



Study of growth and biochemical alteration in *Clarias batrachus* due to cadmium (Cd) toxicity effect

Chinmayee Pattanayak¹, Bhaskar Behera^{2*}

¹Ph.D. Scholar, C/O- Dr. Bhaskar Behera (Associate Professor and Head), P.G. Department of Bioscience and Biotechnology, Fakir Mohan University, Vyasa Vihar, Nuapadhi, Balasore, Odisha, India.

^{2*} Associate Professor and Head, PG. Department of Bioscience and Biotechnology, Fakir Mohan University, Vyasa Vihar, Nuapadhi, Balasore, Odisha, India.

Abstract

Due to Cadmium pollution, Cd enters into all the food chains including animal human food chain via air, water, soil, and food contamination. Cadmium is highly toxic beyond its permissible limits of exposure and has acute and chronic effects on aquatic animal as well as terrestrial animals including human beings and environment. The present investigation was undertaken to evaluate Cd toxicity, LC₅₀ and its impact on growth & the various biochemical parameters like glycogen, glucose, protein, lipid and amino acid content in nine different tissues of exposed catfish (*Clarias batrachus*) in laboratory conditions for 14 days to sub lethal and lethal concentrations of Cd. First, the acute toxicity experiment was conducted to evaluate LC₅₀ of Cadmium chloride (CdCl₂) on *Clarias batrachus* which was found to be 450 ppm for 96 hours. *Clarias batrachus* (average length of 8.5±0.5cm and average weight of 13.5±0.5g) was exposed to sub-lethal concentration (140 ppm) and lethal (450 ppm) concentrations of Cd for 14 days had been observed. All biochemical components were decreased in all the tissues except glucose which increased significantly in the lethal concentration. The growth of the control and Cd sub-lethal concentration treated catfish was nearly same, but retarded growth was observed in the lethal concentration of CdCl₂ treated fish. The sub-lethal concentration is host friendly. The exposed fish showed significant behavioural changes like rapid opercular movement and frequent gulping of air. The present result clearly indicate that the changes of these parameters due to lethal concentration of Cd may provide an early warning signal to us for their effects in fishes, aquatic animals, terrestrial animals and human beings by biomagnifications. Thus the discharge of effluents containing Cadmium into the water resources may be threats to aquatic fauna and flora as well as human beings.

Keywords: Cadmium; *Clarias batrachus*; Lethal concentration; Toxicity.

1. Introduction

Now- a- days pollution increases due to industrialisation, technological development and advance living style maintainance of the society. Domestic, industrial and other man-made activities are responsible for discharging toxic heavy metals to the terrestrial environment which are getting biomagnified at higher trophic levels, contaminating the entire food chain (Turgut *et al.*, 2004)^[24]. Heavy metals due to pollution enter into water ways which affect living organisms via air, water, soil, and food contamination. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (International Occupational Safety and Health Information Centre 1999)^[11].

Cadmium is used in electroplating or galvanizing due to its noncorrosive and cumulative nature. It is also used as colour pigment for paints, plastics, and as a cathode material for nickel-cadmium batteries. Cadmium concentration has been increasing in the upper soil because it is found in insecticides, fungicides, sludge, and commercial fertilizers which are routinely used in agriculture, dental alloys, electroplating, motor oil, and exhaust are other sources of Cd pollution. 10% of total Cd in the environment is derived from natural sources, whereas remaining 90% is derived from anthropogenic activity (Okada *et al.*, 1997^[18], Kumar and Singh, 2010^[12]). Anthropogenic activities like;

smelting operations, use of phosphate fertilizers, pigment, smokes, automobiles etc. have contributed to the entry of cadmium into human and animal food chain (WHO, 1992, Okada *et al.*, 1997, Kumar *et al.*, 2007, Kumar and Singh, 2010)^[26,18,13, 12].

Fish are used as bio-indicators, playing an important role in monitoring heavy metals pollution (Authman *et al.*, 2015)^[3].

The walking catfish (*Clarias batracus*) commonly called as "magur" is a air breathing species of freshwater found primarily in South east Asia, Indian sub-continent and one of the most important fish species for aquaculture as well as for its high economic value and also for its high nutritive value and serve as staple food for the vast population in almost all over India (Rajput & Singh, 2012)^[21]. Thus *Clarias batracus* can be a good model to study responses of various environmental pollution.

If the Cd accumulated such fish will be consumed by the man, Cd will be deposited in the body soft tissues and affect the health. The research related to following field was wanting. Therefore the present investigation was undertaken to evaluate Cd toxicity, LC₅₀ and its impact on growth & the various biochemical parameters like glycogen, glucose, protein, lipid and amino acid content in nine different tissues of exposed catfish (*Clarias batrachus*) in laboratory conditions for 14 days to sub lethal and lethal concentrations of Cd.

