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ELIMINATION OF COPPER FROM Cu-CONTAMINATED FISH BY LONG-TERM EXPOSURE TO EDTA AND FRESH WATER

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A few reports deal with the effects of complexing agents, such as the heavy metal pollution-inhibiting agents, on aquatic animals. Sprague and Ramsay (1965) showed the influence of nitrilotriacetic acid (NTA) on zinc- and copper poisoning of brook trout. Nishikawa and Tabata (1969) studied removal of the toxicity of waste water from copper mines by ethylenediaminetetraacetic acid (EDTA) using water flea and dace. However, few reports refer to the total or partial removal of heavy metals, except for a report on decreased Cd levels in Cd-contaminated fish by short-term (Muramoto 1980a) or by long-term exposure (Muramoto 1982a) to EDTA or fresh water, and on the effects of complexans (EDTA, NTA and DTPA) on the toxicity of heavy metals (Cd, Zn, Cu and Pb) to fish (Muramoto 1980b). This paper reports on the effects of long-term treatment with EDTA or fresh water on removing copper from Cu-contaminated fish.

MATERIALS AND METHODS

Experimental conditions

Ten carp (*Cyprinus carpio* L.), between 18.0 and 19.0 g in weight and 9.0 to 10.5 cm long, were kept in a 60-ℓ glass container. After they had been kept for 3 months in Cu-containing water [0.01 ppm or 0.05 ppm with Cu ($CuSO_4 \cdot 5H_2O$)], they were placed into fresh water or complexan, the tetrasodium salt of ethylenediamintetraacetic acid (EDTA), at three times the molar concentration of the metals. Each group of ten carp was taken for analysis of metals on the 30th, 60th and 90th day after beginning treatment. Water (tap water) was changed twice a week. The fish were fed on No. 4 feed (Japan Combined Feed Co.), containing not more than 0.5 μg/g Cu, every other day. The water temperature was maintained at 16.5–18.5 °C throughout the experimental period. The water characteristics (mg/ℓ) were: Ca, 4.7; Na, 3.6; K, 0.87; SO_4, 2.9; PO_4, 0.005; NO_3–N, 0.19; NH_4–N, 0.04; Fe, 0.04; alkalinity as $CaCO_3$, 15.3; Cl, 0.01; Cu, 0.005; Zn, 0.10; and Pb, 0.06. The pH was 6.8–6.9.

Analysis

Fish were dissected into three parts: viscera, gills and other parts

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(whole body except viscera, gills). Each sample was dissolved at 400 °C for 24-h in an electric muffle furnace. The ash sample was dissolved in HNO_3–$HClO_4$ (2 : 1), and made up to a fixed volume by adding 0.1 N HCl. This solution was used for analysis of metals. Cu and Cd were determined using an atomic absorption spectrophotometer after application of the APDC-MIBK extracting method.

RESULTS AND DISCUSSION

Two fish died during the experimental period; one on the 91st day after beginning the experiment in the 0.01 ppm Cu group and the other

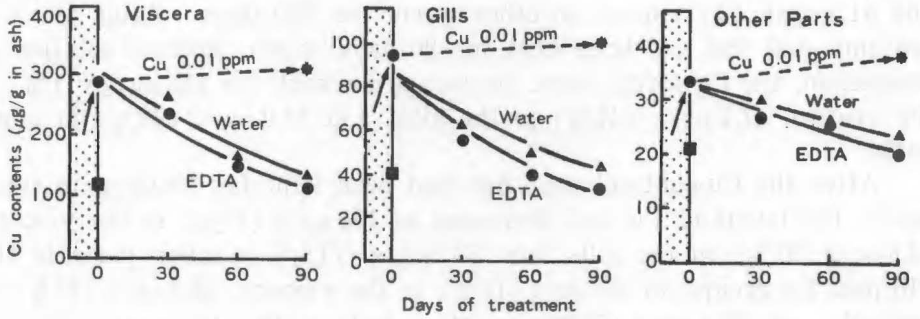
TABLE 1. The content of metals (Cu and Cd) in three dissected parts of fish after treatment with complexan (EDTA) or fresh water

