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Metal Interactions During Accumulation and Elimination of Zinc and Cadmium in Tissues of the Freshwater Fish *Tilapia nilotica*

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Cadmium is often present together with zinc in metal-polluted aquatic environments. Zinc has an antagonistic and protective action in the uptake and toxic effects of cadmium, probably because of Zn-induced synthesis of metallothionein that detoxifies Cd by firmly binding this metal (Hemelraad et al. 1987). Similar effects have been observed in the interaction of heavy metals such as selenium and mercury(Lucu and Skreblin 1981).

Since the toxic effects and accumulation of different combinations of various metals on organisms depend on the metal and organism under consideration, a substantial amount of research is done in this field for freshwater(Hemelraad et al. 1987, Hutchinson and Sprague 1989) and marine organisms (Weis 1980, Negilskiet al. 1981, Moraitou-Apostolopoulou and Verriopoulos 1982).

Studies of interrelationships between heavy metals are of great significance in environmental evaluations, since contamination is rarely limited to a single compound It is therefore important to study the uptake and elimination rates of combinations of metals in aquatic environments (Lucu and Skreblin 1981, Wicklund et al. 1988). The aim of this study was to determine the effects of metal interaction on the accumulation and elimination of zinc and cadmium in the liver, gill and muscle tissues of *Tilapia nilotica.*

MATERIALS AND METHODS

Freshwater fish *T. nilotica* were obtained from pools and acclimatized in the laboratory for one month, at 25±1°C which was also the temperature of the experimental conditions. After this period the mean length and weight of the animals were 11.9±1.87 cm and 46.5±2.15 gr., respectively. The water used for the experiments had a pH value of 8.1±0.5 , dissolved oxygen of 7.1±0.3 mg/L, total hardness of 180.5±5.54 $CaCO_3$ mg/L, and total alkalinity of 274.2±9.7 $CaCO_3$mg/L)

A total of 9 aquariums sized 40x100x40 cm in height were divided into three groups of 3 aquariums each. These were filled with 120 L. of test solution or tap water. Eighteen fish were put in each aquarium Two aquariums of the first group contained 0.1 and 1.0 ppm Cd ($CdCl_2$, H_2O) solutions and two aquariums of the second group contained 1.0 and 10.0 ppm Zn ($ZnCl_2$ H_20) solutions and two aquariums of the third group contained 0.1 ppm Cd + 1.0 ppm Zn and 1.0 ppm Cd + 10.0 ppm Zn solutions. The third aquarium of each group was used as a control The test media were changed every two days to replenish either the Cd or Zn. Six fish from each aquarium were taken out on day 10, and the Cd and Zn concentration in their tissues was measured for accumulation experiments. The remaining metal-contaminated fish were kept in clean freshwater for 15 and 30 days for the elimination experiments. For each exposure period and concentrations, six fish were analyzed to determine Cd and Zn concentrations.

After each test period fish were dissected and the liver, gills and muscle were removed. The dry weight of the dissected tissues was determined after being kept at 105°C for 48 hr. Dried tissues

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were digested with 3.0 ml. of a mixture of HNO_3, and $HCLO_4$ in a 2: 1 ratio (Muramoto 1983). Acid digestion was carried out at 120°C for three hours. After dilution with distilled water, cadmium and zinc concentrations of tissues were measured using a Perkin Elmer Atomic Absorption Spectrophotometer, calibrated with C-5524 Sigma Standards. Detection limit of the spectrophotometer was 0.028 ppm and 90% recovery was obtained during measurement. Data analyses were carried out using Student Newman Keul's Test (SNK).

RESULTS AND DISCUSSON

Mean and standard errors of cadmium and zinc concentrations in the tissues for each exposure concentration are given in tables 1 and 2.In these tables data shown with different letters indicate statistical differences at P<0.01 level. In all concentrations tested, no mortality was observed within 10 days exposure period

In both exposure concentrations, Cd accumulations in the tissues of fish exposed to Cd+Zn mixtures for 10 days were lower when compared with those exposed to Cd only (Table 1, P<0.01).In all concentrations tested, highest levels of cadmium were found in the liver of *T. nilotica,* followed by the gill and muscle tissues.

