Individual and Combined Effects of Heavy Metals on Serum Biochemistry of Nile Tilapia *Oreochromis niloticus*

Özgür Fırat · Ferit Kargın

Abstract Changes in serum biochemistry in response to single- and combined-metal exposure were studied in a freshwater fish *Oreochromis niloticus*. Fish were exposed to 5.0 mg/L Zn, 1.0 mg/L Cd, and 5.0 mg/L Zn+1.0 mg/L Cd mixtures for 7 and 14 days to determine levels of biochemical parameters and metals in blood serum. The individual and combined effects of metals caused an increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and in levels of albumin, transferrin, ceruloplasmin, cortisol, glucose, and total protein, whereas they caused a decrease in cholesterol levels. At both exposure periods, increased ALT activity of fish exposed to Cd was higher compared with the Zn and Zn+Cd groups, respectively. The decreased cholesterol level was higher in the Cd alone, and for Cd in combination with Zn, than in Zn alone at 14 days. Zn or Cd levels increased in the blood serum of fish exposed to metals individually or in combination. When fish were exposed to the mixtures of Zn+Cd, concentrations of these metals in their serum were lower than in fish exposed to individual metals. One metal blocks or even antagonizes the gill epithelium absorption of the other and thereby limits the distribution of the metal in blood. The results indicate that biochemical parameters in fish blood can be used as an indicator of heavy-metal toxicity.

Ö. Fırat (✉)
Faculty of Science and Letters, Department of Biology,
University of Adiyaman, 02040 Adiyaman, Turkey
e-mail: ofirat@adiyaman.edu.tr

F. Kargın
Faculty of Science and Letters, Department of Biology,
University of Cukurova, 01330 Adana, Turkey

Contamination of aquatic environments by heavy metals, whether as a consequence of acute or chronic events, constitutes an additional source of stress for aquatic organisms (Kori-Siakpere and Ubogu 2008). The impact of contaminants on aquatic ecosystems can be assessed by the measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky et al. 1997). Blood parameters are increasingly being used as indicators of the physiologic or sublethal stress response in fish to endogenous or exogenous changes (Cataldi et al. 1998).

Biologic changes in fish related to exposure or effects of contaminants are called "biomarkers" (Peakall 1994). Prominent among these biomarkers are physiologic variables, such as serum levels of metabolites (Adams et al. 1990; DiGiulio et al. 1995) and ions (Martinez and Souza 2002); levels of hormones, such as cortisol (Benguira and Hontela 2000); and biochemical variables, such as enzyme activities (De la Tore et al. 2000).

Alteration of blood biochemistry may be indicative of unsuitable environmental conditions (temperature, pH, oxygen concentration) or the presence of stressing factors, such as toxic chemicals (Barcellos et al. 2004). It is known that physiologic and biochemical parameters in fish blood and tissues can change when exposed to heavy metals (Cicik and Engin 2005). Previous studies have shown that certain metals can cause either increased or decreased levels of serum protein, cortisol, glucose, and cholesterol, as well as changes in serum enzyme activity, depending on metal type, fish species, water quality, and length of exposure (Ruparelia et al. 1989; Gopal et al. 1997; Vaglio and Landriscina 1999; Monteiro et al. 2005).

Measurement of serum biochemical parameters can be especially useful to help identify target organs of toxicity as well as the general health status of animals and has been

advocated to provide early warning of potentially damaging changes in stressed organisms (Folmar 1993; Jacobson-Kram and Keller 2001). Plasma and serum reflect the physiologic state of an animal because they are the products of intermediate metabolism (Artacho et al. 2007). Serum enzymes (ALT, AST) are known to be important serum markers to investigate the health of an animal species. Specific serum proteins, such as albumin, transferrin, and ceruloplasmin, are considered nonenzymatic antioxidants because they play a major role in metal binding and delivery to tissues. Other serum parameters, such as cortisol, glucose, total protein, and cholesterol, are commonly used as stress indicators. Nile tilapia *Oreochromis niloticus* are widely cultured in tropical and subtropical regions of the world and are an important source of protein for humans. Because of its easy handling, culture, and maintenance in the laboratory, and because it responds promptly to environmental alterations, *O. niloticus* is also a well-established model for toxicologic research (Almeida et al. 2002; Garcia-Santos et al. 2006). Therefore, the main purpose of this study was to determine individual and combined effects of Zn and Cd on serum enzyme (ALT and AST) activities and levels of specific proteins (albumin, transferrin, ceruloplasmin), metabolites (cortisol, glucose, total protein, cholesterol), and metals of *O. niloticus*.

