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Toxic Effects of Morpholineum-4-Morpholine Dithiocarbamate and Heavy metals (Cu and Zn) On the Indian Major Carp *Catla catla*: A Biochemical Analysis

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ABSTRACT

The present investigation intended to evaluate the independent and combined toxicological effects of synthetic fungicide Morpholine derivative of dithiocarbamate (MMDTC) and two metallic compounds viz. copper sulphate and zinc sulphate on the biochemical constituents like total proteins, total sugars and total lipids in the muscle, liver, brain and kidney of *Catla catla.* Biochemical studies were carried out in both the control (untreated) healthy fish and the fish exposed to sub-lethal concentrations of MMDTC, $CuSO_4$, $ZnSO_4$, MMDTC +$CuSO_4$,, MMDTC + $ZnSO_4$ and $CuSO_4$+ $ZnSO_4$ on 4th ,7th ,14th, 21st, and 28th day of exposure.

Keywords: MMDTC, $CuSO_4$, $ZnSO_4$, synergistic toxicity, *Catla catla.*

INTRODUCTION

General health of the fish is influenced by the physiological activities occurring in the body. The metabolic activities in the liver are crucial in determining the physiological state of the organism. Any toxic substance reaches soon the liver and causes deleterious effect resulting in disturbed physiological activities. The total carbohydrate, protein and lipid contents of the tissues are crucial for the normal health of the organism because there appears a balanced distribution of these three biochemical constituents; a reduction in one should be compensated by others. If not, the entire metabolic processes become abnormal showing its effects on various physiological activities and may even lead to death. Due to this reason, studies on tissue biochemistry have formed basic necessity to evaluate the health profiles of the organisms under stress.

Dithiocarbamate fungicides are used generally as seed dressing and protection of fruit, vegetable and ornamental crops from a variety of fungal diseases[1]. In an aquatic system pesticide concentration increases as it moves up the food chain because of biomagnifications and the animals accumulate non-biodegradable pesticides from the corresponding environment[2]. Pesticides acting as stress factor affecting the fish has been well documented[3].

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Copper and Zinc are essential trace elements required for the body, but at higher level toxic in nature. Exposure to copper can induce stress responses such as changes in the fish's ion regulation, olfaction and swimming performance[4,5]. A significant reduction in three biochemical constituents viz. protein,lipid and sugars of *Tilapia* under the influence of zinc has also been reported[6]. Several investigators have reported the toxicological effect of zinc in decreasing the protein content of common carp[7,8]. Elevated levels of heavy metals can cause death and mutation in animal populations.

In an aquatic environment the pollutants reach easily as runoff from pesticide residues and heavy metals like copper, mercury, nickel, lead and zinc pollute the aquatic system through industrial and municipal wastes. In such a scenario, the metal compounds react with the chemical pesticides to form complexes that may have entirely different properties than the original one.

The toxic kinetics of the pollutant may inflict some pathogenic effect on the organism depending on the rate of metabolic transformation and elimination. There is paucity of information on how the fish reacts to cumulative toxic synergism.

Hence an attempt has been made in the present study to know the effects of a synthetic fungicide, Morpholineum-4-Morpholine Dithiocarbamate (MMDTC), and two heavy metal compounds, Copper sulphate ($CuSO_4.5H_2O$) and Zinc sulphate ($ZnSO_4$) acting independently and combined on the important biochemical constituents of one of the commercially important Indian Major Carp, *Catla catla.*

MATERIALS AND METHODS

Maintenance of fish Stock and feeding

Catla fingerlings of the same size (10-12 cm in length and 6-8 g in weight) were procured from culture ponds of Tamil Nadu Fish Seed Farm, Poondi, Thiruvallur district and brought to laboratory in oxygen packs. The fish were acclimatized and maintained in ferro-cement tanks (3'L× 2'W×2'H) filled with bore water. The stock fish were fed with pelleted feed prepared with rice bran, groundnut oil cake, tapioca powder and mineral mixture[9]. The fish were fed daily with pelleted feed at 5% body weight in two split doses, in the morning and evening. Feeding was started one day after the fish were stocked and stopped 24 hr prior to experiment.

Selection of experimental Fish

Healthy fish without any observable pathological symptoms were chosen for the experiments and were maintained in disinfected glass aquarium tanks (2'L x 1'W x 1'H) filled with water at rate of 2 litres per fish. During the period of experimentation, the room temperature fluctuated from 30-32^{0}C. The water used for the experiment had dissolved oxygen content of 4.4 - 4.8 ml/l and salinity of 0.82 – 0.84 ppm. The pH of water was in the range of 7.2 – 7.4.

Procurement of toxicants

Morpholineum-4-Morpholine Dithiocarbamate (MMDTC) has insecticidal and fungicidal properties and used as a pesticide. It was prepared by mixing Morpholine and methyl alcohol in 1:2 proportions. Keeping the mixture in ice bath (<10^{0}C), carbon disulphide is added drop by drop with continuous agitation. Later the mixture is evaporated in room temperature to get amorphous powder of MMDTC.

Copper sulphate ($CuSO_4.5H_2O$) 'AR' grade supplied by Sarabhai Merck and Zinc sulphate 'AR' Grade supplied by Qualigen were used for experiment.

