Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(4): 818-821

Received: 13-05-2017
Accepted: 14-06-2017

Sunita Rani
PhD Scholar, Department of Zoology, CCS Haryana Agricultural University, Hisar- 125004, India

Rajender Kumar Gupta
Professor, Department of Zoology, CCS Haryana Agricultural University, Hisar- 125004, India

Jyoti Yadav
PhD Scholar, Department of Zoology, CCS Haryana Agricultural University, Hisar- 125004, India

Correspondence
Sunita Rani
PhD Scholar, Department of Zoology, CCS Haryana Agricultural University, Hisar- 125004, India

Heavy metal induced alterations in acetylcholinesterase activity of Indian major carps

Sunita Rani, Rajender Kumar Gupta and Jyoti Yadav

Abstract

The present study was conducted in 2012 to envisage the impact of heavy metals *viz.* cadmium (0.02, 0.04 and 0.06 ppm) and zinc (0.02, 0.04 and 0.06 ppm) alone and in combinations (Cd +Zn) at 0.02+0.02,0.04+0.04 and 0.06+0.06 ppm concentrations. The analysis of alteration in behavior, morphology and acetylcholinesterase activity was analyzed in three carps *viz. Labeo rohita, Catla catla* and *Cirrhinus mrigala.* Maximum reduction in enzyme activity upto 9.21%, 12.42% and 15.80% has been observed in *C. catla, L. rohita* and *C. mrigala* respectively, when treated with Cd (0.06 ppm). When the fishes were exposed to Zn (0.06 ppm), reduction upto 4.08%, 10.60% and 13.75% has been observed in *C. catla, L. rohita* and *C. mrigala* respectively. It may also be noted that the maximum inhibition (18.67%) of enzyme activity was observed in *C. mrigala* when it was exposed to Cd+Zn (0.06+0.06 ppm).

Keywords: Acetylcholinesterase, major carps, enzyme, zinc, cadmium

1. Introduction

The amplification of pollution levels in soil has not only threatened the soil ecosystem but also influences aquatic ecosystems. Processes like electroplating, metallurgy, tannery, chemical battery manufacturing, mining etc. release heavy amounts of heavy metals in the environment, thereby resulting in soil pollution [1]. Often referred as devils in disguise, the heavy metals are the outcomes of several industrial and agrarian activities [2]. Heavy metals do not disintegrate rapidly; hence persist longer in environment in comparison to other unsafe organic compounds [3]. Heavy metals have received much attention in eco-toxicological research because of their increasing input, extended persistence, widespread toxicity to biota, their tendency to accumulate and biological magnification in freshwater flora and fauna. Longer persistence of heavy metals, thus leads to damage of soil fauna for many years. These heavy metals enter aquatic ecosystems with run-away water and even the slightest dose of heavy metals may disturb metabolism of fishes [4]. Physiological functions such as nutrition, survival, growth and reproduction are affected negatively due to heavy metal exposure [5]. About 94% of total fish production is contributed by Indian major, common and Chinese [6]. As estimated by FAO [7], the production of *C. mrigala* and *L. rohita* production is about 302,025 tonnes and 1,133,233 tonnes respectively. Cd and Zn at higher levels pose threat to the survival of organisms and hence possess ecotoxicological importance. Acetylcholinesterase enzyme activity has been looked upon as the early warning biomarker for the presence of pollution. Therefore, the present study was carried out to envisage the impact of heavy metals (Cadmium and Zinc) on the cholinesterase enzyme of Indian major carps *viz. Labeo rohita, Catla catla* and *Cirrhinus mrigala.*

2. Materials and methods

2.1 Procurement of species: Fish specimens of *Labeo rohita, Catla catla* and *Cirrhinus mrigala* approximately about 4 to 6 inches having age of six months were procured from the local fresh water ponds of Hisar, Hansi and Sirsa.

2.2 Experimental set up: Fishes were then disinfected with 0.1% $KMnO_4$ solution and acclimatized for seven days at room temperature in a large laboratory tub. Thereafter, the fishes were transferred to the plastic tubs with 100 liters capacity 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 [8]. Then the fishes were treated with different doses of heavy metals as shown in Table 1. 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 cholinesterase estimation. In addition, changes in fish behavior and certain morphological parameters were also recorded in fishes exposed to the heavy metal treatments.

