The Bioscan 10(2): 613-616, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

ZINC AND CADMIUM INDUCED CHANGES IN THE PROTEOLYTIC AND AMYLOLYTIC ENZYME ACTIVITY IN INDIAN MAJOR CARPS

SUNITA RANI*, R. K. GUPTA AND KANIKATEHRI
Department of Zoology,
CCS Haryana Agricultural University Hisar - 125 004, INDIA
e-mail: s.sonavickey@gmail.com

KEYWORDS
Zinc
Cadmium
Enzyme activity
Indian major carps

Received on :
04.02.2015

Accepted on :
10.05.2015

***Corresponding author**

ABSTRACT
The present study was conducted during 2012 to investigate the individual and combined effect of heavy metals (Zinc and Cadmium) on proteolytic and amylolytic enzyme activity of most commonly cultured fish species of Indian major carps; *Labeorohita, Catlacatla*and *Cirrhinusmrigala*at three doses i.e. 0.02, 0.04 and 0.06 ppm, respectively. The effect on respective enzyme activities was found in a dose dependent manner. Cd and Zn treatments (0.06 ppm) showed a maximum reduction of 15.21 and 8.84% over control in the proteolytic activity in *L. rohita* out of the three tested fish species. Likewise, the amylolytic activities showed maximum reduction in *C. mrigala*with a decrease of 19.40 and 13.43% over control subjected to the same treatments, respectively. The enzyme activity depicted maximum reduction (20.40% in proteolytic activity in *C. catla* and 38.8% in amylolytic activity in *C. mrigala*) under heavy metal treatment in combination Cd+Zn (0.06+0.06 ppm). The results thus reveal that Cadmium and Zinc in aquatic environments could significantly (P**d"**0.05) reduce enzyme activity in the aquatic fauna thus constituting a core group of aquatic pollutants.

INTRODUCTION

Heavy metal contamination usually causes depletion in food utilization in fish and such disturbance may result in reduced fish metabolic rate and hence causing reduction in their growth. Fishesare widely used to evaluate the health of aquaticecosystems because pollutants build up in the foodchain and are responsible for adverse effects and deathin the aquatic systems.Biochemical parameters assessed in fish may be a useful tool by providing quantitative measurement of metal impact as well as valuable information of ecological relevance on the effects of metals (Oner *et al.,* 2009). Due to the bioaccumulative and non-biodegradable properties, heavy metals constitute a core group of aquatic pollutants. Over the last few decades, there has been a particular interest in determining the levels of heavy metals in the marine and fresh water canals and attention was drawn to the measurement of their contamination levels in public food supplies, particularly in fish. Today, the heavy metals are termed as 'devils in disguise' but the economic reasons impel us to keep them using. Heavy metals are one of the major components of industrial effluents, which have attained an alarming status as water pollutants over the last few decades.

Heavy metals have long been recognized as serious pollutants of the aquatic environment. Several studies have reported that heavy metals are extremely toxic to fish and other aquatic organisms (Shewta*et al.*, 2012). Early life stages of fish appear to be especially susceptible to this form of pollution. 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 (Brungs, 1977). 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 (Rashed, 2001). 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 (Cicik and Engin, 2005; Vinodhini and Narayanan, 2009). 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*. Kumar and Dahiya (2013) reported decreased levels of liver and serum proteins while increased cholesterol in fish subjected to Zinc toxicity.

Hematological parameters are used as an index to detect physiological changes and assess structural and functional status of health during stress conditions in fish. Fish blood is sensitive to pollution induced stress and enzyme activity is considered as sensitive biochemical indicator before hazardous effects occur in fish. The complex of unspecified biochemical indicators of blood reveals the general effect of pollutants on fish and makes it possible to forecast the consequence of long-term exposure to chemical pollutants. Thus the present study aimed to investigate the effect of individual and combined effect of heavy metals (Zn and Cd) on proteolytic and amylolytic enzyme activity of most commonly cultured fish species of Indian major carps:

*Labeorohita, Catlacatla*and*Cirrhinusmrigala*.

MATERIALS AND METHODS

The present study was carried out during 2012 in the Department of Zoology, CCS Harynana Agricultural University, Hisar, with three most commonly cultured fresh water fish species: *C. catla*,*L. rohita*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 was carried out 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 time 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.