2. Materials and Methods

Healthy specimens of *Clarias batrachus* (average length of 8.5 ± 0.5 cm and average weight of 13.5 ± 0.5 g) was selected as an experimental animal model in the present study and which was collected from the local fish hatchery Balaramgadi, Balasore and healthy and disease free fishes were selected for experiment and was cleaned by using 0.1% KMnO_4 to avoid dermal infection for 15 minutes and then the fishes were placed in large plastic tubs of 25 litres capacity with non-chlorinated normal tap water to acclimatize in the laboratory conditions of Department of Bioscience and Biotechnology, Fakir Mohan University, Balasore, Odisha for 15 days before the experiment. All the precautions were followed according to APHA *et al.*, (1998)^[2]. For maintaining the fish. The fishes were feed with 3% of body weight daily once with commercial fish feed. Water quality was maintained with optimum pH (7.2 ± 0.1), temperature ($29 \pm 2^\circ\text{C}$), dissolved oxygen (8.0 ± 0.3 mg/L), bicarbonates (95.0 ± 5.0 mg/L), total suspended solids (TSS= 43 ± 6 mg/L), total hardness (41 ± 6) and other physical parameters. Water was changed daily containing fish excreta and undigested food and dead fish was

removed out. 12hr light and 12hr dark photoperiod was maintained. Water of all plastic tubs containing fish was aerated continuously by aquarium air pump. Cadmium chloride salt (CdCl_2 , Molecular weight-183.32) is used as test compound in this toxicological experiment. LC_{50} (lethal, in which 50% mortality was observed) and sub-lethal (the last CdCl_2 concentration in which no mortality observed) dose of Cd was evaluated by screening catfish with the exposure time (96 Hours) and different concentration of Cd reflected on mortality and growth index (Graph -1).

First evaluate the LC_{50} and sub-lethal dose of Cd of catfish by screening of each 22 numbers of plastic tubs containing 10 healthy fishes/10 liters of water for 96 hours including control. In each 21 numbers plastic tub fishes were exposed with different concentration of Cd (from 2 ppm to 1000 ppm) under the standard laboratory conditions and after 24, 48, 72 and 96 hours duration the fish mortality was recorded in comparison with one control tub (No Cd exposure). The test medium and dead fishes were removed immediately after finding the sub-lethal and LC_{50} values.

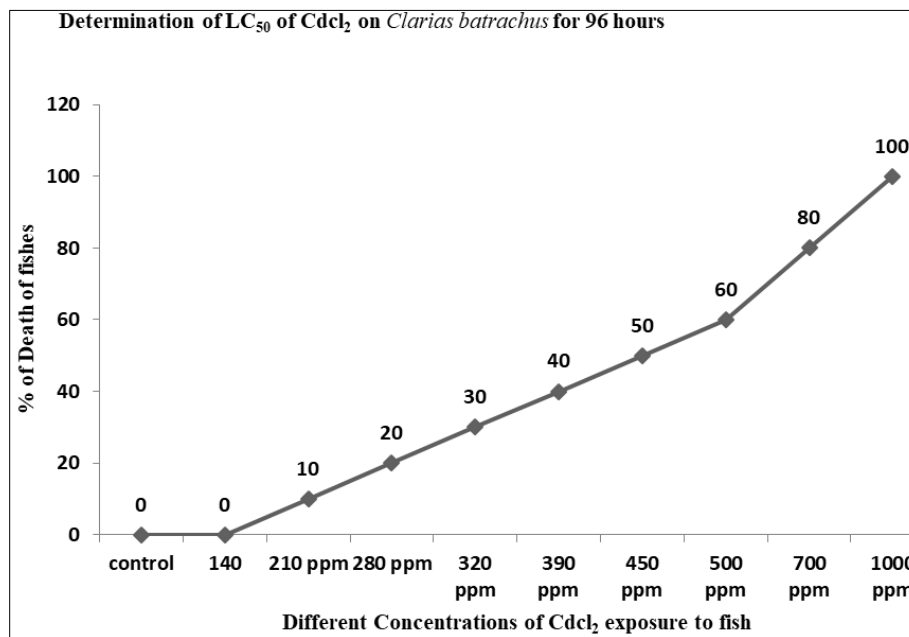


Fig 1: Showing determination of LC_{50} (lethal) and sub lethal concentration of Cadmium chloride on *Clarias batrachus* (Linn.) for 96 hours

The catfishes was exposed to sub-lethal concentrations (140 ppm), lethal (450 ppm) concentrations of Cd and control (0 ppm) in 3 different plastic tubs for 14 days to observe the changes in growth and the biochemical parameters like glycogen, glucose, protein, lipid and amino acid content in nine different tissues i.e. gill, liver, kidney, muscle, heart, brain, ovary, testis and stomach. One fish in 0th day and one fish from each tub were sacrificed for the biochemical analysis after 14th days of exposure of CdCl_2 . The glycogen, glucose, proteins, lipids, and amino acids contents were estimated by methods of Carroll *et al.* (1956)^[6], Seifter *et al.* (1950)^[22], Lowry *et al.* (1951)^[15], Moore and Stein (1954)^[17] and Pande *et al.* (1963)^[19] respectively.

