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Elimination of Cadmium from Cd-Contaminated *Tilapia zilli* in Media Containing EDTA and Freshwater: Changes in Protein Levels

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Cadmium is not an essential metal for aquatic organisms. It enters the aquatic environment through anthropogenic sources such as industry and agriculture. Kay et al. (1986) indicated that in the USA and Belgium 40-50 % of *Salmo gairdneri* contained 1-20 µg Cd/L.

Various agents (EDTA, NTA and DTPA) are known to reduce metal accumulation in tissues of aquatic animals. Several authors indicated that these agents reduce metal accumulation in vertebrates and invertebrates (Muramoto 1980 ; O'Brien et al. 1990). Sunda et al. (1978) showed that EDTA and NTA reduced cadmium toxicity in aquatic organisms.

The aim of this study was to investigate Cd elimination from the tissues of Cd-contaminated *Tilapia zilli* and changes in protein levels in the tissues after exposures to cadmium, EDTA and freshwater.

MATERIALS AND METHODS

Tilapia zilli were obtained from pools and kept under laboratory conditions (25 ± 1°C) for two months. When used, the fish were of 9.65 ± 1.74 cm in mean length and 12.74 ± 2.34 g in mean weight.

Experiments were carried out in 3 glass aquaria 40x100x40 cm in height. The first two aquaria were filled with 0.1 and 1.0 ppm Cd and the third one with tap water and was used as the control. Thirty fish were then added into each aquarium. After exposure to either 0.1 or 1.0 ppm Cd ($CdCl_2H_2O$) for 30 days, six fish were removed from the tanks to measure the tissue levels of cadmium and protein. The remaining Cd-contaminated fish were than kept in clean freshwater or freshwater containing EDTA (0.3 and 3.0 ppm) (Muramoto 1982) for 1.5 and 30 days. For each exposure period and concentration, six fish were analyzed to determine Cd and protein concentrations. The test media were changed every third day to replenish either the Cd or EDTA. All fish were fed an artificial food containing no cadmium.

After each test period fish were dissected and the liver, gills and muscle were removed. One portion of the tissues was used to measure Cd concentrations. A method similar to that of Mason et al. (1986) was adopted in the preparation of samples for atomic absorption spectrophotometry. The dry weight of the dissected tissues was determined after being kept at 105°C for 48 hr. Dried tissues were digested with 3.0 ml of a mixture of HNO_3 and $HClO_4$ in a 2:1 ratio. Acid digestion was carried out at 120°C for three hours. After dilution with distilled water, cadmium concentrations of the tissues were measured using a Perkin Elmer Atomic Absorption Spectrophotometer, calibrated with C-5524 Sigma Standards. Detection limit of the spectrophotemeter was 0.028 ppm and 90% recovery was obtained during measurements. The remainder of the tissues were used to measure total protein concentrations by the Folin-Lowry methods (Plummer 1971). Student Newman Keul's Test (SNK) was used to determine the effects of freshwater and EDTA on the elimination of Cd from the tissues and changes in protein levels.

RESULTS AND DISCUSSION

Mean cadmium and protein concentrations and their associated standard deviations are given in Tables 1 and 2, respectively, for each exposure concentration and period.

Cadmium accumulation in the tissues increased significantly after exposure to 0.1 and 1 ppm cadmium for 30 days ($P<0.01$).The highest accumulation occurred in the liver followed by the gill and muscle. Cadmium concentrations in the tissues of control animals were below the detection limit of the AAS used in this study.

Elimination of cadmium in both freshwater -and EDTA- containing media increased with increasing exposure period ($P<0.01$). The greatest cadmium elimination was in the gill in both media. In all experimental conditions, the elimination of Cd from *T. zilli* was greater in EDTA-containing medium than in clean water without EDTA.

Protein concentrations in the liver and gills of *T.zilli* were higher in animals exposed to 0.1 and 1 ppm Cd for 30 days than in control animals. However, it was lower in the muscle of metal-exposed animals than in control animals. Conversely, in the elimination experiments protein concentrations were higher in the liver and gill and, lower in animals exposed to freshwater or EDTA than in control animals ($P<0.01$).