Materials and Methods

Fish and Experimental Design

O. niloticus (80.49 ± 0.9 g of weight, 17.12 ± 0.8 cm of total length, as mean $\pm$ SE) obtained from Cukurova University Fish Culture Farm were transferred to the laboratory. Fish were acclimatized to laboratory conditions in glass tanks for 1 month before exposure. The laboratory was illuminated for 12 h with fluorescent lamps (daylight 65/80 W). Experimental tanks contained 120 L dechlorinated and gently aerated tap water with the following parameters: temperature $21.09 \pm 0.22°C$, pH 8.12 ± 0.08, dissolved oxygen 7.38 ± 0.06 mg/L, alkalinity 202.9 ± 6.113 mg/L $CaCO_3$, and total hardness 349.12 ± 2.24 mg/L $CaCO_3$. Fish were divided into 4 groups each containing 10 fish. Fish in group I were held in tap water as controls, and the other groups were exposed to the following metal concentrations for 7 and 14 days: 5.0 mg/L Zn ($ZnCl_2$); 1.0 mg/L Cd ($CdCl_2 \cdot H_2O$); or 5.0 mg/L Zn+1.0 mg/L Cd. The Zn and Cd concentrations were selected based on 96-h LC_{50} values (60 mg/L for Zn and 16 mg/L for Cd) of *O. niloticus*. 1/12 and 1/16 values of LC_{50} were used for Zn and Cd, respectively. Throughout the experiments, control and experimental fish were fed daily with commercial fish food (Pinar Yem, Turkey) at approximately 3% body weight. Fish were maintained in static renewal conditions. Water and metals were completely replaced every 2 days by transferring fish to freshly prepared metals solutions (Dutta and Arends 2003).

Serum Preparation and Analysis

At the end of the each experimental duration, five fish were removed from the aquaria and used as replicates. Blood samples were taken from the caudal vein of each fish as described by Congleton and La Voie (2001). This blood was collected in anticoagulant-free centrifuge tubes. Serum was obtained by centrifugation of blood at 3000 rpm for 10 min. Serum samples were then stored at –80°C until analysis.

Biochemical parameters in the serum samples were analyzed using biochemical analyzers (Modular Roche DPP, Modular Roche E170; Hitachi Ltd, Tokyo, Japan). Reactants for all measurements in the analyses were supplied by Roche Diagnostics (Mannheim, Germany).

Enzyme Activity

ALT and AST activities were determined using ultraviolet (UV) test technique (Bergmeyer et al. 1985). The products of ALT and AST activities, pyruvate and oxaloacetate, were used to oxidise NADH to NAD^+. The decrease rate of the photometrically determined NADH is directly proportional to the rate of formation of pyruvate or oxaloacetate and thus to ALT or AST activity, respectively.

Specific Protein Level

Albumin (Hubbuch 1991), transferrin (Lizana and Hellsing 1974), and ceruloplasmin (Wolf 1982) levels were measured by immunoturbidimetric assay. In this assay reaction, antialbumin, transferrin, and ceruloplasmin antibodies react with the antigen in the sample to form antigen/antibody complexes, which after agglutination is measured turbidimetrically.

Metabolite Level

Glucose levels were determined by enzymatic UV test, which has been described by Schmidt (1961). Enzymatic hexokinase catalyzes the reaction between glucose and adenosine triphosphate to form glucose-6-phosphate and adenosine diphosphate. In the presence of NAD, the enzyme glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphogluconate. The increase in NADH concentration is directly proportional to glucose concentration and can be measured spectrophotometrically at 340 nm.

Cholesterol levels were determined by enzymatic colorimetric test (Abell et al. 1952). In this method, cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase. Free cholesterol is then oxidized by cholesterol oxidase to produce hydrogen peroxide, which forms a red chromophore when combined with 4-aminophenazone and phenol. Formation of this chromophore is measured at 520 nm at 37°C and is directly proportional to the cholesterol concentration of the sample.

Total protein was measured using colorimetric test. Operation of the kit was based on the method described by Weichselbaum (1946). Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Color intensity is directly proportional to protein concentration, which can be determined photometrically.

Cortisol levels were determined using electrochemiluminometric assay. The test kit was prepared in accordance with the method described by Chiu et al. (2003). Serum cortisol assay is a competitive polyclonal antibody immunoassay that employs a magnetic separation step followed by electrochemiluminescence quantitation.

Metal Levels

Metal levels in blood serum were measured according to Ghazaly (1991). Serum samples were precipitated with 6% tricholoroacetic acid (1:5 v/v), and Zn and Cd concentrations were determined in the resulting supernatant by inductively coupled plasma (Perkin Elmer Optima 5300 DV).

