The Bioscan 11(3): 1473-1476, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

BIOCHEMICAL ALTERATIONS IN BLOOD SERUM IONS OF INDIAN MAJOR CARPS INDUCED BY ZINC AND CADMIUM TOXICITY

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KEYWORDS
Metal ions
Blood serum
Toxicity
Heavy metals
Indian major carps

Received on :
10.09.2015

Accepted on :
21.04.2016

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ABSTRACT
The aim of the present investigation was to determine the effects of heavy metals such as Zinc and Cadmium in aquatic system of Indian major carps *Catlacatla*, *Labeorohita* and *Cirrhinusmrigala* during 2011-12 at CCS HAU, Hisar. The fishes were subjected to treatments at 0.02, 0.04 and 0.06 ppm of Zinc and Cadmium, individually as well as in combination. A maximum decline of 13.31% in Potassium ion concentration was observed in *L. rohita* subjected to combined Cadmium and Zinc treatment at 0.06 ppm. The same treatments depicted a maximum decline of 23.95% and 25.73% in chloride and calcium ions respectively, in *C. mrigala*. It is evident from our study that the heavy metal treatments significantly altered the ion concentration in serum of tested fish species in a dose dependent manner and acted synergistically in combination.

INTRODUCTION

Today, the heavy metals are termed as 'devils in disguise' but the economic reasons impel us to keep them using. The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents. The metals are of special concern because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms. Industrial wastes constitute the major source of metal pollution in natural water. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants anddrainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life (Karbassi *et al.*, 2006).The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment (Yang and Rose, 2003).

Water born metals may alter the physiological and biochemical parameters in fish blood and tissues. The reaction and survival of aquatic animals depend not only on the biological state of the animals but also on the toxicity, and type and time of exposure to the toxicant. Cadmium and Zinc are nonessential heavy metals; however, they are considered as one of the most toxic water contaminants and could cause toxicity at each level in organisms, from populations and communities to cell elements. Even at sublethal concentration, heavy metals have a cumulative polluting effect and could cause serious disturbances in fish metabolism such as abnormal behavior, locomotor anomalies or anorexia also affecting the blood cells (Vinodhini and Narayanan, 2009). Kumar and Dahiya (2013) reported decreased levels of liver and serum proteins while increased cholesterol in fish subjected to Zinc toxicity. Hematological and biochemical profile in fish is proved to be a sensitive index for the evaluation of fish metabolism under metallic stress. However, there are no studies demonstrating the change in blood ion concentration of Indian major carps exposed to a mixture of heavy metals such as Cadmium and Zinc. Hence the aim of this study was to investigate the individual and combined effects of these heavy metals in Indian major carps *Catlacatla*, *Labeorohita* and *Cirrhinusmrigala*.

MATERIAL AND METHODS

The present study was carried out during 2011-12 in the Department of Zoology, CCS Haryana Agricultural University,Hisar on three most commonly cultured fresh water fish species: *L. rohita, C. catla* and *C. mrigala*. Fish specimens of 4 to 6 inches having approximate age of six months were procured from the local fresh water ponds of Hisar, Hansi and Sirsa. Fishes were disinfected with 0.1% $KMnO_4$ solution and acclimation for seven days at room temperature in a large laboratory tub. Fishes were transferred to the plastic tubs with capacity of 100 liters for various treatments for 60 days. Physico-chemical characteristics (temperature, pH, dissolved

oxygen, alkalinity and free carbon dioxide) of the aquarium water were monitored at an interval of 15 days following standard methods of water analysis of APHA, 1998).

Three replicates were maintained for each treatment and in each replicate 10 fishes of approximately equal size and weight were maintained. After 60 days fishes were dissected and the blood samples were collected from the caudal vein for biological and enzymological estimations.

Fish Serum collection

Blood samples were collected from both the control and experimental fish that survived under 60 days toxicant exposure period. The blood samples were taken from caudal vein of Indian major carps. Blood in the Ependorff tube was incubated at room temperature for coagulation. The serum of blood (the fluid portion) was collected into centrifuging tubes by carefully avoiding the clot formed and centrifuged for 10 min at 3,000 rpm for 5 min. The serum was taken in new micro centrifuge tubes and stored at "40ºC until further analyzed for metal ions. At least three replicates were taken in clean sterilized test tubes.

