Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

Independent and successive effect of two environmental stressors on the nutritional content of *Labeo rohita*

Senthilkumaar P, Velmurugan K, Sarojini S, Silambarasan K

School of Enzymology and Environmental Toxicology, P.G. & Research Department of Zoology, Sir Theagaraya College, Chennai-600 021, Tamil Nadu, India

***Corresponding Author:** School of Enzymology and Environmental Toxicology, P.G. & Research Department of Zoology, Sir Theagaraya College, Chennai-600 021, Tamil Nadu, India, E-mail: drsenthilkumaar@yahoo.co.in.*

Abstract

Evaluating the toxic effect of an organochlorine pesticide, endosulfan and heavy metal lead on the fingerlings of Indian carp *Labeo rohita* was the prime aim. Individual and successive exposure of the two toxicants was assessed. 96 hr LC_{50} studies on pesticide and heavy metal resulted in the sub lethal concentration of 4 ppm and 87.5 ppm respectively. Post exposure estimation of nutritional parameters; total proteins, total carbohydrates and total lipids on muscle, liver, brain and kidney of the fish was done for individual toxicants. The impairment of metabolism was found to be more on lead exposure compared to endosulfan. Successive exposure study of the two environmental stressors was carried out to consider the combination effect on the nutritional status of the test fish.

Keywords: *Labeo rohita*; Endosulfan; Lead nitrate; Proteins; Carbohydrates and Lipids.

Abbreviations: **SLC -** Sub Lethal Concentration; AST - Aspartate amino transferase; ALT - Alanine amino transferase.

Introduction

Organisms are rarely exposed to individual chemicals in the environment. Research has shown that many compounds can enter the environment, disperse and persist to a greater extent. Some compounds, such as pesticides, are intentionally released and others, such as industrial by products, are released through regulated and unregulated industrial discharges to water and air resources (Kolpin *et al*., 2002; Battaglin and Fairchild, 2002). Hence, aquatic organisms are often exposed to mixtures of toxicants because it is believed that regardless of where the pollution occurs, it will eventually end up in the aquatic environment (Firat *et al.,* 2011). Their mechanisms of action are unknown and there is a great need to bridge the gap between our understanding of the toxic effects of exposure to individual xenobiotics and those effects from exposure to mixtures of such chemicals (Junghans, 2004; Olmstead and LeBlanc, 2005). Contamination by pesticide and heavy metal in aquatic system is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated foods (Ribeiroa *et al*., 2005; Ling *et al*., 2011). The effect of pollution on the inhabiting species was found to be more in fresh water than in marine water (Koyama, 1997). Hence, fingerlings of one of the Indian major carps, *Labeo rohita* (rohu) was selected to assess the independent and combined (successive) toxic effects of a commonly used pesticide, endosulfan and an industrial heavy metal effluent lead nitrate.

Materials and methods

Collection and maintenance of fish

The fingerlings of the Indian major carp, *Labeo rohita* were collected from the Tamil Nadu Fish Seed Farm Poondi, Thiruvallur Dist. They were acclimatized to laboratory condition for three days. During this period they were fed with groundnut oil cake and the water was also renewed alternatively after feeding. The environmental parameters like the temperature, pH, salinity and dissolved oxygen of the water used were well within the acceptable range.

Methodology

Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

Table 1 Determination of 96 hr lc_{50} for endosulfan in rohu						
S. No	Conc. (ppm)	Percentage mortality				Cumulative % mortality
		DAY 1	DAY 2	DAY 3	DAY 4	
1	10	0	0	0	0	0
2	12	0	0	10	10	20
3	14	0	10	10	20	40
4	16	0	10	20	20	50
5	18	10	10	20	25	65

Table 2 Determination of 96 hr lc_{50} for lead nitrate in rohu						
S. No	Conc. (ppm)	Percentage mortality				Cumulative % mortality
		DAY 1	DAY 2	DAY 3	DAY 4	
1	150	0	0	0	0	0
2	200	0	0	10	10	20
3	250	0	0	10	20	30
4	300	0	10	10	20	40
5	350	10	10	10	20	50