Data Analysis

Data are presented as mean $\pm$ SE. For statistical analyses, one-way analysis of variance was used, followed by Student Newman-Keuls test using SPSS version 10.0 statistical software (SPSS, Chicago, IL). Differences were considered significant at $p < 0.05$.

Results and Discussion

In the present study, no mortality was observed during exposure to concentrations of Zn, Cd, and the Zn+Cd mixture during 14 days.

Enzyme Activity

Compared with controls at 7 and 14 days, increased serum ALT and AST activities were observed in *O. niloticus* exposed to concentrations of Zn, Cd, and Zn+Cd (Figs. 1, 2). At both exposure periods, increased ALT activity in fish
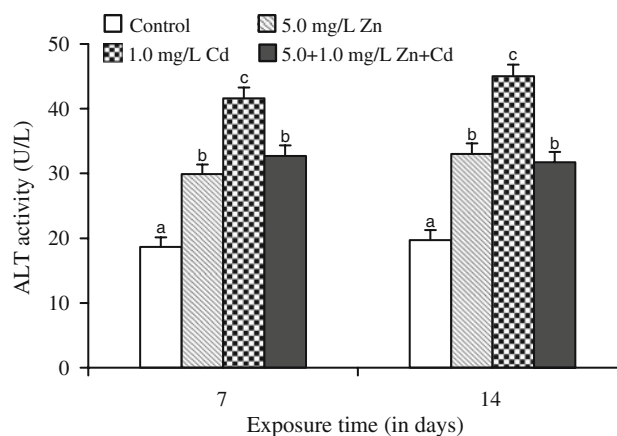
Fig. 1 Serum ALT activity in *O. niloticus* exposed to metals for 7 and 14 days. Data are expressed as mean $\pm$ SE ($N = 5$). Different letters indicate significant differences among groups at the same time ($p < 0.05$). No significant alteration of serum ALT and AST activities was found between 7 and 14 days of single or combined metal exposure
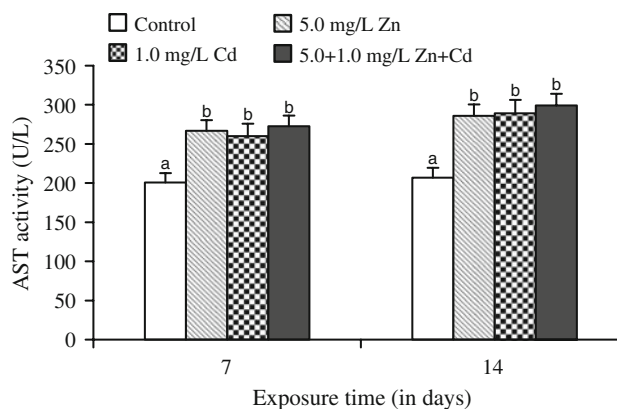
Fig. 2 Serum AST activity in *O. niloticus* exposed to metals for 7 and 14 days. Data are expressed as mean $\pm$ SE ($N = 5$). Different letters indicate significant differences among groups at the same time ($p < 0.05$). No significant alteration of serum ALT and AST activities was found between 7 and 14 days of single or combined metal exposure

exposed to Cd was higher compared with fish exposed to Zn and Zn+Cd, respectively. Similarly, increased ALT and AST activities have been observed in plasma and serum of fish *Oncorhynchus mykiss* exposed to Cu (Nemcsok and Hughes 1988) and *Sparus aurata* (Vaglio and Landriscina 1999) exposed to Cd.

ALT and AST are frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle, and gills (De la Tore et al. 2000). It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage (Palanivelu et al. 2005). Harvey et al. (1994) concluded that blood levels of ALT and AST may increase because of cellular damage in the liver and

that high levels of these enzymes in serum are usually indicative of disease and necrosis in the liver of animals. Therefore, increased ALT and AST activity in the serum of *O. niloticus* is caused mainly by leakage of these enzymes from liver cytosol into the bloodstream as a result of liver damage caused by metal exposure.

Specific Protein Level

Serum albumin and transferrin levels increased for both exposure periods, whereas ceruloplasmin levels increased at 14 days in both individual and combined treatments (Table 1). Metals taken up from the gill are released to the blood for transfer to the other organs, such as the liver and kidney, for use in metabolism, or they are sequestered. It is known that serum proteins are recognized as major participants in metal transport in both vertebrates and invertebrates. Vertebrates possess metal-specific proteins—such as serum albumin, transferrin, ceruloplasmin, transcobalamin, and nickeplasmin—which are responsible for the transport of Zn, Fe, Cu, Co, and Ni, respectively (Nair and Robinson 1999). Zn and Cd are transferred mainly by serum albumin (Li et al. 1996), transferrin, and α_2-macroglobulin (Carson 1984) in human plasma. De Smet et al. (2001) reported that Cd in plasma of *Cyprinus carpio* was able to bind metal-transferring proteins of 60 and 70 kDa. Albumin-like proteins, with a molecular mass of approximately 66 kDa, are considered major binding proteins of Cd and Zn in *O. mykiss* (Jasim Chowdhury et al. 2003).

