

## ESTIMATION OF PROTEIN IN THE DETERGENT INDUCED TOXICITY FISH, *CATLA CATLA*

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### Abstract

To understand the effect of Detergent on vital organs of fresh water fish *Catla catla* was selected. The biochemical studies of (Muscle protein) studies have been carried out.  $LC_{50}$  value was found out before the experiment by following methods (Saptami Moitra and Verma 1997). The female fishes were divided into two groups, one is control (N=10) another is experimental group (N=10). The experimental groups of fishes were treated with sub lethal concentration of Detergent at a rate of 0.0113ml/l (or) 11.3 $\mu$ l/l i.e. . considered as 1/3 of  $LC_{50}$  values. In the beginning the experimental group of fishes showed very active and tried to escape from the aquarium, but day by day they were not showed active in movement, function and low intake of food throughout the experimental period. 0<sup>th</sup>, 7<sup>th</sup> day of experiment 5 fishes from each group were dissected out for the analysis of biochemical study. In the 0<sup>th</sup> day of both the group of fishes were showed  $8.5 \pm 0.4$ mg/g concentration of protein in the muscle. In the 7<sup>th</sup> day of experiment the concentration of protein in the control group of fishes were showed  $10.25 \pm 0.4$ mg/g but fishes treated with Detergent were more decreased value of protein  $6.25 \pm 10$  mg/g in muscle.

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### INTRODUCTION

India is one of the largest fish producing nations and ranks ninth among all the nations in fisheries (Jhingran, 1983). India is endowed with rich and diverse fishery resources due to its long coastline, extensive river systems, reservoirs, tanks, ponds, estuaries, swamps etc. However, a matter of great concern is that most of these water bodies which are of paramount importance for fisheries have been under increasing threat due to aquatic pollution (Kumaraguru, 1995). Population explosion, rapid industrialization, consequent urbanization and advancements in the field of agriculture have brought about severe environmental deterioration and degradation (Kassim, 1993).

Though the green revolution had pushed up agricultural productivity and made our nation self-reliant, the indiscriminate use of synthetic fertilizers, pesticides etc., have inflicted tremendous damage to the ecosystem (Venkataramani, 1996). Agricultural fertilizers are widely used in aquaculture to enhance the natural productivity of a pond by stimulating the production of phytoplankton which serve as feed for fishes (Jhingran, 1983). The fertilizers used for the augmentation of productivity of ponds in aquaculture belong to two categories – inorganic and organic. Organic fertilizers include manures of liquid origin, guana, offal, farmyard manure, sewage, plant material, etc. (Jhingran, 1983). Inorganic fertilizers include limestone, urea, ammonium sulphate and phosphate.

Among the inorganic fertilizers used in aquaculture, ammonium sulphate and urea are widely utilized throughout the world. In India, ammonium sulphate is extensively used as a fertilizer and its application to fish ponds has been found to stimulate the growth of bottom fauna leading to sustained production of Zooplankton (Jhingran, 1983) also showed that ponds fertilized with ammonium sulphate promoted better growth and survival of major carp spawn. However, excessive use of ammonium sulphate results in contamination of water bodies with ammonia (Varadachari, 1992) which may have detrimental effects on fishes.

Today's world is more dependent on environment resources for its development. But mans activities are continuously polluting all such resources and hindering their beneficial usage

Pollution is thus direct to indirect change is any components of the biosphere. Any substance which causes pollution is called pollutants. A pollutant may thus include any chemical or geochemical substance biotic component or product or physical facts that is released intentionally or

unintentionally by man into the environment is a concentration that may have adverse, harmful or unpleasant effect.

A pollutant has also been defined as “any solid, liquid or gaseous substance present in such concentration as may be or tend to be injurious to environment” industries and agricultures are the two arms of nation's economy and health. However in recent years these two arms are infected with evil called “pollution” (Reddy, 2002).

Various principle pollutants which pollute our air, water, land are deposits matter, gases, acids, droplets, metals, florides, agrochemicals, compels organic substances, photo chemicals, oxidants solids wasters, detergents, radio active wastes and noise. It has become essential to provide farmers and crofters and those involved in activities such as agriculture contractors and companies, involved in spreading organic measures to land, with practical guidance on how to prevent pollution.