Fishes were dissected and digestive tract was removed. It was homogenized in 5 volumes (v/w) of ice cold distilled water. The contents were centrifuged at 4ºC at 10000 rpm and the supernatants were taken for analysis. Proteolytic enzyme activity was assayed according to Walter (1984) using Bovine Serum Albumin (BSA) as 1% substrate and expressed in terms of mg of tyrosin/mg of protein/h. Amylase enzyme activity was assayed according to Sawhney and Singh (2000) in which the increase in reducing power of buffered starch solutions was estimated and expressed in terms of mg of maltose liberated/ mg of protein/h.

Statistical Analysis

The data so obtained was statistically analyzed using OPSTAT software. Analysis of variance (ANOVA) was carried out and means were compared using student's t-test to check the statistical significance of data (Pd"0.05).

RESULTS

The results of the present investigation have been presented in Tables 2-3. All the fish species *viz. C. catla*,*L. rohita*and*C. mrigala* exhibited a dose dependent decrease in the specific protease and amylase activities with respect to control on exposure to heavy metals. Both the heavy metals i.e. Cd and Zn (individual and combined) have been found to impose toxic effects on the fish, thereby reducing the specific protease as well as amylase activities with respect to the control.

Specific proteolytic activity

In the fish *C. catla,* theleast toxicity was caused by Zn (0.02 ppm) and the specific protease activity was reduced by 2.72% as compared to the control. Cd+Zn (0.06 ppm) induced maximum reduction in the specific protease activity *i.e.* up to 20.40% (Table 2). A similar trend was recorded in *C. mrigala,* where a minimum reduction of 1.36% in the specific protease enzyme activity was induced by Zn at 0.02 ppm and maximum (13.60%) was induced by Cd+Zn (0.06 ppm). In fish *L. rohita,* maximum reduction of 3.40%was caused by Zn (0.02 ppm) and minimum reduction of 17% was induced by Cd+Zn (0.06 ppm). When all the three fish species were exposed to Zn at 0.06 ppm, maximum reduction was almost similar in fish *L. rohita, C. catla* and *C. mrigala i.e.* 8.84, 7.40 and 4.08% respectively. The heavy metal Cd (0.06 ppm) showed a maximum reduction of 12.24%, 15.21% and 10.20% in the specific protease activity in fish species *C. catla, L. rohita*and *C. mrigala,* respectively. Zn with Cd (0.02 ppm) induced a minimum reduction of 14.96%, 14.96% and 12.24%, respectively in the specific protease activity (Table 2). When all the three fishes were exposed to the mixture of both the heavy metals *i.e.* Cd and Zn, maximum reduction was almost similar in fish *C. mrigala* i.e.13.60 % at 0.06 ppm. While in fish *C. catla* maximum reduction in specific protease activity was 20.40% (Table 2).

Specific amylase activity

In the fish *C. catla,* maximum reduction (20.83%) in the specific amylase activity was caused by Cd+Zn (0.06 ppm) as compared to the control. Zn (0.02 ppm) induced minimum reduction (5.55%) in the specific amylase activity (Table 3). A similar trend was there in *C. mrigala and L. rohita,* where a maximum reduction of 38.80% and 21.01%, respectively was caused by Cd+Zn (0.06 ppm). While minimum reduction induced by Zn (0.02 ppm) was 10.44% and 10.14%, respectively. The heavy metal Cd (0.06 ppm) showed a maximum reduction of 15.27%, 15.21% and 19.40% in the specific amylase activity in fish species *C. catla,L. rohita*and *C. mrigala,* respectively. Zn with Cd (0.02 ppm) induced a minimum reduction of 18.75%, 18.11% and 20.89%, respectively in the specific amylase activity (Table 3). When all the three fishes were exposed to Zn, maximum reduction was almost similar in fish *L. rohita, C. catla* and *C. mrigala i.e.,* 11.11%, 11.59 and 13.43%, respectively at 0.06 ppm. In the fishes exposed to the heavy metal treatment in combination, the specific amylase enzyme activity was more as compared to the treatments of heavy metals alone.