2.1 Statistical analysis

All the results obtained were subjected to statistical analysis to find out the significant difference between all the treatments. For comparing control fish, sub lethal and lethal concentrations exposed fish; ANOVA test was performed by using Graph pad Instat Version 3.0. Level of significance was set at $p < 0.05$.

3. Results

The changes over 0th day on 14th days in growth with respect to body length and body weight, glycogen, glucose, total protein, lipid and amino acid content in mg/g of the wet tissue of different

organs like the gill, liver, kidney, muscle, heart, brain, ovary, testis and stomach of the control fish and the fish exposed to sub-lethal concentrations (140 ppm), lethal (450 ppm) concentrations of Cd for 14TH days were estimated and expressed in terms of Mean ± SD are given below in the table - 1. All the results obtained were subjected to ANOVA among

control, sub-lethal and lethal concentration of CdCl₂ exposed fish to find out the significant difference between all the treatments. Significant p-value has been given below in the table - 1 in Bold.

Table 1: Shows the growth and biochemical compositions of 0th day and after 14th days of *Clarias batrachus* control fish and sub-lethal and lethal concentration of Cadmium Chloride (CdCl₂) exposed fish for 14TH days with ANOVA analysis were given below

Table – 1		0TH Day		14TH Days					
Fish Tissues	Mean ± SD	Control	Sub-lethal	Lethal	Control vs Sub-lethal	Control vs Lethal	Sub-lethal vs Lethal	ANOVA	
		Mean ± SD	Mean ± SD	Mean ± SD					
Growth	In Weight (gram.)	13.5 ±0.393	18.98 ±0.804	18.532 ±0.844	14.633±0.13	>0.05	<0.001	<0.001	<0.0001
	In Length (c.m.)	8.5 ±0.4	11.9 ±0.2	11.6 ±0.2	9.1±0.1	>0.05	<0.001	<0.001	<0.0001
Glycogen Content in mg/g of the wet tissue	Liver	9.31±0.18	9.52±0.09	9.45±0.28	6.17±0.25	>0.05	<0.001	<0.001	<0.0001
	Kidney	8.48±0.13	8.78±0.27	8.71±0.08	5.41±0.19	>0.05	<0.001	<0.001	<0.0001
	Gill	7.81 ±0.21	8.1 8±0.17	8.11 ± 0.08	4.71 ± 0.38	>0.05	<0.001	<0.001	<0.0001
	Muscle	8.61±0.27	9.08±0.2	8.91±0.18	5.91±0.29	>0.05	<0.001	<0.001	<0.0001
	Heart	7.14±0.17	7.64±0.33	7.59±0.32	4.07±0.19	>0.05	<0.001	<0.001	<0.0001
	Brain	6.31±0.24	6.91±0.32	6.85±0.37	3.45±0.4	>0.05	<0.001	<0.001	<0.0001
	Ovary	5.59±0.32	6.07±0.16	6.01±0.17	2.47±0.18	>0.05	<0.001	<0.001	<0.0001
	Testis	4.67±0.23	5.07±0.15	5.02±0.16	2.11±0.18	>0.05	<0.001	<0.001	<0.0001
Glucose Content in mg/g of the wet tissue	Liver	10.622±0.095	10.952±0.425	10.881±0.477	19.177±0.6819	>0.05	<0.001	<0.001	<0.0001
	Kidney	1.329±0.197	1.839±0.51	1.774±0.115	6.693±0.226	>0.05	<0.001	<0.001	<0.0001
	Gill	3.681 ±0.239	3.74 ±0.18	3.75 ± 0.09	5.136 ± 0.197	>0.05	<0.001	<0.001	<0.0001
	Muscle	10.939±0.491	11.43±0.38	11.58±0.35	15.29±0.48	>0.05	<0.001	<0.001	<0.0001