Table 1. Cadmium levels (μg/g dry wt.)in the tissues of *Tilapia nilotica* exposed to Cd alone and Cd-Zn mixture for 10 days.

Concentrations (ppm)	Liver X±Sx	*	Gill X±Sx	*	Muscle X±Sx	*
Control	ND		ND		ND	
0.1 ppmCd	17.69±1.15	a	14.46±1.00	a	4.37±0.25	a
0.1 ppmCd+1.0 ppmZn	14.55±1.06	b	11.50±1.05	b	3.01±0.32	b
1.0 ppmCd	32.89±1.72	a	25.35±1.22	a	6.94±0.32	a
1.0 ppmCd+10.0 ppmZn	26.78±1.65	b	21.26±1.35	b	5.07±0.25	b

*=SNK: Letters a and b show differences between Cd and Cd+Zn mixture. Data shown with different letters are statistically significant at the P<0.01 level.
X±Sx: Mean ±Standard Errors.
ND : Not Detectable.

Table 2. Zinc levels (μg/g dry wt.) in the tissues of *Tilapia nilotica* exposed to Zn alone and Zn-Cd mixture for 10 days.

Concentrations (ppm)	Liver X±Sx	*	Gill X±Sx	*	Muscle X±Sx	*
Control	23.33±1.50	a	43 77±2.05	a	14.77±1.10	a
1.0 ppmZn	32.94±1.72	b	74.62±2.75	b	23.60±1.15	b
1.0 ppmZn+0.1ppmCd	30.80±2.10	b	75.00±2.25	b	23.53±1.32	b
Control	23.33±1.50	a	43.77±2.05	a	14.77±1.10	a
10.0 ppmZn	46.48±2.12	b	79.48±2.74	b	28.52±1.17	b
10.0 ppmZn+1.0 ppmCd	64.88±2.81	c	87.62±2.38	c	33.72±1.71	c

*=SNK: Letters a,b and c show differences among Control, Zn and Zn +Cd mixture. Data shown with different letters are statistically significant at the P<0.01 level.
X±Sx: Mean ±Standard Errors.

Zinc accumulation in the tissues of fish exposed to high levels of Cd+Zn mixture (1.0 ppm Cd + 10.0 ppm Zn) for 10 days increased when compared to those exposed to high level of Zn only (10.0 ppm Zn). In all concentrations tested the highest accumulation of zinc after 10 days of exposure to the zinc was observed in the gills, and the lowest accumulation in muscle.

Accumulation and elimination of Cd and Zn in the tissues of *T. nilotica* are given in figures(1 to 4).Significant elimination of cadmium occurred in the gill in all concentrations tested (fig. 1 and 2, $P<0.01$), while the muscle did not show any significant elimination($P>0.05$).Significant elimination of cadmium was detected in liver Cd+Zn mixtures ($P<0.01$) but not in Cd only at the end of 30 days. Cadmium levels in the gill and liver declined by 70% and 40% respectively at the end of 30 days of elimination period

In all concentrations tested significant elimination of zinc was detected in the gill (fig. 3 and 4, $P<0.01$) while the liver elimination occured in 10.0 ppm Zn and the 10.0 ppm Zn plusl.0 ppm Cd mixture ($P<0.01$). Muscle did not show any significant elimination of zinc at the end of 30 days of elimination period in any of the exposures ($P>0.05$).

Metal accumulation in tissues of fish is dependent upon exposure dose and time as well as other factors such as temperature, age of fish, interaction with other metals, water chemistry and metabolic activity the of fish (Pagenkopf 1983, Heath 1987, Goyer 1991). Accumulation of *cadmium* whole tissues of *T.nilotica* decreased in the presence of zinc. In a study carried out *with Mytilus edulis planulatus* cadmium accumulation was reduced in the presence of zinc (Elliot et al.1986). Hemelraad et al. (1987) found that the presence of zinc caused a decrease in the accumulation of cadmium in freshwater clams *Anadonta cygnea.* Different effects of zinc on the accumulation of Cd in various tissues of *Pandalus montagui* was observed by Ray et al. (1980).