Proteins are the most important compounds in serum, with albumin and globulin being the major serum proteins, and heavy metals may be involved in the normal working of these molecules (De Smet and Blust 2001). Most serum proteins are synthesized in the liver (Burtis et al. 1996; Ziak et al. 2002). Increased concentrations of serum albumin, transferrin, and ceruloplasmin in *O. niloticus* may result from increased protein synthesis in the liver in response to single and combined Zn and Cd exposure.

Serum albumin is the most abundant protein in the circulatory system (its redox modification modulates its physiologic function) as well as a biomarker of oxidative stress (Fabisiak et al. 2002). It is known that albumin, transferrin, and ceruloplasmin in vertebrates serum act as nonenzymatic antioxidants. Thus, the induction of these specific serum proteins by Zn and Cd may have an important role in protecting against metal damage. Halliwell and Gutteridge (1999) suggested that binding metal in an inactive form prevents metal ion–catalysed degradation of peroxides, thereby preventing hydroxyl production. They concluded that this type of antioxidant mechanism is effective and is still used in biologic systems, e.g, albumin acts as a sacrificial antioxidant. It has been suggested that ceruloplasmin may function as a circulating scavenger of superoxide anion radicals, thus protecting cells and tissues from the injurious effects of free radicals by inhibiting lipid peroxidation and DNA damage (Goldstein et al. 1979; Gutteridge 1983).

Metabolite Levels

After 7 and 14 days, the individual and combined effects of metals caused an increase in serum cortisol, glucose, and total protein levels and a decrease in cholesterol levels (Table 2). The decreased cholesterol level was higher in fish exposed to Cd alone, and to the Zn+Cd combination, than to Zn alone.

Cortisol plays an important role in ion regulation, energy metabolism, and metal detoxification by way of metallothionein induction in fish (Fu et al. 1990; Wendelaar Bonga 1997). Cortisol is a nonspecific indicator of stress that is released to the blood by way of stimulation of the hypothalamus–pituitary–interrenal (HPI) axis by heavy-metal exposure (Dethloff et al. 1999). Cortisol is not stored in the interrenal tissue but rather is synthesized on demand (Sumpter 1997); thus, in this study increased circulating cortisol levels must be a function of *de novo* stimulation of the HPI axis in response to heavy-metal stress. An increased cortisol concentration has been recognized as the main hormonal response to stressors and is widely used as a stress-response indicator (Barton and Iwama 1991).

Table 1 Specific-serum protein levels of *O. niloticus* under Zn, Cd, and Zn+Cd exposures[a,b]

Metal concentrations (mg/L)	7 days	14 days
Serum albumin (g/dL)		
0.0	0.87 ± 0.02 **ax**	0.91 ± 0.03 **ax**
5.0 Zn	1.10 ± 0.03 **bx**	1.20 ± 0.02 **bx**
1.0 Cd	1.15 ± 0.02 **bx**	1.27 ± 0.04 **bx**
5.0 Zn+1.0 Cd	1.18 ± 0.01 **bx**	1.29 ± 0.02 **bx**
Transferrin (mg/dL)		
0.0	0.21 ± 0.017 **ax**	0.23 ± 0.015 **ax**
5.0 Zn	0.29 ± 0.024 **bx**	0.38 ± 0.014 **by**
1.0 Cd	0.31 ± 0.022 **bx**	0.42 ± 0.010 **by**
5.0 Zn+1.0 Cd	0.32 ± 0.029 **bx**	0.45 ± 0.033 **by**
Ceruloplasmin (mg/dL)		
0.0	12.04 ± 0.49 **ax**	11.79 ± 0.13 **ax**
5.0 Zn	12.94 ± 0.10 **ax**	13.62 ± 0.43 **bx**
1.0 Cd	12.80 ± 0.13 **ax**	13.73 ± 0.33 **bx**
5.0 Zn+1.0 Cd	12.97 ± 0.11 **ax**	14.12 ± 0.22 **bx**

[a] Values are expressed as mean ± SE ($N = 5$)

[b] Letters "a" and "b" indicate differences between groups at the same time, and letters "x" and "y" indicate differences between times for the same group ($p < 0.05$)

Table 2 Serum metabolite levels of *O. niloticus* under Zn, Cd, and Zn+Cd exposures[a,b]

