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IMPACT OF HEAVY METALS ON CHANGES IN METABOLIC BIOMARKERS OF CARP FISH, *CIRRHINUS MRIGALA*

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Abstract: The Indian Major carp fish, *Cirrhinus mrigala* were exposed to 1/10th sub lethal concentration of three toxic heavy metals – Cadmium chloride ($CdCl_2$), Lead chloride ($PbCl_2$) and Mercuric Chloride ($HgCl_2$) for a period of 3, 7, 15, 30 and 45 days to study their effect on biochemical and metabolic enzyme biomarkers. The glycogen contents in gill, liver and muscle tissues of carp fish, *Cirrhinus mrigala* were found to be decreased. The maximum percentage decrease (65.57%) was observed on day 45 in liver tissue under mercuric chloride toxic stress. The succinate dehydrogenase (SDH), malate dehydrogenase (MDA), cytochrome C-oxidase and protein contents in three tissues were gradually decreased, whereas lactate dehydrogenase (LDH), aspartate amino transferase (AAT) and alanine transferase (AIAT) showed a constant and gradual elevation throughout the experimental period of 45 days under the treatment of heavy metals intoxicated fish. It is observed that the increased / decreased percentage was very much pronounced by $HgCl_2$) in liver tissue in particular. These variations have assumed that the fish yields high energy demands to overcome the stress indicated by the existence of hypoproteinamea in all tissues of fish.

Keywords: *Cirrhinus mrigala*; Three Heavy metals; Metabolic markers; Glycogen; Dehydrogenases; Transaminases; Hypoproteinamea.

INTRODUCTION

It is well known that Heavy metal pollutants presence in water may induce severe ecological consequences, generating reorganizations of the biogenesis, changing it and consequently affecting aquatic ecosystems integrity (Vosyliene and Jankaite, 2006). The fishery culture is facing a constant decline in fish stock, both in coastal and inland water resources on account of constantly increasing water pollution and causing proved to be carcinogenic or produce teratogenic effects. The two most important factors that contribute to the deleterious effects of heavy metals are pollutants are their indestructible nature through bioremediation unlike organic pollutants and their tendency to accumulate in environment especially in the bottom sediments of aquatic habitats in association with organic and inorganic matter. For healthy fish production, it is very important to evaluate the harmful effects of heavy metals (Cunha et al., 2007).

Cadmium (Cd), Lead (Pb) and Mercury (Hg) are the heavy metals, which are highly toxic and carcinogenic to all animals including many aquatic organisms, because of their high toxicity, non-biodegradability and biomagnifications tendencies (Reddy *et al.,* 1991). The heavy metals may accumulate in the body of fish either through "water path" or "food path" (Feldlight et al., 2008). Heavy meals enter into human body through various routes including fish, poultry and livestock food products; ultimately disturb the "balance of nature" causing serious health hazards. Once absorbed in to the body, inorganic metals are capable reacting with a variety of binding sites (Leland and Kawabara, 1984). High concentration of heavy metals potentially interacts with DNA and cause mutations in living systems (Creed, 1973). Little research has been conducted on the pathophysiological and biochemical biomarkers underlying adaptations to heavy metals, in particular, cadmium (Cd), lead (Pb) and mercury (Hg) in Indian Major carps. Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sub lethal effects in fish exposed to different toxic substances. The aim of this study is to investigate the effect of exposure to sub lethal concentrations of $CdCl_2$, $PbCl_2$ and $HgCl_2$ on biochemical and metabolic biomarkers of commercially important an highly cultivable Indian major carp, *Cirrhinus mrigala*.

MATERIALS AND METHODS

Test animal:

The carp fish species *Cirrhinus mrigala* (9.5±0.50 cm in length and 12.4 ±1.5 g in weight were obtained from the Government fish breed and culture farm. Immediately they were transferred to our laboratory and put in large aerated water containing tanks. The unchlorinated tap water supplied from reservoir was aerated and used throughout the experimental period. Healthy and active fish were separated and maintained at least for ten days and fed on commercial and formulated dry pelleted feed. The physico-chemical

parameters of used water, throughout the experimental period, were as indicated in Tables according to APHA, 1998.

LC_{50} Determination:

The 96 hours median lethal concentration (LC_{50}) of Cadmium chloride ($CdCl_2$), Lead chloride ($PbCl_2$) and Mercuric chloride ($HgCl_2$) to *Cirrhinus mrigala* was determined by Proibit Analysis.

