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Effect of Complexans (EDTA, NTA and DTPA) on the Exposure to High Concentrations of Cadmium, Copper, Zinc and Lead

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The synthetic complexans including NTA have been widely used in all industrial areas as chelating agents for metal ions owing to their versatility (POLLARD 1966, WALLACE 1971). Today, their effects on aquatic life through their interaction with the heavy metals in environmental water have become of such great importance that proper study is essential. The biological investigation using brook trout by SPRAGUE (1968) indicated that NTA was effective against copper- and zinc-poisoning as a pollution-inhibiting agent. NISHIKAWA & TABATA (1968) conducted some experiments on elimination of the toxicity of waste water from copper mines with EDTA using water fleas and dace. This report is an examination of the effect of complexans on the toxicity of short exposures to high chemical concentrations of heavy metals. The heavy metals used were cadmium, zinc, lead and copper. Mortality every 24-h and the content of metal in each of three areas of fish — viscera, gills and other parts — were determined both in the groups kept in water containing metal alone and in those whose aqueous environments contained complexan in mole concentrations three times that of the heavy metal.

MATERIALS AND METHODS

Experimental conditions

The carp (*Cyprinus carpio* L.) 8.0 + 0.5 cm, 11.5 + 2.0 g, were kept in groups of 8 - 10, each in a 60-L glass aquaria at 14.5 - 16.5°C throughout the experiment. There were altogether 49 such groups: 12 kept in three relatively high concentrations of each of the metals, Cu, Cd, Pb and Zn, alone; 36 groups in environments each containing only one of the three complexans, the tetrasodium salt of ethylenediamine tetraacetic acid (EDTA), the trisodium salt of nitrilotriacetic acid (NTA), and the pentasodium salt of diethylenetriamine pentaacetic acid (DTPA) were used. Each of these was added to the environments of 13 of the groups at three timed the molar concentration of the metal in each. Mortality checks were made at intervals of 24-h by eye, and dead fish were immediately frozen and kept in a freezer at -20°C.

Analysis

Frozen fish were thawed for analysis and dissected into three parts: viscera, gills, and all other parts. Each of these was incinerated at 450°C for 24-h in an electric muffle furnance and the ash weight was determined. The ash sample was dissolved in the mixed acid HNO_3-$HClO_4$(2:1), and made up to a fixed volume by addition of 0.1N-HCl.

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Zn was determined using an atomic absorption spectrophotometer after application of the APDC-MIBK extracting method.

RESULTS

Concentration of heavy metals and mortality

Fig. 1 shows the percentage of mortality after each 24-h period. From the relation between the concentration of each metal and the mortality, the order of the toxicity of the metals of high concnetration within 48-h was seen to be as follows: Cu, Cd, Zn, Pb. All of the fish receiving 20 ppm of Pb survived after 48-h, whereas in case of the other metals, Cd, Zn and Cu, the mortality exhibited a tendency to increase moderately with increase in metal concentration. Cu particularly showed high toxicity; all the fish receiving 0.6 ppm of Cu died within 24-h. No effect of differing pH values on mortality was observed in any of the experimental groups within 48-h.

