Bull. Environm. Contam. Toxicol. 26, 641-646 (1981)

Influence of Complexans (EDTA, DTPA) on the Toxicity of Cadmium to Fish at Chronic Levels

S. Muramoto

Institute for Agricultural and Biological Sciences, Okayama University, 2-20-1 Chu-o, Kurashiki, 710 Japan

There are a few reports on the effects of the complexing agents, as heavy metal pollution-inhibiting agents, to the aquatic animals. SPRAGUE(1968) reported on the influence of NTA on the zinc-and copper poisoning to brook trout. NISHIKAWA & TABATA(1969) conducted some experiments on eliminations of the toxicity of waste water from copper mines with EDTA using water fleas and dace. However, this in an area which has not so far as been clarifies in investigations on metal complexan toxicity and accumulation. In the previous paper (MURAMOTO 1981a, 1981b), the effects of complexans(EDTA,NTA and DTPA) on the metal-toxicity(Cd,Cu,Zn and Pb) at lethal levels and of complexan(EDTA) on the removing cadmium from the Cd-polluted fish were reported. This reports is examination of influence of complexans (EDTA and DTPA) on the metal toxicity and on the metal accumulation in viscera, gills and other parts of the fish exposing cadmium at low concentrations, long term(3 months). Vertebrae deformed fish induced by cadmium were observed with eye. The vertebrae of the fish were then examined by X-ray photography.

MATERIALS AND METHODS

Experimental conditions

Ten carp(*Cyprinus carpio* L.) 10-12 g, 7-9 cm, were kept in a 60-L glass container at 18.0-20.5 °C throughout experiment. There were altogether 12 such groups: 3 kept in relatively low concentrations of cadmium($CdCl_2.2.5H_2O$); 8 groups in environments each containing not only one of the two complexans, the pentasodium salt of diethylenetriamine pentaacetic acid(DTPA) and the tetrasodium salt of ethylenediamine tetraacetic acid(EDTA) were used. Each of these the was added to the environments of 6 of the groups at three times the molar concentration of the metal in each. The fish were fed feed No.4(Japan Combined Feed Co., Ltd) containing not more than 0.05 µg/g Cd. Feed was administered every other days. Test water was prepared with tap water and changed twice a week. Water characteristics(mg/L) were: Ca 4.6; Mg 1.7; Na 3.2; K 0.77; SO_4 3.2; Cl 2.6; Alkalinity as $CaCO_3$ 16.9; Dissolved Solid 42.6; Cd 0.001; Cu 0.04; Zn 0.07; Pb 0.07. pH was 6.8-7.0. Dead fish were immediately frozen and kept in a freezer at -20 °C.

0007-4861/81/0026-0641 $01.20

Analysis

Frozen fish were thawed for analysis and dissected into three parts: viscera, gills, and other parts(including bone tissues). After determination of raw weight, each sample was dried at 60 °C for 24-h in a hot-air drier and then the dry weight was determined. It was then incinerated at 450 °C for 24-h in an electric muffle furnance and ash weight was determined. The ash samole was dissolved in the mixed acid HNO_3-HCl(2:1), and made up to a fixed volume by addition of 0.1N-HCl. Cd was determined using an atomic absorption spectrophotometer after application of the APDC-MIBK extracting method. Fish exposed to Cd were conviniently devided into two groups: (1) the deformed fish(vertebral anomalies), and (2) the normal fish. Deformed fish were subjected to X-ray photography using SOFTEX(Japan SOFTEX Co.) to photograph the skeleton.