### INDIAN MAJOR CARP, *CATLA CATLA*

*Catla* is endemic to the riverine system in northern India, Indus plain and adjoining hills of Pakistan, Bangladesh, Nepal and Myanmar, and has been introduced later into almost all riverine systems, reservoirs and tanks all over India. As the species breeds in the riverine ecosystem, its ready seed availability has helped in establishing its aquaculture in the peripheral region of the riverine system in these countries. The natural distribution of *catla* seems to be governed by temperature dependency rather than latitude and longitude. The minimum tolerance temperature limit is ~14 °C. The use of *catla* as a component in pond culture was a traditional practice in the eastern Indian states, spreading to all other Indian states only during the second half of the 20th century. Its higher growth rate and compatibility with other major carps, specific surface feeding habit, and consumer preference have increased its popularity in carp polyculture systems among the fish farmers in India, Bangladesh, Myanmar, Laos, Pakistan and Thailand. The collection of riverine seed was the only source for culture until the 1950s. Success in the induced breeding of the species in 1957 assured subsequent seed supply, thus revolutionising this form of polyculture in India and other south-east Asian countries. The species has also been introduced elsewhere, including Sri Lanka, Israel, Japan, and Mauritius. (Hamilton, 1822)

### DETERGENT

There are several factors which dictate what compositions of detergent should be used, including the material to be cleaned, the apparatus to be used, and tolerance for and type of dirt. For instance, all of the following are used to clean glass. The sheer range of different detergents which can be used demonstrates the importance of context in the selection of an appropriate glass-cleaning agent.

### SURFACTANTS

Are organic chemicals, obtained through complex chemical reactions, from oil or fat raw materials. They have wetting, emulsifying and dispersing properties, enabling the removal of dirt ("soil") from fabrics and keeping the soil suspended in the washing water. Detergents usually contain several types of surfactants such as soaps (anionic), alkylbenzenesulphonate (anionic), ethoxylated fatty alcohols (non-ionic). The mixture is carefully balanced to control foaming and provide the appropriate washing efficiency (for the required washing temperatures, types of fabric and water hardness), at a price the consumer is willing to pay. However, surfactant efficiency is very much reduced in hard water and their detergent properties are not complete even in soft water.

Surfactants used in household and various industries, are rather toxic; therefore, the accumulation of these compounds in the environment through wastewaters has challenged the problem of their biodegradation. In this research, an attempt was made to assess the toxic effect of various surfactants and the likely products of their biodegradation on the acetoclastic methanogens of an anaerobic microbial community. Among the substances investigated, cationic surfactants were found to be most toxic to methanogens: 154 mg/l alkamon DS and 345 mg/l catamin AB induced a 50% inhibition of methanogenesis (Victoria *et al.*, 1999).

Surfactants are of considerable importance in the field of detergents and in cosmetics. Of the anionic, nonionic, and cationic surfactants the most important products — as far as sales volume is concerned — belong to the anionic type. Anionic and nonionic surfactants taken orally are of low toxicity according to acute toxicity tests as well as long term studies, while certain cationics are moderately toxic. In local application, compatibility with skin and mucous membranes is strongly dependent on

concentration. Surfactant action on biological systems can largely be explained on the basis of physico-chemical properties of the surfactants. Some surfactants show pharmacological activity (Glohuber, 2004).

Oya *et al.* (2007) studied the effectiveness of surface tension on surfactants risk assessment.  $\gamma(\text{tox})$  was defined as surface tension at a point where acute aquatic toxicity of a surfactant emerges. *Oryzias latipes*, *Daphnia magna*, and *Podocopa* were used for acute aquatic toxicity test of 7 surfactants and 3 detergents.  $\gamma(\text{tox})$  values were plotted on surface tension curves, and the effect of water hardness on toxicity and surface tension were examined.

The acute and sublethal chronic effects of sodium dodecyl sulphate (SDS) on the survival, metabolism, and growth of juveniles of *Centropomus parallelus* were investigated at three different salinities (Rocha *et al.*, 2007).

The toxic effects of four commercial detergents (two washing powders and two cakes) are reported in this paper on behavior, mortality and RBC counts of a freshwater fish *Gambusia affinis* (Sexena *et al.*, 2005).

In this study, feral leaping mullet (*Liza saliens*) liver microsomal 7-ethoxyresorufin O-deethylase (EROD), and cytosolic glutathione S-transferases (GSTs) activities were investigated using 7-ethoxyresorufin, 1-chloro-2,4-dinitrobenzene (CDNB), and ethacrynic acid (EA) as substrates, respectively. The average EROD activity was found as  $1139 \pm 175$  pmol resorufin/min/mg protein (Sen and Semiz, 2007).

Erickson *et al.* (2006) shows the Effects of exposure-water pH on chemical uptake at rainbow trout (*Oncorhynchus mykiss*) gills were investigated for nine weakly acidic, chlorinated phenols with different ionization constants and hydrophobicities and for a moderately hydrophobic, nonionizable reference chemical (1,2,4-trichlorobenzene).