	Heart	3.768±0.193	3.961±0.411	4.025±0.212	6.522±0.599	>0.05	<0.001	<0.001	<0.0001
	Brain	1.531±0.337	1.714±0.154	1.715±.045	5.221±0.199	>0.05	<0.001	<0.001	<0.0001
	Ovary	1.676±0.256	1.932±0.2	1.924±0.089	3.932±0.182	>0.05	<0.001	<0.001	<0.0001
	Testis	1.114±0.037	1.151±0.04	1.147±0.025	3.041±0.074	>0.05	<0.001	<0.001	<0.0001
Lipid Content in mg/g of the wet tissue	Liver	17.253±0.242	17.756±0.261	17.437±0.081	4.103±0.328	>0.05	<0.001	<0.001	<0.0001
	Kidney	7.395±0.394	7.732±0.354	7.643±0.432	3.448±0.509	>0.05	<0.001	<0.001	<0.0001
	Gill	14.77±0.559	14.81 ±0.46	14.63 ± 0.46	5.44 ± 0.37	>0.05	<0.001	<0.001	<0.0001
	Muscle	33.298±0.313	33.955±0.622	33.516±0.37	13.967±0.653	>0.05	<0.001	<0.001	<0.0001
	Heart	4.289±0.322	4.599±0.555	4.392±0.385	0.982±0.175	>0.05	<0.001	<0.001	<0.0001
	Brain	5.234±0.223	5.744±0.575	5.614±.606	3.018±0.244	>0.05	<0.001	<0.001	<0.0001
	Ovary	3.425±0.414	3.802±0.555	3.738±0.516	1.009±0.026	>0.05	<0.001	<0.001	<0.0001
	Testis	2.096±0.215	2.244±0.227	2.118±0.103	0.642±0.078	>0.05	<0.001	<0.001	<0.0001
Protein Content in mg/g of the wet tissue	Liver	56.545±0.534	57.106±0.804	56.82±0.49	38.26±0.75	>0.05	<0.001	<0.001	<0.0001
	Kidney	48.489±0.242	48.726 ±0.72	48.612 ±0.717	23.135 ±0.679	>0.05	<0.001	<0.001	<0.0001
	Gill	31.212±0.375	31.47 ±0.58	31.46 ± 0.92	14.5 ± 0.77	>0.05	<0.001	<0.001	<0.0001
	Muscle	62.645±0.634	62.717±0.722	62.67 ±0.92	34.23±0.56	>0.05	<0.001	<0.001	<0.0001
	Heart	36.063±0.596	36.604 ±0.717	36.334±0.62	18.648±0.841	>0.05	<0.001	<0.001	<0.0001
	Brain	35.772±0.561	35.867±0.813	35.82± 0.955	17.284± 0.632	>0.05	<0.001	<0.001	<0.0001
	Ovary	14.682±0.671	14.79 ±0.76	14.69±0.78	6.88 ±0.81	>0.05	<0.001	<0.001	<0.0001
	Testis	6.491±0.49	6.704±0.525	6.586±0.582	2.938±0.424	>0.05	<0.001	<0.001	<0.0001
Amino Acid Content in mg/g of the wet tissue	Liver	25.931±0.602	26.302 ±0.401	26.133±0.698	15.016±0.702	>0.05	<0.001	<0.001	<0.0001
	Kidney	83.468±0.459	83.696 ±0.931	83.591±0.748	39.631±0.6011	>0.05	<0.001	<0.001	<0.0001
	Gill	77.14±0.371	1.839±0.808	1.774±0.816	6.693±0.64	>0.05	<0.001	<0.001	<0.0001
	Muscle	53.484±0.483	53.546 ±0.728	53.542 ± 0.753	22.653 ± 0.649	>0.05	<0.001	<0.001	<0.0001
	Heart	92.445±0.456	93.242 ±0.454	93.129±0.452	58.621±0.614	>0.05	<0.001	<0.001	<0.0001
	Brain	70.786±0.498	70.982 ±0.849	70.979 ±0.873	35.701±0.692	>0.05	<0.001	<0.001	<0.0001
	Ovary	57.228±0.357	57.464±0.751	57.423±.769	29.339 ±0.524	>0.05	<0.001	<0.001	<0.0001
	Testis	30.546±0.439	30.651 ±0.638	30.568 ±0.669	15.891±0.576	>0.05	<0.001	<0.001	<0.0001
Stomach	19.193±0.474	19.358 ±0.464	19.288 ±0.522	10.854 ±0.551	>0.05	<0.001	<0.001	<0.0001	
Stomach	33.441±0.4365	33.739 ±0.627	33.59 ±0.689	14.779 ±0.576	>0.05	<0.001	<0.001	<0.0001	

