The maximum level of Cd attained in adductor muscle, mantle and byssus gland was 10.23, 7.7 and 9.2 ppm. At the end of the depuration period the levels were 4.35, 4.2 and 1.2 ppm respectively.

100% mortality was observed in clams on 5th day after the start of the experiment. The initial concentration was 0.18 ppm and the final concentration was 0.76 ppm.

**Lead**—Lead was found to be the least toxic of the three metals under study. The gills and liver show similar trends of accumulation with maximum concentration of Pb accumulated being 3.2 and 2.84 ppm respectively. However, liver of *Tilapia* was able to purge Pb very efficiently as compared to gills (Fig. 3 A-C). The pattern of accumulation of Pb by muscles was irregular.

The uptake of Pb by *P. viridis* shows maximum accumulation in the adductor muscle 15.93 ppm, followed by mantle 12.92 ppm and byssus gland 7.06 ppm (Fig. 3 D-F).

Mortality in clams was observed only on the 18th day after the start of the experiment caused by high levels of Pb 18.8 ppm in the soft parts of the clams.

## Discussion

The result in Table 1 and Figures 1-3 suggest that Hg is accumulated in greater amounts than Cd and Pb, except in the liver of *T. mosambica*. The retention of Hg in the muscles of *Tilapia* suggest the possible linkage with protein and subsequent methylation of Hg. Cunningham and Tripp<sup>12</sup> observed that the concentration of Hg declined only during the first 18 to 30 days of the experiments on depuration, while for the next 160 days, the concentration of Hg in mussels remained constant, indicating a permanent fixation of Hg after methylation.

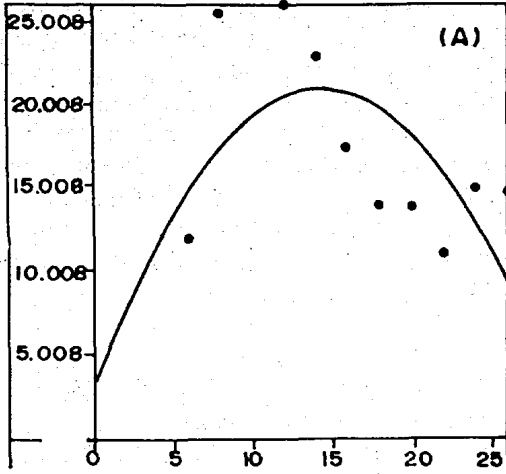
Pringle *et al.*<sup>13</sup> proposed that the rate of loss is determined by the rate of uptake. The results here suggest that the rate of loss is directly related to the time of exposure and the concentration of the particular metal reached in the various tissues. Schulz Baldez<sup>2</sup> had indicated that in uptake and depuration studied the loss of Pb indicates the presence of exchange equilibrium. He also pointed out that the rate of uptake may always exceed that of loss so that a permanent fixation of Pb could occur. In the gills of *T. mosambica* the rate of loss is much slower than the rate of uptake.

Table 2—Depuration rate ( $\mu\text{g}/\text{days}$ ) of Hg, Cd and Pb in different tissues of *T. mosambica* and *P. viridis*