Experimental Design

Fish were divided into groups of ten each and exposed to the different toxicants *viz*. MMDTC, $CuSO_4$ and $ZnSO_4$ independently and synergistically. In the synergistic toxicity experiment, the fish were exposed to two toxicants together (combined synergism).

Experimental Groups

Fingerlings of *Catla catla* were treated with pesticide and heavy metals individually and synergistically to determine 96 hr LC_{50} values for the test toxicants. Groups of fish were maintained in separate tanks and considered as experimental groups which were categorized as follows:

Group I: Independent Toxicity

1. MMDTC (DTC)
2. Copper sulphate ($CuSO_4$)
3. Zinc sulphate ($ZnSO_4$)

Group II: Combined Synergism

4. DTC + $CuSO_4$
5. DTC + $ZnSO_4$
6. $CuSO_4$ + $ZnSO_4$

Determination of LC_{50}

To determine LC_{50} for the different groups based on the cumulative percentage mortality at the end of 96 h of experimentation the standard graphic method was followed. One fourth of LC_{50} values obtained from the above experiments was taken as the sub-lethal concentration (SLC). Apparently normal and healthy catla fingerlings were exposed to respective SLC of MMDTC (DTC), $CuSO_4$, $ZnSO_4$ for independent toxic analysis, DTC + $CuSO_4$ DTC + $ZnSO_4$. $CuSO_4$ + $ZnSO_4$ for combined toxic analyses. Suitable controls were maintained in normal bore well water for all the experimental groups, without dissolving the chemicals.

Biochemical Analyses

Biochemical parameters like total carbohydrates, total proteins and total lipids were analyzed on zero day control and on 4th, 7th, 14th, 21st and 28th day after exposing the fish with the toxicants in the experimental groups of independent toxic exposure and combined synergistic toxic exposure.

For the third group involving successive synergistic toxic exposure, these analyses were done on zero day and 7th day post exposure.

Four different tissues Muscle, Liver, Brain and Kidney were dissected out carefully and weighed using K-Roy Single pan electrical balance for biochemical investigation. The dissected tissues were kept in an ice box till taken out for homogenization.

Colorimetric method was followed for the biochemical analyses using Spectronic-21 (Bausche and Lomb) spectrophotometer. Total sugars was estimated by anthrone method[10] and the total protein content in the tissues was done by folin phenol method[11] while for the estimation of lipids the method of Bligh and Dyer [12] was followed.

Statistical Analysis

The data obtained were subjected to statistical analysis to arrive at Arithmetic Mean, Standard Deviation and standard error of mean using statistical package software SPSS version 16. To test the significance of differences observed between control and experimental groups, the data was subjected to students 't" test.

RESULTS

The analysis of 96 h LC_{50} by arithmetic graphic method, showed 16 ppm (Fig. 1), 4.8 ppm (Fig.2) and 160 ppm (Fig.3) concentrations of MMDTC, $CuSO_4$ and $ZnSO_4$ respectively. Further, the SLC (1/4th of LC_{50} value) for MMDTC, $CuSO_4$ and $ZnSO_4$ were calculated as 4 ppm, 1.2 ppm and 40 ppm respectively for independent toxicity.

The LC_{50} value for synergistic toxicity were calculated as 10ppm of MMDTC with 4.4ppm of $CuSO_4$ (Fig.4), 5ppm of MMDTC and 24ppm of $ZnSO_4$ (Fig.5) and 2.6 ppm of $CuSO_4$ with 10ppm of $ZnSO_4$ (Fig. 6). ¼ th these values were calculated as SLC.

The total sugar (Table 1), protein (Table 2) and lipid (Table 3) content in the muscle, liver, brain and kidney tissues of *C. catla* healthy untreated and treated with MMDTC, $CuSO_4$ and $ZnSO_4$ for independent toxic effects and with MMDTC and $CuSO_4$, MMDTC and $ZnSO_4$ and $CuSO_4$ with $ZnSO_4$ for syngergistic toxic effects were recorded at 4th, 7th, 14th, 21stand 28th days.

The mean value of the carbohydrate content in muscle of the fishes treated with MMDTC was significantly higher on 7th day, when compared to control group. In liver the elevated level of the sugars was noticed on 21st day ($p<0.001$). A significant increase in levels of sugar content were observed in brain and kidney tissues on 14th, 21st and 28th day. The heavy metal $CuSO_4$ exposed fish recorded a significant increase of the sugar content in muscle on 4th and 7th day. In liver a higher significant value of the average was recorded on 7th and 14th day. A

Table 1: Independent and combined toxic effects of MMDTC, $CuSO_4$, $ZnSO_4$, MMDTC+ $CuSO_4$, MMDTC + $ZnSO_4$ $CuSO_4$ + $ZnSO_4$on total sugar (Mg/G wet Wt) of *Catla catla*