Determination of concentration of sodium and potassium ions

Sodium and Potassium ion concentration in the serum was estimated with a Micro Flame Photometer (Elico CL 361, India) by direct reading (Richards, 1954).The readings were recorded and corresponding concentrations were determined from the standard curves prepared by using NaCl and KCl.

Determination of chloride ions

The chloride ions were determined by Mercury Thiocyanate Method (Miller, 1984) using Sigma kit. Samples were prepared as per instructions and absorbance read at 505 nm against blank. The amount of chloride ions present in samples was calculated as per the formula:

Chloride conc. (mMol/ L) = (Absorbance of Test/Absorbance of Standard) X100

Determination of calcium ions

The calcium ions in serum samples were measured by feeding the samples into fully automatic auto analyzer by Arsenazo method (Farrell, 1984). Concentration was determined from the standard curve prepared by using $CaCl_2$.

Statistical analysis

The data so obtained were subjected to statistical analysis. One way ANOVA was applied and student's t-test was done to find out significant difference between various treatments (pd£0.05).

RESULTS

ISubjected to different treatments, all the fish species *viz*. *C. catla, L. rohita*and*C. Mrigala* exhibited a dose dependent decrease in Sodium, Calcium and Chloride ions while Potassium ions were found to increase with respect to control (Tables 2-5). In *L. rohita*, maximum decline in the Sodium ion concentration was observed to be 9.58% exposed to Cadmium at 0.06 ppm with a minimum reduction of 2.89% exposed to Zinc at 0.02 ppm as depicted in Table 2. Similar results were obtained in *C. mrigala* and *C. catla*, where maximum reduction was caused by Cadmium (0.06 ppm) *i.e*. 11.90% and 8.18%, respectively. Zinc (0.02 ppm) induced minimum reduction in Sodium ions in *C. mrigala* and *C. catla i.e*. 2.25% and 2.69%, respectively (Table 2).

In contrast to Sodium ions, the Potassium ion concentration showed a significant increase in all the three fish species after exposure to heavy metals (Table 3). In fishes*C. mrigala* and *L. rohita*, maximum increase was caused by Cadmium treatment (0.06 ppm) *i.e*. 3.34% and 1.37%, respectively. Zinc treatment however (0.02 ppm) induced minimum increase in Potassium contents in *C. mrigala* and *C. catla* (0.49% and 0.32%, respectively). Similar results were obtained in *C. catla*, where maximum increase in the potassium ions in blood serum was observed to be 2.71% following Cadmium treatment at 0.06 ppm and a minimum increase of 0.85% following the Zinc treatment at 0.02 ppm (Table 3).

As presented in Tables 4 and 5, Chloride and Calcium ions in *L. rohita* depicted maximum decline (11.58% and 11.35%, respectively) following Cadmium treatment at 0.06 ppm and a minimum reduction (8.82% and 11.40%, respectively) following Zinc treatment at 0.02 ppm. Similar results were obtained for Calcium and Chloride ions in *C. mrigala*, where maximum reduction was caused by Cadmium at 0.06 ppm. Heavy metal Zn (0.02 ppm) induced minimum reduction in the respective ions concentration in blood serum of *C. mrigala* and *C. catla i.e*. 15.81% and 15.12%, respectively. The results presented in Tables 2-5 depict that both the heavy metals in combination (Cadmium 0.06 ppm + Zinc 0.06 ppm) proved to be more effective than the individual treatments.

DISCUSSION

Studies on the toxicity of metals for fish have been focused on the effects of short term exposures to metals at relatively high concentrations; rather than investigating the toxic impact of long-term exposures to metal mixtures at environmentally realistic concentrations. Under conditions of acute, high-dose metal exposure, the maintenance of branchial osmo regulation and gas exchange is of prime importance for the survival of the fish; whereas under conditions of sublethal, chronic metal intoxication, the adaptive capacity of internal metal accumulating organs may gain importance. Many workers have reported significant alteration in the hematological and biochemical parameters (such as haemoglobin, number of blood cells, enzyme concentration, following short and long exposure periods to heavy metal treatments, individually or in combination (Rani *et al.*, 2015;Vinodhini and Narayanan, 2009; Kumar and Dahiya, 2013; Younis *et al.*, 2012).