Cu Accumulation	Days of treatment (30 / 60 / 90)	pH	Metal contents (μg/g in ash) Cu A	Cu B	Cu C	Cd A	Cd B	Cd C
Cu 0.01 ppm	→ EDTA →	7.1	237	55.7	27.3	5.52	3.17	1.72
	→ EDTA →	7.2	149	39.4	26.5	2.91	3.01	1.99
	→	7.1	118	33.0	20.4	2.20	1.85	0.99
	→ Water →	6.9	261	62.4	30.0	6.29	3.22	1.87
	→ Water →	7.0	167	49.2	26.3	5.51	2.99	1.15
	→	7.0	137	43.8	23.7	3.52	1.64	0.84
	—	7.0	289	93.6	33.3	7.15	3.41	1.33
→	→	7.0	308	101	39.6	7.61	5.04	1.89
Cu 0.05 ppm	→ EDTA →	7.2	451	65.7	29.4	7.77	3.38	1.46
	→ EDTA →	7.2	249	50.7	24.5	3.30	4.14	1.19
	→	7.1	112	47.2	23.0	2.46	2.05	0.29
	→ Water →	7.0	545	98.7	31.8	5.14	4.92	1.47
	→ Water →	7.0	319	59.4	29.9	4.27	3.38	1.31
	→	7.0	127	48.4	25.1	3.71	2.35	0.88
	—	7.0	1020	114	48.0	12.7	7.14	1.51
→	→	7.0	1430	197	51.5	14.3	7.78	2.85
Control (tap water)		6.9	117	41.0	22.9	3.69	1.56	0.18
Fish at the beginning		6.9	89.9	34.8	19.3	2.56	1.19	0.40
Complexan (EDTA 1.1 ppm)		7.1	92.3	41.9	17.1	6.78	1.08	0.73

A, viscera; B, gills; C, other parts.

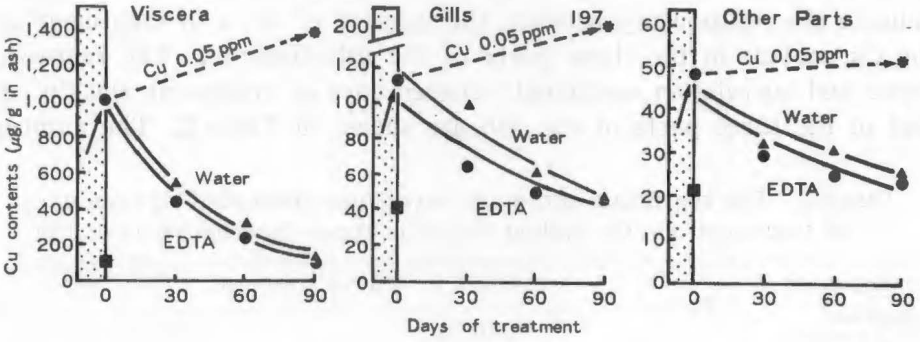
on the 175th in the 0.05 ppm Cu group. No deformed fish was observed. Cu content tended to rise with increase in the exposure time to Cu containing water (Table 1).

The changes in Cu levels with the passage of the time in the three parts of the fish treated with complexan or fresh water after exposure to 0.01 ppm or 0.05 ppm Cu containing water for 3 months are shown in Figs. 1 and 2. As pre-treatment, fish were exposed to 0.01 ppm Cu for



▨ : exposure to 0.01 ppm Cu for 3 months.
■ : control fish.

FIG. 1. Changes in Cu levels with the passage of time in three dissected parts of fish after treatment with complexan or fresh water.



▨ : exposure to 0.05 ppm Cu for 3 months.
■ : control fish.

FIG. 2. Changes in Cu levels with the passage of time in three dissected parts of fish after treatment with complexan or fresh water.

3 months (Muramoto 1982b). The levels of Cu in ash increased to 289 μg/g (3.2 times of the beginning contents), 93.6 μg/g (2.7 times) and 33.3 μg/g (1.7 times) in the viscera, gills and other prats, respectively (Fig. 1). After Cu-contaminated fish had been kept for 90 days in an aqueous solution of complexan (EDTA), the level of Cu had decreased to 118 μg/g

(41%) in the viscera, 33.0 μg/g (35%) in the gills, and 20.4 μg/g (61%) in other parts. However, after fish had been exposed to 0.01 ppm Cu for 180 days, the levels of Cu had reached 308 μg/g (3.4 times), 101 μg/g (2.9 times) and 39.6 μg/g (2.1 times) in the viscera, gills and other parts, respectively. Also in the 0.05 ppm Cu group (Fig. 2), Cu reached 1020 μg/g (11.3 times) in viscera, 114 μg/g (3.3 times) in the gills and 48.0 μg/g (2.5 times) in other parts with exposure to Cu for 90 days and 1430 μg/g (15.9 times) in the viscera, 197 μg/g (5.7 times) in the gills, and 51.5 μg/g (2.7 times) in other parts for 180 days. After the Cu-contaminated fish had been kept for 90 days in the aqueous solution of complexan, the Cu levels were decreased markedly to 112 μg/g (11%) in the viscera, 47.2 μg/g (41%) in the gills, and 23.0 μg/g (48%) in other parts.