The laboratory conditions:

The physico-chemical parameters of the laboratory water, during experimental period were as follows: Temperature: 28± 20C; 12 h: 12h light and dark period; pH: 7.5±0.1; salinity: 0.25±0.02%.

Test chemicals:

Cadmium chloride ($CdCl_2$), Lead chloride ($PbCl_2$) and Mercuric chloride ($HgCl_2$) are used as test toxic heavy metals for cadmium, lead and mercury respectively during the present research work.

The LC_{50} values:

The Median lethal concentration (LC_{50}) for 96 hours of three heavy metals to *Cirrhinus mrigala* was determined by Proibit Analysis (Finney, 1971) as: Cadmium chloride: 5.22 mg/L; Lead chloride: 6.11 mg/L and Mercuric chloride: 4.65 mg/L.

Test periods:

A large group of acclimated fish, ready for experimentation, further divided in to three main groups exposed to sub lethal concentrations of $CdCl_2$, $PbCl_2$ and $HgCl_2$ respectively. Each group further was divided in to six batches of ten individuals in each group. Of these, the first five batches (1–5) constitute the experimental, each of which received daily 1/10th of LC50 of related heavy metal for 3, 7, 15, 30 and 45 days. The sixth batch was served as control.

Table.1: Variations in glycogen content in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure periods (in days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	9.75±0.36	8.32±0.73 (-14.67)	7.64±0.68 (-21.64)	6.47±0.18 (-33.64)	5.45±0.49 (-44.10)	4.95±0.23 (-49.23)
	Liver	12.63±1.34	11.96±0.51 (-11.876)	10.17±0.86 (-19.477)	8.55±0.44 (-32.304)	7.94±0.58 (-37.133)	6.49±0.37 (-49.40)
	Muscle	10.52±0.75	9.51±0.42 (-9.600)	8.19±0.66 (-22.148)	6.92 ±0.39 (-34.221)	6.36±0.43 (-39.54)	4.95±0.49 (-45.438)
$PbCl_2$	Gill	9.63±0.42	8.49±0.66 (-11.838)	7.98±0.53 (-17.133)	6.43±0.44 (-33.229)	5.62±0.61 (-41.641)	4.64±0.52 (-51.817)
	Liver	12.25±1.26	11.63±0.48 (-5.061)	10.06±0.43 (-17.877)	8.74±0.63 (-28.653)	7.62±0.39 (-37.795)	6.35±0.39 (-48.16)
	Muscle	10.68±0.66	1015±0.39 (-4.963)	9.06±0.52 (-15.168)	7.36±0.54 (-31.086)	6016±0.59 (-42.322)	5.69±0.45 (-46.72)
$HgCl_2$	Gill	9.69±0.43	9.08±0.42 (-6.295)	8.16±0.36 (-15.789)	6.81±0.39 (-29.729)	5.34±0.62 (-44.892)	4.25±0.36 (-56.140)
	Liver	13.46±1.39	12.17±0.66 (-9.718)	10.78±0.44 (-12.611)	8.71±0.54 (-35.386)	7.06±0.59 (-47.626)	6.54±0.49 (-51.42)
	Muscle	10.97±1.48	10.06±0.42 (-8.295)	9.34±0.49 (-14.859)	8.35±0.39 (-23.883)	7.43±0.62 (-32.269)	6.15±0.62 (-43.94)

Values are expressed in mg/g wet weight of tissue. Each value is mean ± SD of six individual observations.
Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05