TABLE 1

Numbers of dead fish and surviving fish and ratio of ash weight/dry weight(%) after 3 months in environments containing known concentrations of cadmium with, and without complexan treatment

Experimental groups (ppm)	pH	Numbers of fish		Ash weight/dry weight(%)		
		Dead	Survival	Viscera	Gills	Others
Cd 0.01	7.1	0	10(1)	6.1	11.9	13.8
				(6.6)	(12.1)	(10.7)
Cd 0.01 + EDTA 0.12	7.1	0	10	6.4	14.8	16.8
Cd 0.01 + DTPA 0.13	7.3	0	10	6.0	15.2	19.4
Cd 0.05	7.0	3	7(1)	6.2	10.9	20.8
				6.9	24.1	17.1
				(11.4)	(10.5)	(10.0)
Cd 0.05 + EDTA 0.60	7.2	1	9	6.9	14.1	17.1
				7.2	20.5	22.2
Cd 0.05 + DTPA 0.67	7.4	1	9	6.4	14.4	17.9
				6.6	19.6	19.1
Cd 0.1	7.2	3	7(1)	7.2	12.7	16.7
				5.6	12.7	20.0
				(4.6)	(8.1)	(8.0)
Cd 0.1 + EDTA 1.2	7.3	1	9	6.6	11.1	15.4
				6.4	19.0	18.9
Cd 0.1 + DTPA 1.3	7.5	0	10	6.9	12.0	19.1
EDTA 2.1	7.1	0	10	6.5	14.9	14.7
DTPA 2.3	7.2	0	10	6.8	14.3	19.4
Control	7.0	0	10	6.7	13.6	20.8

Figures in parenthesis : Deformed fish,
Underlined figures : Dead fish

TABLE 2

Metal concentrations(μg/g in ash) of each parts of fish treated with cadmium with, and without complexan treatment

Experimental group (ppm)	Metal concentration					
	Viscera		Gills		Others	
	Dead	Survivor	Dead	Survivor	Dead	Survivor
Cd 0.01		101		109		9.0
		613		120		8.4
Cd 0.01 + EDTA 0.12		96.2		80.0		8.3
Cd 0.01 + DTPA 0.13		82.3		94.3		7.4
Cd 0.05	490	720	3412	1110	14.8	34.2
		635		245		39.4
Cd 0.05 + EDTA 0.60	120	419	283	554	16.6	25.9
Cd 0.05 + DTPA 0.67	60.0	325	31.3	334	8.1	11.0
Cd 0.1	350	851	2690	1240	37.9	64.9
		1030		684		35.3
Cd 0.1 + EDTA 1.2	943	407	50.2	538	25.6	24.1
Cd 0.1 + DTPA 1.3		338		417		15.4
EDTA 2.1	5.7		0.4		1.5	
DTPA 2.3	2.1		0		0.6	
Control	3.3		2.1		0.8	

Underlined figures : Deformed fish

RESULTS

The number of dead and surviving fish and ratio of ash weight/dry weight(%) after 3 months (100 days) in environments containing known concentrations of cadmium are shown in Table 1. In the group containing 0.01 ppm Cd alone, no fish died; but in 0.05 ppm Cd the mortality was 10 % in the 24th day and 30 % on the 37th day; and in 0.1 ppm Cd, the mortality was 30 % on the 76th day after the begining ofthe test. Addition of complexans, however, produced decreases in mortality of fish to the toxicity of cadmium. One extraordinary phenomenon was observed: there was one deformed fish with a bent dorsum in each of the Cd environments, 0.01 ppm, 0.05 ppm and 0.1 ppm. This phenomenon will be discussed later.

The ratio of ash weight/dry weight(%) in deformed fish, especially other parts(including bone tissues), was one half that of the control because of depression of the ash weight, which suggested that Ca release from bones is effected by long exposere to cadmium. The ratio in the gills of the fish exposed to Cd alone tended to be less than with Cd plus EDTA and DTPA. The results of analysis of the metal concentrations(μg/g in ash) of each parts of the fish treated with

metals either with or without complexans are shown in Table 2. The fish exposed to cadmium had higher levels of cadmium in their viscera, gills and other parts than the control fish. The cadmium levels tended to rise with increases in the concentration of cadmium in the breeding water. However, there were marked differences in the viscera or gills of the deformed and normal fish. In contrast, the complexan-plus-metal groups generally exhibited striking increases with concentration of metal in each part of the fish in comparison with the metal alone.