Alkylphenol-polyethoxylates (APnEO,  $n = 1-40$ ) are a major group of surfactants and are normally present in raw sewage. Many of the products of the biodegradation of these compounds are both persistent and present in substantial quantities in effluent and in river water. They report here on the use of an in vitro bioassay to determine the oestrogenic potencies of these compounds to fish. The bioassay is based on the fact that the synthesis of vitellogenin by hepatocytes is oestrogen dependent (Jobling and Sumpter, 2000).

## MATERIALS AND METHODS

To study the sub lethal effects of organo phosphates detergents on to the *Catal catla* was selected as an experimental animal. Totally 60 number of healthy fishes or females were selected. The body weight of the fishes are ranging between 20-30gm and total length of 10-15cm were collected from the local lake of Poondi at Thiruvallur District.

The fishes were brought to the laboratory with care and acclimatized for laboratory condition. After acclimatization, the fishes were transferred to the plastic tub of 10litre capacity. They were grouped into 5, each tub containing 10 fishes along with control group at room temperature. The fishes were treated with detergents to observe the LC50 and the biochemical analysis and histological changes. Each group was exposed to gradually increased concentration (i.e)  $10\mu\text{l/l}$  (0.01ml/L) to  $50\mu\text{l/l}$  (0.05ml/L) for 4 days (i.e) 24,48,72 and 96 hours. The biochemical parameter such as protein was analyzed by adopting the method of Lowry *et al.*, (1951) and histological methods followed by Humason, (1972).

To detergent the fishes two glass aquarium of 20 liter capacity were used to the present investigation. The fishes were divided into two group namely control group NO: (N=10) and experimental group NO2 (N=10). The first aquarium is a control group and second aquarium is a experimental group.

The second aquarium (Experimental group) was treated with detergent at a sub-lethal concentration of 0.0113ml/l of water. Where as the control group fishes, were not treated with detergent. Both the group of fishes (Control and experimental group) were fed with commercial fish feed at a rate of 15gm./kg. of fish/day. Throughout the experimental period the water in both aquariums was changed. The fecal matter and left out food materials were removed at every 27hrs using pillar. To estimate the protein content on 0<sup>th</sup> and 7<sup>th</sup> day (Lowery *et al.*, 1951) of muscle tissue was collected both the experimental and control group fishes.

## BIOCHEMICAL ANALYSIS

### ESTIMATION OF PROTEIN

For the analysis of biochemical parameters in the 0<sup>th</sup> day and 7<sup>th</sup> day of the exposure of the both the experimental and control group of fishes were sacrificed. The muscle tissues was taken and analyzed by the methods of Lowery *et al.* (1951), with crystalline bovine serum albumin (BSA) as the standard.

Proteins form a complex with copper; the protein – copper complex reacts with Folin-Ciocalteu reagent to give a blue colour, which is due to the reduction of phosphomolybdate by tryptophane present in their protein. The intensity of the colour is proportional to the amount of protein being estimated.

0.5 ml of samples were taken in the test tubes and made up to 5.0 ml with distilled water and subsequently, 5.0 ml of alkaline copper reagent was added. The contents were mixed well and allowed to stand at room temperature for 10 minutes. 0.5 ml of 1 N Folin-Ciocalteu reagent was then added and mixed well. After 20 minutes, the intensity of the blue colour developed was read at 500 nm against a reagent blank. The concentration of protein was calculated by using the following formula

$$\frac{\text{O.D. of unknown}}{\text{O.D. of known}} \times \text{standard concentration.}$$

Following the values were expressed as mg/100 mg of tissue, and mg/ml of blood.

## RESULTS

The LC<sub>50</sub> experiments shown in the Table. 1 and Fig. 1. LC 50 values were found to be 0.0113ml/1 (or) 11.3 µl/l.

### BIOCHEMICAL CHANGES

The biochemical analysis (Protein) of control and experimental group of fishes of *Catla catla* are shown in Table. 1 and Fig. 1.

It is understood from the Table. 2 and Fig. 2. From the above results it is understood that the protein content in the muscle of the control fishes showed highest activity at 7<sup>th</sup> day ( $10.25 \pm 0.4\text{mg/g}$ ) whereas the lower value was observed at 0<sup>th</sup> day i.e. ( $8.5 \pm 0.4\text{mg/g}$ ) in the control group.

When compared to experimental group of fishes which are administered with detergent showed same protein level at 0<sup>th</sup> day ( $8.5 \pm 0.4\text{mg/g}$ ) in the muscle. But 7<sup>th</sup> day of experiment the fishes showed very lowest value of muscle protein i.e., ( $6.25 \pm 0.1\text{mg/g}$ ) Table. 2 and Fig. 2.