Metal concentrations (mg/L)	7 days	14 days
Glucose (mg/dL)		
0.0	50.67 ± 0.66 **ax**	47.02 ± 0.24 **ax**
5.0 Zn	67.33 ± 0.32 **bx**	63.09 ± 0.55 **bx**
1.0 Cd	80.89 ± 1.01 **bx**	75.80 ± 1.45 **bx**
5.0 Zn+1.0 Cd	80.65 ± 1.16 **bx**	79.11 ± 1.76 **bx**
Cortisol (µg/dL)		
0.0	10.51 ± 1.01 **ax**	9.69 ± 2.04 **ax**
5.0 Zn	22.72 ± 2.13 **bx**	17.49 ± 2.14 **by**
1.0 Cd	26.92 ± 2.11 **bx**	20.04 ± 2.08 **by**
5.0 Zn+1.0 Cd	28.84 ± 2.42 **bx**	19.29 ± 2.15 **by**
Total protein (g/dL)		
0.0	3.22 ± 0.03 **ax**	3.24 ± 0.01**ax**
5.0 Zn	4.20 ± 0.03 **bx**	4.29 ± 0.02 **bx**
1.0 Cd	4.17 ± 0.03 **bx**	4.28 ± 0.02 **bx**
5.0 Zn+1.0 Cd	4.19 ± 0.01 **bx**	4.30 ± 0.02 **bx**
Cholesterol (mg/dL)		
0.0	214 ± 1.45 **ax**	216 ± 2.19 **ax**
5.0 Zn	172 ± 0.67 **bx**	176 ± 1.86 **bx**
1.0 Cd	170 ± 2.10 **bx**	131 ± 2.13 **cy**
5.0 Zn+1.0 Cd	168 ± 1.15 **bx**	132 ± 3.33 **cy**

[a] Values are expressed as mean ± SE ($N = 5$)

[b] Letters "a," "b," and "c" indicate differences between groups at the same time, and letters "x" and "y" indicate differences between times for the same group ($p < 0.05$)

An increase in blood glucose is common in animals encountering a stressful situation, and it is one of the major effects of the secretion of catecholamine and corticosteroid hormones that occurs in such situations (Brown 1993). Increased serum glucose levels in fish under stress has been reported by Cicik and Engin (2005). Hyperglycemic response, as illustrated in the present study, is an indication of a disruption in carbohydrate metabolism, possibly caused by enhanced glucose 6-phosphatase activity in the liver, increased breakdown of liver glycogen, and synthesis of glucose from extrahepatic tissue proteins and amino acids. Increased blood glucose may indicate disrupted carbohydrate metabolism caused by enhanced breakdown of liver glycogen, which possibly is mediated by increased adrenocorticotrophic and glucagon hormones and/or decreased insulin activity (Raja et al. 1992).

Stress is an energy-demanding process, and the animal mobilizes energy substrates to cope with stress metabolically (Vijayan et al. 1997). Glucose is one of the most sensitive indices of an organism's stress: Its high concentrations in blood indicate that the fish is under stress and intensively using energy reserves, i.e, glycogen in liver and muscles (Vosyliene 1999). The stress hormone cortisol has been shown to increase glucose production in fish by

gluconeogenesis as well as glycogenolysis and likely plays an important role in the stress-associated increase in plasma glucose concentrations (Iwama et al. 1999). Plasma glucose and cortisol levels, used as stress indicators, increased during a waterborne copper–exposure period are thus significantly correlated with each other (Monteiro et al. 2005). In our work, cortisol and glucose levels, both of which are important pathways for stress recovery, may have increased to cope with the increased energy demand that occurs during metal-induced stress. Increases in cortisol and glucose levels have been reported in *Prochidolus lineatus* (Martinez et al. 2004) and *O. niloticus* (Monteiro et al. 2005) in response to Pb and Cu, respectively.

Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma 1998). Total serum protein, i.e, the majority of serum proteins synthesized in the liver, is used as an indicator of liver impairment (Yang and Chen 2003). The increase in serum total protein caused by treatment with metals was mainly related to the increased albumin, transferrin, and ceruloplasmin observed in this study. Exposure to heavy metals resulted in increased total protein levels of plasma and serum of fish *O. mossambicus* (Ruparelia et al. 1989) and *C. carpio* (Gopal et al. 1997).