Table.2: Variations in Succinate dehydrogenase levels (SDH) in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	0.645±0.036	0.623±0.015 (-3.411)	0.546±0.028 (-29.302)	0.425±0.026 (-34.108)	0.409±0.18 (-36.589)	0.376±0.016 (-41.705)
	Liver	0.889±0.059	0.817±0.059 (-8.098)	0.766±0.065 (-13.836)	0.715±0.08 (-19.572)	0.628±0.053 (-29.359)	0.415±0.074 (-53.32)
	Muscle	0.495±0.026	0.461±0.018 (-6.868)	0.416±0.028 (-15.959)	0.388 ±0.018 (-21.616)	0.349±0.016 (-29.495)	0.318±0.016 (-35.76)
$PbCl_2$	Gill	0.635±0.021	0.603±0.009 (-5.039)	0.589±0.012 (-7.244)	0.51119±0.022 (-18.267)	0.463±0.009 (-27.087)	0.372±0.0261 (-41.42)
	Liver	0.878±0.042	0.813±0.022 (-7.403)	0.762±0.008 (-13.212)	0.63±0.009 (-27.790)	0.566±0.013 (-35.535)	0.418±0.042 (-52.39)
	Muscle	0.489±0.036	0.441±0.006 (-9.816)	0.413±0.005 (-15.542)	0.388±0.008 (-20.654)	0.351±0.011 (-28.221)	0.326±0.039 (-33.33)
$HgCl_2$	Gill	0.665±0.042	0.611±0.003 (-8.120)	0.586±0.012 (-11.879)	0.523±0.010 (-21.353)	0.462±0.012 (-30.526)	0.286±0.028 (-41.95)
	Liver	0.869±0.049	0.753±0.015 (-13.348)	0.619±0.008 (-28.768)	0.545±0.007 (-37.284)	0.438±0.008 (-49.597)	0.396±0.36 (-54.43)
	Muscle	0.516±0.38	0.472±0.008 (-8.527)	0.442±0.012 (-14.341)	0.418±0.004 (-18.992)	0.384±0.010 (-25.581)	0.328±0.018 (-36.43)

Values are expressed in µ moles of formazan formed / mg protein/h. Each value is mean ± SD of six individual observations.
Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05

Table.3: Variations in Lactate dehydrogenase levels (LDH) in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	0.072±0.011	0.075±0.014 (-4.167)	0.081±0.018 (+12.500)	0.085±0.016 (+18.056)	0.092±0.015 (+27.778)	0.103±0.004 (+43.055)
	Liver	0.318±0.019	0.342±0.016 (-7.547)	0.378±0.024 (+18.867)	0.396±0.036 (+24.528)	0.422±0.029 (+32.704)	0.468±0.036 (47.169)
	Muscle	0.097±0.008	0.102±0.006 (-5.155)	0.109±0.007 (+12.371)	0.114±0.004 (+17.525)	0.119±0.008 (+22.680)	0.129±0.026 (32.989)
$PbCl_2$	Gill	0.079±0.012	0.084±0.009 (-6.329)	0.094±0.013 (+18.987)	0.102±0.008 (+29.114)	0.109±0.012 (+37.975)	0.115±0.019 (45.569)
	Liver	0.316±0.016	0.347±0.018 (-9.810)	0.379±0.021 (+19.937)	0.419±0.034 (+32.594)	0.442±0.045 (+39.843)	0.471±0.022 (49.051)
	Muscle	0.092±0.006	0.096±0.008 (-4.347)	0.099±0.009 (+7.608)	0.108±0.010 (+17.391)	0.112±0.012 (+21.739)	0.119±0.018 (29.348)
$HgCl_2$	Gill	0.076±0.012	0.082±0.014 (-7.895)	0.095±0.012 (+25.00)	0.109±0.011 (+43.421)	0.116±0.015 (+52.631)	0.121±0.028 (59.211)
	Liver	0.32±0.018	0.354±0.016 (-8.588)	0.418±0.054 (+28.221)	0.473±0.052 (+45.092)	0.518±0.054 (+58.895)	0.547±0.036 (+67.791)
	Muscle	0.095±0.012	0.099±0.011 (-4.211)	0.108±0.014 (+13.684)	0.112±0.04 1 (+17.894)	0.118±0.032 (+24.211)	0.146±0.027 (+53.684)

Values are expressed in µ moles of formazan formed / mg protein/h. Each value is mean ± SD of six individual observations. Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05