Days of Exposure	Muscle	Liver	Brain	Kidney
		MMDTC (SLC-4ppm)		
Control	0.97 ± 0.11	26.25 ± 1.1	1.13 ± 0.15	2.86 ± 0.4
4th day	0.55 ± 0.13*	5.77 ± 2.13*	1.56 ± 0.1*	4.67 ±1.16*
7th day	2.18 ± 0.72*	26 ± 1.6[a]	2.38 ± 0.37*	1.87 ± 0.23*
14th day	0.45 ± 0.02*	26.35 ± 1.12[a]	3.05 ± 0.34*	6.65 ± 0.44*
21st day	0.57 ± 0.13*	31 ± 1.1*	2.26 ± 0.44*	4.22 ± 0.37*
28th day	1.07 ± 0.15[a]	21.55 ± 0.56*	3.91 ± 0.45*	3.21 ±0.3[d]
Days of		**$CuSO_4$ (SLC-1.2ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	0.97± 0.11	26.25 ± 1.1	1.13 ± 0.15	2.86 ± 0.4
4th day	3.05 ± 1.25*	12.3 ± 3.01*	11.28 ± 2.55*	27.94 ± 2.7*
7th day	1.47 ± 0.28*	32.11 ± 2.45*	1.62 ± 0.23*	13.66 ± 0.88*
14th day	0.44 ± 0.02*	31.71 ± 1.25*	1.46 ± 0.3[b]	1.06 ± 0.09*
21st day	0.44 ± 0.03*	15.28 ± 0.48*	0.84 ± 0.03*	0.63 ± 0.03*
28th day	1.28 ± 0.27[b]	15.21 ± 0.52*	2.25 ± 0.2*	10.62 ± 0.8*
Days of		**$ZnSO_4$ (SLC-40ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	0.97± 0.11	26.25 ± 1.1	1.13 ± 0.15	2.86 ± 0.4
4th day	0.88 ± 0.11[a]	9 ± 0.49*	2.46 ± 0.63*	3.16 ± 0.93[a]
7th day	1.09 ± 0.18[a]	32.98 ±1.9*	0.41 ± 0.02*	4.43 ± 0.56*
14th day	0.48 ± 0.08*	2.03 ± 0.42*	0.83 ± 0.05*	1.12 ± 0.1*
21st day	0.44 ± 0.02*	4.33 ± 0.52*	4.1 ±0.31*	2.05 ± 0.32*
28th day	0.51 ± 0.02*	3 ± 0.22*	0.44 ± 0.04*	1.02 ± 0.1*
Days of		**MMDTC+ $CuSO_4$ (SLC-2.5ppm+1.1ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	0.97± 0.11	26.25 ± 1.1	1.13 ± 0.15	2.86 ± 0.4
4th day	0.65 ± 0.09*	3.55 ± 0.42*	3.09 ± 0.6*	4.53 ± 0.5*
7th day	0.46 ± 0.04*	5.96 ± 0.6*	0.47 ± 0.05*	0.76 ± 0.1*
14th day	0.45 ± 0.04*	34.32 ± 3.33*	1.15 ± 0.34[a]	1.15 ±0.36*
21st day	2.7 ± 0.6*	55.69 ± 2.3*	14.63 ± 2.3*	24.66 ± 1.97*
28th day	6.54 ± 1.75*	42.56 ± 5.81*	20.77 ± 4.03*	24.82 ± 4.78*
Days of		**MMDTC+$ZnSO_4$ (SLC-1.25ppm+6ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	0.97± 0.11	26.25 ± 1.1	1.13 ± 0.15	2.86 ± 0.4
4th day	0.42 ± 0.05*	2.65 ± 0.6*	1.85 ± 0.59[b]	2.68 ± 0.49[a]
7th day	0.46 ± 0.05*	1.87 ± 0.47*	5.21 ± 0.4*	8.07 ± 0.64*
14th day	0.85 ± 0.08[c]	25.75 ± 2.5[a]	3.34 ± 0.51*	3.2 ± 0.51[a]
21st day	1.18 ± 0.52[a]	4.09 ± 0.54*	2.9 ± 0.5*	1.28 ± 0.28*
28th day	3.41 ± 0.66*	79.97 ± 1.95*	7.73 ± 1.4*	4.73 ± 1.04[b]
Days of		**$CuSO_4$+ $ZnSO_4$ (SLC-0.65ppm+2.5ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	0.97± 0.11	26.25 ± 1.1	1.13 ± 0.15	2.86 ± 0.4
4th day	0.44 ± 0.07*	2.86 ± 0.34*	3.8 ± 0.36*	15.65 ± 0.75*
7th day	0.44 ± 0.03*	23.4 ±1.6*	4.15 ± 1.21*	6.65 ±0.87*
14th day	5.83 ± 1.8*	36.27 ±3.64*	8.38 ± 1.9*	9.72 ± 1.85*
21st day	0.49 ± 0.08*	1.07 ±0.437*	0.5 ± 0.04*	0.76 ± 0.04*
28th day	3.07 ± 0.79*	9.9 ± 2.131*	2.13 ± 0.56*	1.01 ± 0.26*

Student t -Test * P<0.001; [a] P N.S; [b] P<0.01; [c] P<0.02; [d] p<0.05

Table 2: Independent and combined toxic effects of MMDTC, $CuSO_4$, $ZnSO_4$, MMDTC+ $CuSO_4$, MMDTC + $ZnSO_4$, $CuSO_4$ + $ZnSO_4$ on total protein (Mg/G wet Wt) of *Catla catla*