Cholesterol is an essential structural component of cell membranes as well as the outer layer of plasma lipoproteins and is the precursor of all steroid hormones (Yang and Chen 2003). In the present study, metal exposure caused decreased serum cholesterol concentrations, indicating hypocholesteremia. This response may have been caused by the inhibitory effects of metals on cholesterol synthesis. Dutta and Haghighi (1986) suggested that heavy metals inhibit cholesterol synthesis in fish. Agrahari et al. (2007) suggested that hypocholesteremia observed after exposure to monocrotophos in *C. carpio* may be caused by hydropic changes in tissues and that the bioaccumulated pesticide inhibited the conversion of esterified cholesterol to free cholesterol. Decreased serum cholesterol levels were reported in *Lepomis macrochrus* (Dutta and Haghighi 1986) and *O. mossambicus* (Ruparelia et al. 1989) in response Hg and Pb exposure, respectively.

Metal Level

Zn or Cd levels in blood serum of fish exposed to metals individually or in combination increased compared with controls during 7 and 14 days (Table 3). We observed that when fish were exposed to the mixtures of Zn and Cd, concentrations of these metals in their serum were lower than in fish exposed to individual metals. It is possible that one metal blocks or even antagonizes gill epithelium absorption of the other and thereby limits distribution of the metal in blood. Serum Zn and Cd levels increased with

Table 3 Serum metal levels (µg/mL) of *O. niloticus* under Zn, Cd, and Zn+Cd exposures[a,b]

Metal concentrations (mg/L)	7 days	14 days
Zn		
0.0	49.9 ± 2.9 **ax**	53.8 ± 1.7 **ax**
5.0 Zn	98.7 ± 2.2 **bx**	129.3 ± 1.9 **by**
5.0 Zn+1.0 Cd	68.4 ± 2.1 **cx**	93.6 ± 1.5 **cy**
Cd		
0.0	NA	NA
1.0 Cd	0.121 ± 0.002 **ax**	0.179 ± 0.003 **ay**
5.0 Zn+1.0 Cd	0.079 ± 0.003 **bx**	0.115 ± 0.003 **by**

NA not available

[a] Values are expressed as mean ± SE ($N = 5$)

[b] Letters "a," "b," and "c" show the differences between groups at the same time, and letters "x" and "y" show differences between times for the same group ($p < 0.05$)

increasing exposure periods in all concentrations tested. Metal accumulation in fish tissues depends on exposure dose and time as well as other factors, such as interaction with other metals, water chemistry, and fish metabolic activity (Heath 1995). Metals levels increased in blood plasma of *Tilapia zilli* (Ghazaly 1991) and *O. mossambicus* (Pelgrom et al. 1995a) exposed to Pb and Cu, respectively.

The results of our work suggest there is a positive relation between contents of specific serum proteins and metal concentrations in blood. Under metal exposure, increased serum albumin, transferrin, and ceruloplasmin levels may be associated with increased blood metal levels. Ceruloplasmin levels of *O. mossambicus* increased after Cu exposure (Pelgrom et al. 1995b).

In conclusion, the results of the present study demonstrate that exposure to heavy metals affect serum biochemistry, with all biochemical parameters levels except cholesterol increasing in response both to Zn and Cd alone as well as to combined Zn+Cd exposure. Changes in blood parameters of fish are possible as a result of metal-induced stress. Therefore, these parameters can used as indicators of heavy-metal toxicity.

References

Abell LL, Levy BB, Brodie BB, Kendall FE (1952) A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 195:357–366

Adams SM, Shugart LR, Southworth GR, Hinton DE (1990) Application of bioindicators in assessing the health of fish populations experiencing contaminant stress. In: McCarthy JF, Shugart LR (eds) Biomarkers of environmental contamination. Lewis, Boca Raton, FL, pp 333–353

Agrahari S, Pandey KC, Gopal K (2007) Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). Pestic Biochem Physiol 88:268–272

Almeida JA, Diniz YS, Marques SFG, Faine LA, Ribas BO, Burneiko RC et al (2002) The use of oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ Int 27:673–679

Artacho P, Soto-Gamboa M, Verdugo C, Nespolo RF (2007) Blood biochemistry reveals malnutrition in black-necked swans (*Cygnus melanocoryphus*) living in a conservation priority area. Comp Biochem Physiol A 146:283–290

Barcellos LJG, Kreutz LC, de Souza C, Rodrigues LB, Fioreze I, Quevedo RM et al (2004) Hematological changes in jundia (*Rhamdia quelen* Quoy and Gaimard *Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. Aquaculture 237:229–236

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 10:3–26

Benguira S, Hontela A (2000) Adrenocorticotrophin and cyclic adenosine 3′,5′-monophosphate-stimulated cortisol secretion in interrenal tissue of rainbow trout exposed in vitro to DDT compounds. Environ Toxicol Chem 19:842–847

Bergmeyer HU, Horder M, Rej R (1985) International federation of clinical chemistry (IFCC) scientific committee. J Clin Chem Clin Biochem 24:481–495