After the Cu-contaminated fish had been kept for 90 days in fresh water, the levels of Cu had decreased to 137 μg/g (47%) in the viscera, 43.8 μg/g (47%) in the gills, and 23.7 μg/g (71%) in other parts in the 0.01 ppm Cu group; to 127 μg/g (12%) in the viscera, 48.4 μg/g (42%) in the gills, and 25.1 μg/g (52%) in other parts in the 0.05 ppm Cu group. Therefore, fresh water treatment for 90 days tended to reduce the levels of Cu in the three parts of the fish as almost the same to those of the EDTA treatment.

As a method of estimating the degree of Cu eliminated by complexan or fresh water after long-term treatment, an equation was needed to indicate the relationship between the number of days of treatment and the Cu content in the three parts of the fish dissected. The regression curve and correlation coefficient between days of treatment and Cu content in the three parts of the fish are shown in Table 2. The numbers

TABLE 2. The regression curve and correlation coefficient between days of treatment and Cu content in ash in three dissected parts of fish

Cu treatment (exposed 3 months)	Parts	After treatment	
		EDTA	Water
Cu 0.01 ppm	Viscera	$\log Y = -0.01504X + 5.670$ ($r = -0.988$)	$\log Y = -0.00895X + 5.720$ ($r = -0.974$)
	Gills	$\log Y = -0.01158X + 4.453$ ($r = -0.977$)	$\log Y = -0.00839X + 4.454$ ($r = -0.968$)
	Others	$\log Y = -0.00500X + 3.501$ ($r = -0.962$)	$\log Y = -0.00384X + 3.508$ ($r = -0.999$)
Cu 0.05 ppm	Viscera	$\log Y = -0.02407X + 6.001$ ($r = -0.998$)	$\log Y = -0.02262X + 6.977$ ($r = -0.993$)
	Gills	$\log Y = -0.00968X + 4.611$ ($r = -0.938$)	$\log Y = -0.01026X + 4.785$ ($r = -0.976$)
	Others	$\log Y = -0.07965X + 3.755$ ($r = -0.926$)	$\log Y = -0.00669X + 3.788$ ($r = -0.944$)

of days of EDTA treatment required for reduction to two-thirds, one-half and one-third of the levels of Cu after exposure to Cu containing water for 3 months were calculated. In the 0.01 ppm Cu group, the periods were estimated approximately as 41.8, 69.2 and 108 days for viscera; 27.6, 52.5 and 87.5 for gills; and 219, 138 and 80.2 days for other parts. In the 0.05 ppm Cu group, the periods were 15.8, 27.7 and 44.6 days for viscera; 29.0, 58.7 and 44.6 days for gills; and 36.3, 72.4 and 123 days for other parts.

Also these periods for the treatment with fresh water were estimated at 51.3, 83.4 and 129 days for viscera; 29.5, 73.8 and 122 days for gills; and 287, 181 and 106 days for other parts in the 0.01 ppm Cu group. In the 0.05 ppm group, they were 20.1, 32.8 and 50.8 days for viscera; 44.3, 72.3 and 112 days for gills; and 48.3, 91.3 and 152 days for other parts.

After the fish had for three months in Cu-containing water, the Cd content also had increased in the three parts of the fish. The Cd contents were about 3 times in the 0.01 ppm Cu group compared with the fish at the beginning of the experiment and 4–6 times in 0.05 ppm Cu group. The levels of Cd was also decreased by subsequent treatment with complexan.

The results of the present experiments made it clear that the protein of the fish combined with metals loosely (Lloyd 1960, Muramoto 1980a, 1981), in view of the decrease of copper content from Cu-contaminated fish following treatment by either fresh water or complexan.

SUMMARY

The changes of the Cu and Cd concentrations in the bodies of the Cu-contaminated fish exposed for long periods to fresh water and EDTA were determined.

1) The fish exposed to Cu-containing water for 90 days (Cu-contaminated fish) had 3.2, 2.7 and 1.7 times the Cu content for viscera, gills and other parts in the 0.01 ppm Cu group compared with fish at the beginning of the experiment, and had 11.3, 3.3 and 2.5 times for viscera, gills and other parts in the 0.05 ppm Cu group, respectively.

2) The fresh water and EDTA treatment for 90 days in removing heavy metals from Cu-contaminated fish tended to reduce the levels of Cu in the fish as the almost equal to those of the fish at the beginning of experiment.

3) After the fish had been kept for 90 days in Cu-containing water, the Cd content had also increased in the three parts of fish. Treatment with complexan or fresh water after the accumulation of Cd had an

effect on the decrease of Cd content in the bodies of the Cu-contaminated fish.

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