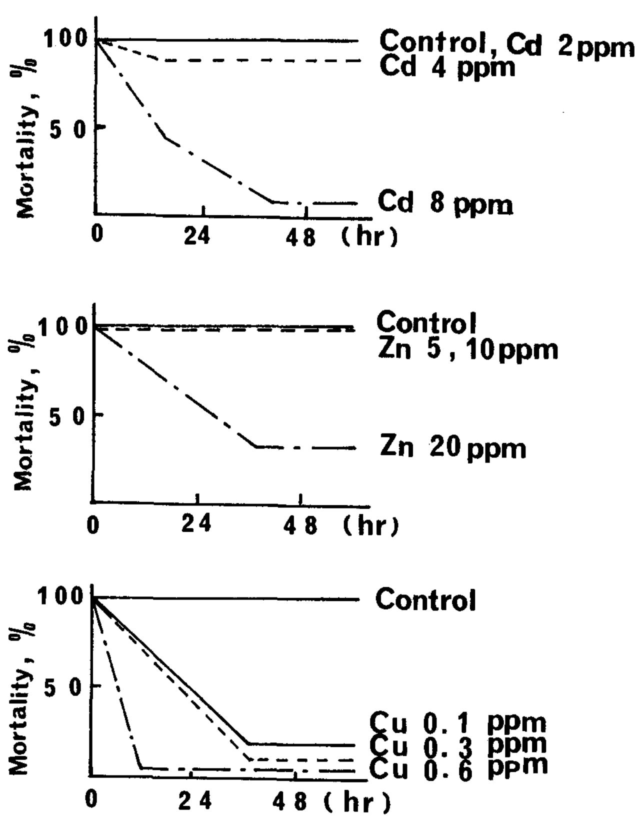

Fig. 1. The periodical change of mortality of fish exposed to each heavy metal.

Comparison of the concentration of accumulated metal between the metal-alone groups and control

The concentrations of metals accumulated in each part of the bodies of the fish survived after 48-h are shown in Tables 1a,1b. The results of the comparison of the metal content of the ash in the metal-alone groups with that of the control (normal fish) for viscera, gills, and other parts was as follows : 795 - 1460, 36 - 129 and 22 - 75 times for Cd, 3.3 - 4.2, 8.4 - 21 and 1.8 - 1.9 times for Cu, 8.9 - 415, 4.2 - 35 and 0.9 - 6.8 times for Pb, 1.1 - 1.6, 0.8 - 1.0 and 0.8 - 1.5 times for Zn, respectively.

TABLE 1a

The concentrations (μg/g in ash) of metals accumulated in each parts of the bodies of the fish surviving after 48-h.

Metal	Experimental group (mg/L)	pH	Viscera	Gills	Other parts
	Zn 5	6.8	9720	9000	1720
	Zn 5 + EDTA 104	7.1	8170	3310	2160
Zn	Zn 5 + NTA 63	7.1	9230	8590	1910
	Zn 5 + DTPA 116	7.5	8380	8850	1400
	Zn 10	6.9	10800	9110	2120
	Zn 10 + EDTA 208	7.1	7110	7780	2080
($ZnCl_2$)	Zn 10 + NTA 126	7.2	6020	6720	2110
	Zn 10 + DTPA 232	8.2	9400	8390	1950
	Zn 20	6.8	15200	7460	2990
	Zn 20 + EDTA 415	7.4	8700	4500	2600
	Zn 20 + NTA 252	7.6	7310	8780	2480
	Zn 20 + DTPA 462	9.4	7520	5080	2390
	Control	6.9	9220	9370	2050
	Cd 2	7.3	1750	107	24
Cd	Cd 2 + EDTA 24	7.4	50	3	0.7
	Cd 2 + NTA 15	7.4	137	0.4	2
	Cd 2 + DTPA 27	7.4	43	10	2
	Cd 4	7.2	3110	216	18
	Cd 4 + EDTA 48	7.3	74	4	2
	Cd 4 + NTA 29	7.3	154	16	6
($CdCl_2 \cdot 2\frac{1}{2} H_2O$)	Cd 4 + DTPA 34	7.6	73	3	3
	Cd 8	7.1	3210	388	60
	Cd 8 + EDTA 96	7.4	63	9	4
	Cd 8 + NTA 59	7.4	262	27	10
	Cd 8 + DTPA 108	7.6	181	12	0.2
	Control	6.9	2.2	3.0	0.8

DISCUSSION

Correlation of the metal concentration between contents of water and in fish

In the metal-alone groups, there was a positive correlation between the concentration of each of the metals in the water and degree of metal accumulation in the fish. Significance was recognized in the following parts: viscera and other parts in the Zn-treated groups; gills in the Cd-treated groups; viscera and other parts, and gills in the Pb-treated groups. In contrast, the complexan-plus-metal groups generally exhibited striking decreases of metal concentration in each part of the fish in comparison with the metal-alone ones; the concentration gradient of the accumulated metals decreased in all parts except in the viscera of the Cu-plus-DTPA, Cd-plus-NTA and Cd-plus-DTPA groups. This indicates inhibition of the metal accumulation by the complexans.

TABLE 1b

The concentrations (μg/g in ash) of metals accumulated in each parts of the bodies of the fish survived after 48-h.

