ACTA BIOL. SZEGED. 38. pp. 11–18 (1992)

METALS EFFECT ON FISH TISSUES
II. THE EFFECT OF CHRONIC ZINC AND CADMIUM TREATMENT ON YOUNG TILAPIA TISSUE ENZYMES AND LIPID PEROXIDATION

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(Received: May 12, 1992)

Abstract

Metals effect on liver and muscle tissues of young Tilapia *(Oreochromis niloticus)* were studied under separate and simultaneous effects of $ZnCl_2$ and $CdCl_2$ during 120 days, with special regards to the respective ion containing active site of the enzymes. Besides the enzymes, changes in lipid peroxidation value in both tissues were also studied.

Key words: Young Tilapia, chronic treatment, zinc chloride and cadmium chloride, tissue enzyme activity, lipid peroxidation.

Introduction

Due to industrial development and the extensive use of chemicals in agriculture, heavy metals are widely distributed in aquatic systems and can affect fish populations, reducing their growth, reproduction and causing significant mortality (VINIKOUR et al., 1980). Similarities in chemical reactivity of cadmium (Cd) and zinc (Zn) lead to similar metabolic pathways in biological systems. Whereas, Zn is an important essential element, acting as co-factor for many enzymes, necessary for DNA synthesis and others. Cd is best known for its toxicity and metabolic antagonism of Zn and other essential elements. Anemia, bone demineralizaton and kidney damage are principal adverse effects of Cd ingested in moderate amount, its higher levels can lead to death (VALLEE et al., 1972). In general Cd can inhibit several key enzymes metabolic processes and it can reduce their ability in osmoregulation. It has been suggested that the environmental contamination of Cd found even in low concentration in the nature, interferes

with the basal metabolism of birds and mammals (SUZUKI et al. 1990a, b, c; MENENDER-BOTET et al. 1991). The aim of the present work was to study the effects of different concentrations of cadmium and zinc (both used in plant protection) on the activity of metabolic enzymes, protein values and level of the lipid peroxidation of tilapia *(Oreochromis niloticus)*.

Materials and Methods

Fish: Two separate experiments were carried on Tilapia *(Oreochromis niloticus)* fingerlings weighing about 4—5 g/fish in average, obtained from the Marjout Fish Farming Company, Alexandria Governorate, Egypt. They were kept in glass jars (105 litre capacity) at temperature of 28 °C and fed for seven days on basic diet. At the start of the experiment healthy fish were weighed and distributed in the experimental jars. Fourteen jars of 105 litres capacity each were used in the experiment and filled with tap water stored for two days before use. Water was changed every three days (about third part changed every day). Water temperature was thermostatically controlled at 28 °C±1 °C.

The formulated basic diet consisted of fish meal, soybean meal, yellow corn and bone meal, fish oil, vitamins and minerals, and was used as control diet RADY et al., (1992) without the addition of stock sol. of A or B.

Experimental diets: Stock solutions of trace metals: cadmium chloride ($CdCl_2$ and zinc chloride ($Zn\ Cl_2$) of 100 ppm concentrations were prepared. They were prepared by dissolving 0.203 g of $CdCl_2$ hydrate with 5 ml of conc. HNO_3 in 1 litre of distilled waater (stock solution A) and one gram of $ZnCl_2$ and 20 ml conc. HCl in one litre of distilled water (stock solution B) suggested by CHAPMAN et al (1961).

The experimental diets were prepared by the addition of 5, 10 and 15 ml from stock solution A per kg of basic diet to give diet 5, 10 and 15 ppm of cadmium, respectively, and 10, 20 and 30 ml of stock solution B added 1 kg of basic diet to give diets of 10, 20 and 30 ppm of zinc concentration. Mixture of $CdCl_2$ (solution A) and $ZnCl_2$ (solution B) were prepared by adding 5,10 and 15 ml of solution A and 30, 20 and 10 ml of solution B respectively.

Feeding Strategy: Fish in every jar were fed on experimental diet (two jars) for each diet at a rate of 8 per cent on the basis of live body weight, first week, then feeding level was reduced to 6 per cent from the third week on, and to 4 per cent from the 7th week. (Daily feed was divided into 100 equal portions and offered for each jar and adjusted every 7-day intervals to the fresh body weight.) The glass jars were cleaned daily to prevent accumulation of faeces. Specimens of different treatments were collected and taken alive to the laboratory in aerated plastic bags.

The apparent examination showed that all fishes were somatically healthy and parasite-free.

Fish sampling: At the end of the experiments five fish from each jar were taken sacrificed by vertebral rupture, then liver and parts from the muscle tissues were rapidly removed. A part of each tissue were weighted and homogenized with 0,64 per cent sodium chloride for biochemical analysis.