Healthy fish without any observable pathological symptoms were chosen for the experiments. Fishes were divided into groups of ten each and exposed to the toxicants viz. endosulfan ($C_9H_6Cl_6O_3S$) and lead nitrate ($PbNO_3$) independently and successively. In the independent toxicity experiment, fingerlings of *Labeo rohita* were exposed to the test toxicants individually and in the successive study, it was exposed to one toxicant for four days followed by the second toxicant for three days.

Independent toxicity

Initially the fingerlings of rohu were treated with endosulfan and lead nitrate individually to determine 96hr LC_{50} values for the test toxicants. Based on the range finding bioassay, five different concentrations of investigative toxicants were prepared by dissolving in distilled water. Suitable controls without toxicant were maintained for all the experimental groups. Mortality was recorded for each day and tabulated to arrive at the cumulative per cent mortality at the end of 96hr for the different groups and 96hr LC_{50} value was arrived using Finney (1971) probit analysis method.

One fourth of LC_{50} values obtained from the above experiments was taken as the Sub Lethal Concentration (SLC). Apparently normal and healthy fingerlings of rohu was exposed to these concentrations of the toxicant in groups for further experimental studies and sacrificed on 4th, 7th, 14th, 21th and 28th day of post exposure to study the biochemical parameters; total proteins, total carbohydrates and total lipids of fish muscle, liver, brain and kidney.

In the experiment involving the study of successive synergistic effect of the toxicants, the fingerlings of rohu was first treated with endosulfan for four days and later with the second toxicant for another three days. The biochemical estimations were carried in tissues; muscle, liver, brain and kidney at the end of 7th day after successive exposure of the toxicants and the results were compared with zero day control. The carbohydrates, proteins and lipids, were estimated

Table 3 Total proteins (mg/g wet wt.)							
MUSCLE							
TOXICANT		**CONTROL**	**4th DAY**	**7th DAY**	**14th DAY**	**21st DAY**	**28th DAY**
$C_9H_6Cl_6O_3S$	**MEAN**	44.09	41.90	29.45	24.44	11.40	42.92
	S.D.	1.28	1.11	1.34	1.19	1.41	0.88
	P	-	0.001	0.001	0.001	0.001	NS
LIVER							
	MEAN	20.27	18.96	15.38	12.12	10.20	18.72
	S.D	1.43	1.31	1.02	0.59	1.35	1.08
	P	-	NS	0.001	0.001	0.001	0.001
BRAIN							
	MEAN	9.91	6.82	4.77	2.54	1.32	7.08
	S.D	0.94	1.12	0.89	0.82	0.20	1.23
	P	-	0.001	0.001	0.001	0.001	0.001
KIDNEY							
	MEAN	15.06	13.82	10.06	7.74	5.26	13.82
	S.D	1.75	1.78	1.27	1.25	1.06	1.78
	P	-	NS	0.001	0.001	0.001	NS

Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

Table 4 Total carbohydrates (mg/g wet wt.)							
MUSCLE							
TOXICANT		CONTROL	4th DAY	7th DAY	14th DAY	21st DAY	28th DAY
$C_9H_6Cl_6O_3S$	MEAN	3.87	2.06	1.74	0.77	0.61	1.32
	S.D.	0.30	1.48	0.92	0.07	0.08	0.20
	P	-	0.001	0.001	0.001	0.001	0.001
LIVER							
	MEAN	20.11	22.15	24.35	26.50	26.85	19.38
	S.D	1.24	1.07	1.05	0.91	1.04	2.50
	P	-	0.001	0.001	0.001	0.001	0.001
BRAIN							
	MEAN	1.83	1.00	0.99	0.71	0.56	1.43
	S.D	0.44	0.21	0.15	0.06	0.09	0.59
	P	-	0.001	0.001	0.001	0.001	0.001
KIDNEY							
	MEAN	5.75	3.65	2.13	1.81	0.46	4.45
	S.D	1.07	0.40	0.21	0.58	0.12	0.85
	P	-	0.001	0.001	0.001	0.001	0.001