Brown JA (1993) Endocrine responses to environmental pollutants. In: Rankin JC, Jensen FB (eds) Fish ecophysiology. Chapman and Hall, London, UK, pp 276–296

Burtis CA, Ashwood ER, Tietz NW (1996) Tietz fundamentals of clinical chemistry. Saunders, Philadelphia, PA

Carson SD (1984) Cadmium binding to α_2-macroglobulin. Biochem Biophys Acta 791:370–374

Cataldi E, Di Marco P, Mandich A, Cataudella S (1998) Serum parameters of adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): Effects of temperature and stress. Comp Biochem Physiol A 121:351–354

Chiu SK, Collier CP, Clark AF, Wynn-Edwards KE (2003) Salivary cortisol on ROCHE Elecsys immunoassay system: Pilot biological variation studies. Clin Biochem 36:211–214

Cicik B, Engin K (2005) The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L., 1758). Turk J Vet Anim Sci 29:113–117

Congleton JL, La Voie WJ (2001) Comparison of blood chemistry values for samples collected from juvenile Chinook salmon by three methods. J Aquat Anim Health 13:168–172

De la Tore FR, Salibian A, Ferrari L (2000) Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. Environ Pollut 109:227–278

De Smet H, Blust R (2001) Stress responses and changes in protein metabolism in carp *Cyprinus carpio* during cadmium exposure. Ecotoxicol Environ Saf 48:255–262

De Smet H, Blust R, Moens L (2001) Cadmium-binding to transferrin in the plasma of the common carp *Cyprinus carpio*. Comp Biochem Physiol C 128:45–53

Dethloff GM, Schlenk D, Khan S, Bailey HC (1999) The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Arch Environ Contam Toxicol 36:415–423

DiGiulio RT, Benson WH, Sanders BM, VanVeld PA (1995) Biochemical mechanisms: metabolism, adaptation and toxicity. In: Rand GM (ed) Fundamentals of aquatic toxicology: Effects, environmental fate and risk assessment. Taylor and Francis, London, UK, pp 523–562

Dutta HM, Arends DA (2003) Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish. Environ Res 91:157–162

Dutta HM, Haghighi AZ (1986) Methylmercuric chloride and serum cholesterol levels in blugill *Lepomis macrochirus*. Bull Environ Contam Toxicol 36:181–185

Fabisiak JP, Sedlov A, Kagan VE (2002) Quantification of oxidative/nitrosative modification of CYS (34) in human serum albumin using a fluorescence-based SDS-PAGE assay. Antioxid Redox Signal 4:855–865

Folmar LC (1993) Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects. Environ Toxicol Chem 12:337–375

Fu H, Steinebach OM, van den Hamer CJA, Balm PHM, Lock RAC (1990) Involvement of cortisol and metallothionein-like proteins in the physiological responses of tilapia (*Oreochromis mossambicus*) to sublethal cadmium stress. Aquat Toxicol 16:257–270

Garcia-Santos S, Fontainhas-Fernandes A, Wilson JM (2006) Cadmium tolerance in the Nile tilapia (*Oreochromis niloticus*) following acute exposure: Assessment of some ionoregulatory parameters. Environ Toxicol 21:33–46

Ghazaly KS (1991) Influences of thiamin on lead intoxication, lead deposition in tissues and lead hematogical responses of *Tilapia zilli*. Comp Biochem Physiol C 100:417–421

Goldstein IM, Kaplan HB, Edelson HS, Weissmann G (1979) Ceruloplasmin. J Biol Chem 254:4040–4045

Gopal V, Parvathy S, Balasubramanian PR (1997) Effect of heavy metals on the blood protein biochemistry of the fish *Cyprinus carpio* and its use as a bio-indicator of pollution stress. Environ Monit Assess 48:117–124

Gutteridge JM (1983) Antioxidant properties of ceruloplasmin towards iron- and copper-dependent oxygen radical formation. FEBS Lett 27:37–40

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Clarendon Press, Oxford, UK

Harvey RB, Kubena LF, Elissalde M (1994) Influence of vitamin E on aflatoxicosis in growing swine. Am J Vet Res 55:572–577

Heath AG (1995) Water pollution and fish physiology. CRC, Boca Raton, FL, pp 141–170

Hubbuch A (1991) Results of the multicenter study of tina-quant albumin in urine. Wien Klin Wochenschr Suppl 189:24–31

Iwama GK, Vijayan MM, Forsyth RB, Ackerman PA (1999) Heat shock proteins and physiological in fish. Am Zool 39:901–909

Jacobson-Kram D, Keller KA (2001) Toxicology testing handbook. Marcel Dekker, New York, NY