In this study, accumulation of zinc in tissues of *T. nilotica* increased in the presence of Cd. However, this increase was observed only at the highest concentrations of Zn +Cd mixture used in the experiments. Elliot et al. (1986) studied metal interaction during accumulation by the mussel *Mytilus edulis planulatus,* and found that zinc accumulation was increased in the presence of Cd. However, this increase was only at the highest concentrations of both metals.

The toxic effects and accumulation of different combinations of various metals on organisms depend not only on the metals of the mixture but also on the organism tested. Cadmium and zinc indicated clear *eases* of synergism to the copepod *Tisbe holothuriae* (Verriopoulos and Dimas 1988) while the same metals acted as antagonists in the crabs *Uca pugilator* (Weis 1980).

In this Study, an antagonistic effect of zinc on cadmium accumulation was determined in the *T. nilotica.* Many studies of Cd-Zn interaction in aquatic organisms have concentrated on the antagonistic effect of zinc, in other words, on the protective effect of zinc against the toxicity of cadmium (Eisler and Gardner 1973, Weis 1980, Hemelraad et al 1987, Verriopoulos et al. 1987). These researchers have suggested that zinc has an antagonistic effect on cadmium accumulation either by the inhibition of cadmium uptake *from* the gills or by the acceleration of the transport of the ingested cadmium to the internal organs. The antagonistic effect of zinc on the cadmium accumulation *in T nilotica tissues can be* partially explained by the competition of these two metals for the binding sites on the protein carriers such as metallothionein or at the uptake sites on the gills.

Several factors influence the elimination of metals from the tissues of aquatic animals. These include time, temperature, interacting agents, age of fish, metabolic activity of animals and biological half lives of metals (Larson et al. 1985, Heath 1987, Rao et al.1988, Douben 1989, Woo et al. 1993, Kargm 1996, Nielsen and Andersen 1996).Elimination routes of metals from fish are generally through gill, bile, urine, skin, and via mucus (Varanasi and Markey 1978, Heath 1987)Metal elimination routes are more numerous than uptake routes, however metal accumulation is more rapid than metal elimination, probably due to the presence of metal binding proteins in tissues.

In the present study the gills showed fastest elimination rates of metals in all concentrations tested, whereas significant elimination of Zn from the liver was seen only in high Zn concentrations and Cd+Zn mixtures, and muscle did not eliminate the metals after 30 days of