Table 5 Total lipids (mg/g wet wt.)							
MUSCLE							
TOXICANT		CONTROL	4th DAY	7th DAY	14th DAY	21st DAY	28th DAY
$C_9H_6Cl_6O_3S$	MEAN	3.66	2.65	2.13	1.03	0.95	2.69
	S.D.	0.41	0.82	0.21	0.28	0.50	1.05
	P	-	NS	0.001	0.02	0.001	NS
LIVER							
	MEAN	14.60	11.50	9.15	8.36	6.96	12.61
	S.D	1.34	1.13	1.18	1.00	1.81	1.03
	P	-	NS	0.001	0.001	NS	NS
BRAIN							
	MEAN	19.00	16.34	14.67	12.15	11.95	17.35
	S.D	1.15	0.72	1.20	1.26	1.79	1.20
	P	-	NS	0.001	0.001	0.001	NS
KIDNEY							
	MEAN	10.68	8.27	6.76	5.14	4.85	6.96
	S.D	1.09	0.74	0.93	0.99	0.35	1.81
	P	-	0.001	0.001	0.001	0.001	0.001

Table 6 Total proteins (mg/g wet wt.)							
MUSCLE							
TOXICANT		CONTROL	4th DAY	7th DAY	14th DAY	21st DAY	28th DAY
$PbNO_3$	MEAN	44.09	42.21	40.44	39.14	36.69	41.35
	S.D.	1.28	1.29	1.26	1.68	0.97	1.08
	P	-	0.001	0.001	0.001	0.001	0.001
LIVER							
	MEAN	20.27	15.48	13.29	9.78	6.01	18.76
	S.D	1.43	1.90	1.32	1.38	1.37	1.48
	P	-	0.001	0.001	0.001	0.001	NS
BRAIN							
	MEAN	9.91	7.84	6.32	3.13	2.11	5.77
	S.D	0.94	1.11	0.72	0.91	0.99	1.25
	P	-	0.02	0.001	0.001	0.001	0.001
KIDNEY							
	MEAN	15.06	13.64	12.04	9.86	4.85	12.12
	S.D	1.75	0.80	0.98	0.79	1.17	0.59
	P	-	NS	0.02	0.001	0.001	0.001

Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

Table 7 Total carbohydrates (mg/g wet wt.)							
MUSCLE							
TOXICANT		**CONTROL**	**4th DAY**	**7th DAY**	**14th DAY**	**21st DAY**	**28th DAY**
$PbNO_3$	**MEAN**	3.87	2.86	1.90	1.21	0.60	1.02
	S.D.	0.30	0.25	0.09	0.07	0.06	0.09
	P	-	0.001	0.001	0.001	0.001	0.001
LIVER							
	MEAN	20.11	21.95	22.35	22.93	23.15	19.75
	S.D	1.24	1.41	1.46	1.23	0.85	1.02
	P	-	0.001	0.001	0.001	0.001	0.001
BRAIN							
	MEAN	1.83	1.39	0.71	0.66	0.61	1.46
	S.D	0.44	0.10	0.06	0.08	0.10	0.10
	P	-	NS	0.001	0.001	0.001	NS
KIDNEY							
	MEAN	5.75	3.10	2.81	1.55	1.43	4.92
	S.D	1.07	0.63	0.14	0.57	0.13	1.02
	P	-	0.001	0.001	0.001	0.001	NS