Table.4: Variations in Malate dehydrogenase levels (MDH) in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 day
$CdCl_2$	Gill	0.064±0.003	0.058±0.004 (-9.376)	0.051±0.012 (-20.312)	0.048±0.011 (-25.000)	0.043±0.020 (-32.812)	0.039±0.005 (-39.63)
	Liver	0.245±0.015	0.215±0.013 (-12.245)	0.198±0.006 (-19.183)	0.178±0.044 (-27.347)	0.159±0.21 (-35.120)	0.136±0.043 (-44.489)
	Muscle	0.226±0.014	0.214±0.014 (-5.309)	0.202±0.005 (-10.612)	0.186±0.031 (-17.699)	0.164±0.012 (-27.434)	0.145±0.037 (-35.398)
$PbCl_2$	Gill	0.065±0.003	0.061±0.04 (-6.154)	0.55±0.05 (-15.385)	0.050±0.03 (-20.000)	0.045±0.06 (-30.769)	0.041±0.008 (-36.923)
	Liver	0.235±0.016	0.212.0.05± (-9.787)	0.203±0.06 (-13.617)	0.186±0.05 (-20.851)	0.166±0.02 (-29.361)	0.139±0.018 (-40.851)
	Muscle	0.211±0.014	0.202±0.06 (-4.265)	0.189±0.012 (-10.427)	0.0169±0.014 (-19.905)	0.152±0.013 (-27.962)	0.135±0.035 (-36.019)
$HgCl_2$	Gill	0.067±0.004	0.061±0.44 (-8.955)	0.056±0.015 (-13.433)	0.050±0.016 (-25.373)	0.046±0.08 (-31.343)	0.037±0.007 (-44.776)
	Liver	0.257±0.018	0.231±0.015 (-10.526)	0.219±0.012 (-14.785)	0.185±0.019 (-28.016)	0.149±0.026 (-42.023)	0.126±0.038 (-48.988)
	Muscle	0.231±0.005	0.216±0.08 (-7.042)	0.207±0.013 (-10.389)	0.183±0.022 (-20.779)	0.162±0.024 (29.870)	0.133± 0.029 (-42.424)

Values are expressed in µ moles of formazan formed / mg protein/h. Each value is mean ± SD of six individual observations. Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05.

Table.5: Variations in Cytochrome c-oxidase levels in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	1.26±0.036	1.234±0.009 (-3.291)	1.206±0.030 (-5.485)	1.183±0.021 (-7.288)	1.413±0.016 (-10.737)	1.025±0.020 (-19.671)
	Liver	3.115±0.054	3.005±0.012 (-3.531)	2.846±0.013 (-8.635)	2.731±0.014 (12.327)	2.470±0.025 (-20.706)	2.206± 0.013 (-29.181)
	Muscle	2.574±0.062	2.484±0.007 (-3.496)	2.348±0.008 (-8.780)	2.201±0.006 (-14.491)	2.015±0.016 (-21.717)	1.878±0.009 (-27.039)
$PbCl_2$	Gill	1.266±0.032	1.228±0.014 (-3.002)	1.196±0.012 (-5.529)	1.164±0.011 (-8.057)	1.22±0.022 (-11.374)	1.022±0.011 (-19.273)
	Liver	3.214±0.046	3.108±0.022 (-3.298)	2.968±0.031 (-7.654)	2.745±0.024 (-14.592)	2.469±0.018 (-23.179)	2.235±0.017 (-30.406)
	Muscle	2.526±0.042	2.316±0.0015 (-8.314)	2.263±0.026 (-10.412)	2.098±0.014 (-16.944)	1.975± 0.011 (-21.813)	1.855±0.019 (-27.564)
$HgCl_2$	Gill	1.276±0.025	1.234±0.002 (-3.291)	1.208±0.003 (-5.329)	1.164±0.021 (-8.777)	1.132±0.016 (-14.400)	1.012±0.022 (-20.689)
	Liver	3.243±0.039	3.062±0.011 (-5.581)	2.992±0.015 (-7.739)	2.674±0.019 (-17.545)	1.963±0.021 (-39.469)	1.556±0.006 (-52.019)
	Muscle	2.543±0.52	2.473±0.025 (-2.753)	2.421± 0.014 (-4.797)	2.366±0.022 (-6.960)	1.878± 0.032 (-26.150)	1.596± 0.015 (-37.239)

Values are expressed in µ moles of formazan formed / mg protein/h. Each value is mean ± SD of six individual observations. Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05.

Tested Tissues:

After expiry of each specified heavy metal exposure period, the individuals were sacrificed by cervical dislocation of gill, liver and muscle tissues were isolated quickly and were placed in deep freezer at -40°C till further use.

Tested Parameters:

The glycogen content of selected tissues was estimated by the method of Carrol et al. (1956). The activity levels of Succinate dehydrogenate (SDH), Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) (Nachlas et al., 1960), Transaminases (Rietman and Frankel, 1957) and Cytochrome-C- oxidases (Oda et al., 1958) were estimated. Protein content was determined by using Folin phenol reagent (Lowry et al., 1951). The results were subjected to statistical treatment. Mean, standard deviation and analysis of variance (ANOVA) was carried out.