DISCUSSION

Heavy metals have been reported to accumulate in the tissues of aquatic fauna. Mahananda *et al.* (2013) reported accumulation of lead and nickel (gill, kidney, liver, muscle, ovary, testis) of *Channa punctatus* (Bloch) when the test fishes were exposed to different concentrations of lead (5, 10, 15 and 20ppm) and nickel (10, 20, 30 and 40ppm) the amount of accumulation in all the tissues under investigation were noted to be significantly higher, progressive and directly proportional to the change in heavy metal concentration in the medium. Digestive enzymes are responsible for the breakdown of food particles in the alimentary canal thus influencing growth rate and food utilization by fishes. Various histochemical studies (Khillar and Wagh, 1988; Khare, 1993) have suggested that rupture of cells and deformation of tissues

Table 1: Heavy metal treatments given to fish species along with control

Sr. 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: Effect on specific proteolytic activity in the intestine of fresh water fishes exposed to different heavy metals

Fish species	Proteolytic Activity (mg of tyrosin/mg of protein/h)									CD(Pd*0.05)
	Control	Cd (ppm)			Zn (ppm)			Cd+Zn (ppm)		
	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	1.47±0.01	1.34±0.08(8.84)	1.31±0.03(10.88)	1.29±0.05(12.24)	1.43±0.01(2.72)	1.38±0.05(6.12)	1.36±0.04(7.4)	1.25±0.03(14.96)	1.24±0.06(15.64)	1.17±0.04(20.40)	0.14
Labeorohita	1.37±0.01	1.18±0.06(12.92)	1.17±0.02(13.60)	1.16±0.07(15.21)	1.32±0.04(3.40)	1.29±0.06(5.44)	1.24±0.02(8.84)	1.15±0.06(14.96)	1.14±0.03(15.64)	1.12±0.02(17)	0.14
Cirrhinusmrigala	1.26±0.03	1.23±0.06(2.04)	1.14±0.02(8.16)	1.11±0.07(10.20)	1.24±0.01(1.36)	1.21±0.03(3.40)	1.20±0.02(4.08)	1.08±0.02(12.24)	1.07±0.03(12.92)	1.06±0.05(13.60)	0.13

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

Table 3: Effect on specific amylase activity in the intestine of fresh water fishes exposed to different heavy metals

Fish species	Amylolytic activity (mg of maltose/mg of protein/h)									CD(Pd*0.05)
	Control	Cd (ppm)			Zn (ppm)			Cd+Zn (ppm)		
	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	1.44±0.04	1.26±0.05(12.5)	1.25±0.03(13.19)	1.22±0.06(15.27)	1.36±0.01(5.55)	1.32±0.02(8.33)	1.28±0.07(11.11)	1.17±0.08(18.75)	1.16±0.02(19.44)	1.14±0.05(20.83)	0.16
Labeorohita	1.38±0.06	1.20±0.03(13.04)	1.19±0.05(13.76)	1.17±0.06(15.21)	1.24±0.06(10.14)	1.23±0.01(10.86)	1.22±0.05(11.59)	1.13±0.03(18.11)	1.11±0.05(19.56)	1.09±0.05(21.01)	0.15
Cirrhinusmrigala	1.34±0.01	1.12±0.03(16.41)	1.10±0.06(17.91)	1.08±0.01(19.40)	1.20±0.06(10.44)	1.18±0.03(11.94)	1.160.03(13.43)	1.06±0.03(20.89)	1.04±0.04(22.38)	0.82±0.01(38.80)	0.11

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

affected the functional activity of the digestive enzyme and this might interfere with digestion. In the present investigation effect of Cd and Zn (alone and in combination) were studied on the activities of protease and amylase enzymes in *C. catla*, *L. rohita* and *C. mrigala*. The results revealed considerable decrease in activities of both the enzymes subjected to different treatments. The effect of heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}) on activities of carp trypsin, alpha-chymotrypsin, carboxypeptidase A and lipase were studied by Kotorman (2000). When the fishes were treated with single heavy metal, Cd (0.06 ppm), maximum reduction was registered for both the enzymes, in accordance with our findings. Toxic effects in combination of heavy metals were found to be more severe than single heavy metal treatment. Likewise, in our study also, Cd in combination with Zn showed maximum reduction of protease and amylase enzyme activity. Cd has received considerable attention because of its high toxicity and the fact that there is no known function in cells for these elements; even high concentrations of Cd can result in enzyme inhibition (Gould and Karolus, 1974). Eichhorn (1975) has pointed out that heavy metals can also bind with sites other than the active sites of the enzyme molecule and can produce both beneficial and adverse effects depending upon the concentration of the heavy metals. According to Hodson (1988) the inhibitory action of the heavy metals on enzyme is due to the binding of metals with enzyme protein. Heavy metal ions can displace metals situated at the active site of the enzyme and inhibit the enzyme activity. Supporting our findings, Sayed *et al*. (2011) reported adverse effect of 4-nonylphenol stress on AST, aspartate aminotransferase and ALT,alanine aminotransferase activities in *Clariasgariepinus*.

