

## Decrease in Cadmium Concentration in a Cd-Contaminated Fish by Short-Term Exposure to EDTA

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Heavy metals, especially cadmium in aqueous environments, are accumulated in aquatic plants and animals. There are many reports (SPRAGUE & RAMSAY 1965, MOUNT 1968, PIKERING & GAST 1972) which refer to the toxicity and accumulation of heavy metals in aquatic organisms (SKIDMORE et al. 1972, EATON 1974, DAVIES et al. 1975, MURAMOTO 1978). Experiments on the elimination in fish in which heavy metals have accumulated, and have led to the postulate that the protein (LLOYD 1960) of the fish combine with metals relatively loosely. Accordingly, the present experiment studied the effects of short periods of fresh-water and EDTA treatment in removing heavy metals from Cd-contaminated fish.

### MATERIALS AND METHODS

Each group of 10 carp (*Cyprinus carpio* L.), 14 - 16 g and 10 - 11.5 cm, was kept in a 60-L glass container. After the fish had been kept for 3 months in Cd-containing water, 0.05 mg Cd/ L ( $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ), they were treated with fresh water or complexan, the tetrasodium salt of ethylenediamine tetraacetic acid (EDTA), at three times molar concentration of the each metals. Ten carps were picked on the 3rd, 7th and 14th day after the beginning of treatment. The water (tap water) was changed twice a week. The fish were kept on feed NO.4 (Japan Combined Feed Co., Ltd.) containing not more than 0.05  $\mu\text{g/g}$  Cd, and these feeds were administered every other day. The water temperature was maintained at 16.0 - 18.0 °C throughout the experimental period. Water characteristics (mg/L) were: Ca 4.6; Mg 1.7; Na 3.2; K 0.77;  $\text{SO}_4$  2.9;  $\text{PO}_4$  0.003;  $\text{NO}_3\text{-N}$  0.22;  $\text{NH}_4\text{-N}$  0.04; Fe 0.04; alkalinity as  $\text{CaCO}_3$  16.9; Cl 2.5;  $\text{SiO}_2$  14.5; Dissolved solid 42.6; Cd 0.001; Cu 0.03; Zn 0.09; and Pb 0.07. pH was 6.8 - 7.1.

Fish were thawed for analysis and dissected into three parts: viscera, gills and other parts. Each sample was incinerated at 450 °C for 24-h in an electric muffle furnace. The ash sample was dissolved in  $\text{HNO}_3\text{-HClO}_4$  (2:1), and made up to a fixed volume by addition of 0.1N-HCl. This solution was used for the analysis of metals. Cd, Cu and Zn were determined using an atomic absorption spectrophotometer after application of the APDC-MIBK extracting method. Deformed fish were subjected to X-ray photography using SOFTEX (JAPAN SOFTEX Co.) to photograph the skeleton.

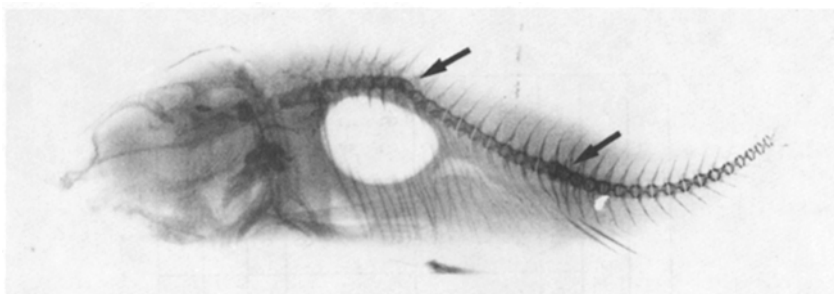


Fig. 1 Magnified X-ray photograph of a fish exposed to 0.05 mg/L Cd. Arrow indicates the deformed vertebrae.

## RESULTS

Two fish deaths were incurred in the tank containing 0.05 mg/L Cd, one each on the 31st and 95th day after the beginning of experiments. One deformed fish was observed with a vertebra damage in the Cd environment on the 90th day after beginning of experiments. (Fig. 1).