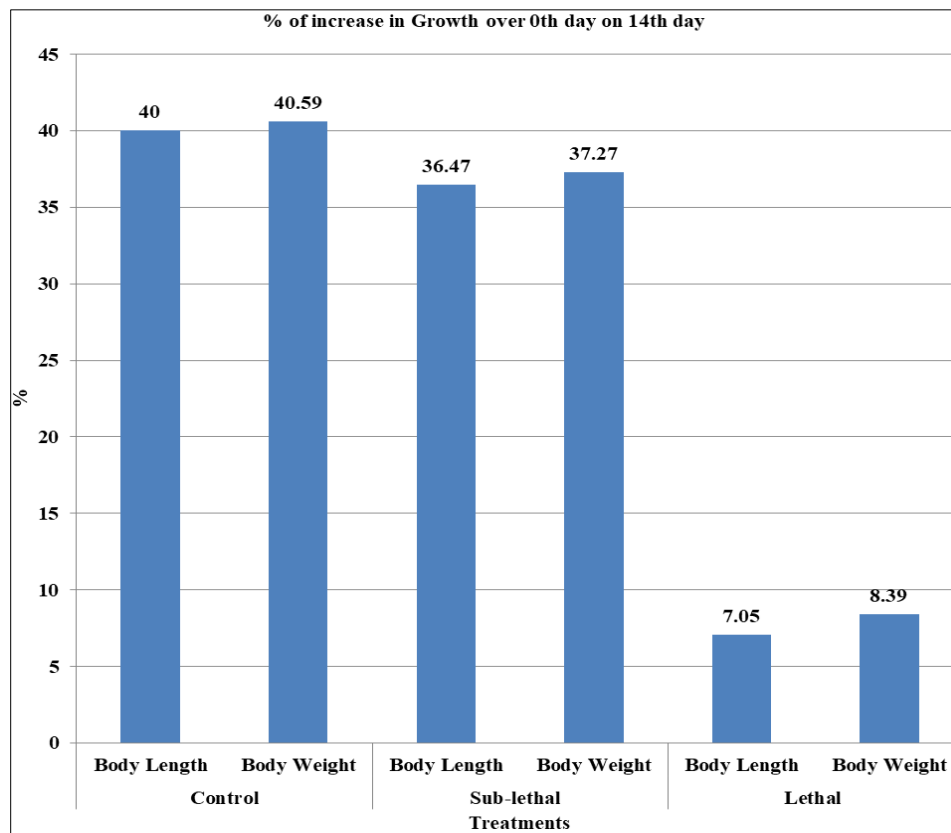


Fig 2: Showing the Changes in % of increase in growth with respect to body length and body weight over 0th day on 14th days of *Clarias batrachus* control fish and exposed fish to sub-lethal and lethal concentration of Cadmium Chloride (CdCl₂) for 14th days.

Table 2: Changes in biochemical compositions in % increase (↑) or % decrease (↓) over 0th day on 14th days of *Clarias batrachus* control fish and exposed fish to sub-lethal and lethal concentration of Cadmium Chloride (CdCl₂) for 14th days were given below

Biochemical Components	Tissues↓	Control	Sub-lethal	Lethal	Biochemical Components	Tissues↓	Control	Sub-lethal	Lethal
Glycogen (%) ↓	Liver	2.26	1.5	-33.73	Total proteins (%) ↓	Liver	0.99	0.49	-32.34
	Kidney	3.53	2.71	-36.2		Kidney	0.49	0.25	-52.29
	Gill	4.73	3.84	-39.69		Gill	0.83	0.79	-53.54
	Muscle	5.45	3.48	-31.36		Muscle	0.11	0.04	-45.36
	Heart	7	6.3	-43		Heart	1.5	0.75	-48.29
	Brain	9.51	8.56	-45.32		Brain	0.26	0.13	-51.68
	Ovary	8.59	7.51	-55.81		Ovary	0.73	0.05	-53.14
	Testis	8.57	7.49	-54.82		Testis	3.28	1.46	-54.74
	Stomach	9.68	8.63	-53.35		Stomach	1.43	0.78	-42.09
Glucose (%) ↑	Liver	3.11	2.44	80.54	Amino acids (%) ↓	Liver	0.27	0.15	-52.52
	Kidney	20.51	16.25	338.6		Kidney	1	0.64	-40.3
	Gill	1.6	1.87	39.53		Gill	0.12	0.11	-57.65
	Muscle	4.49	5.86	39.78		Muscle	0.86	0.74	-36.59
	Heart	5.12	6.82	73.09		Heart	0.28	0.27	-49.56
	Brain	11.95	12.02	241.02		Brain	0.41	0.34	-48.73
	Ovary	15.27	14.8	134.61		Ovary	0.34	0.07	-47.97
	Testis	3.32	2.96	172.98		Testis	0.86	0.49	-43.45
	Stomach	9.19	9.63	83.01		Stomach	0.89	0.45	-55.81
Lipids (%) ↓	Liver	2.92	1.07	-76.22	Lipids (%) ↓	Brain	9.74	7.26	-42.34
	Kidney	4.56	3.35	-53.37		Ovary	11.01	9.14	-70.54
	Gill	0.27	-0.95	-63.17		Testis	7.06	1.05	-69.37
	Muscle	1.97	0.65	-58.05		Stomach	7.63	1.77	-71.36
	Heart	7.23	2.4	-77.1		-	-	-	-

Discussion

The fish showed normal behaviour such as well co-ordinate with active movements, static equilibrium, active swimming,

normal gill movement, free gulping of air at the surface water, horizontal hanging in the water with natural body colour and zero mortality rates were observed in the control group. During

the exposure of cadmium chloride for 14 days, a number of behavioural changes were observed i.e. fishes frequently coming to the surface of water, tried to jump out of the water, loss of equilibrium, erratic and darting swimming movements, rapid gill movement, vertical hanging, fading for their body colour, increased opercular movements, being lethargic and sluggish, excess mucus secretion all over the body and restlessness. Finally fish stays motionless and open their mouth prior to death in the bottom of the container throughout the tenures. The growth of the control and Cd sub-lethal concentration treated catfish was nearly same, but retarded growth was observed in the lethal concentration of CdCl₂ treated fish (graph-2). The sub-lethal concentration is host friendly.