No mortality was observed within the 30 d exposure period to 0.1 and 1 ppm cadmium. Abel and Papatsoglou (1986) reported that *Tilapia aurea* survived a wide range of cadmium concentration, however, fish mortality increased with increasing exposure concentration of cadmium. They reported that the 35 d LC50 values of *T. aurea* ranged between 0.7 and 20 mg Cd/L.

Cadmium accumulation in tissues of fish increases with increasing exposure periods and concentrations (Honda et al. 1983 ; Amiard et al. 1987). Results of

this study also showed that cadmium accumulation increased with increasing exposure concentrations. Cadmium generally accumulates to high levels in organs which have high metabolic activities such as the liver and kidney (Brown et al. 1986 ; Kay et al. 1986). In this study the highest accumulation also occurred in the liver, followed by gill and muscle.

Studies carried out on fish showed that Cd uptake from food was an important route of uptake (Haesloop and Schirmer 1985). During the experiments conducted here, fish were fed twice daily with artificial fish food. Cadmium in the medium may have been adsorbed on to the food, and, therefore, data in Table 1 could also indicate a route of uptake for the cadmium.

Table 1. Cadmium concentration (μg/g dry wt.) in the tissues of Cd-accumulated fish after treatment with EDTA or freshwater.

	N	Exposure Period (Days)	Muscle $\bar{X} \pm S\bar{x}$	Gill $\bar{X} \pm S\bar{x}$	Liver $\bar{X} \pm S\bar{x}$
Control	6		ND	ND	ND
0.1 ppm Cd	6	30	7.21 ± 0.47 **a*s***	30.41 ± 1.45 **a*s***	42.76 ± 1.68 **a*s***
0.3 ppm EDTA	6	15	4.41 ± 0.11 **b**	8.41 ± 0.67 **b**	21.30 ± 1.65 **b**
	6	30	3.59 ± 0.14 **b**	4.67 ± 0.18 **c**	14.99 ± 1.22 **c**
Clean Water	6	15	4.82 ± 0.23 **t**	14.23 ± 0.82 **t**	30.47 ± 1.05 **t**
	6	30	4.35 ± 0.32 **t**	7.83 ± 0.54 **x**	20.63 ± 1.17 **x**
1.0 ppm Cd	6	30	12.14 ± 1.47 **a s**	44.26 ± 1.09 **a s**	74.18 ± 1.47 **a s**
3.0 ppm EDTA	6	15	5.03 ± 0.25 **b**	12.78 ± 0.33 **b**	51.13 ± 0.90 **b**
	6	30	4.31 ± 0.33 **b**	8.83 ± 0.61 **c**	42.13 ± 0.85 **c**
Clean Water	6	15	8.31 ± 0.67 **t**	20.37 ± 1.05 **t**	61.24 ± 0.95 **t**
	6	30	6.42 ± 0.69 **t**	11.20 ± 0.78 **x**	50.21 ± 0.84 **x**

*=SNK :Letters a, b and c show the differences among , Cd and EDTA; and s, t and x among Cd and freshwater. Data shown with different letters are significantly different at the P<0.01 level.

$\bar{X} \pm S\bar{x}$:Mean ± Standard Error

N :Number of fish in each group

ND :Not detectable (<0.028 ppm)

Cadmium elimination from the tissues of Cd-contaminated fish increased with increasing treatment with EDTA. Muramoto (1982) reported that Cd-contaminated *Cyprinus carpio* eliminated high levels of Cd after treatment with freshwater and EDTA, suggesting that elimination effects of EDTA could be due to complexation of EDTA with Cd adsorbed or precipitated on the surface of cells. EDTA also causes loose binding of metals with proteins which could lead also to higher elimination of Cd from the fish.

Cadmium concentrations in the tissues significantly decreased after 30 days of exposure to EDTA. In fish treated with 0.1 ppm Cd, tissue Cd concentrations decreased 51% in the muscle, 77 % in the liver and 85% in the gills. These concetrations in fish treated with 1 ppm Cd were 77% in the muscle, 44% in the

liver and 80% in the gills. In both groups, the highest Cd elimination was observed in the gills. This is possibly due to the gills having direct contact with the environment.