Table 8 Total lipids (mg/g wet wt.)							
MUSCLE							
TOXICANT		**CONTROL**	**4th DAY**	**7th DAY**	**14th DAY**	**21st DAY**	**28th DAY**
$PbNO_3$	**MEAN**	3.66	2.98	2.10	1.58	0.61	2.61
	S.D.	0.41	0.55	0.58	0.24	0.10	1.00
	P	-	NS	0.001	0.001	0.001	NS
LIVER							
	MEAN	14.60	12.84	8.55	7.57	4.12	13.37
	S.D	1.34	1.20	0.56	0.90	0.77	1.60
	P	-	NS	0.001	0.001	0.001	NS
BRAIN							
	MEAN	19.00	16.70	14.34	13.15	11.99	15.21
	S.D	1.15	1.24	0.86	0.89	1.60	0.86
	P	-	NS	0.001	0.001	0.001	0.001
KIDNEY							
	MEAN	10.68	8.02	6.82	3.90	2.48	7.22
	S.D	1.09	0.69	1.12	0.85	0.72	0.90
	P	-	0.001	0.001	0.001	0.001	0.001

Table 9 Combination effect (successive) total proteins									
TOXICANTS		MUSCLE (C)	MUSCLE (T)	LIVER (C)	LIVER (T)	BRAIN (C)	BRAIN (T)	KIDNEY (C)	KIDNEY (T)
$C_6H_6Cl_6O_3S$ + $PbNo_3$	MEAN	44.09	15.43	20.27	10.29	9.91	4.05	15.06	9.89
4d + 3d	S.D	1.28	1.26	1.43	1.32	0.64	1.11	1.75	1.21
	P	-	0.001	-	0.001	-	0.001	-	0.001

Table 10 Combination effect (successive) total carbohydrates									
TOXICANTS		MUSCLE (C)	MUSCLE (T)	LIVER (C)	LIVER (T)	BRAIN (C)	BRAIN (T)	KIDNEY (C)	KIDNEY (T)
$C_6H_6Cl_6O_3S$ + $PbNo_3$	MEAN	3.87	0.70	20.11	15.65	1.83	0.34	5.75	1.40
4d + 3d	S.D	0.30	0.09	1.24	1.43	0.44	0.07	1.07	0.14
	P	-	0.001	-	0.001	-	0.001	-	0.001

Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

Table 11 Combination effect successive) total lipids									
TOXICANTS		MUSCLE (C)	MUSCLE (T)	LIVER (C)	LIVER (T)	BRAIN (C)	BRAIN (T)	KIDNEY (C)	KIDNEY (T)
$C_6H_6Cl_6O_3S$ + $PbNo_3$	MEAN	3.66	0.97	14.60	6.98	19.00	11.34	10.68	4.31
4d + 3d	S.D	0.41	0.16	1.34	1.18	1.15	0.86	1.09	0.84
	P	-	0.001	-	0.001	-	0.001	-	0.001

by methods of Carrol *et al*., (1956), Lowry *et al.,* (1951) and Jayaraman (2003) respectively. Data analysis was carried out using SPSS statistical package to arrive at mean, standard deviation and test of significance (P value).

Results and discussion

Determination of 96hr LC_{50} values for the test toxicants and fixing of sub lethal concentration

For endosulfan the five different concentrations used to decide 96hr LC_{50} were in the range of 10-18 ppm. No mortality was recorded at 10 ppm concentration of the pesticide and 20% and 40% mortality was recorded at 12 and 14 ppm concentration respectively. 50% mortality was recorded at 16 ppm concentration. 96hr LC_{50} for the pesticide, endosulfan was noted as 16 ppm by graphical method. SLC was calculated as 4ppm being $1/4^{th}$ of LC_{50} value (Table 1).

The fish was exposed to lead nitrate in the range of 150-400 ppm to determine 96hr LC_{50} concentration. With regard to mortality rates, there was no evidence of mortality at 150 ppm. 20% mortality was noticed at 200 ppm concentration and the death rate of the test fish increased at higher concentrations. 50% mortality was recorded in the experimental group subjected to 350 ppm of the chemical. 87.5 ppm being $1/4^{th}$ of LC_{50} value was taken as SLC for lead nitrate toxicity studies (Table 2).