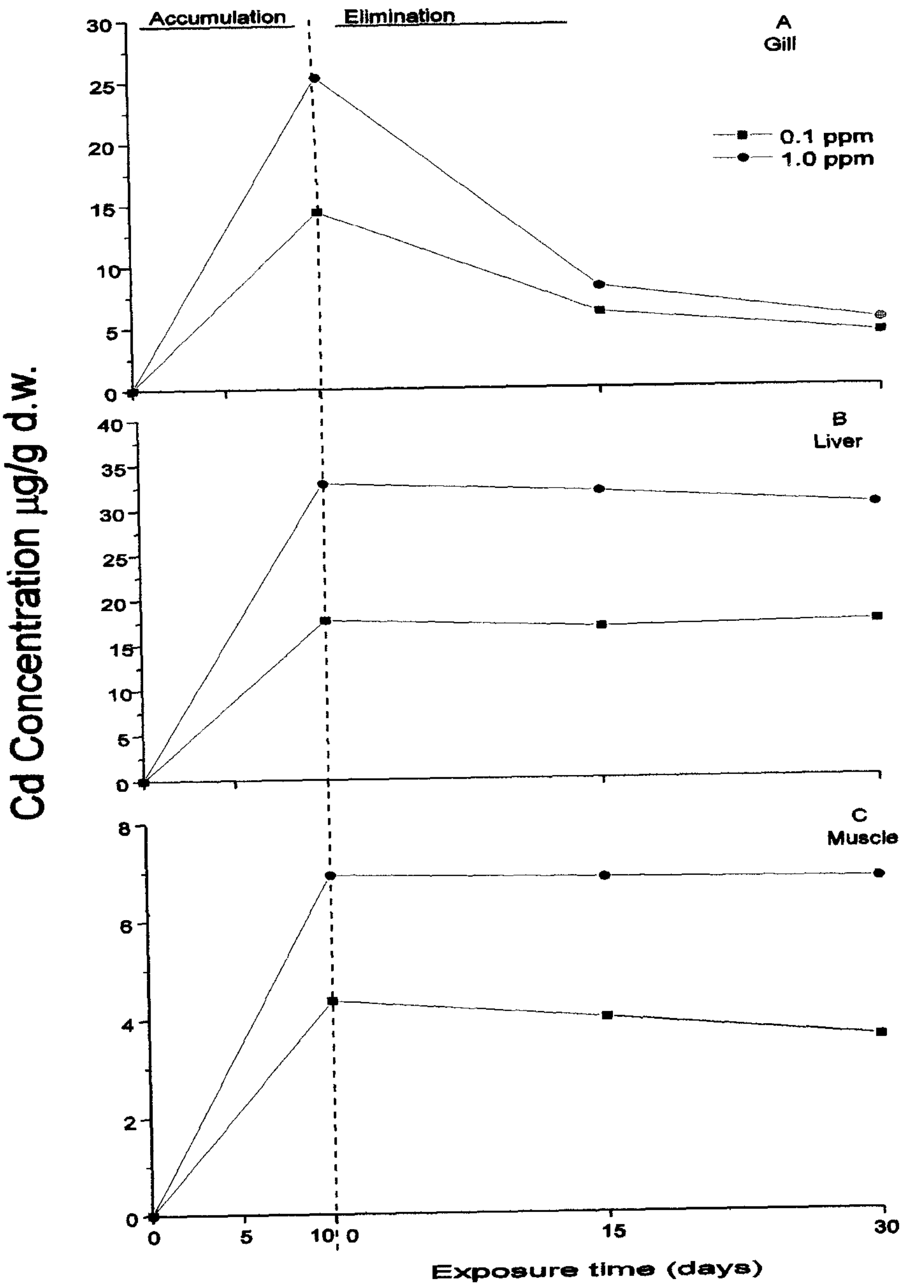

Figure 1. Cadmium accumulation (10 day) and elimination (30 day) in the gill (A), liver (B) and muscle (C) tissues of *Tilapia nilotica* exposed to cadmium alone.