DISCUSSION

Comparison of metal concentrations in various parts of deformed and normal fish

The Cd concentraions in each parts of the deformed fish compared with that in the control fish (Fig. 1). In the normal fish, the Cd concentrations decreased in the order: viscera > gills > other parts. That in the gills was 1.01-1.54 times of value in the viscera; whereas in the deformed fish the order was: gills > viscera > other parts; and the Cd concentration had particularly high levels in the viscera, namely, 1.51-5.11 times the concentration in the gills. The fact that no deaths among them occurred at any time during tne experiment were interesting. The Cd deposit in the gills of fish which were exposed to Cd at low concentrations for long periods, however, was not so high as to cause their deaths, but it is presumed that the accumulation of

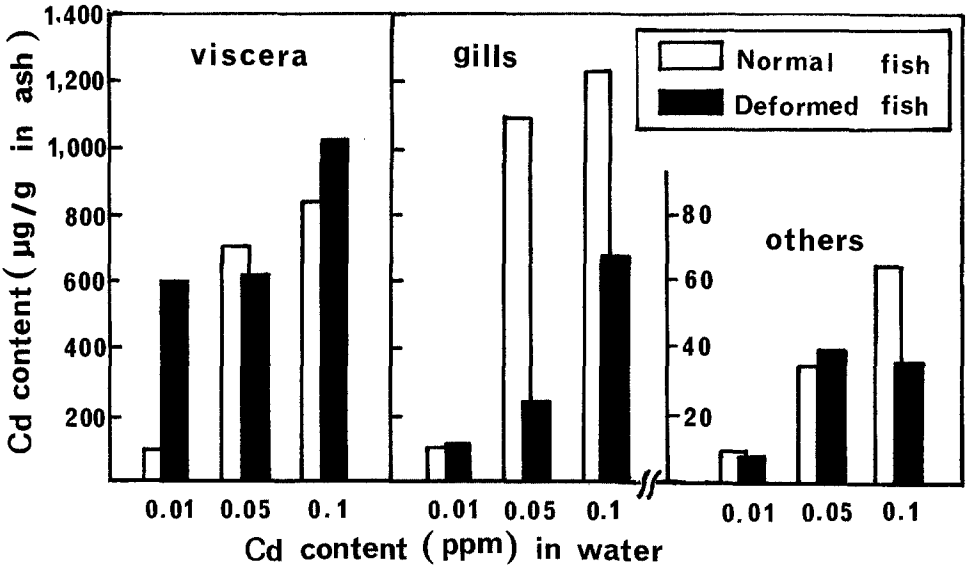

Fig. 1 Comparison on metal concentrations(μg/g in ash) in each parts of deformed and normal fish

Cd in the viscera caused functional disorders in the digestive or circulatory system, finaly leading to death and deformation.

Morphological anomalies induced by cadmium

Deformations caused by heavy metal, especially cadmium, have been described by the present authors(MURAMOTO et al.1972, MURAMOTO 1979) in relation to vertebral anomalities in carp(*Cyprinus carpio* L.). In other reports, PIKERING et al.(1972) reported a case of malformation in the fathead minnow due to cadmium, whilist EATON(1974) described a similar effect in bluegill, NAKAMURA(1975) in dace(*Tribolondon nakonensis*) and FUJIMAGARI et al.(1975), in guppies. As for caused due to lead, DAVIES et al.(1975) reported the development of vertebral scoliosis in rainbow trout(*Salverinus fontinalis*). For zinc, BENGTSSON(1974) reported on vertebral fracturies in minnow (*Phoxinus phoxinus*).