	Days	14	16	18	20	22	24	26
<i>Tilapia</i>								
Hg	Muscles	0.046	0.023	0.00	-0.02	-0.046	-0.069	-0.092
	Gills	0.06	-0.28	-0.63	-0.97	-1.32	-1.66	-1.01
	Liver	0.00	-0.0008	-0.0002	-0.0002	-0.0003	-0.0004	-0.00048
Cd	Muscles	-0.003	-0.02	-0.036	-0.053	-0.069	-0.005	-0.102
	Gills	-0.003	-0.022	-0.04	-0.050	-0.077	-0.095	-0.114
	Liver	0.76	0.52	0.28	0.04	0.19	-0.44	-0.67
Pb	Muscles	-0.012	-0.016	-0.02	-0.025	-0.03	-0.034	-0.038
	Gills	0.051	0.015	-0.02	-0.056	-0.091	-0.127	-0.162
	Liver	0.01	-0.03	-0.07	-0.12	-0.16	-0.21	-0.39
<i>Mussel</i>								
Hg	Byssus	0.016	-0.295	-0.607	-0.919	-1.221	-1.543	-1.355
	Mantle	0.029	-0.502	-1.032	-1.565	-2.09	-2.628	-3.159
	A. Muscle	0.776	0.106	-0.563	-1.232	-1.902	-2.572	-3.242
Cd	Byssus	-0.058	-0.265	-0.472	-0.678	-0.885	-1.092	-1.299
	Mantle	0.079	-0.048	-0.177	-0.305	-0.434	-0.562	-0.691
	A. Muscle	0.069	-0.118	-0.306	-0.493	-0.681	-0.868	-1.056
Pb	Byssus	-0.043	-0.135	-0.226	-0.317	-0.408	-0.499	-0.590
	Mantle	0.193	-0.055	-0.304	-0.552	-0.801	-1.049	-1.297
	A. Muscle	0.192	-0.049	-0.29	-0.531	-0.773	-1.014	-1.255

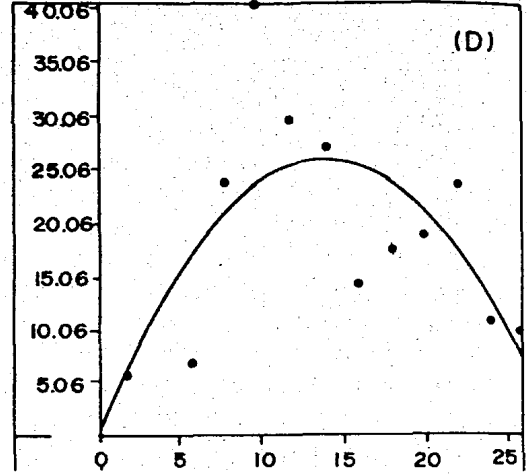
$$C_{Hg} = 3.0277 + 2.4775 T - 0.0863 T^2 (\pm 7.644)$$

$$T_{max} = 14.37$$



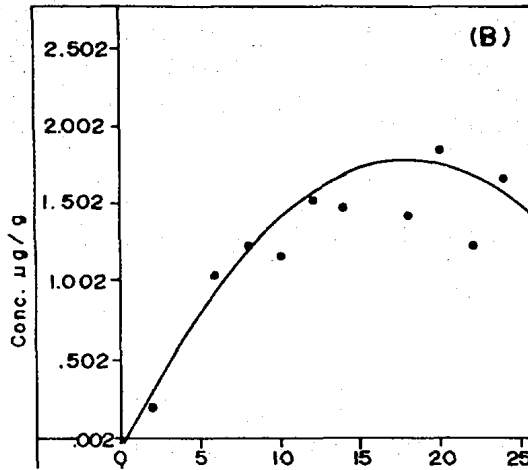
$$C_{Hg} = 0.3746 + 3.751 T - 0.13297 T^2 (\pm 7.6086)$$

$$T_{max} = 14.0$$



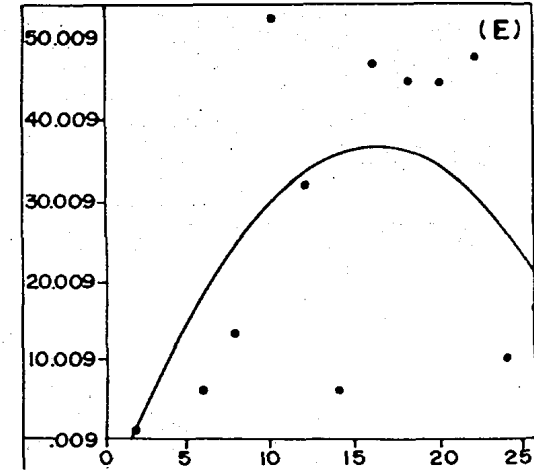
$$C_{Hg} = 0.0978 + 0.20897 T - 0.0038 T^2 (\pm 0.38)$$