Days of		**MMDTC (SLC-4ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	85.38 ± 0.75	72.31 ± 6.8	53.71 ± 6.63	66.84 ± 4.28
4th day	80. 05 ± 3.79	106.21 ± 7.82	59.41 ± 6.22	131.46 ± 0.79
7th day	172.63 ± 2.74	154.23 ± 2.36	136.42 ± 1.84	194.73 ± 3.17
14th day	91.75 ±1.05	76.41 ± 1.33	60.25 ± 1.04	109.36 ±1.24
21st day	120.56 ± 1.59	117.84 ± 0.93	123.86 ± 1.09	203.17 ± 1.49
28th day	82.32 ±1.56	83.52 ± 1.92	94.85 ± 1.22	114.23 ±1.90
Days of		**$CuSO_4$ (SLC-1.2ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	85.38 ± 0.75	72.31 ± 6.8	53.71 ± 6.63	66.84 ± 4.28
4th day	82.21 ± 2.018	38.21 ±1.41	27.52 ± 2.05	48.25 ± 1.18
7th day	92.08 ± 2.70	104.91 ± 2.61	87.04 ± 0.91	161.27 ± 1.29
14th day	96.88 ± 1.38	87.31 ±1.2	71.56 ± 0.91	121.39 ± 3.46
21st day	61.01 ± 1.27	55.95 ±1.5	50.17 ± 1.25	94.34 ± 2.89
28th day	118.79 ± 1.72	89.33 ±1.68	88.7 ± 2.17	166.31 ± 1.92
Days of		**$ZnSO_4$ (SLC-40ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	85.38 ± 0.75	72.31 ± 6.8	53.71 ± 6.63	66.84 ± 4.28
4th day	99.78 ± 1.86	35.24 ±1.18	19.93 ± 1.54	8.61 ± 0.92
7th day	101.67 ± 0.58	112. 3 ± 1.37	65.93 ± 1.93	197.32 ± 1.2
14th day	30.44 ± 1.02	29.89 ± 0.84	64.51 ± 2.55	35.87 ± 0.6
21st day	65.69 ± 1.42	106.11 ± 2.25	102.28 ± 1.44	107.4 ± 0.99
28th day	72.01 ± 2.42	65.6 ± 2.01	75.12 ± 2.6	104.45 ± 2.8
Days of		**MMDTC+ $CuSO_4$ (SLC-2.5ppm+1.1ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	85.38 ± 0.75	72.31 ± 6.8	53.71 ± 6.63	66.84 ± 4.28
4th day	30.79 ± 0.68	46.65 ± 1.2	32.01 ± 0.99	61.11 ± 0.82
7th day	77.64 ± 3.42	96.13 ± 2.5	93.52 ± 3.85	124.26 ± 4.19
14th day	107.32 ± 4.32	76.09 ± 5.13	83.81 ± 5.07	144.53 ± 5.25
21st day	152.95 ± 2.63	72.59 ± 4.3	60.83 ± 3.63	72.74 ± 2.83
28th day	144 ± 3.87	113.69 ±3.67	144.18 ± 7.62	144.08 ± 3.44
Days of		**MMDTC+$ZnSO_4$ (SLC-1.25ppm+6ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	85.38 ± 0.75	72.31 ± 6.8	53.71 ± 6.63	66.84 ± 4.28
4th day	25.38 ± 0.66	71.67 ± 0.74	37.75 ± 1.25	117.15 ± 3.55
7th day	97.44 ± 3.21	128.22 ± 4.83	124.06 ± 4.58	125.65 ± 9.44
14th day	124.59 ± 2.84	87.21 ± 2.9	84.39 ± 3.16	114.36 ± 2.75
21st day	168.35 ± 3.2	125.23 ± 6.67	153.25 ± 4.71	112.84 ± 4.26
28th day	67.33 ± 1.343	90.15 ± 3.69	40.39 ± 2.88	100.06 ± 3.77
Days of		**$CuSO_4$+ $ZnSO_4$ (SLC-0.65ppm+2.5ppm)**		
Exposure	**Muscle**	**Liver**	**Brain**	**Kidney**
Control	85.38 ± 0.75	72.31 ± 6.8	53.71 ± 6.63	66.84 ± 4.28
4th day	102.84 ± 4.44	77.31 ± 1.05	48.59 ± 1.13	101.29 ± 0.74
7th day	108.62 ± 1.64	102.89 ± 1.56	82.39 ± 1.98	133.35 ± 3.68
14th day	120.08 ± 3.52	108.65 ± 4.26	108.48 ± 3.34	228.89 ± 5.46
21st day	174.88 ± 4.99	152.01 ± 6.69	161.79 ± 5.01	180.23 ± 5.12
28th day	112.35 ± 3.83	113.12 ± 3.58	101.5 ±2.28	96.3 ± 3.99

Table 3: Independent and combined toxic effects of MMDTC, $CuSO_4$, $ZnSO_4$, MMDTC+ $CuSO_4$, MMDTC + $ZnSO_4$ $CuSO_4$ + $ZnSO_4$on total lipid (Mg/G wet Wt) of *Catla catla*