Digestive enzyme activity appeared to be affected by feeding behaviour, biochemical composition and ambient stress in the fresh water teleost (Kuzumina, 1996). So another reason for decrease in digestive enzyme activity might be altered feeding behaviour *i.e.* the fishes did not feed well under heavy metal toxicity stress. Mehrotra (1996) reported a decrease in absorption rate due to damaged villi of intestine. Golovanova*et al*. (1999) studied *in vitro* effect of Cd (0.5-50 mg/L) on the total amylolytic activity in burbot (*Lotalota*), crucian carp (*C. auratus)* and common carp (*C. carpio*); sucrase activity in blue bream and total proteolytic activity in burbot and piper were significantly decreased by Cd at 50 mg/L, caused a significant decrease in total proteolytic activity in pipe and had no effect on either protease or carbohydrase activities in other fish species. Chaudhary and Sultana (2002) reported the impact of nickel chloride and lead chloride on amylase activity of fresh water bivalve, *Parreysiacylindrica*, which reduced after acute treatment of both the heavy metals. Dinodia *et al*. (2003) also investigated the effects of Cd toxicity on proteolytic enzyme activity in fresh water carps and it was reported that proteolytic enzyme activity declined maximally in fish *Cirrhinusmrigala* from 26% at 2.5 ppm to 55.60% at 5.0 ppm.Nevalennyi and Bednyakov (2004) reported the influence of Cd ions at 0.25 mg/l on the alpha-amylase and maltase activities of carp, *Cyprinuscarpio*. Maltase activity decreased to 45% of the control by day 10 and then increased on day 30 and again decreased to 30% of control at the end of experiment. Alpha-amylase was at 43% of the control on day 40 and increased to 92% of control on day 40 and

increased to 92% of control on day 50. Different concentrations (0.01-100 mg metal/L) of $CuSO_4$ and $ZnSO_4$ influenced carbohydrase activity of fish intestinal mucosa *in vitro*. Toxic effects were found to depend on fish species and concentration of metals, in agreement with our findings (Kuzumina *et al.,* 2004). For all investigated species (carp, bream and roach) more toxic e ffect of Cu in comparison to Zn was shown. The degree of enzyme activity reduction at high concentration of Cu (10 and 25 mg/L) for different species was variable; 35-40% in carp and bream, 55-60% in perch and 75-80% in pike as compared to control.

The present study thus revealed that even allowable concentrations of Cadmium and Zinc in aquatic environments could considerably reduce enzyme activity in the aquatic fauna; and both the metals acted synergistically, in consonance with the findings of Samanta *et al.* (2010). 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 ban the waste discharge so as to provide a healthy environment for aquatic fauna to perish.

REFERENCES

APHA (American Public Health Association. 1998. Standard methods for the examination of water and waste water. *APHA, AWWA, WPFC, 16Ed,* New York.

Brungs, W. A., McCormick, J. H., Neiheisel, T. W., Spehar, C. E. and Stokes, G. N. 1977. Effects of pollution on freshwater fishes. *J. W.P.C.F, Washington DC.* **49:** 1425-1493.

Chaudhary, R. T. and Sultana, M. 2002. Effect of nickel chloride and lead chloride on amylase activity of fresh water bivalve. *Parreysia cylindrical.* **21(3):** 341-342.

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

Dinodia, G. S., Gupta, R. K., Jain, K. L. and Yadava, N. K. 2003. Cadmium toxicity and its effect on the proteolytic enzyme activity in some fresh water carps. *Proceedings of 3rd Interaction*, December 17-18, 2003. pp. 195-199.

Eichorrn, G. L. 1975. Active sites of biological macromolecules and other interactions with heavy metals. *In "Ecological Toxicology Research: Effect of heavy metals and organohalogen compounds"* (A.D. McIntyre and C.F. Mills, Eds.),V. Plenum, New York.

Firat, O. and Kargin, F. 2010. Biochemical alterations induced by Zn and Cd individually or in combination in the serum of *Oreochromisniloticus. Fish Physiol Biochem.* **36(3):** 647-53.