$$T_{max} = 18$$



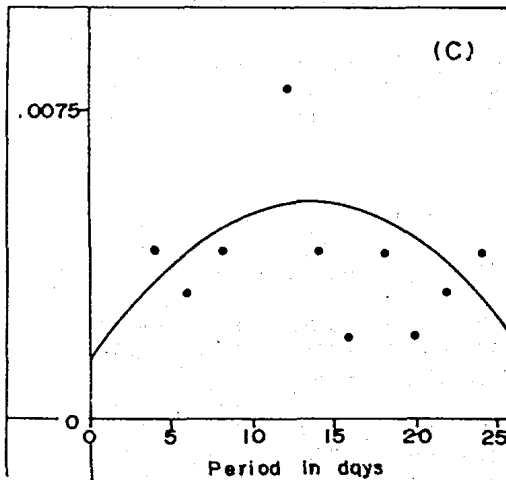
$$C_{Hg} = -8.0944 + 5.4633 T - 0.1674 T^2 (\pm 14.97)$$

$$T_{max} = 16.3$$



$$C_{Hg} = 0.0014 + 0.0056 T - 0.00002 T^2 (\pm 0.00211)$$

$$T_{max} = 14.13$$



$$C = -1.5126 + 2.2007 T - 0.07803 T^2 (\pm 3.265)$$

$$T_{max} = 14$$

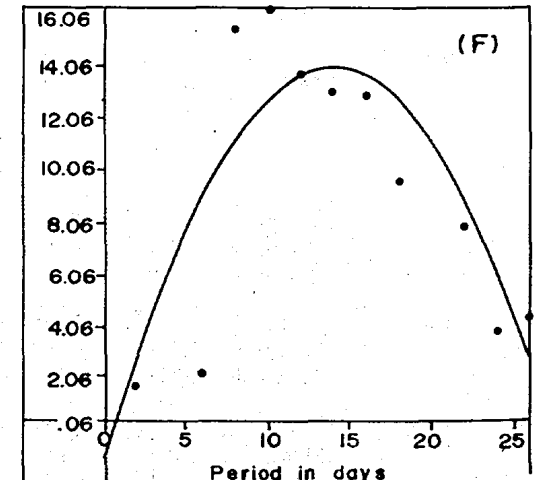


Fig. 1—Uptake and loss of Hg in various tissues of *T. mosambica* (A, gills; B, muscle; C, liver; D, mantle; E, adductor muscle and F, byssus gland)