Ions are very important for any organism because they are involved in most biological processes such as respiration, muscle contraction, absorption, nerve impulses transmission,

Table 1: Following treatments were given to the test fish species along with control

S. No.	Treatment	Dose (ppm)
1.	Cd	0.02, 0.04 and 0.06
2.	Zn	0.02, 0.04 and 0.06
3.	Cd + Zn	0.02+0.02,0.04+0.04 and 0.06+0.06

Table 2: Sodium ion concentration (mEq·l) in the serum of fresh water fishes subjected to different treatments

Fish species	Treatments (ppm) Control	Cd		Zn			Cd+Zn			CD (pd″ 0.05)
	0	0.02	0.04	0.06	0.02	0.04	0.06	0.02+0.02	0.04+0.04	0.06+0.06	
Catlacatla	131.99±0.31	126.61±0.51(4.07)	125.08±0.07(5.23)	121.19±0.1(8.18)	128.44±0.28(2.69)	126.20±0.45(4.38)	123.44±0.22(6.48)	124.62±0.15(5.58)	120.35±0.11(8.82)	116.41±0.13(11.80)	0.83
Labeorohita	130.00±0.19	120.78±0.12(7.03)	119.55±0.07(7.97)	117.44±0.07(9.58)	126.20±0.45(2.89)	123.44±0.22(5.00)	121.63±0.11(6.38)	118.32±0.35(8.91)	118.01±0.32(9.14)	116.34±0.02(10.42)	0.71
Cirrihinu smrigala	128.72±0.36	115.54±0.14(10.23)	114.64±0.33(10.93)	113.40±0.12(11.90)	125.82±0.25(2.25)	122.86±0.14(4.55)	121.22±0.12(5.82)	112.40±0.19(12.68)	110.49±0.07(14.16)	108.34±0.14(15.83)	0.73

Mean ± S. E. (3 Observations); Values in parenthesis are per cent reduction over control

Table 3: Potassium ion concentration (mEq·l) in the serum of fresh water fishes subjected to different treatments

Fish species	Treatments (ppm) Control	Cd		Zn			Cd+Zn			CD(p d″0.05)
	0	0.02	0.04	0.06	0.02	0.04	0.06	0.02+0.02	0.04+0.04	0.06+0.06	
Catlacatla	4.05±0.01	4.12±0.003(1.72)	4.14±0.005(2.22)	4.16±0.025(2.71)	4.07±0.003(0.85)	4.08±0.008(0.90)	4.11±0.003(0.16)	4.16±0.003(2.88)	4.19±0.006(3.62)	4.24±0.01(4.69)	0.03
Labeorohita	4.13±0.01	4.17±0.008(1.12)	4.17±0.003(1.04)	4.18±0.008(1.37)	4.14±0.008(0.32)	4.15±0.005(0.56)	4.16±0.008(0.80)	4.40±0.02(6.53)	4.50±0.017(9.03)	4.68±0.01(13.31)	0.04
Cirrihinusmrigala	4.29±0.02	4.42±0.008(2.04)	4.44±0.01(3.27)	4.45±0.01(3.34)	4.31±0.07(0.49)	4.32±0.02(0.64)	4.41±0.04(2.63)	4.52±0.02(4.91)	4.53±0.01(5.27)	4.66±0.01(8.04)	0.09

Values are expressed as Mean ± S. E. (3 Observations); Values in parenthesis are per cent increase over control

Table 4: Chloride ion concentration (m mol/l) in the serum of fresh water fishes subjected to different treatments

Fish species	Treatments (ppm) Control	Cd		Zn			Cd+Zn			CD(pd ″0.05)
	0	0.02	0.04	0.06	0.02	0.04	0.06	0.02+0.02	0.04+0.04	0.06+0.06	
Catlacatla	112.72±2.7	105.62±3.4(6.30)	104.03±3.4(7.72)	103.64±3.7(8.06)	106.80±3.4(5.25)	106.60±3.3(5.43)	106.24±3.8(5.75)	101.94±4.5(9.57)	101.90±5.5(9.60)	100.22±2.6(11.09)	NS
Labeorohita	109.42±4.5	97.31±6.0(11.06)	96.80±3.0(11.53)	96.74±3.3(11.58)	99.76±3.6(8.82)	98.32±2.9(10.13)	97.90±3.2(10.52)	95.94±1.9(12.31)	94.80±3.9(13.35)	92.81±1.6(15.17)	NS
Cirrihinusmrigala	108.22±3.6	88.78±3.2(17.96)	88.55±2.3(18.17)	88.03±2.8(18.65)	91.85±3.2(15.12)	91.79±2.9(15.18)	90.79±2.4(16.10)	85.35±4.4(21.13)	85.25±2.8(21.22)	82.30±2.6(23.95)	9.27