The present study is mainly concerned with the effect of Cd heavy metal pollution on catfish quality. Biochemical alteration in the body of a fish gives an indication of pollution and help to understand the mode of action and type of pollutant. The analysis of biochemical components of fresh water fish has been done for their nutritive values. These fish serve as protein rich food for human beings. The obtained result illustrate highly significant depletion of glycogen, protein, lipid, amino acid content of catfish exposed to lethal concentration of heavy metal for 14th days compared to that of the control and sub lethal concentration and whereas increase in the glucose content of catfish exposed to lethal concentration of heavy metal for 14th days compared to that of the control and sub lethal concentration. There was no significant difference seen between the control and sub lethal concentration exposed fish which is visible in the table- 1 and 2.

Glycogen and Glucose

Glycogen is one of the immediate fuel reserves and an important constituent which can be used during stress condition. Under hypoxic conditions; fish derive the energy by anaerobic breakdown of glucose which is available to the cells with the increased glyco genolysis. The observed depletion of glycogen in the present study explains the increased demand of these molecules to provide energy for the cellular biochemical process under toxic manifestations (Martin and Arivoli, 2008)^[6]. Similar results were observed in *Thalimile crenata*, *Anabas testudineus*, *Anabas scandens* and *Catla catla* when exposed to lead nitrate, copper, mercury chloride and cadmium chloride respectively (Candravathy and Reddy, 1991 Villalan *et al.*, 1988 and Sobha *et al.*, 2007;)^[5, 23, 25]. Glucose is a primary source of energy for living organisms. In energy metabolism, glucose is the most important source of energy in all organisms. In animals glucose arises from the breakdown of glycogen in a process known as glycogenolysis.

The present result data clearly indicated that at the end of 14 days exposure, the glycogen content was decreased in all the tissues whereas the glucose content of different tissues showed an increase in catfish exposed to lethal concentration of heavy metal compared to that of the control and sub lethal concentration. The glucose level of all the tissues was increased whereas tissue glycogen was decreased in exposed fish makes it

clear that the glycogen reserves are being used to meet the stress caused (Sobha *et al.*, 2007)^[23]. Due to stress, in fish Serum glucose levels was increased (Bedii and Kenan, 2005; Chowdhury *et al.*, 2004; Almeida *et al.*, 2001)^[4, 7, 1]. Our report was agreed with the earlier reports.

Protein and Amino Acid

The proteins are the most abundant biological macro molecules and are extremely versatile in their function and interaction during the metabolism of protein, amino acids, enzymes and coenzymes. Proteins are also involved in major physiological events, therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are mainly involved in the architecture of the cell. Liver is rich in proteins and the centre for various metabolisms. During cadmium stress, the proteolysis was intended to increase the role of proteins in the energy production and also a decrease in the level of protein and amino acid content (De smet and Blust 2001 and Sobha *et al.* 2007)^[8, 23]. Due to toxicant poisoning, a continuous stress could reduce the protein markedly suggested by many workers. In the present study, depletion in both protein content and amino acid content had been observed in the different tissues of the fish *Clarias batrachus* as a result of Cadmium toxicity with the lethal concentration. When an animal is under stress condition, they needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative sources of energy are protein, lipid and amino acid to accomplish the impending energy demand.

Lipid

Fatty acid composition and lipid metabolism depend on peroxidation in animals. In the present study it was showed that lipid levels decreases in the tissues of fish exposed to cadmium. Toxic heavy metals in fish can damage the positive effects of the omega 3 fatty acid in fish and may lead to heart disease. The cadmium had reduced effects on palmitoleic acid, oleic acid; linoleic acid belongs to unsaturated fatty acid in muscle tissue. The changes in the distribution of lipids should be associated to a change in the turnover of lipids in a medium high of oxidative stress which is known to modify the properties of membranes. The lipid levels was also decreased in the tissues of the fish exposed to the lethal cadmium chloride concentration was agreed with earlier researchers (Sobha *et al.*, 2007; Fabien Pierron *et al.*, 2007; Levesque *et al.*, 2002 and Dubale and Punita shah, 1981)^[23, 10, 14, 9].

In case of lethal concentrations Cd heavy metal treated fish for 14th days, bio molecules like glycogen, total proteins, free amino acids and lipids of the 9 tissues were decreased in the order glycogen: M>L>K>G>H>B>S>T>O; Total Proteins: L>S>M>H>B>K>O>G>T; Free amino acids: M>K>T>O>B>H>L>S>G and Lipids: B>K>M>G>T>O>S>L>H respectively whereas Glucose increased in the order for 14th days: k>B>T>O>S>L>H>M>G.