The Cd-exposed fish had 91, 38 and 35 times the Cd content for viscera, gills and others (Table 1). In the case of zinc and copper however, the tissue metal content in each case parts of fish was to be almost equal to, or more than that of the fish at beginning of experiment. Complexan-treated fish showed a tendency to decrease Cd in various sites in their fish bodies, such as 0.37, 0.36 and 0.64 times, for viscera, gills and others, respectively and those in the fresh-water treated fish, 0.83, 0.67 and 0.71 times, respectively.

## DISCUSSION

Fig. 2 shows that the Cd in the viscera reached a level of 320  $\mu\text{g/g}$  (in the ash) as a result of exposure to Cd-containing water for three months, but after fish had been kept for three days in an aqueous solution of complexan, the level decreased to 214  $\mu\text{g/g}$  (approximately 70 %), and 125  $\mu\text{g/g}$  (approximately 40 %) after seven days. A state of equilibrium was reached thereafter with little change at 14 days.

The fresh-water treatments tended to reduce the levels of Cd in the fish as the treatment progressed, although this was slow. The efficacy of elimination classified by the parts of the fish was order: gills, viscera and other parts.

As for change in the amount of Zn (Table 1), the levels decreased markedly in the viscera and gills of the fish after three months of Cd accumulation. An antagonism has been seen between the amount of cadmium in water and that of Zn in fish. Treatment with complexan after the accumulation of Cd reduced to the levels as in the control fish only on the 14th day.

Also the levels of Cu differ from those of Cd and Zn. After the fish had kept for three months in Cd-contaminated water, the Cu content had increased to about twice that in the gills of the control fish, but was 0.9 times that in the viscera, a low value. The treatment with complexan tended gradually to increase the Cu levels

TABLE 1. The contents of metals ( Cd, Zn, Cu ) of each parts of the Cd-accumulated fish after treated with complexan or with fresh water

Cd accumulation	Days of treatment			pH	Heavy metal contents (µg/g in ash)								
	3	7	14		Cd			Zn			Cu		
					viscera	gills	others	viscera	gills	others	viscera	gills	others
Cd 0.05 ppm (exposed 3 months)	→	→	→	7.2	214	152	18	1411	1517	500	105	53	40
	→	→	→	7.2	125	138	15	1354	1560	428	89	59	37
	→	→	→	7.2	117	61	18	1449	1743	380	103	55	38
	→	→	→	7.0	311	149	21	1193	972	475	102	46	39
Control (tap water)	→	→	→	7.0	277	139	18	1287	889	413	98	39	45
	→	→	→	7.0	264	113	20	1328	1464	413	105	69	43
	→	→	→	7.1	320	169	28	1064	922	370	103	36	31
Fish at the beginning	→	→	→	7.0	3.7	0.4	0.3	1418	1580	425	116	51	36
	→	→	→	7.0	3.5	4.4	0.8	1135	1537	343	85	35	28

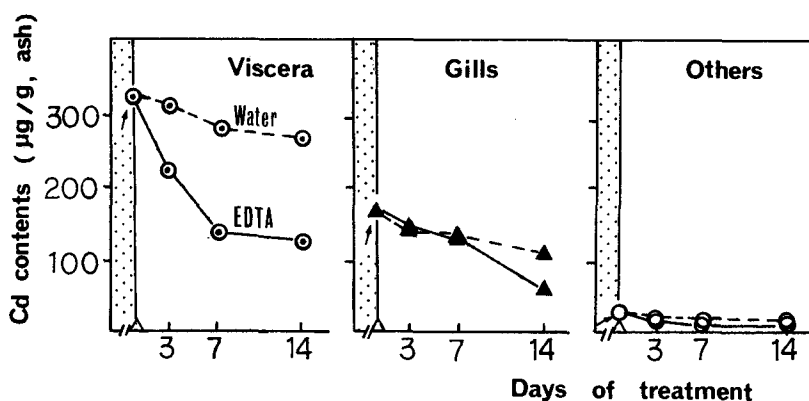


Fig. 2 Changes of Cd levels with the passage of time in each of the three parts of the fish after treatment with complexan or fresh water.

▨ : exposure to 0.05 mg/L Cd for 3 months.  
 Δ : control fish.

in both the viscera and gills, showing a slightly antagonistic relation to Cd. By contrast, in the treatment with fresh water, the Cu content changed little in either the gills or the viscera, and was approximately the same as immediately after the end of the Cd accumulation period.

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