Biochemical assays: Total protein was determined by the method of LOWRY et al. (1951), for preparation of calibration curve bovine serum albumin was used.

Lipid peroxidation (LP): Malondialdehyde (MDA) was used as an indicator for lipid peroxides. It was determined by the method described by PLACER et al (1966). Calibration curve was prepared by using malondialdehyde diethyl acetate (Merck, Germany).

Alkaline and acid phosphatase (AlPh-as; EC 3.1.3.1) (AcPh-ase; EC 3.1.3.1) activities were measured activities by the method of BERGMEYER (1974). Phenol released through enzyme hydrolysis

from phenyl phosphate was determined under defined conditions, colorimetrically at 400 nm. Time and pH were also measured.

Lactate dehydrogenase (LDH; EC 1.1.1.27) activity was measured also colorimetrically by the method of ANON (1971). The method is based on reduction of pyruvate by incubation with the enzyme in the presence of NADH. The reaction was arrested by adding dinitrophenyl hydrazine to the solution which reacts wiht the remaining pyruvate, the colour produced was measured at 510 nm.

Glutathione S-transferase (GSH-S-Tr-ase; EC 2.51.18) enzyme activity expressed as μmol of 4-chloro-1,3 dinitrobenzene (CDNB) conjugated /minute/ mg protein according to the method of VESSEY et al. (1984).

Results

In Fig. 1 it can be seen that AlPh-ase activity of liver tissues is increasing steadily after the effect of $ZnCl_2$, while it is gradually decreasing affected by $CdCl_2$, both in the function of concentration. Combination of the two metals, however, do not show any characteristic effect.

AcPh-ase activities are elevated by increased $ZnCl_2$ concentrations, while activating effect of $CdCl_2$ is decreasing along with its concentrations increases. The same characteristic is demonstrated by the simultaneous effects of the two metals. etc. of $CdCl_2$ is demonstrated in Figure 2, which is prevailing, though to a lesser extent upon the treatment with the two metals.

Effect of $ZnCl_2$ and $CdCl_2$, respectively and in combination on GSH-S-Tr-ase activity can also be seen in Fig. 2. Here, Zn has an inhibitory effect, as well as that of the higher concentrations of $CdCl_2$.

Liver parenchyma LP is decreased by $ZnCl_2$, while increased by $CdCl_2$ in the function of the concentration of the latter.

Changes in the activities of the same enzymes in muscle tissues are shown in Figs 3 and 4. Here, striking are the low enzyme activities of muscle-tissues and that all enzyme activities were increased at low- while inhibited at higher concentration of $CdCl_2$.

No noticeable is the LP increasing effect of the highest concentration of $CdCl_2$ in muscle tissues.

The results in Table 1 showed that most of Cd^{2+} an Zn more accumulated mainly, in the liver. Increasing the level of Cd^{2+} and Zn in the diet significantly increased its accumulation in liver and muscle. Zn supplementation greatly reduced Cd^{2+} in the tissues of tilapia.

Discussion

Cadmium occurs widely in the nature in close association with Zn. Cadmium as a human pollutant (Steel industry, waste incineration, volcanic action, Zn production etc...) continuously added to soil, water and air. The main feature of Cd^{2+} toxicity are (i) accumulation in soft tissues (liver and kidney) (ii) inter