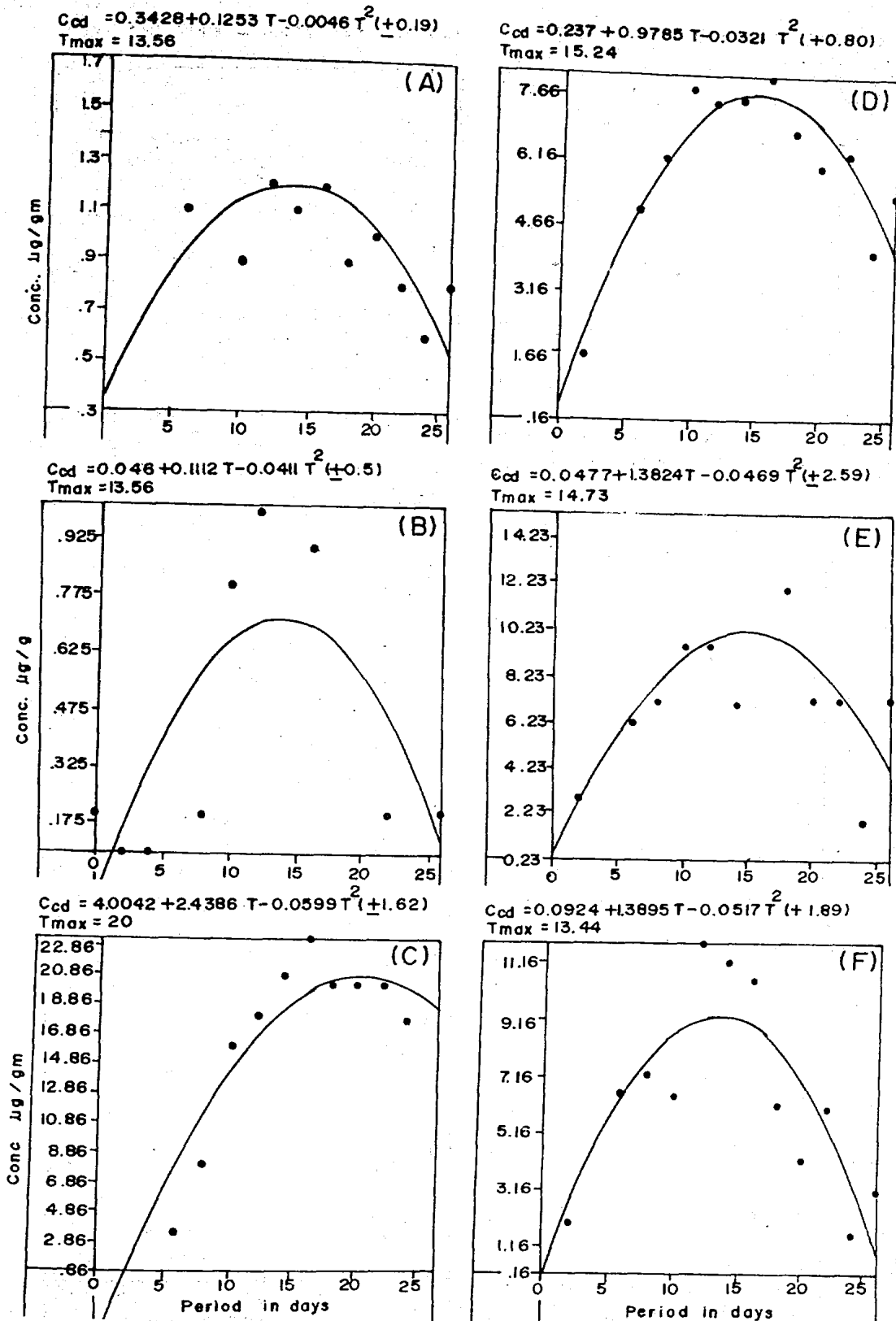


Fig. 2—Uptake and loss of Cd in various tissues of *T. mosambica* and *P. viridis* (A, gills; B, muscle; C, liver; D, mantle; E, adductor muscle and F, byssus gland)