Independent toxic effects

The total protein content in muscle, liver, brain and kidney of rohu exhibited a decreasing trend in all the pesticide exposure periods. With reference to total carbohydrate content,with the exception of liver, other test organs presented a declining trend. The total lipid content also reduced during the exposure days. The independent toxic effects of lead nitrate also exhibited a similar picture. Most of the mean values were significant for both the toxicants (Tables 3 to 8).

Combination effects (successive)

The total protein content in muscle, liver, brain and kidney of *Labeo rohita* distinctly decreased on successive exposure of two environmental stressors. The total carbohydrate content in all the test organs including the liver showed a marked decrease. The total lipid content also revealed a decreasing trend during the exposure days. Most of the values obtained were significant (Tables 9 to 11).

Concoctions of chemicals present in aquatic environments may elicit toxicity due to additive or synergistic effects among the constituents or vice versa, the adverse outcome may be reduced by antagonistic interactions (Dondero *et al*., 2011; Cassee *et al*., 1998). Assessment of biochemical parameters; proteins, carbohydrates and lipids are important to indicate the susceptibility of organ systems to these pollutants (Fahmy, 2012). In the present independent exposure study of heavy metal lead on *Labeo rohita* exhibited less deviation from the control with reference to nutritional content in the muscle. This may be due to the low affinity of muscle tissue for heavy metals compared with other tissues (Korai *et al*., 2008). Generally, the impairment of metabolism was found to be more on lead exposure compared to endosulfan. The finding is attributed to the fact that the test fish is highly sensitive to lead (Abdullah *et al*., 2007). Suneetha *et al.,* (2010) reported decrease in intensity as well as disappearance of some protein subunits compared to the control in liver, brain, gill and muscle of *Labeo rohita* exposed to endosulfan. The decreased protein levels in pesticide stressed tissues strongly suggest the toxicant induced proteolysis to meet the increased energy demand

Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

(Saravanan *et al*., 2000; Kumar *et al.,* 2005). Increase of aspartate amino transferase (AST) and alanine amino transferase (ALT) in the organophospate or organochlorine stressed tissues of *Labeo rohita* is another indication of incorporation of amino acids by way of amino transferase activities into the Kreb's cycle to overcome the acute strain posed by the pesticide (Tilak *et al*., 2005; Saravanan, 2010).

Heavy metals are metabolic inhibitors of animals and lead can significantly decrease the total free aminoacids (Pugazhendy *et al*., 2005; Ghosi and Mukhopadhyay, 2000). In accordance with the referred reports, the present study on independent toxicity study of endosulfan and lead on *Labeo rohita* exhibited decreased protein levels suggesting proteolytic activity owing to toxicity stress.

Increase in serum glucose levels in fish under stress was reported by Bedii and Kenan (2005) and Chowdhury *et al*., (2004) This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (Almeida *et al*., 2001). The detoxifying role of the liver was evident in the present study as it alone depicted a slightly elevated level of total carbohydrates during the post exposure of individual toxicants. The lipid content decreased in all the test tissues during the experimental period for both the toxicants justifying the utilization of energy depot to meet the requirement of more energy for detoxification process and also to balance the hindrance of normal metabolism.

Continuous exposures of two different test xenobiotics were tried on *Labeo rohita* to study the combination effect (successive) on its nutritional content. Additional stress during the recovery period was notable in the total proteins, total carbohydrates and total lipids of the fish. Mostly the test organs exhibited additive effect. The question of potentiation does not arise since endosulfan and lead nitrate are known toxicants. Synergistic effect and antagonism was not evident in the present study.

Conclusion

In a polluted environment, the accumulation of toxicants in the fingerlings is a continuous process and this stress will markedly affect the nutritional value of *Labeo rohita* especially in terms of its protein content which the fish is known to provide. The loss is much more in combination effect (successive) and additive outcome was evident when the fish was in continuous stress. Pesticide and heavy metal tend to accumulate even at the fingerlings stage and usually gets magnified in the adults. If this condition prevails, the effect of the toxicant may result in production of less nutritive fish foods. Unless scientific monitoring of water bodies and the quality of aqua wealth, particularly the edible ones is undertaken, fish food health hazards are in the waiting for the present and future human population.