Table 1: Treatments given to the test fish species along with control

Sr. No.	Treatment	Concentrations (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

2.3 Assessment of water quality
For the determination of water quality parameters, water samples were obtained in replicate of 3 from each treatment. Water temperature (ºC), Dissolved oxygen, pH, alkalinity and the free carbon dioxide were recorded at an interval of 15 days during treatment period.

2.4 Enzymatic estimation
The fishes that survived 60 days after treatment were used for blood sampling. The blood samples were collected in eppendorf tubes and allowed to stand for half an hour to allow coagulation. Thereafter, blood sample were centrifuged at 3000 rpm for 10 minutes. After collecting serum, it was then stored at -20̊ C and used for enzymatic estimation by the method described by Ellman *et al.* [9].

2.5 Statistical analysis
The data represented are the mean of triplicates and were subjected to one way ANOVA to find out significant differences between different treatments using OPSTAT software developed by Department of Statistics, CCS Haryana Agricultural University, Hisar. CRD was used as experimental design.

3. Results and discussion

Table 2: Physico-chemical characteristics of water used during the experiment

Sr. No.	Parameter	Value
1	Temperature (ºC)	26-34
2	Dissolved oxygen (mg/L)	6.0-8.3
3	Hydrogen ion concentration	6.7-7.5
4	Alkalinity (mg/L)	151-157
5	Free carbon dioxide (mg/L)	1.5-3.2

3.1 Morphological and behavioral changes induced due to heavy metal exposure
Changes in body color and other apparently visible symptoms like fin erosion, body deformity, descaling and hemorrhage was observed at fortnightly intervals in fishes exposed to Cd and Zn at different concentrations as shown in Table 3. Depigmentation due to heavy metal exposure at higher doses in fishes exposed to Cd and Zn alone and in combinations has been observed. Fin blackening, body deformities and blood oozing can also be justified by the findings of Rani *et al.* [10] and Murthy *et al.* [11]. The heavy metal exposed fishes while swimming, exhibited jerky and whirling movements, restlessness and sometimes these were found surfacing and engulfing air. The behavioral changes like erratic restless movements can be the result of acetylcholinesterase activity inhibition of fishes due to heavy metal induced stress as stated by Rani *et al.* [10]. Fishes exposed to Cd and Zn (0.06 ppm), individually as well as in combination, exhibited restless and showed surfacing in every 2 to 5 min. Engulfing air and repeated surface visiting has also been documented by Thenmozhi *et al.* [12]. *C. catla*, being a surface feeder was seen with higher surfacing in most of the treatments. However, loss of equilibrium, uncoordinated swimming movements and easily excitable conditions were observed in all heavy metals exposed fishes. Increased swimming activity and breathing movements were observed in all the fish species on exposure to different treatments of heavy metals alone as well as in combinations. Increased fin movement alongwith delayed responses have been observed in treated fishes. The feeding response of the treated fishes was also distinctly low and these fishes in general abstained themselves from feeding in higher dose levels. Stress induced changes such as excessive secretion, anorectic condition and increased distance between gills and operculum due to exposure of nickel, copper and Zinc in *Poecilia reticulate* has previously been documented by Khunyakari *et al.* [13].