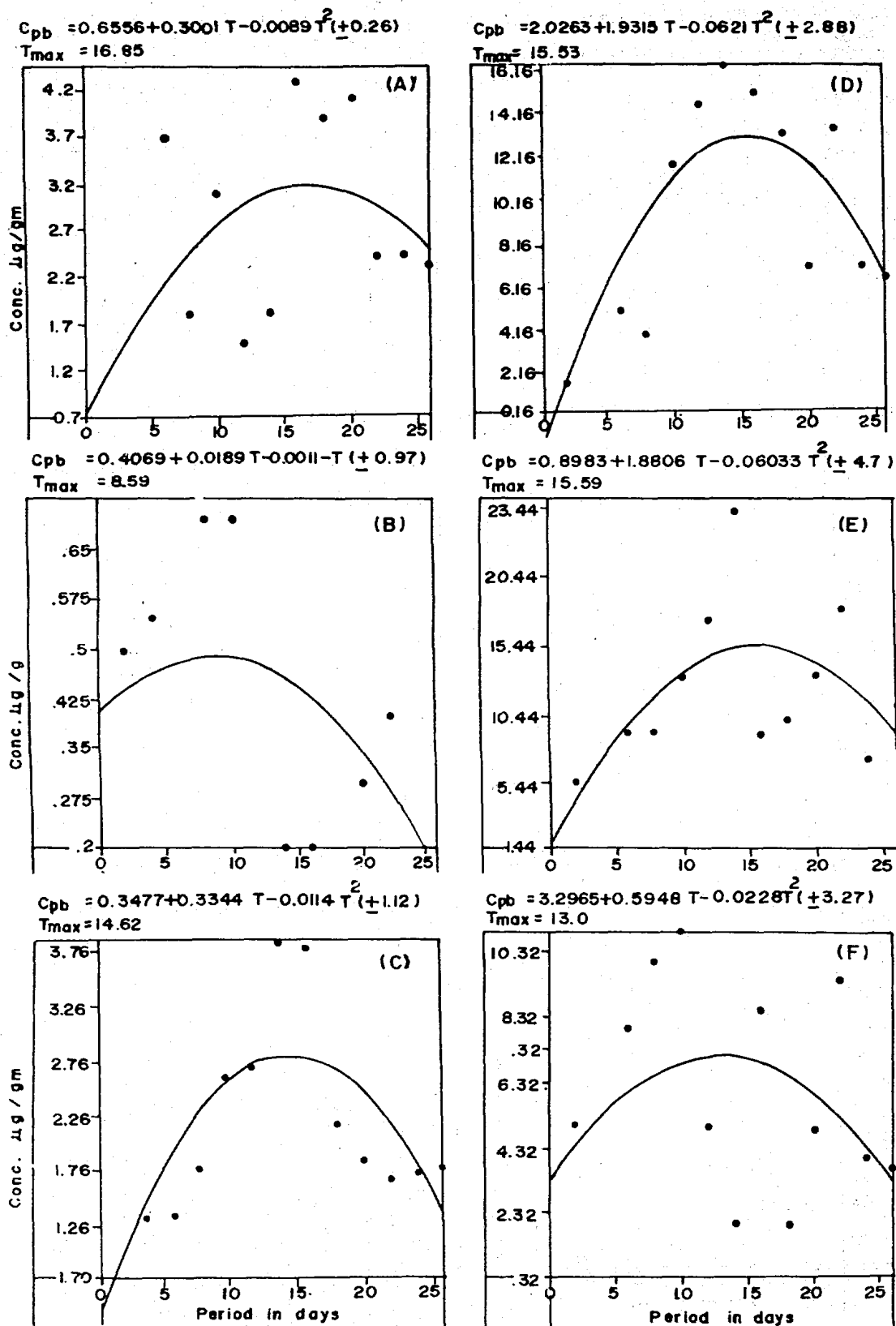


Fig. 3—Uptake and loss of Pb in various tissues of *T. mosambica* and *P. viridis* (A, gills; B, muscle; C, liver; D, mantle; E, adductor muscle and F, byssus gland)

A steady state of uptake and loss must exist in principle but is seldom so, because of the affinity of certain metals towards muscles of organisms. Hg has been found to have an affinity towards muscles of Tilapia and the adductor muscles of *P. viridis* with no affinity towards liver of Tilapia. This finding was also evident in tissues of a wide variety of fishes analysed from the Indian coast. Higher concentration of Hg was observed in the muscles while liver of most of the fishes gave non-detectable Hg values<sup>8,14</sup> similarly Cd has no affinity towards muscles and gills but shows a positive correlation in terms of uptake in liver of Tilapia suggesting a probable incorporation of Cd with metallothionein which is not released on exposure to natural sea water. These findings corroborate well with field results where highest concentrations of Cd were observed in the liver of fishes collected from the Indian coast. An exceptionally high concentration of 20.0 ppm wet weight was observed in the liver. Pb is purged fairly well from muscles and liver of Tilapia, however retention was observed in the gills which may be due to the affinity of Pb towards bony tissues suggesting fixation of Pb. Similar trends were observed in field studies<sup>14</sup>. However, Pb concentrations in the kidney of some of the fishes collected at field was also high. In general it was observed from field and laboratory studies that higher concentrations of metals were recorded mostly in those tissues which are not normally consumed, viz. liver, kidney and gills, with the exception for Hg which showed greater affinity towards muscle.

It can be expected that over extended period of depuration the concentrations of these elements may come down further, however incorporation into

tissues after extended period of exposure may lead to bio-magnification to higher levels. Another important observation was the emergence of certain tissues indicative of selective absorption and retention of particular metal, thus making them an ideal monitoring tool in future monitoring programmes.

#### Acknowledgement

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