Golovanova, I. L., Kuzmina, V. V., Gobzhelian, T. E., Davlov, D. F. and Chuiko, G. M. 1999. *In vitro* effects of cadmium and DDVP dischlorovos on intestinal carbohydrase and protease activities in fresh water teleost. *Comp. Biochem. Physiol.* **122(1):** 21-25.

Gould, E. and Karolus, J. 1974. Physiological response of the cunner, *Tautogloabrusadspersus*, to cadmium. Observations on the biochemistry, U.S. Department of Commerce, Washington, D.C., *NOAA Technical Report NMFS SSRF.* **21:** 681.

Hodson, P. V. 1988. The effect of metal metabolism on uptake, disposition and toxicity in fish. *Aquat. Toxicol.* **38:** 257-276.

Khare, P. 1993. Endosulfanrogar, mercuris chloride and lead nitrate induced histopathological and histochemical changes with stomach and intestine of *Mystuscavasius*. Ph.D. Thesis, Baraktullah University, Bhopal.

Khillar, V. K. and Wagh, S. B. 1988. Acute toxicity of pesticides in the fresh water fish *Barbus stigma*. Histopathology of the stomach. *Uttar Pradesh J. Zool.* **8:** 176-179.

Kotoraman, M., Laszla, K., Nemcsok, J. and Simon, L. 2000. Effects of Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} on activities of some digestive enzymes in carp *(Cyprinuscarpio). J. Environ. Sci. Health.* **35(9):** 1517-1526.

Kumar, A. and Dahiya, T. 2013. The impacts of zinc on liver and serum proteins and cholesterol levels of fish (*Cirrhinusmrigala*). *The Bioscan.* **8(3):** 1057-1059.

Kuzumina, V. V. 1996. Influence of age on digestive enzyme activity in some fresh water teleosts. *Aquaculture.* **148:** 25-37.

Kuzumina, V. V., Golovanova, I. L., Shishin, M. M. and Smirnova, E. S. 2004. Influence of heavy metals on feeding behaviour and digestive process. *The sixth Syrian, Egyptian conference on the* chemical and petroleum engineering. Al Baath University Homes, Syria. 9.

Mahananda, H. B., Behera, R. and Behera, M. K. 2013. Heavy metal bio-accumulation in some selected tissues of *Channa punctatus* (Bloch). *The Ecoscan.* Special issue. **4:** 23-26.

Mehrotra, A. 1996. Studies on the toxic effects of pesticides and heavy metals on the intestine of *Nandusnandus (Ham.).* Ph.D Thesis, Baraktullah University, Bhopal.

Nevalennyi, A. N. and Bednyakov, D. A. 2004. Influence of cadmium ions on the activities of enzymes responsible for membrane digestion in carp. *Russian J. Ecology.* **35(2):** 128-130.

Oner, M., Atli, G. and Canli, M. 2009. Effects of metal (Ag, Cd, Cr, Cu, Zn) exposures on some enzymatic and non-enzymatic indicators in the liver of Oreochromisniloticus. *Bullet. Environ. Contam. Toxicol.* **82(3):** 317-21.

Rashed, M. N. 2001. Cadmium and lead levels fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. *Environ Monit Assess.* **68:** 75-89.

Samanta, P., Senapati, T., Mukherjee, A. K., Mondal, S., Haque, S. and Ghosh, A. R. 2010. Effectiveness of almix in controlling aquatic weeds and fish growth and its consequent influence. *International J. Life Sciences.* **3:** 691-700.

Sawhney, S. K. and Singh, R. 2000. In Introductory Practical Biochemistry. Narosa Publishing House. p. 452.

Sayed, A. H., Mekkawy, I. A., Mahmoud, M. 2011. Effects of 4-nonylphenol on metabolic enzymes, some ions and biochemical blood parameters of the African catfish *Clariasgariepinus* (Burchell, 1822) *African J. Biochemistry Research.* **5(9):** 287-297.

Shewta, A., Hosetti, B. B. and Dube, P. N. 2012. Toxic Effects of Zinc Cyanide on Some Protein Metabolites in Fresh water fish, *Cirrhinusmrigala (Hamilton). Int. J. Environ. Res.* **6(3):** 769-778.

Vinodhini, R. and Narayanan, M. 2009. The impact of toxic heavy metals on the hematologicalparameters in common carp (*CyprinusCarpio*L.) *Iran. J. Environ. Health. Sci. Eng.* **6(1):** 23-28.

Walter, H. E. 1984. Methods of enzymatic analysis. Verlag Chemie, *Weinchem.* p. 238.