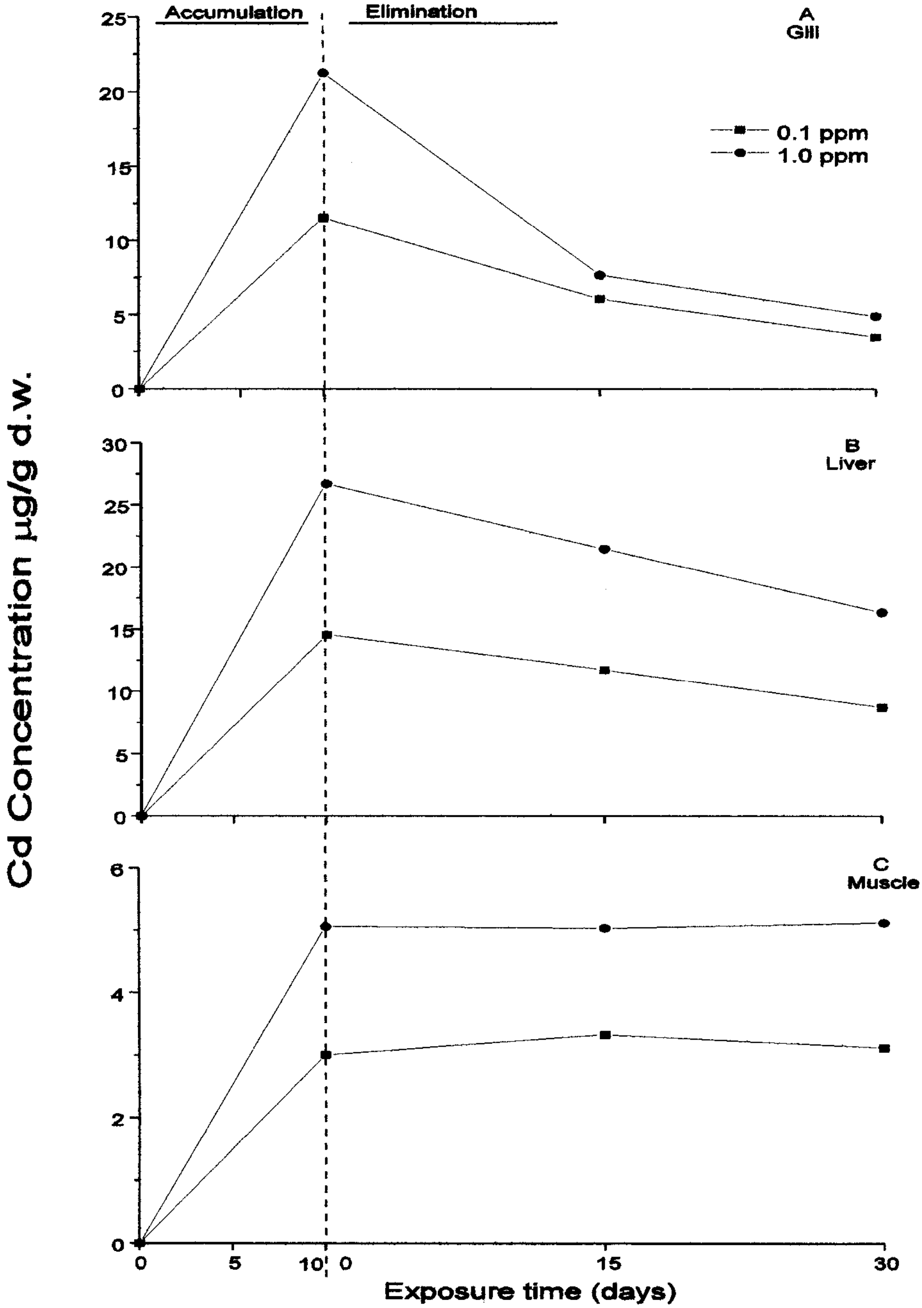

Figure 2, Cadmium accumulation (10 day) and elimination (30 day) in the gill (A), liver (B) and muscle (C) tissues of *Tilapia nilotica* exposed to Cd+Zn mixture.