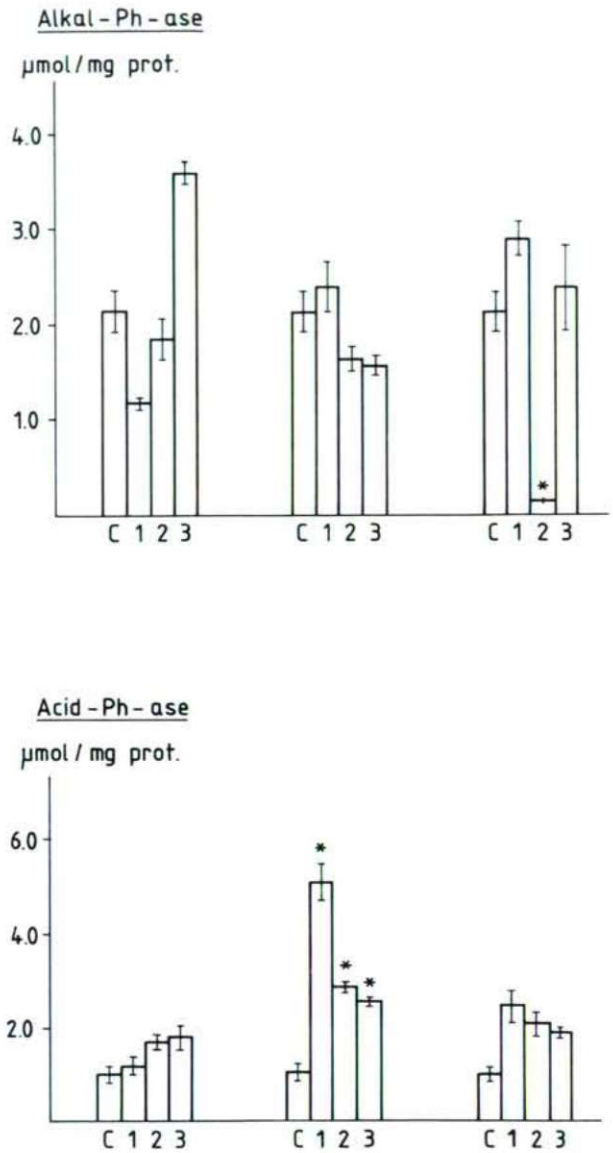

Fig. 1. Effects of metal treatments on liver tissue enzyme activities, first row of columns demonstrate the effect of 10, 20 and 30 ppm $ZnCl_2$ on AlPh-ase and AcPh-ase activities in tilapia liver compared with the control. The second row of columns shows the influence of $CdCl_2$ of 5, 10 and 15 ppm, respectively, on the enzymes. The third row of columns illustrate the simultaneous effect of $ZnCl_2$ and $CdCl_2$ (1 = 30 ppm $ZnCl_2$+5 ppm $CdCl_2$; 2 = 20 ppm $ZnCl_2$+10 ppm $CdCl_2$; 3 = 10 ppm $ZnCl_2$+15 ppm $CdCl_2$; 3 = 10 ppm $ZnCl_2$+15 ppm $CdCl_2$) treatment on enzyme activities.

*and** = p 0.01 and P 0.001 significances, respectively.

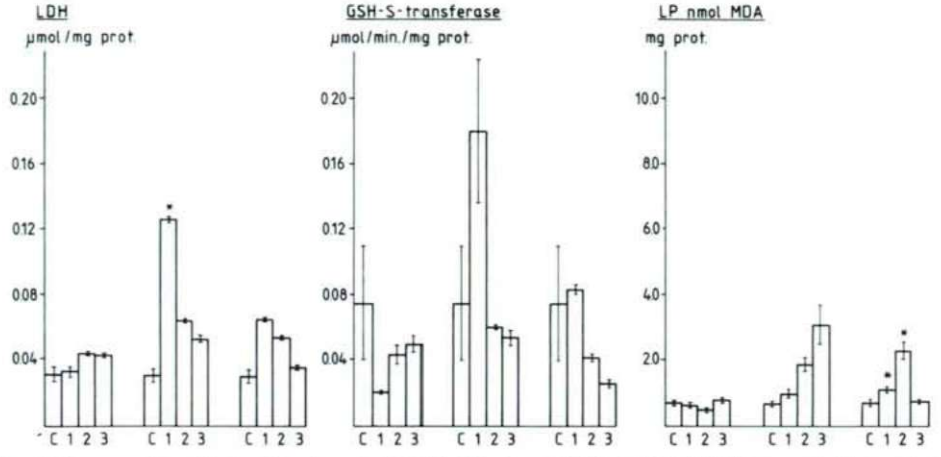

Fig. 2. Sums up the effects of treatments listed in Fig. 1 on LDH and GSH-S-Tr-ase activities and on LP in liver homogenates.

action with other divalent cations (mainly with Zn) (iii) Long half-live time (iv) Very slow elimination. Its metabolism and transport is bound to metallothionein.

As to follow up our eaarlier investigatins with carp (RADY et al. 1988) in the present work it was studied how $ZnCl_2$ and $CdCl_2$, respectively, at three different concentrations, and in combination at various defined concentrations, affect liver and muscle biochemical parameters of young *Tilapia*.

It could be observed that upon the treatment with the two metals, either

Table 1. The distribution of cadmium and zinc in liver and muscle of tilapia *(Orochromis niloticus)* μg/gm w. t.