Days of Exposure	Muscle	MMDTC(SLC-4ppm) Liver	Brain	Kidney
Control	31.62 ± 0.72	55.37 ± 1.81	42.11 ± 0.91	23.97 ± 1.21
4th day	8.63 ± 1.85 *	8.38 ± 1.08*	7.55 ± 1.39*	4.76 ± 0.55*
7th day	19.76 ± 1.17 *	102.56 ± 1.61*	85.24 ± 2.06*	143.19 ± 2.31*
14th day	61.61 ± 1.14 *	147.66 ± 1.39*	151.96 ± 1.23*	294. 81 ± 2.04*
21st day	118.4 ± 0.91 *	141.78 ± 1.59*	175.76 ± 1.79*	181.03 ± 1.26*
28th day	56.99 ±1.01 *	139.34 ± 1.38*	143.28 ± 1.81*	141.76 ± 1.36*
Days of Exposure	**Muscle**	**$CuSO_4$ (SLC-1.2ppm) Liver**	**Brain**	**Kidney**
Control	31.62 ± 0.72	55.37 ± 1.81	42.11 ± 0.91	23.97 ± 1.21
4th day	3.71 ± 0.24 *	33.75 ± 4.71*	10.3 ± 3.04*	2.7 ± 0.5*
7th day	114.67 ± 0.92 *	202.8 ± 1.77*	144.03 ± 2.17*	244.7 ± 4.71*
14th day	71.42 ± 0.85*	138.37 ± 0.85*	184.25 ± 5.72*	143.43 ± 1.59*
21st day	47.19 ± 0.84*	184.37 ± 1.67*	242.39 ± 1.62*	196.69 ± 2.41*
28th day	41.01 ±1.64 *	83.7 ± 2.67*	117.08 ± 1.53*	170.42 ± 1.34*
Days of Exposure	**Muscle**	**$ZnSO_4$ (SLC-40ppm) Liver**	**Brain**	**Kidney**
Control	31.62 ±0.72	55.37 ± 1.81	42.11 ± 0.91	23.97 ± 1.21
4th day	1.41 ± 0.25*	20.87 ± 0.86*	7.01 ± 0.13*	1.91 ± 0.29*
7th day	31.84 ± 0.72 [a]	110.92 ± 1.13*	181.44 ± 0.89*	85.44 ± 1.16*
14th day	47.48 ± 0.66 *	113.89 ± 1.78*	137.6 ± 1.25*	103.83 ± 1.34*
21st day	77.01 ± 1.21 *	131.23 ± 0.9*	242.16 ± 2.13*	191.13 ± 2.2*
28th day	76.13 ± 2.78*	99.93 ± 2.12 *	156.33 ± 3.16*	340.14 ± 1.31*
Days of Exposure	**Muscle**	**MMDTC+ $CuSO_4$ (SLC-2.5ppm+1.1ppm) Liver**	**Brain**	**Kidney**
Control	31.62 ±0.72	55.37 ± 1.81*	42.11 ± 0.91	23.97 ± 1.21
4th day	6.07 ± 0.12 *	27.84 ± 0.76*	5.13 ± 0.19*	3.11 ± 0.638*
7th day	27.18 ± 2.79 *	66.67 ± 2.06*	70.81 ± 1.95*	53.17 ± 2.63*
14th day	45.63 ±2.52 *	83.67 ± 1.82*	88.65 ± 3.58*	69.01 ± 2.67*
21st day	76.92 ± 3.29*	126.84 ± 5.47*	61.32 ± 4.71*	97.48 ± 3.41*
28th day	48.51 ± 4.49 *	96.24 ± 3.9*	172.52 ± 2.98*	100.73 ± 4.81*
Days of Exposure	**Muscle**	**MMDTC+$ZnSO_4$ (SLC-1.25ppm+6ppm) Liver**	**Brain**	**Kidney**
Control	31.62 ±0.72	55.37 ± 1.81	42.11 ± 0.91	23.97 ± 1.21
4th day	5.91 ± 0.45*	23.64 ± 0.72*	4.65 ± 0.44*	2.81 ± 0.41*
7th day	28.6 ± 3.47[c]	65.67 ± 4.59*	82.6 ± 5.44*	53.46 ± 3.75*
14th day	49.09 ± 4.07 *	96.44 ± 1.95*	134.31 ± 3.21*	93.48 ± 5.26*
21st day	81.88 ± 7.47 *	67.31 ± 3.75*	244.31 ± 4.02*	96.28 ± 3.80*
28th day	143.87 ± 3.09*	164.54 ± 2.35*	211.89 ± 2.14*	301.95 ± 5.78*
Days of Exposure	**Muscle**	**$CuSO_4$+ $ZnSO_4$ (SLC-0.65ppm+2.5ppm) Liver**	**Brain**	**Kidney**
Control	31.62 ±0.72	55.37 ± 1.81	42.11 ± 0.91	23.97 ± 1.21
4th day	4.62 ± 0.46 *	22.85 ± 0.79*	3.84 ± 0.5*	2.06 ± 0.33*
7th day	53.27 ± 3.53 *	75.68 ± 2.78*	94.56 ± 1.96*	108.02 ± 3.56*
14th day	57.51 ± 3.13 *	158.26 ± 2.85*	167.08 ± 3.69*	6.41 ± 1.07*
21st day	48.46 ± 3.36 *	53.84 ± 4.06[a]	115.72 ± 5.11*	86.5 ± 3.85*
28th day	38.93 ±1.87 *	42.89 ± 1.89*	66.95 ± 4.03*	38.24 ± 6.65*