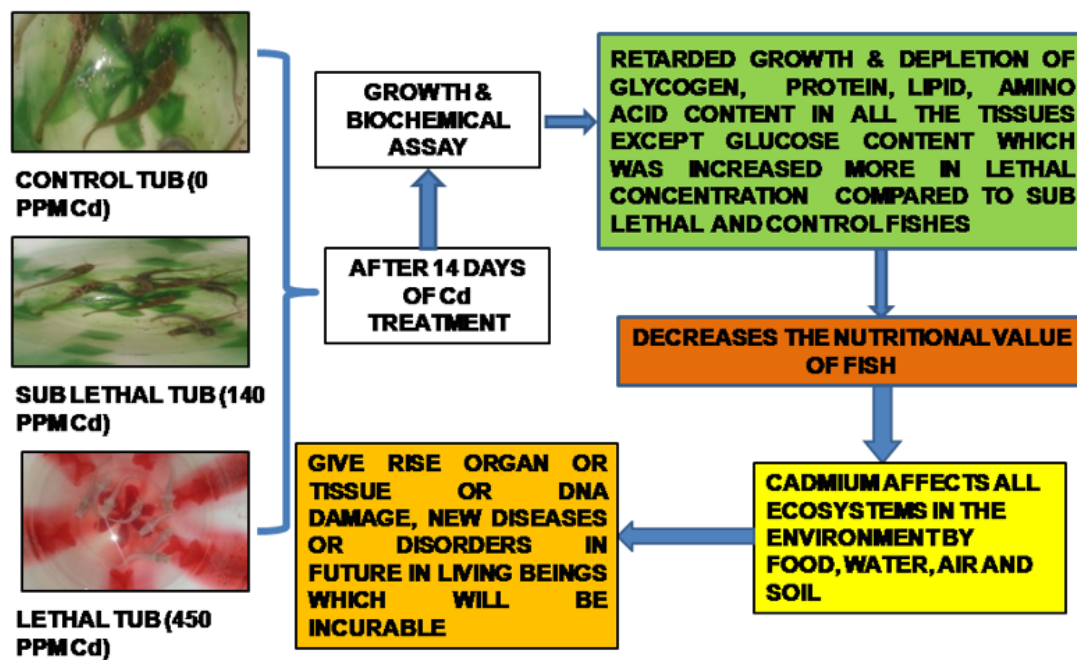


Fig I: Shows Growth and Biochemical alteration in *Clarias batrachus* due Lethal Concentration of Cadmium (Cd); Chinmayee Pattanayak¹ and Bhaskar Behera^{2*}

Conclusions

Small amounts of Heavy metal are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (poisoning). As fish is staple food for human, the accumulations of metals in fish decrease their nutritional value. The present result clearly indicate that the changes of these parameters due to lethal Cadmium concentration may provide an early warning signal to us for their effects in fishes, aquatic animals, terrestrial animals and human beings by biomagnifications. Cadmium is highly toxic beyond its permissible limits of exposure and long exposure of cadmium produces a wide variety has acute and chronic effects on aquatic animal as well as terrestrial animals including human beings and environment. The growth of the control and Cd sub-lethal concentration treated catfish was nearly same, but retarded growth was observed in the lethal concentration of Cd treated fish. The sub-lethal concentration is host friendly. Exposure to lethal concentrations of Cadmium chloride resulted biochemical alteration. The changes suggest that the treated fish were faced with a serious metabolic crisis and the Cadmium chloride is toxic to *Clarias batrachus* (Figure – I). All biochemical components were decreased in all the tissues except glucose which was increased significantly. All the biochemical changes were an indicator of damages to catfish by the Cadmium in comparison to control catfish. The present result clearly indicate that the discharge of effluents containing Cadmium chloride into the water resources may be threats to aquatic fauna and flora as well as humans. The finding of the present study also provide a better understanding of the toxicological end point of aquatic pollution and to ascertain a safer level of these chemicals in the aquatic environment and protection of aquatic habitats. If the heavy metal pollution will not be minimized, it will affect all ecosystems in the environment by biomagnifications which may give rise organ or

tissue or DNA damage, new diseases or disorders in living beings in future which will be incurable.