Values are expressed as Mean ± S. E. (3 Observations); Values in parenthesis are per cent reduction over control

Table 5: Calcium ion concentration (μg/ml) in the serum of fresh water fishes subjected to different treatments

Fish species	Treatments (ppm) Control	Cd		Zn			Cd+Zn			CD(pd ″0.05)
	0	0.02	0.04	0.06	0.02	0.04	0.06	0.02+0.02	0.04+0.04	0.06+0.06	
Catlacatla	52.85±0.59	48.99±0.35(7.67)	48.05±0.11(9.52)	47.65±0.34(10.33)	51.33±0.07(3.01)	50.13±0.01(5.40)	49.51±0.08(6.62)	47.37±0.06(10.88)	47.280.55(11.07)	46.77±0.11(12.08)	0.92
Labeorohita	50.14±0.01	46.3±0.07(7.65)	45.00±0.01(10.23)	44.44±0.11(11.35)	44.42±0.23(11.40)	45.82±0.005(8.61)	45.29±0.09(9.67)	45.08±0.03(10.07)	44.29±0.09(11.66)	44.25±0.03(11.74)	0.28
Cirrihinus mrigala	49.59±0.38	44.21±0.06(10.83)	43.41±0.28(12.45)	42.97±0.01(13.34)	41.74±0.30(15.81)	41.61±0.15(16.09)	41.44±0.18(16.43)	40.27±0.09(18.78)	37.89±0.07(23.58)	36.82±0.16(25.73)	0.61

Values are expressed as Mean ± S. E. (3 Observations); Values in parenthesis are per cent increase over control

osmo regulation, acid-base balance and excretion in fish. Several changes in serum ion concentrations were measured for different fish species under different types of stresses to indicate osmo regulatory disruptions (Fernandes, 2007; Fast, 2009). Carrera *et al*. (2007) studied the effect of 4-nonylphenol on osmoregulation referring to its action and its ecological significance. The current study reported a significant effect of Cadmium and Zinc treatments; both individually and in combination, on the metal ions concentration the blood serum of tested fish species in a dose dependent manner. These results are in agreement with the findings of (Chezhian and Sivakumari, 2006) who stated similar decrease in sodium and chloride levels of fingerlings of *Cyprinuscarpio*exposed to heavy metals. Firat and Kargin (2010) observed changes in serum biochemistry and metal concentration in response to single and combined-metal exposure at 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 in freshwater fish *Oreochromisniloticus*. Supporting our findings, Sayed *et al*. (2011) reported that some ions decreased significantly (HCO^{3-} and Na^{+}) or insignificantly (Cu^{+2}, Cl^{-} and Ca^{+2}) and others increased significantly (Fe^{+2}) or insignificantly (K^{+}) under 4-nonylphenol stress on *Clariasgariepinus*. McCormick *et al*. (2005) indicated that estrogenic compounds may cause a general shift toward increased capacity for ion uptake (Na^{+}, Cl^{-} and Ca^{+2}). Calcium imbalance seems to cause cell death through inhibition of the calcium pump in the endoplasmic reticulum. Histopathological alterations in the liver of rainbow trout as necrosis, cell number decrease and vacuolation were mediated by inhibition of calcium pump after exposure to Nonyphenol (Uguz, 2003).It is thus evident from our study that the heavy metal treatments, individually as well as in combination, significantly altered the ion concentration in serum of tested fish species in a dose dependent manner. This may, in turn, disturb the electrolyte balance in fish body, resulting in difficulties in osmoregulation and thus challenging survival in the polluted water bodies. It is hence recommended to prevent the water bodies from getting polluted and limit/ ban the waste discharge so as to provide a healthy environment for aquatic fauna to perish.

ACKNOWLEDGEMENT

We are thankful to Dr. V. P. Sabhlok (Professor and Head, Zoology) for providing necessary facilities. Help rendered by Mr. Mastermohan during the fish sample collection and financial help in the form of Rajiv Gandhi National Fellowship from UGC, New Delhi is gratefully acknowledged

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