3.2 Acetylcholinesterase activity in blood serum of fishes exposed to Cd and Zn
The acetylcholinesterase activity in blood serum in fishes exposed to the heavy metals individually as well as in combination as compared to the control has been represented in Table 4. In *C. catla* exposed to Cd (0.06 ppm), reduction upto 9.21% in cholinesterase activity has been observed while this inhibition (15.80%) in enzyme activity was highest in *C. mrigala* at same exposure. However, the range of acetylcholinesterase inhibition was from 11.81% (Cd 0.02%) to 12.41% (Cd 0.06%). In the fish *C. mrigala* minimum toxicity (13.55%) was induced by Zn at 0.02 ppm and maximum (15.80%) by Cd at 0.06 ppm. *L. rohita* exposed to Zn at 0.02 ppm showed the least reduction of 10% in blood serum cholinesterase activity, whereas the maximum reduction of 12.42% on exposure to Cd at 0.06 ppm. Likewise, in fish *C. catla*, where maximum reduction was about 9.21% when exposed to Cd at 0.06 ppm and minimum reduction of 2.10% induced by Zn at 0.02 ppm. The results clearly showed the dose dependent significant reduction in enzyme activity due to heavy metal exposure at all concentrations. It is also worth noticing that *C. mrigala* faced a maximum level of enzymatic inhibition, marking the greater sensitivity of it towards heavy metal pollution. The toxic effects of these metals enhanced slightly when the fishes were exposed to heavy metals in combination. In the fish *L. rohita,* Cd in combination with Zn at 0.06 ppm induced 14.84% while Cd in combination with Zn at 0.02 ppm induced least toxicity i.e. 13.43%. In the fish *C. mrigala,* maximum reduction (16.01%) in the cholinesterase activity in blood serum was induced by a combination of Cd and Zn at (0.06 ppm) and minimum (18.67%) by a combination of Cd and Zn at (0.02 ppm). In fish *C. catla* maximum reduction of 11.78% was induced by Cd in combination with Zn at 0.06 ppm and minimum reduction of 9.41% was induced by the combination of Cd with Zn (0.02 ppm). Cd was found to be more toxic to fish as compared to Zinc. Acetylcholinesterase enzyme is vital for food orientation, prey location and

predator escaping [14]. The findings related to acetylcholinesterase inhibition due to pollution can be justified by the findings of Rajni and Revathy [15] and Shoaib *et al.* [16]. Thus, heavy metals by inhibiting acetylcholinesterase enzyme activity poses threat to fish survival.

Table 3: Effect of cadmium and Zinc on various physical and behavioral changes in Indian major carps

Treatment (ppm)	Physical characteristics				Behavioral changes
	Body colour / shape	**Scale erosion**	**Fin colour**	**Hemorrhage**	**Swimming pattern**
Control	Normal	Nil	Normal	Nil	Normal swimming
Cadmium 0.02	Shiny reddish	Slight	Black patches were observed	Normal	Abnormal swimming
Cadmium 0.04	Blackish colour	Moderate	Fin blackening were observed	Mildred patches on the body	Slight Fast swimming
Cadmium 0.06	Reddish and whitish	Moderate	Fin become black	Mild blood oozing	Fast swimming
Zinc 0.02	Normal	Slight	Normal	Normal	Normal swimming
Zinc 0.04	Shiny reddish	Slight	Black patches	Mild blood oozing	Normal swimming
Zinc 0.06	Body deformity	Moderate	Fin blacking	Blood oozing	Abnormal swimming
Cadmium + Zinc 0.02 + 0.02	Shiny Reddish	Slight	Black patches were observed	Mildred patches on the body	Abnormal swimming
Cadmium + Zinc 0.04 + 0.04	Dark Blackish colour	Moderate	Fin blackening were observed	Mild blood oozing	Fast swimming
Cadmium + Zinc 0.06 + 0.06	Dull Reddish and whitish	Moderate	Fin become black	Blood oozing from different part of body	Fast swimming

Table 4: Mean value of cholinesterase (IU/L) in the blood serum in fishes exposed to different heavy metals

Fish species	Heavy metals (ppm)										CD ($p \leq 0.05$)
	Control	**Cd**			**Zn**			**Cd+Zn**			
	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**	
Catla catla	168. 9 ±0.79	157.00 ±0.57 (7.04)	154.00 ±0.57 (8.81)	153.33 ±0.88 (9.21)	165.33 ±1.33 (2.10)	162.33 ±0.33 (3.88)	162.00 ±0.57 (4.08)	153.00 ±0.57 (9.41)	149.33 ±0.33 (11.58)	149.00 ±0.57 (11.78)	2.36
Labeo rohita	165.19 ±0.47	145.67 ±1.20 (11.81)	145.33 ±0.33 (12.02)	144.67 ±1.45 (12.42)	148.67 ±0.88 (10.00)	147.67 ±0.88 (10.60)	147.47 0.88 (10.60)	143.00 ±0.57 (13.43)	141.67 ±0.88 (14.24)	140.67 ±0.88 (14.84)	2.68
Cirrihinus mrigala	162.72 ±0.25	139.33 ±0.33 (14.37)	138.67 ±2.02 (14.78)	137.00 ±0.57 (15.80)	140.67 ±0.88 (13.55)	140.33 ±01.33 (13.75)	139.66 ±0.66 (14.16)	136.67 ±0.57 (16.01)	133.00 ±1.52 (18.26)	132.33 ±0.66 (18.67)	3.09