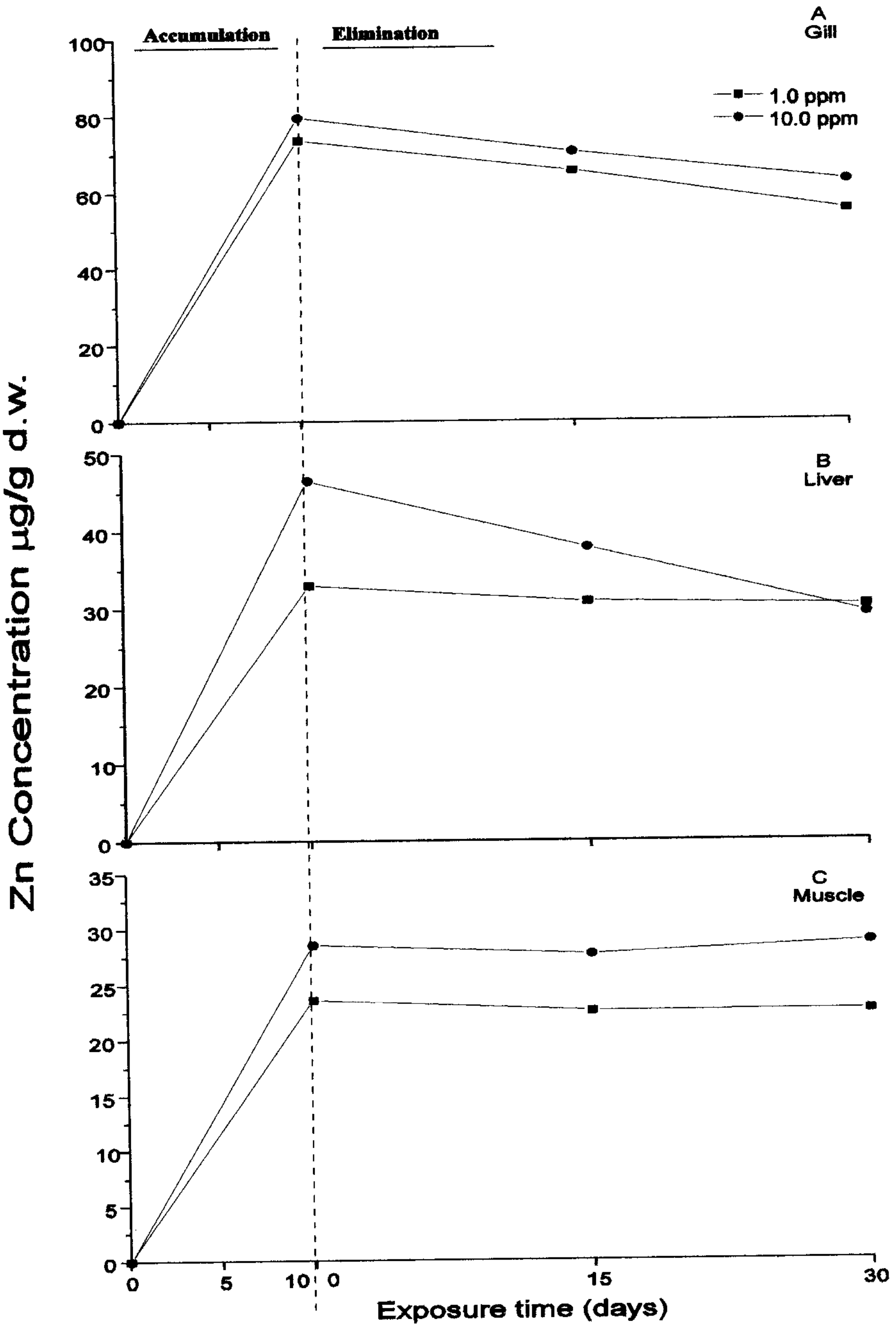

Figure 3. Zinc accumulation (10 day) and elimination (30 day) in the gill (A), liver (B) and muscle (C) tissues of *Tilapia nilotica* exposed to zinc alone.