Student t -Test * P<0.001; [a] P N.S; [b] P<0.01; [c] P<0.02; [d] p<0.05

significant increase over the values of control fish on 4th, 7th and 28th day was observed in the sugar content of brain and kidney. When the fishes were exposed to $ZnSO_4$ the sugar content of muscle and liver was greatly reduced on all the exposure groups except on 7th day, where the significant increase was observed. However, a significant increase were recorded on 4th and 21st day in the brain tissues and 4th and 7th day in the kidney tissues. MMDTC when combined with $CuSO_4$, and $ZnSO_4$ revealed the reduction of the sugar content in the muscle tissues on 4th, 7th and 14th day. However, in liver tissues a significant increase in the average of the sugar content was observed on 14th, 21st and 28th day in fishes exposed to MMDTC with $CuSO_4$, while MMDTC with $ZnSO_4$ brought about a significant decline on 4th, 7th and 21st day. In brain and kidney tissues the synergistic effect of MMDTC and $CuSO_4$ revealed significantly elevated level of sugars on 4th, 21st and 28th day,while MMDTC with $ZnSO_4$ significantly decreased the sugar content in kidney tissues on 21st day. Exposure of $CuSO_4$ in addition to $ZnSO_4$ resulted in a significant decrease in the carbohydrate content of the muscle and liver tissue on 4th, 7th and 21st day. The additive effect of these two compounds effected elevated level of carbohydrates from 4th to 14th day in the brain and kidney.

The Protein content in the muscle of catla fingerlings exposed to MMDTC was significantly decreased on 4th day and 28th day while in the liver, brain and kidney tissues of treated fish showed a significant increase in the protein content on all the exposure periods. The heavy metal $CuSO_4$ treated fish revealed significant depletion of protein on 4th and 21st day in the muscle, liver, brain tissues and on 4th day in the kidney tissues. A reduction in the protein content was recorded in the muscle and liver tissues of $ZnSO_4$ exposed fish on 14th and 28th days, while in brain and kidney tissues an increase was observed on 7th. 21st and 28th day.

The protein content in the muscle and liver tissues of combined toxic synergistic group MMDTC and metals viz. $CuSO_4$ and $ZnSO_4$ reduced on 4th day and continued to rise on subsequent days up to 21st day and again dropped on the 28th day. In brain and kidney tissues the protein content of MMDTC+$CuSO_4$ exposed group showed a significant decrease on the 4th day and MMDTC with $ZnSO_4$ group showed a significant increase from 7thto 21st days. When the two metallic compounds were combined together the fish recorded progressively increased protein content in all the tissues viz. muscle, liver , brain and kidney compared to the control.

Exposure to the toxicants MMDTC, $CuSO_4$ and $ZnSO_4$ independently and in combination with one another had effected a very significant reduction in the lipid content in all the tissues on the early period of exposure (4th day). However, the lipid content of the muscle on the 7th day decreased in the fish exposed to MMDTC independently and in combination with $CUSO_4$ and $ZnSO_4$ while an increased trends was observed in groups of fish treated with the heavy metals both independently and combined together. Contrastingly,the lipid content of liver showed a decreasing trend in the fish exposed to $CuSO_4$ and $ZnSO_4$ together on 21st and 28th day. The lipid content of brain and kidney tissues showed significant elevation in the lipid content in all the treated groups on all the exposure periods.

DISCUSSION

Pesticides are mainly used against pests of crop and disease causing vectors, but their improper use in agricultural practice has posed a serious threat to human life and his environment. Any variation in the environment acts as a stress on the organisms. When a pesticide or any pollutant reaches the aquatic ecosystem, the fish are exposed to severe stress and as a natural instinct, the fish tend to adapt themselves by reacting suitably to overcome the stress. When two toxicants act together, the stress shall be more and severe and cause irreparable damage.

In the present investigation, total sugar content in the muscle,liver, brain and kidney revealed a mixed trend in the different experimental groups and different days of the exposure. There was an immediate fall in the sugar content of the 4 th day exposed fish in the muscle and liver, showing a state of recovery on the 7 th day. The recovery trend was normal in DTC exposed fish, and slow in the $CuSO_4$ treated fish. In the synergistic experimental groups, recovery occurred from 21st day onwards. In the brain and kidney of the 4th day experimental groups the