Metal	Experimental group (mg/L)	pH	Viscera	Gills	Other parts
Cu	Cu 0.1	7.1	265	202	40
	Cu 0.1 + EDTA 2.1	7.3	211	42	71
	Cu 0.1 + NTA 1.3	7.3	107	40	25
	Cu 0.1 + DTPA 2.4	7.3	117	44	37
($CuSO_4 \cdot 5H_2O$)	Cu 0.3	7.1	344	511	41
	Cu 0.3 + EDTA 6.3	7.5	155	75	30
	Cu 0.3 + NTA 3.9	7.4	202	60	38
	Cu 0.3 + DTPA 7.2	7.5	173	146	30
	Cu 0.6	7.1	-	-	-
	Cu 0.6 + EDTA 12.6	7.5	253	77	44
	Cu 0.6 + NTA 7.8	7.4	157	117	52
	Cu 0.6 + DTPA 14.4	7.7	434	202	42
	Control	6.9	81	24	22
Pb	Pb 2	7.1	1850	548	50
	Pb 2 + EDTA 13.1	7.3	236	68	1
	Pb 2 + NTA 7.9	7.5	339	216	1
	Pb 2 + DTPA 14.5	7.7	82	165	1
($Pb(NO_3)2$)	Pb 10	6.9	42200	3170	165
	Pb 10 + EDTA 65.5	7.6	158	963	1
	Pb 10 + NTA 39.5	7.6	187	588	2
	Pb 10 + DTPA 72.5	8.4	122	224	1
	Pb 20	6.9	86000	4560	359
	Pb 20 + EDTA 131	7.8	686	645	1
	Pb 20 + NTA 79	7.7	399	298	1
	Pb 20 + DTPA 145	9.0	690	312	1
	Control	6.9	207	131	53

As shown in these results, addition of the complexans resulted in the decrease of the tissue concentrations of Cd, Zn, Pb and Cu. It is considered that the heavy metals were not present merely as metal ions but formed comlexes with the complexans, since the complexans were added at three times mole of the metal and therefore complex formation occurred in preference to the binding of the metals with the tissue proteins of the fish. It is furthermore assumed that the heavy metals can pass through the fish as metal-complexes, and so are not retained. In addition, it is interesting that, with Cd, addition of the complexans resulted in the next highest decease in tissue concentration, in spite of the stability constant of Cd-complexans was partial, and no significant difference was recognized in the gills.

Comparison of tissue metal concentration in ash between the dead and surviving fish

Table 2 shows the results of the comparison of the tissue metal concentration between the dead fish and the 48-h survivors. In the dead fish, the adsorption and deposition of Zn was particularly marked in the gill tissue (LLOYD 1960). In the environment containing 4 mg/L of Cd, the order of the tissue metal concentrations in the fish which died within the first 24-h and in the surviving ones were gills > viscera > other parts and viscera > gills > other parts, respectively.

TABLE 2

Comparison of the metal content (μg/g in ash) of various sites in bodies of the fish between dead fish and 48-h survivors

Experimental group	Viscera			Gills		
	Dead		Survivor	Dead		Survivor
	0-24 (h)	24-48 (h)	48 - (h)	0-24 (h)	24-48 (h)	48 - (h)
Zn 20 ppm	-	10300 (3)	15200 (7)	-	10700 (3)	7460 (7)
Cd 4 ppm	305 (1)	-	3110 (9)	10100 (1)	-	216 (9)
8 ppm	731 (5)	340 (4)	3210 (1)	1020 (5)	920 (4)	388 (1)
Cu 0.1 ppm	-	323 (3)	265 (5)	-	154 (3)	202 (5)
0.3 ppm	172 (6)	1470 (1)	346 (1)	209 (6)	543 (1)	511 (1)
0.6 ppm	180 (8)	-	-	169 (8)	-	-

( ) : numbers of fish

The former relation was also noticed in the fish which were kept in 8 ppm Cd and died within both the first and second 24-h periods. These results lead to the supposition that the cause of death through Cd adsorption by the gill tissue was asphyxia due to respiratory distress.

With Cu, however, there was not such a marked difference in adsorption of the metal in the gill tissue between the dead fish and 48-h survivors as in the case of Cd and Zn. Consequently, it is considered that the cause of death of these fish was different from that of the fish kept in Cd and Zn environments. Cu may increase its effect on the viscera by passage through the gill tissues.

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