RESULTS AND DISCUSSION

Glycogen levels are found to be the highest in liver tissue of all fish, as it is chief organ of carbohydrate metabolism in animals, followed by muscle tissues. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. The glycogen contents in gill, liver and muscle tissues of fish *Cirrhinus mrigala*, under the heavy metals stress, were decreased compared to that of the fish under control. The maximum decrease (-49.40%) was observe d on day 45, in liver tissue under mercuric chloride intoxication only. A steady decrease in the tissue glycogen content clearly indicates that effects of heavy metals are persistent under prolonged exposure periods. This disruption of biological oxidation process agrees with earlier report of Reddy et al., (1991). This change suggesting that glycogen utilization by anaerobic glycolysis perhaps to meet the energy anaerobic glycolysis perhaps to meet the energy warranted by intoxicated environment. In the present study it is evident that the heavy metals are hepatotoxins from the maximum drop in liver tissue activity. Glycogen depletion is more prevalent under hypoxic conditions that may stimulate phosphorylase activity bringing about a drop in glycogen level. In general, when the animal undergone to stress, then the glycogen reserves were used as substitution of metabolic requirement to meet the energy demands through glycolysis or Hexose Monophosphate Shunt pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. The diminution of glycogen would result in the disruption of enzymes associated with carbohydrate metabolism.

It is observed that there is a gradual decrease in the activity levels of SDH, MDH and Cytochrome–C-oxidase have shown a steady decrease (Tables 2, 4 and 5), however the magnitude of maximum decrease in SDH (-54.43%), MDH (-48.988%) and Cytochrome-c-oxidase (-52.019%) recorded only on 45 day exposure under $HgCl_2$ treatment. LDH activity has showed a gradual increase up to 45 days exposure over the control and the magnitude of increase was recorded only on 45 (+67.791%) and $HgCl_2$ treatment.

Decrease / increase in activity levels of oxidative enzymes are more in liver tissues under three heavy metals intoxication and the maximum percentage increase or decrease was induced by mercuric chloride on day 45 in all the tissues of fish. SDH, MDH and Cytochrome-c-oxidase activities are gradually decreased and it may be due to a gradual impairment of mitochondrial organization.

In living organisms energy produced by the synthesis of ATP from ADP which results in the oxidation of certain precursor compounds such as succinate, lactate, malate etc. Decrease in oxygen supply to tissues which in turn decrease the oxidation of the substrate. Lactate dehydrogenase (LDH), an enzyme located at a strategic point between glycolysis and citric acid cycle, catalyses the reversible oxidation of lactate to pyruvate, serving in the terminal step of gylcolysis. The activity levels of LDH in gills, liver and muscle tissues *Cirrhinus mrigala* increased on intoxication $CdCl_2$, $PbCl_2$ and $HgCl_2$ (Table3) indicating that it may favour anaerobic respiration to meet the energy demands when aerobic oxidations are lowered. Another plausible reason is conversion of lactate to pyruvate by LDH at the exposure of NAD resulting in the increased operation of glycolysis. It is well known that the variations in biochemical parameters are helpful for monitoring the pathological status of fish induced by toxic stress. Transaminase activity levels are being elevated in serum during pathological conditions. The variations of transaminases are tissue specific and species specific, hence they can be used as indicators of toxic pollution. In the present study both AAT and AlAT activities are increased and maximum percentage elevations are recorded on day 45 and the highest elevations were recorded in the liver tissues as +85.29% and +94.52% respectively under $HgCl_2$, stress (Tables 6 and 7). Increased transaminase activity levels may be attributed to cellular damage (Drotman and Lawhorn, 1978), increased plasma membrane permeability (Ramazotto and Cablin, 1978) or altered metabolism of enzymes (Malik *et al.*, 1988).