References

1. Abdullah S, Javed M and Javid A (2007) Studies on Acute Toxicity of Metals to the Fish (*Labeo rohita). Int J Agri & Biol.,* 9(2), 333–337.
2. Almeida JA, Novelli EL, Dal Pai Silva M, Junior RA (2001) Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environ Pollut.,* 114 (2) 169-175.
3. Battaglin WA, Fairchild JF (2002) Potential toxicity of pesticides measured in mid-western streams to aquatic organisms. *Water Sci Technol*., 45, 95-103.
4. Bedii C and Kenan E (2005) The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio. Turk. J. Vet. Anim. Sci.,* 29, 113-117.
5. Carrol WV, Longley RW and Roe JH (1956) The determination of glycogen in the liver and muscle by the use of anthrone reagent. *J. Biol. Chem*., 220, 583-593.
6. Cassee FR, Groten JP, van Bladeren PJ, Feron VJ (1998) Toxicological evaluation and risk assessment of chemical mixtures. *Crit. Rev Toxicol*., 28, 73-101.
7. Chowdhury MJ, Pane EF, Wood CM (2004) Physiological effects of dietary cadmium acclimation and water borne cadmium challenge in rainbow trout: respiratory,

Indian J. Innovations Dev., Vol. 1, No. 6 (June 2012) ISSN 2277 - 5390

ionoregulatory and stress parameters. *Comp. Biochem. Physiol. C Toxicol Pharmacol*., 139(1-3), 163-173.

8. Finney DJ (1971) Probit Analysis, Cambridge University Press. 3rd Edition. pp: 1-333.
9. Fırat O, Cogun HY, Yüzereroğlu TA, Gök G, Fırat O, Kargin F, and Kötemen Y (2011) A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus. Fish Physiol Biochem.,* 37(3), 657–666.
10. Ghosh S and Mukhopadhyay MK (2000) Toxicity of five industrial metals on Gangetic cat fish *Rita rita. Geobios*., 27, 93-96.
11. Jayaraman J (2003) Laboratory manual in biochemistry. New Delhi, New Age International Publisher, edition 1, pp 1-180.
12. Junghans M (2004) Studies on combination effects of environmentally relevant toxicants, Thesis, Institute for Cell Biology, Biochemistry and Biotechnology. University of Bremen, Germany, pp. 1-136.
13. Kolpin DW, Furlong DT, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ. Sci. Technol*., 36, 1202-1211.
14. Koyama J (1997) Difference of heavy metal toxicities for freshwater fish and marine fish. *Bull. Nat. Rest. Ins. Fish. Sci.,* 10, 123-133.
15. Ling, XP, Zhang YH, Lu YH and Huang HQ (2011) Superoxide dismutase, catalase and acetyl cholinesterase: biomarkers for the joint effects of cadmium, zinc and methyl parathion contamination in water. *Environ Technol.,* 32(13), 1463-1470.
16. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with folin phenol reagent. *J. Bio. Chem*., 193, 265-275.
17. Olmstead AW, LeBlanc GA (2005) Toxicity assessment of environmentally relevant pollutant mixtures using a heuristic model. *Integr. Environ. Assess Manag.,* 2, 114-122.
18. Pugazhendy K, Jayanthi C and Kanaga K (2005) Effect of lead intoxication on total free amino acids and glutamate dehydrogenase in liver and muscle tissues of a fresh water fish*, Cyprinus carpio. Ind J Exp Zool.*, 8(2), 361-364.
19. Ribeiroa CAO, Vollaireb Y, Sanchez-Chardic A and Rocheb H (2005) Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aqua Toxicol*., 74 (1), 53-69.