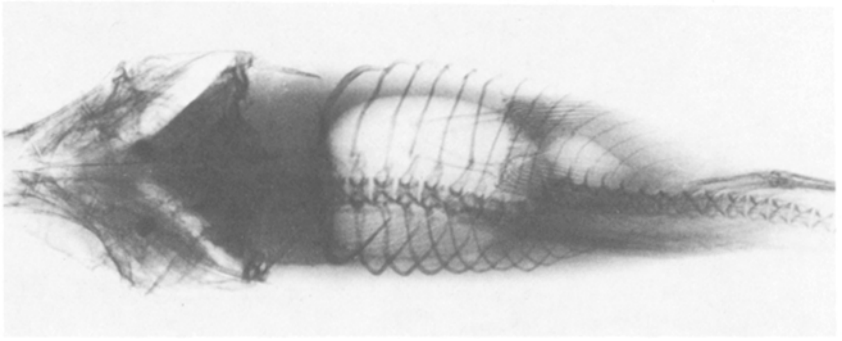

Fig. 2-1 Magnified X-radiograph of deformed carp exposed to 0.05 ppm Cd. (upper side view)

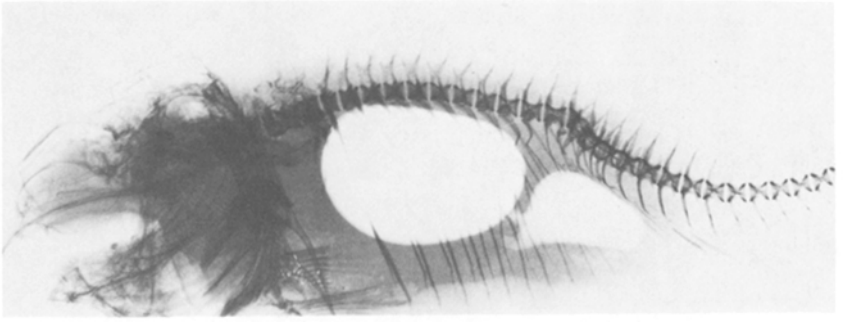

Fig. 2-2 Magnified X-radiograph of deformed carp exposed to 0.05 ppm Cd. (left side view)

In this experiment, deformed fish was observed on the 21st, 51st day, and 90th day in 0.01 ppm, 0.05 ppm, and 0.1 ppm Cd water, respectively. The X-ray photographs of the vertebral column of some fish developed deformity were shown in Figs. 2-1, 2-2.

Striking vertical curvature and tail shrinkage of vertebrae was observed in the deformed specimens. Contracted adhesion of articulations due to compression, collapsed cartilage and crookedness at the ends of small bones suggested osteal decalcification. Since all the deformation occurred in the form of bent vertebral columns, it was presumed that bone tissues softend by decalcification detriorate and become deformed as result of muscular stress following begining movements of the bone tissues, and this leads to bent spines.

ACKNOWLEDGEMENTS

The author is grateful to Drs. J.Kobayashi, F.Morii, I.Aoyama for their helpful advices and H.Yagi for comments on the manuscript and also M.Tokura for his support.

REFERENCES

BEGTSSON,B.E.: Oikos 25, 134(1972)

DAVIES,P.H. GOETTL,Jr., J.P., SINLEY,J.R. & SMITH,N.F.: Water Res. 10, 199(1958)

EATON,J.G.: Trans.Am.Fish.Soc. 103, 729(1974)

FUJIMAGARI,M., OTAWARA,J. & KATAYAMA,S.: Jap.J.Public Health 21, 587 (1974), (in Japanese)

MURAMOTO,S., KOBAYASHI,J., MORII,F., NAKASHIMA,S., HARA,K. & TOKURA, M.: Jap.J.Hyg. 2, 224(1972), (in Japanese)

MURAMOTO,S.: Bull. Environm. Contam. Toxicol. 25, 828(1981a)

MURAMOTO,S.: Bull. Environm. Contam. Toxicol. 25, 941(1981b)

NAKAMURA,M.: Jap.J.Public Health 21, 321(1975), (in Japanese)

NISHIKAWA,K.& TABATA,K.: Bull. Tokai Reg. Fish Res. Lab. 58, 233(1969), (in Japanese)

PIKERING,Q.H. & GAST,M.H.: J. Fish. Res. Bd. Can. 29, 1099(1972)

SPRAGUE,J.B.: Nature 22, 1345(1968)

Accepted February 23, 1981