Elimination of cadmium from the tissues was higher in EDTA than freshwater. These results were also supported by Muramoto (1983) who found higher levels of Cu elimination in EDTA than freshwater.

Table 2. Protein concentration (mg/g wet wt.) in the tissues of Cd-accumulated fish after treatment with EDTA or freshwater.

	N	Exposure Period (Days)	Muscle $\bar{X} \pm S\bar{x}$	Gill $\bar{X} \pm S\bar{x}$	Liver $\bar{X} \pm S\bar{x}$
Control	6		56.39 ± 1.22 **a*s***	37.55 ± 1.55 **a*s***	42.50 ± 0.99 **a*s***
0.1 ppm Cd	6	30	35.29 ± 0.52 **b t**	53.63 ± 0.37 **b t**	56.65 ± 0.47 **b t**
0.3 ppm EDTA	6	15	40.94 ± 0.37 **c**	48.39 ± 0.44 **c**	50.59 ± 1.08 **c**
	6	30	46.65 ± 1.52 **d**	38.96 ± 0.76 **a**	43.52 ± 0.42 **a**
Clean Water	6	15	38.95 ± 0.23 **x**	49.17 ± 0.52 **x**	52.53 ± 0.68 **x**
	6	30	43.33 ± 0.75 **y**	42.79 ± 0.92 **s**	46.71 ± 1.05 **s**
Control	6		56.39 ± 1.22 **a s**	37.55 ± 1.55 **a s**	42.50 ± 0.99 **a s**
1.0 ppm Cd	6	30	26.88 ± 0.50 **b t**	57.45 ± 1.33 **b t**	65.30 ± 1.91 **b t**
3.0 ppm EDTA	6	15	33.07 ± 2.41 **c**	50.86 ± 1.65 **c**	56.76 ± 2.05 **c**
	6	30	40.43 ± 0.59 **d**	40.19 ± 0.76 **d**	45.21 ± 0.62 **d**
Clean Water	6	15	30.50 ± 1.15 **x**	52.24 ± 0.67 **x**	59.83 ± 1.67 **x**
	6	30	36.44 ± 0.62 **y**	44.18 ± 0.68 **y**	51.82 ± 0.84 **y**

*=SNK :Letters a, b, c and d show the differences among control, Cd and EDTA ; and s, t, x and y among control, Cd and freshwater. Data shown with different letters are significantly different at the $P<0.01$ level.

$\bar{X} \pm S\bar{x}$:Mean ± Standard Error

N :Number of fish in each group

In this study, Cd exposure of *T. zilli* caused increases in total protein concentration of the gills and liver, whereas it caused a decrease in protein concentration of the muscle. Similarly, in a study carried out with *Clarias batrachus*, results showed that Cd exposure caused an increase in protein concentration of the liver and a decrease in protein concentration of the muscle (Jana and Shana 1988). Hilmy et al. (1985) found that cadmium exposure increased protein concentration of the gill in *Mugil cephalus*. Cadmium in the liver causes induction of metallothionein, which is a low molecular weight metal-binding protein (Hidalgo et al. 1985 ; Kay et al. 1986). Hilmy et al. (1987) indicated that increases of protein concentrations in the liver of *Clarias lazera* and *T. zilli* after exposure of zinc could have been due to increases in metallothionein induction in the liver. Jana et al. (1986) found that protein concentrations in the muscle of *C. batractus* decreased after exposure to metals. They indicated that this could have been due to destruction of protein synthesis.

Results of this study showed that EDTA increased elimination of Cd from the tissues of Cd-contaminated fish and also decreased protein concentration of the tissues. This could be due mainly to decreases in the induction of metal-binding proteins. Total protein concentration was increased after EDTA treatment, suggesting that disrupted protein synthesis in the muscle due to 30 days of Cd exposure was normalized after EDTA treatment.

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