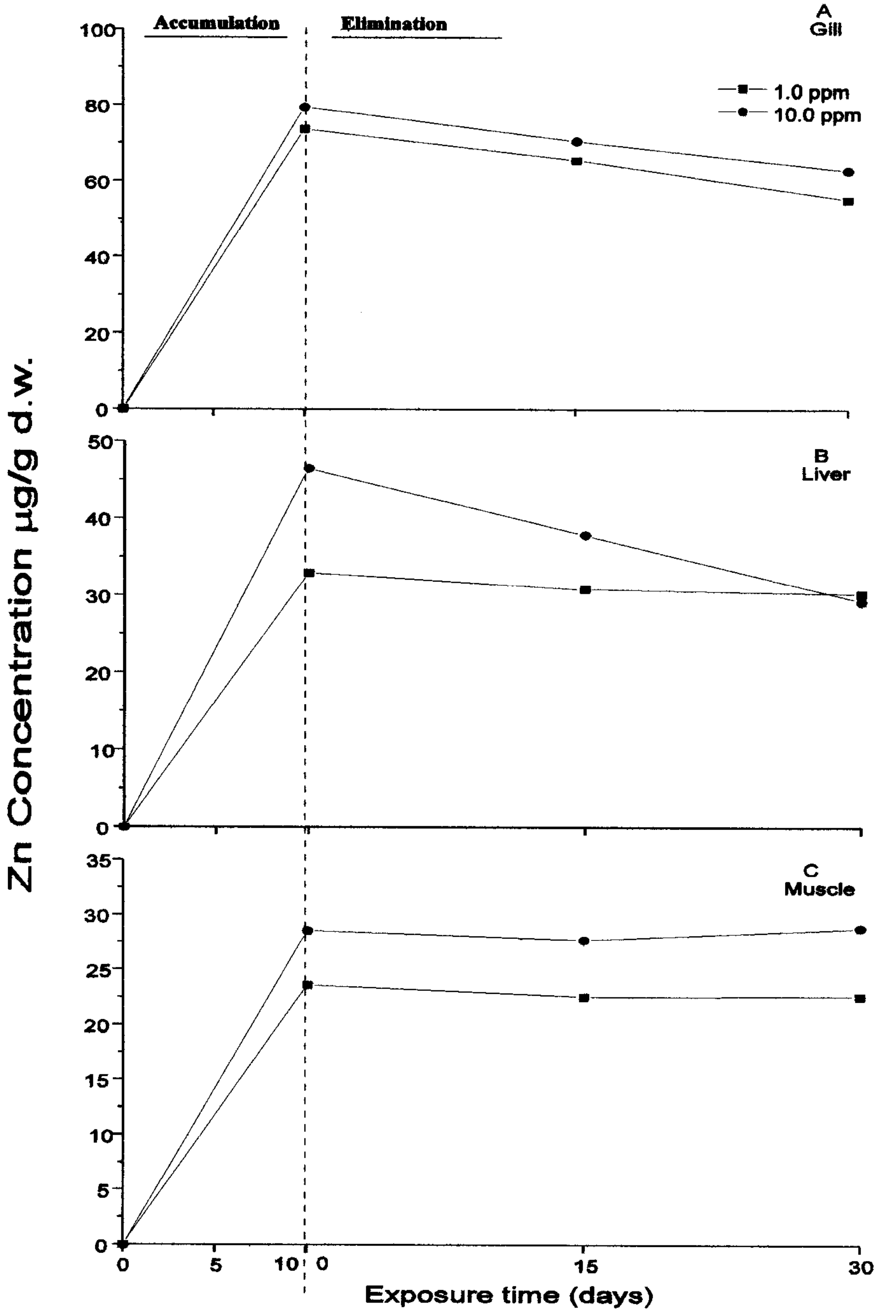

Figure 4. Zinc accumulation (10 day) and elimination (30 day) in the gill (A), liver (B) and muscle (C) tissues of *Tilapia nilotica* exposed to Cd+Zn mixture.

elimination period. Similar patterns of heavy metal elimination were also shown in other studies carried out with aquatic animals (Viarengo et al. 1985, Wicklund et al. 1988, Woo et al. 1993).In all concentrations tested, the highest metal elimination was observed in the gills. This is possibly due to gills having direct contact with the environment. Elimination of metals is relatively low in the liver. This is probably because it is the organ of metal storage and detoxification.

In both mixed exposure concentrations, Zn significantly increased Cd elimination rate in the liver of *T. nilotica.* This is probably due to interactions at sites binding both metals in tissues. Wicklund et al. (1988) studied the effects of Zn on the elimination of Cd in the zebrafish *Brachydenio rerio,* and found that Zn tended to increase the Cd elimination rate in the gills.

Elimination of heavy metals depend on the biological half-life of the metals and differences between various species of water animals. Viarengo et al. (1985) found that *Mytilus galloprovincialis* exposed to Cu and Cd showed different levels of metal elimination from its tissues. Cu is rapidly eliminated from the gill and digestive gland cells, showing a biological half life of 10 days, whereas Cd is released much more slowly from the tissues. Very slow elimination of cadmium and mercury was observed in the clams *Macoma balthica* (McLeese and Ray 1984, Riisgard et al. 1985). In a study carried out with the zebra mussel *Dreissena polymorpha,* metal excretion differed between the two metals (Zn and Ni); more than half of the accumulated zinc was excreted in twenty four hours, while no nickel excretion was evident (Klerks and Fraleigh 1997). In a study carried out with Rainbow trout , the biological half-life was shorter than !6 weeks for liver lead, whereas the biological half-life for liver and kidney cadmium was estimated to be more than a year (Larson et al. 1985).

To study the effects of metal interaction on metal accumulation and elimination in fish it would be useful to locate metal binding proteins and metals in the tissues.

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