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

4. Conclusion

Both cadmium and zinc are toxic to fishes and possess an inhibitory effect on the acetylcholinesterase activity. However, it is also clear that acetylcholinesterase inhibition threatens the survival of fishes. *C. mrigala* was found to be more sensitive to heavy metal exposure as compared with other major carps. Synergistic mode of inhibition of enzyme activity has also been observed as the inhibition levels increased when heavy metals were used in combinations. Cadmium being more toxic as compared to zinc should be minimally and judiciously used in agricultural and industrial processes.

5. Acknowledgement

The authors are deeply obliged to the HOD, Zoology for rendering timely help and facilities to complete the research work.

6. References

1. Vidovic M, Sadibasic A, Cupic S, Lausevic M. Cd and Zn in atmospheric deposit, soil, wheat, and milk. Environmental Resource. 2005; 97:26-31.
2. Vandana S, Keshav S. Toxic effect of herbicide 2, 4-D on the earthworm *Eutyphoeus waltoni* Michaelsen. Environmental Process. 2015; 2:251-260.
3. Brusseau ML. Transport and fate of toxicants in soils. In: Soil Ecotoxicology. 1997; 33-53.
4. Rani S, Gupta RK, Rani M. Heavy metal induced toxicity in fish with special reference to zinc and cadmium. International Journal of fisheries and aquatic sciences. 2015; 3(2):118-123.
5. Bernard F, Brulle F, Dumez S, Lemiere S, Platel A, Nesslany F et al. Antioxidant responses of annelids, Brassicaceae and Fabaceae to pollutants: a review. Ecotoxicology and Environmental Safety. 2014, 04-024.
6. Ramakrishna R, Shipton TA, Hasan MR. Feeding and feed management of Indian major carps in Andhra Pradesh, India. FAO Fisheries and Aquaculture Technical Paper No. 578. Rome, FAO. 2013, 90.
7. FAO, Fishery Information, Data and statistics unit, FAO Fisheries Department, Fishtat Plus: Universal software for fishery statistical time series, 2013.
8. APHA. American water works association and water pollution control federation. (24th Ed.) Washington. American Public Health Association. 1998, 801-823.
9. Ellman GL, Courtney KD, Andres VV, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemistry and Phamocology, 1961; 7:88-95.
10. Rani M, Gupta RK, Yadav J, Kumar S. Assessment of organophosphates induced acetylcholinesterase inhibition in Indian major carps. Journal of Entomology and Zoological Studies. 2017; 5(2):1369-1371.
11. Murthy KS, Kiran BR, Venkateshwarlu M. A review on toxicity of pesticides in Fish. International Journal of Open Scientific Research. 2013; 1:15-36.

12. Thenmozhi C, Vignesh V, Thirumurugan R, Arun S. Impacts of Malathion on mortality and biochemical changes of freshwater fish *Labeo rohita.* Iranian Journal of Environmental Health of Scientific Engineering. 2011; 8(4):387-394.
13. Khunyakari R, Vrushali P, Sharma RN. Effects of some trace heavy metals on *Poecilia reticulates* (Peters). Journal of Environmental Biology. 2001; 22(2):141-144.
14. Miron DS, Crestani M, Shettinger MR, Morsch VM, Baldisserotto B, Tierno MA et al. Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae). Ecotoxicology and Environmental Safety. 2005; 61:398-403.
15. Rajini A, Revathy K. Effect of combination pesticide on acetylcholineesterase activity in freshwater fish *Danio rerio.* International Journal of Pharma and Bio Sciences. 2015; 6(1):1305-1310.
16. Shoaib N, Siddiqui PJA. Impact of organophosphate pesticides, methyl parathion and chlorpyrifos on some tissue enzymes of fish (*Aphanius dispar*). Indian Journal of Geo- Marine Sciences. 2016; 45(7):869-874.