Acknowledgements

We express our deep sense of gratitude to Prof. Bhabatosh Mitra (Ex- H.O.D.) and Prof. Bishnu Prasad Dash (Ex- H.O.D.) of the Department of Bioscience and Biotechnology, Fakir Mohan University, Balasore for having provided us all the laboratory facilities and kind cooperation of all staffs during the study.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Almeida JA, Novelli EL, Dal Pai Silva M, Junior RA. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environ Pollut*. 2001; 114 (2):169-175.
2. APHA AWWA, WPCF. Standard methods for examination of water and Waste waters. 20th edition, APHA, AWWA and WPCF, Washington. 1998
3. Authman MMN, Zaki MS, Khallaf EA, Abbas HH. Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution. *Journal of Aquaculture Research & Development*. ISSN: 2155-9546. 2015; 6(4):1-12. doi:10.4172/2155-9546.1000328.
4. Bedii C, Kenan E. The effects of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio*(L.,1758). *Turk.J.Vet.Anim.Sci*. 2005; 29:113-117.

5. Candravathy VM, Reddy SLN. Lead nitrate exposure changes in carbohydrate metabolism of freshwater fish. *J. Env. Biol.* 1991; 17:75-79.
6. Carroll NV, Longley RW, Roe JH. Glycogen determination in liver and muscles by the use of anthrone. *Journal of Biological chemistry.* 1956; 220:583-593.
7. Chowdhury MJ, Pane EF, Wood CM. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionic regulatory, and stress parameters. *Comp Biochem Physiol C Toxicol Pharmacol.* 2004; 139(1-3):163-173.
8. De Smet H, Blust R. Stress response and changes in protein metabolism in carp *Cyprinus carpio* during cadmium exposure. *Ecotoxicol Environ Saf.* 2001; 48(3):255-262.
9. Dubale MS, Punita Shah. Biochemical alterations induced by cadmium in the liver of *Channa punctatus*. *Environmental research.* 1981; 26 (1):110-118.
10. Fabien Pierron, Magalie Baudrimont, Angélique Bossy, Jean-Paul Bourdineaud, Daniel Brèthes, Pierre Elie and Jean-Charles Massabuau. Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). *Aquatic Toxicology.* 2007; 81(3): 304-311.
11. International Occupational Safety and Health Information Centre Basics of chemical safety, International Labour Organization Conference, Geneva, 1999.
12. Kumar P, Singh A. Cadmium toxicity in fish: An overview. *GERF Bulletin of Biosciences.* 2010; 1(1):41-47.
13. Kumar P, Prasad Y, Patra AK, Swarup D. Levels of Cadmium and Lead in Tissues of Fresh water Fish (*Clarias batrachus* L.) and Chicken in Western UP (India). *Bull. Environ. Contamin. and Toxicol.* 2007; 79:396-400.
14. Levesque HM, Moon TW, Campbell PGC, Hontela A. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquatic Toxicology,* 2002; 60(3-4):257-267.
15. Lowry OH, Rosenbrough NJ, Farr RL, Randall RJ. Protein measurement with the Folin Phenol reagent. *J.Biol.Chem.* 1951; 193: 265-275.
16. Martin DPP, Arivoli S. (Biochemical Study of freshwater fish *Catla catla* with reference to mercury chloride, *Iran. J. Environ. Health Sci. Eng.* 2008; 5 (2):109-116.
17. Moore, S, Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J.Biol.Chem.* 1954; 221: 907.
18. Okada IA, Sakuma AM, MaioFD, Dovidemskas S, Zenebon O. Evaluation of lead and cadmium in milk due to environmental contamination in Paraíba Valley region of South Eastern Brazil., *Revista-de-Saude-Publication.* (1997; 31:140-143.
19. Pande SV, Parvin Khan A, Venkata subramaniam TA. Micro determination of lipids and serum fatty acids. *Analyt. Biochem.* 1963; 6 (5):120-125.
20. Pritchard JB. Aquatic Toxicology: Past, Present and Prospects. *Environmental Health Perspective.* (1993; 100:249-257.
21. Rajput V, Singh SK. Comparative toxicity of Butachlor, Imidacloprid and sodium fluoride on protein profile of the walking catfish *Clarias batrachus*. *Journal of applied pharmaceutical Science.* 2012; 2(6):121-124.
22. Seifter S, Dayton S, Novic B, Muntwyler E. The estimation of glycogen with the anthrone reagent. *Arch. Biochem. Biophys.* 1950; 50:191-200.
23. Sobha K, Poornima A, Harini, P, Veeraiah K. A study on biochemical changes in the fresh water fish, catla catla (hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu University Journal Of Science, Engineering And Technology.* 2007; 1(4):1-11.
24. Turgut C, Katie PM, Cutright TJ. The effect of EDTA and citric acid on phytoremediation of Cd, Cr and Ni from soil using *Helianthus annuus*. *Environ. Pollut.* 2004; 131(1):147-54.
25. Villalan P, Narayanan KR, Ajmal Khan S, Natarajan R. Proximate composition of muscle, hepato pancreas and gill in the copper exposed estuarine crab *Thalamita crenata* (Latreille). *Proceeding of the second national symposium on ecotoxicology, Annamalai University, India,* 1988.
26. WHO Environmental Health Criteria, No. 134, Environmental aspects, *Geneva WHO.*