Treatment	Cadmium		Zinc	
	Muscle	Liver	Muscle	Liver
Control	–	–	0.260	2.43
$CdCl_2$ mg/kg feed				
5	0.245	13.28	0.245	2.320
10	0.265	14.18	0.135	1.820
15	0.318	14.61	0.125	1.500
$ZnCl_2$ mg/kg feed				
10	–	–	0.365	6.070
20	–	–	0.790	7.120
30	–	–	1.180	9.082
$CdCl_2$:$ZnCl_2$ mg/kg feed				
5:30	0.225	10.17	1.250	6.370
10:20	0.240	12.70	0.813	6.330
15:10	0.312	13.57	0.409	5.250

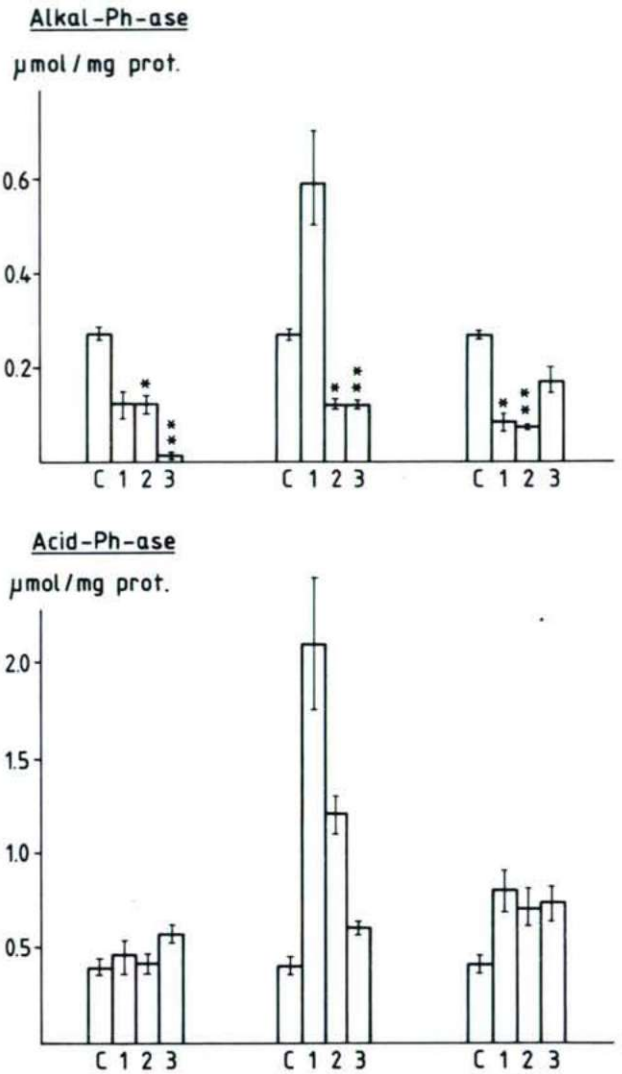

Fig. 3. Effects of treatments detailed in Figure 1 on muscle tissue phosphatases activities.

separately or together, the effect of $CdCl_2$ dominated, which is in line with earlier papers reporting that Cd ion is able to ousting Zn from several important loci of its effect (SUZUKI et al., 1990a; HELLAWELL, 1986 and HEATH, 1987). In additin to lead to significantly enzyme inhibition of white muscle, due to its accumulation increase oxidation of molecular oxygen to superoxide radical, this reaction would act as source of H_2O_2, which inicitiates polymerisation of specific membrane protein.

The present series of investigations might be important from the fact that the enzymes studied contain Zn atom in their active centres.

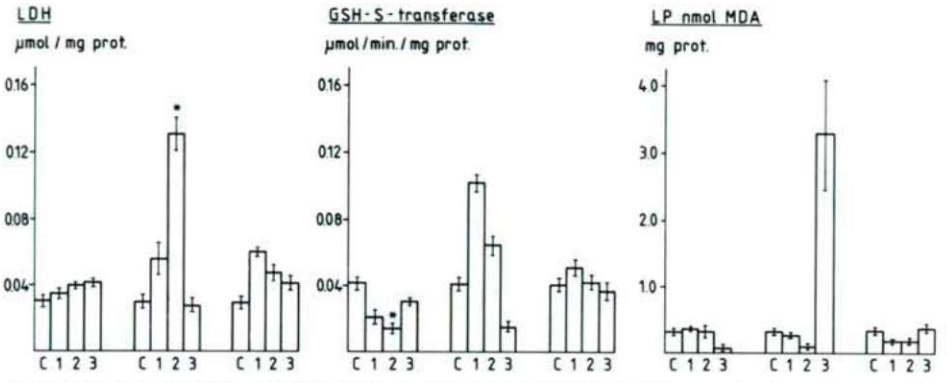

Fig. 4. Changes of LDH and GSH-S-Tr-ase activities and LP values in muscle tissue homogenates upon separate and simultaneous treatments with Zn and Cd.

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