Jasim Chowdhury M, Grosell M, McDonald DG, Wood CM (2003) Plasma clearance of cadmium and zinc in non-acclimated and metal-acclimated trout. Aquat Toxicol 64:259–275

Kori-Siakpere O, Ubogu EO (2008) Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae). Afr J Biotechnol 7:2068–2073

Li H, Sadler PJ, Sun H (1996) Unexpectedly strong binding of a large metal ion (Bi^{3+}) to human serum transferrin. J Biol Chem 271:9483–9489

Lizana J, Hellsing K (1974) Manual immunonephelometric assay of proteins, with use of polymer enhancement. Clin Chem 20:1181–1186

Martinez CBR, Souza MM (2002) Acute effects of nitrite on ion regulation in two neotropical fish species. Comp Biochem Physiol A 133:151–160

Martinez CBR, Nagae MY, Zaia CTBV, Zaia DAM (2004) Acute morphological and physiological effects of lead in the neotropical fish, *Prochidolus lineatus*. Braz J Biol 64:797–807

Monteiro SM, Mancera JM, Fernandes AF, Sousa M (2005) Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochoromis niloticus*. Comp Biochem Physiol C 141:375–383

Nair PS, Robinson WE (1999) Purification and characterization of a histidine-rich glycoprotein that binds cadmium from the blood plasma of the bivalve *Mytilus edulis*. Arch Biochem Biophys 366:8–14

Nemcsok J, Hughes GM (1988) The effect of copper sulphate on some biochemical parameters of rainbow trout. Environ Pollut 49:77–85

Palanivelu V, Vijayavel K, Ezhilarasibalasubramanian S, Balasubramanian MP (2005) Influence of insecticidal derivative (Cartap Hydrochloride) from the marine polychaete on certain enzyme systems of the freshwater fish *Oreochromis mossambicus*. J Environ Biol 26:191–196

Peakall DB (1994) Biomarkers: the way forward in environmental assessment. Toxicol Ecotoxicol News 1:50–60

Pelgrom SMGJ, Lock RAC, Balm PHM, Wendelaar Bonga SE (1995a) Integrated physiological response of tilapia, *Oreochromis mossambicus*, to sublethal copper exposure. Aquat Toxicol 32:303–320

Pelgrom SMGJ, Lock RAC, Balm PHM, Wendelaar Bonga SE (1995b) Effects of combined waterborne Cd and Cu exposures on ionic composition and plasma cortisol in tilapia, *Oreochromis mossambicus*. Comp Biochem Physiol C 111:227–235

Petrivalsky M, Machala M, Nezveda K, Piacka V, Svobodova Z, Drabek P (1997) Glutathione-dependent detoxifying enzymes in rainbow trout liver: search for specific biochemical markers of chemical stress. Environ Toxicol Chem 16:1417–1421

Raja M, Al-Fatah A, Ali M, Afzal M, Hassan RA, Menon M et al (1992) Modification of liver and serum enzymes by paraquat treatment in rabbits. Drug Metab Drug Interact 10:279–291

Ruparelia SG, Verma Y, Mehta NS, Salyed SR (1989) Lead-induced biochemical changes in freshwater fish *Oreochromis mossambicus*. Bull Environ Contam Toxicol 43:310–314

Schmidt FH (1961) Enzymatic determination of glucose and fructose simultaneously. Klin Wochenschr 39:1244–1250

Singh RK, Sharma B (1998) Carbufuran induced biochemical changes in *Clarias batrachus*. Pestic Sci 53:285–290

Sumpter JP (1997) The endocrinology of stress. In: Iwama GK, Pickering AD, Sumpter JP, Schreck CB (eds) Fish stress and health in aquaculture, Society for Experimental Biology Seminar Series 62. Cambridge University Press, Cambridge, UK, pp 95–118

Vaglio A, Landriscina C (1999) Changes in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. Ecotoxicol Environ Saf B 43:111–116

Vijayan MM, Cristina Pereira E, Grau G, Iwama GK (1997) Metabolic responses associated with confinement stress in tilapia: the role of cortisol. Comp Biochem Physiol C 116:89–95

Vosyliene MZ (1999) The effects of heavy metals on haematological indices of fish. Act Zool Lit Hydro 9:76–82

Weichselbaum TE (1946) An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Pathol 16:40–48

Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 7:591–625

Wolf PL (1982) Ceruloplasmin: methods and clinical use. Crit Rev Clin Lab Sci 17:229–245

Yang JL, Chen HC (2003) Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere 53:877–882

Ziak M, Meier M, Novak-Hofer I, Roth J (2002) Ceruloplasmin carries the anionic glycan oligo/poly α2, 8 deaminoneuraminic acid. Biochem Biophys Res Commun 295:597–602