Table.6: Variations in Aspartate Amino Transefvrase levels (AAT) in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	64.21±0.08	66.37± 0.07 (+3.369)	71.09±0.09 (+10.715)	75.12±0.12 (+16.991)	80.93± 0.14 (+26.039)	86.65± 0.14 (+34.95)
	Liver	186.39±0.72	196.49±0.22 (+5.419)	214.37±0.031 (+15.011)	325.52±0.43 (+26.359)	276.43±0.52 (+ 48.307)	316.45±0.39 (+69.78)
	Muscle	58.16±0.32	59.62±0.08 (+2.510)	64.03±0.09 (+10.093)	67.44±0.13 (+15.956)	70.95±0.22 (+21.991)	75.63±0.23 (+30.04)
$PbCl_2$	Gill	66.34±0.09	69.15± 0.11(+4.221)	74.02± 0.16 (+11.576)	80.32± 0.23 (+21.073)	84.07± 0.19 (+26.725)	89.86± 0.15 (+35.45)
	Liver	185.21±0.63	201.34±0.33 (+8.709)	21.97±0.47 (+18.767)	242.34±0.44 (+30.846)	287.40±0.73 (+55.175)	325.36±0.42 (+75.67)
	Muscle	56.43±0.12	58.93±0.52 (+4.430)	63.61±0.32 (+12.723)	67.42±0.21 (+19.475)	71.32± 0.33 (+26.387)	76.25±0.21 (+35.12)
$HgCl_2$	Gill	65.34±0.08	69.95±0.43 (+7.055)	74.35±0.22 (+12.118)	79.87±0.015 (+22.237)	85.17±0.16 (+30.348)	93.46±0.45 (+43.04)
	Liver	192.50±0.39	209.87±0.16 (+9.023)	23.00±17 (+20.519)	254.52±0.23 (+32.218)	301.46±0.34 (+56.602)	356.68±0.34 (+85.29)
	Muscle	55.43±0.13	59.63+0.25 (+7.577)	64.75±0.37 (+16.814)	69.00±0.09 (+24.481)	74.87±0.19 (+35.071)	81.52±0.25 (+47.07)

Values are expressed in µ moles of formazan formed / mg protein/h. Each value is mean ± SD of six individual observations.
Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05

Table.7: Variations in Alanine aminotransferase levels (AIAT) in gill, liver and muscle tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	65.52±0.09	67.34±0.12 (+2.777)	76.51±0.23 (+16.773)	84.37±0.16 (+28.769)	92.52± 0.22 (+41.209)	98.66± 0.19 (+50.579)
	Liver	198.61±0.25	208.36±032 (+4.909)	232.84±0.26 (+17.235)	249.61±0.18 (+30.713)	292.58±0.29 (+47.314)	321.61±0.23 (+61.93)
	Muscle	54.43±0.16	56.32±0.16 (+3.472)	62.19±0.08 (+14.257)	68.36±0.42 (+25.519)	73.18±0.36 (+34.447)	79.52±0.18 (+46.095)
$PbCl_2$	Gill	61.09±0.07	64.86± 0.09 (+6.090)	72.61±0.21 (+18.857)	79.83±0.12 (+30.757)	87.36± 0.42 (+43.002)	95.25± 0.26 (+55.917)
	Liver	201.70±0.09	213.64±0.26 (+5.919)	239.36±0.13 (+18.671)	266.34± 0.21 (+32.047)	315.61±0.34 (+56.475)	365.22±0.35 (+81.070)
	Muscle	5616±0.07	59.34±0.19 (+5.662)	62.29±0.21 (+10.915)	72.26±0.08 (+28.668)	78.62± 0.41 (+39.993)	85.83±0.15 (+54.612)
$HgCl_2$	Gill	64.25±0.16	69.83±0.13 (+8.685)	76.62±0.11 (+19.253)	86.19±0.17 (+34.147)	95.34±0.13 (+48.389)	112.16±0.33 (+74.57)
	Liver	210.52±0.25	231.16±0.27 (+9.804)	259.36±0.36 (+23.199)	306.72±1.16 (+45.696)	349.86±0.83 (+66.188)	409.52±0.37 (+94.52)
	Muscle	56.25±0.16	60.08+0.08 (+6.809)	67.24±0.12 (+19.716)	74.34±0.25 (+33.937)	80.61±0.34 (+42.596)	89.75± 0.38 (+59.56)

Values are expressed in µmoles of pyruvate formed / mg protein/h. Each value is mean ± SD of six individual observations.
Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05.

Table.8: Variations in Protein content in various tissues of Indian Major Carp fish, *Cirrhinus mrigala* exposed to 1/10th sub lethal concentration of three heavy metals.