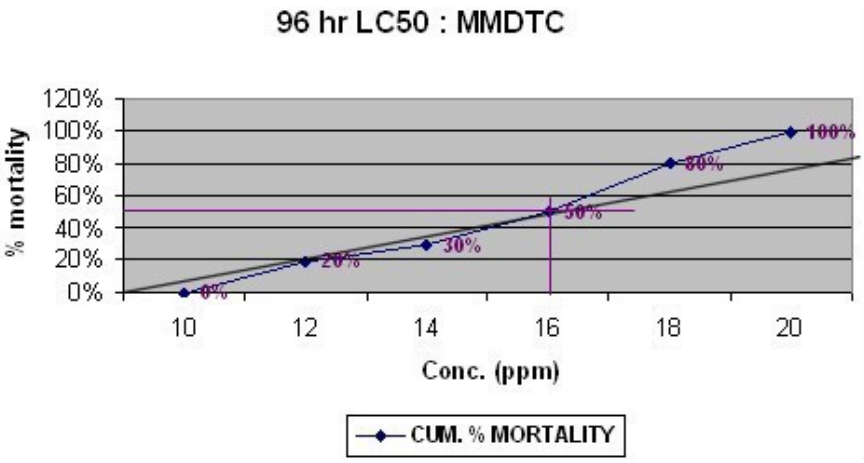

Fig. 1: 96 hr LC50 for MMDTC

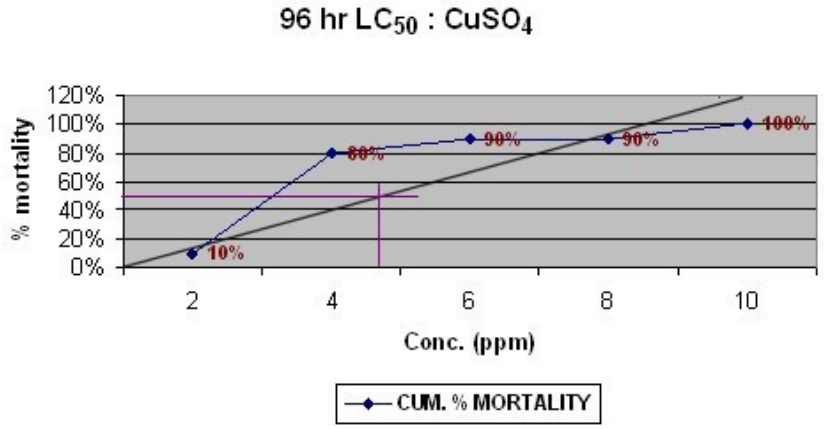

Fig. 2: 96 hr LC50 for $CuSO_4$

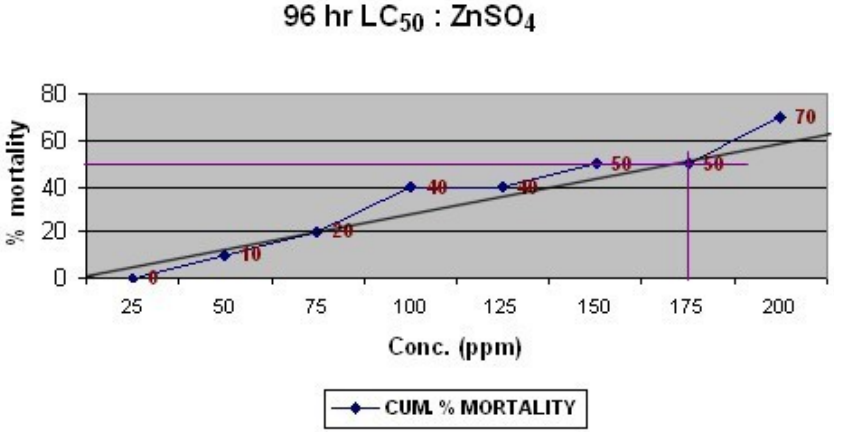

Fig. 3: 96 hr LC50 for $ZnSO_4$

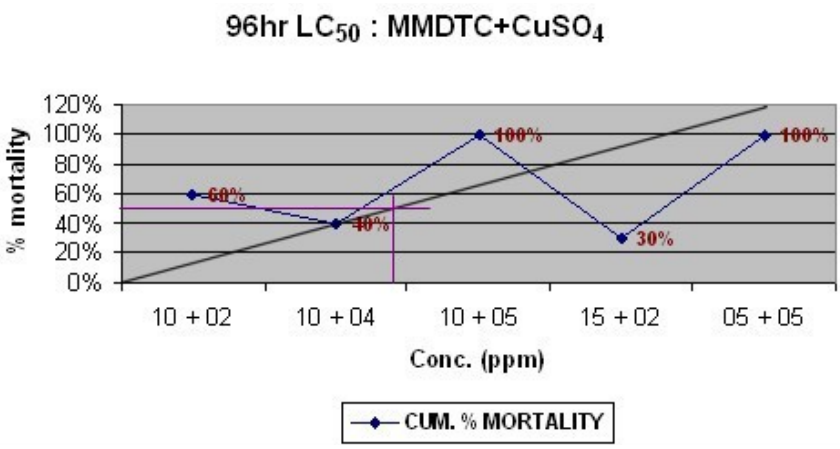

Fig. 4: 96 hr LC50 forMMDTC + $CuSO_4$

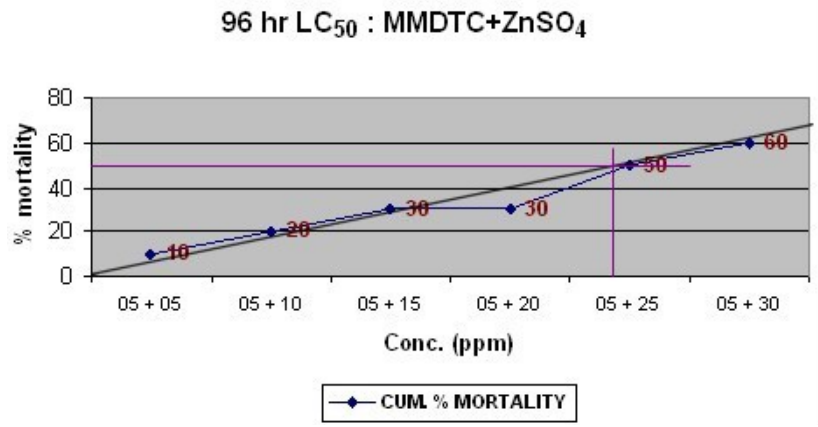

Fig. 5: 96 hr LC50 for MMDTC + $ZnSO_4$

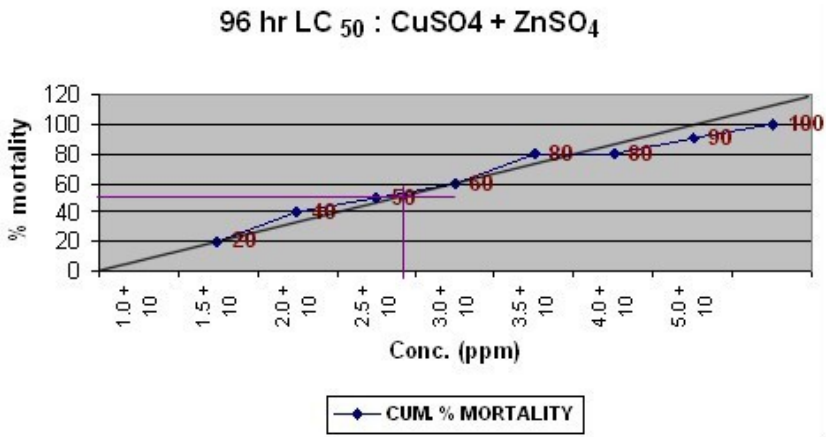

Fig. 6: 96 hr LC50 for $CuSO_4$+ $ZnSO_4$

sugar content was more compared to control groups, which gradually reduced on 7 th day. The increased or decreased trend was more pronounced in the groups of fish exposed to $CuSO_4$ independently and synergistically with MMDTC and $ZnSO_4$ .This shows the severe toxic nature of $CuSO_4$ both independently and in combination with others.

Protein showed a trend of increasing and decreasing activity in the different experimental groups. As far as lipids were concerned there was a reduction in all the tissues of 4 th day experimental group showing trends of recovery on further days of exposure. Increase in the protein content was in accordance with the reduction of sugar content and lipid content suggesting gluconeogenic pathway utilizing lipid and proteins to compensate for the loss of sugars.

Depletion of tissue proteins in fishes exposed to various pesticide toxicants have been reported by many workers. Further, it has been reported that acute or chronic treatment of pesticides cause biochemical alteration in the organs involved in detoxification mechanisms[13,14,15].Decreasing trend in total proteins was reported in the liver,brain and gill tissues of *C. catla* under sub lethal and lethal concentrations of fenvalerate[16].A significant decrease was reported in the protein content in almost all tissues in *Ctenophayngodon idellus* when exposed to sub lethal and lethal concentrations of both the technical and 20% E.C. formulations offenvalerate[17]. Break down and synthesis of protein proceeds simultaneously in all the tissues. But during pesticide stress breakdown of protein occurs which acts as an alternative source of energy. The fish exposed to toxic stress stimulates protein metabolism[18]. During protein metabolism the removal of amino group from different amino acids was observed suggesting the elevated levels of amino acids in the fish exposed to pesticide[19].

The loss of protein in the tissues of fish exposed to heavy metal stress may be due to excessive proteolysis to overcome the metabolic stress. Heavy metals may alter the protein concentration through impairing the synthesis and metabolism of protein, DNA and RNA as well as by altering the activity of lysosomal enzymes. It is possible that pollutant stress influences the conversion of tissue proteins into soluble fractions reaching the blood and hence, decrease in protein content might have observed in the tissues of liver and muscle. The metal binding protein usually binds the metal ions,preventing them from exerting toxic effects through binding to enzymes or other sensitive sites. However, if the rate of influx of metals into the cell exceeds the rate at which metallothionein or metallothionein-like proteins can be synthesized, there may occur a"spill over" of metals from the metallothionein or metallothionein like proteins due to the displacement of essential metals from metalloenzymes by non-essential metals[20].

The changes observed in the protein content in different days of exposure may be due to the influence of exogenous factors like toxic environment. The loss of protein under stress to long period may be attributed to the utilization of amino acid in various catabolic reactions. Another probability is that there might have occurred blocking of the protein synthesis and proteolysis on exposure to chronic period of stress condition. Similar observations of decrease in the protein content were reported in the muscle of *Channa punctatus* treated with heavy metal[21], in the muscle of *Puntius stigma* after exposure with pesticides[22], in the muscle, liver and intestine of *Cyprinus carpio* treated with textile mill effluent[23], in the muscle of *Oreochromis mossambicus* due to the effect of phenol[24], in *Etroplus maculates* exposed to Ekalux[25] and in *Anabas testudineus* under the influence of alloxan monohydrate[26].

Heavy metals are metabolic inhibitors of animals. They exert toxic effects in the organism at tissue, cellular, sub-cellular and molecular levels. At molecular levels metals interact with protein leading to denaturation, precipitation, allosteric effects or enzyme inhibition. Depletion in total protein could be due to augmented proteolysis and possible utilization of their product for metabolic purpose, decline in protein content may be related to impaired food intake, increased energy cost of homeostasis,tissue repair and detoxification mechanism during stress[27].

CONCLUSIONS

The study clearly indicates that the toxic effect of pollutants or toxicants will be more

when they act together in a synergistic manner. The alterations of biochemical profiles of the test organism as observed in the present study may be used as non–specific biomarkers against anthropogenic stress.

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