Heavy Metal	Tissue	Control	Exposure Periods (Days)				
			3 days	7 days	15 days	30 days	45 days
$CdCl_2$	Gill	66.73±0.12	64.07± 0.11 (-3.986)	60.09±0.08 (-9.950)	53.62±0.16 (-19.646)	48.08± 0.09 (-27.948)	41.23± 0.16 (-38.214)
	Liver	89.36±0.15	86.19±0.13 (-4.667)	80.03±0.07 (-10.441)	71.33±0.14 (-20.177)	64.16±0.21 (-29.319)	52.36± 1.24 (-41.405)
	Muscle	78.21±0.11	75.22±0.15 (-3.823)	71.43±0.11 (-8.668)	63.31±0.21 (-17.773)	56.39±0.13 (-27.899)	49.56±0.43 (-36.63)
$PbCl_2$	Gill	64.34±0.10	61.09±0.14 (-5.012)	57.33±0.06 (-10.895)	51.29±0.15 (-20.283)	45.37±0.11 (-29.484)	39.06±1.06 (-39.291)
	Liver	88.36±0.12	83.06±0.21 (-5.998)	78.12±0.05 (-11±589)	70.35±0.21 (-20.382)	61.29±0.18 (-30.636)	50.22± 0.34 (-43.16)
	Muscle	79.61±0.14	76.03±0.04 (-4.157)	69.06±0.03 (-13.252)	62.19±0.13 (-21.881)	56.17± 0.26 (-29.443)	48.63±1.02 (-38.91)
$HgCl_2$	Gill	67.34±0.11	62.17±0.12 (-7.677)	57.00±0.22 (-15.355)	51.43±0.10 (-23.626)	48.41±0.12 (-31.081)	40.15 ±0.57 (-40.377)
	Liver	89.25±0.31	80.13±0.14 (-7.977)	73.82±0.18 (-17.288)	65.08±0.26 (-27.081)	48.49±0.26 (-40.067)	39.25±0.27 (-56.02)
	Muscle	75.33±0.14	70.18±0.42 (-6.836)	65.39± 0.08 (-15.850)	58.76±0.13 (-21.996)	52.67± 0.012 (-30.081)	47.68± 0.28 (-36.705)

Values are expressed in µ moles of formazan formed / mg protein/h. Each value is mean ± SD of six individual observations.
Values in parenthesis indicate percentage changes over the control. Values are significant at p<0.05

The increased activity levels of AAT and AIAT may be considered as a compensatory mechanism for the impaired mitochondrial oxidation (Muralimohan et al., 1989) and also indicate that there is a conversion of amino acids into ketoacids than that of utilized energy synthesis. It is apparent that the increased AAT and AIAT activities are an indication of an adaptive physiological response to combat energy demand. As the liver is the major metabolic centre, the conversion of amino acids into ketoacids was more in liver tissues than other tissues and conversion gradually increased, with increase of mercuric chloride exposure period is justified. The variations in total protein content in gill, liver and muscle tissues of *Cirrhinus mrigala,* exposed to three heavy metals – $CdCl_2$, $PbCl_2$ and $HgCl_2$ for a period of 45 days are represented in Table.8. The protein contents of gill, liver and muscle tissues were showed a gradual decrease with increasing the exposure periods heavy metals. However, *Cirrhinus mrigala* on day 458 only (-51.45). The variation in metabolic calibre of various tissues of fish. The present result suggests a stepped up proteolysis, fixation of ammonia and kepo acids resulting in amino acid formation or may be due to metabolic utilization of the keto acids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free amino acids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation. It could also be due to the production of heat shock proteins or destructive free radicals or could be a part of heavy metal induced apoptosis (Sobha et al., 2007) under the stress of heavy metals intoxication. In conclusion, the present work indicates that the three heavy metals caused considerable and significant changes in the intermediate metabolism of *Cirrhinus mrigala* and maximum changes are recorded in the liver tissue under mercuric chloride ($HgCl_2$) intoxication over 45 days period.

These variations have assumed that the fish yields high energy demand to overcome the stress indicated by the existence of hypoproteinamea in all tissues of fish. However, no mortality was recorded over a 45 day intoxicated period. From the point of fish production, it is recognized that as the protein in the tissues is utilized as energy source resulting in decrease of productivity in culture ponds.

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