The result are in agreement with the work of Kumar and Asari, (1986). They concluded that the marked inhibition DNA, RNA, Protein, Acid and Alkaline phosphatase in the liver of four month old Zebra Fish after 7<sup>th</sup> day of exposure of different concentration of detergent (0.5,0.7,0.9 and 1.1 mg/l).

## DISCUSSION

Detergent a widely used insecticide is know to cause serious metabolic disturbance in non –target species, like fish. and fresh water mussels. Detergent known to effect the nervous system by inhibiting acetyl cholinesterase (ACHE), the enzyme that modulated the amount of the neurotransmitter, acetylcholine (Fukuto, 1972). They are several metabolic routes by which an organism can detoxify organophosphate. In addition, the physiological condition of the organism during toxic impact must be consider to understand the influence of pesticide. In the present study Detergent was chosen to evaluate the influence on the muscle protein of the fish *Catla catla* at sub lethal concentration of Detergent exposure have been analyzed and discussed.

The change of aquatic toxicity with varying water hardness, however, could be explained by the change of surface tension. Aquatic toxicity of LAS (Linear Alkylbenzene Sulphonate) increased and aquatic toxicity of SOAP decreased with an increase of water hardness, but both gamma(tox), values were constant (Oya *et al.*, 2007)

Roacha *et al.* (2007) results of 96 h LC<sub>50</sub> test showed that juveniles of *C. parallelus* were very sensitive to SDS in comparison to other species investigated. For each group of exposure to nominal concentrations of SDS (0.10 and 0.25 mg/L) and the control group (0.0 mg/L), at the different salinities (5, 20, and 30) there were significant differences in the specific growth rate, oxygen

consumption, and ammonia excretion rates, O:N atomic ratio at the different exposure periods (15 and 30 days).

The effect of various detergents on leaping mullet liver EROD, GST-CDNB, and GST-EA activities were studied. It was found that ionic detergents strongly inhibited the EROD activity, whereas much less inhibitions were observed with GST catalyzed activities. Therefore, the CYP1A inhibition potencies of metals and detergents suggest that their contribution to the overall CYP1A induction in polycyclic aromatic hydrocarbons contaminated environmental samples has to be taken into account for better interpretation of environmental studies (Sen and Semiz, 2007).

The gradual decrease of protein from various days of exposure may be due the influence of exogenous factors like toxic environment, Sapna Srivastava, *et al.*, (2004); Khalaf Allah, (1999) reported that the decreased level of protein, globulin and serum enzyme activity in vaccinated *Tilapia nilotica* exposed to sub lethal concentration Detergent.

In the present study the low, level of protein content estimated in the Detergent treated fishes. This may be due to the pollution stress posted to the fishes, mobilization protein from muscle to blood, to compensate to certain acidosis caused by the lactate accumulation (Palnichamy, *et al.*, 2004).

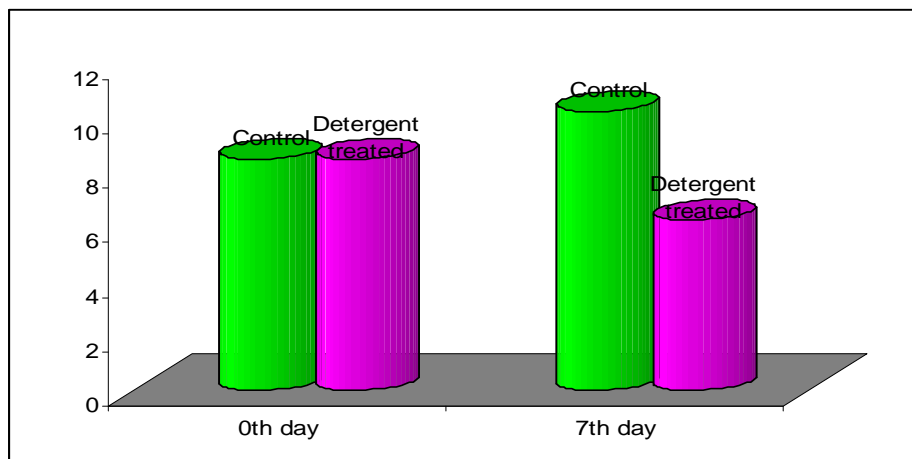
**TABLE. 1: Mortality rate of Fresh water fish *Catla catla* at different concentration of detergents at 96 hrs. exposure.**

S/No.	Concentration of detergent (ml/l)	No. of fishes exposed	No. of fishes dead	Percentage of Mortality %
1	0.01	10	1	10%
2	0.02	10	3	30%
3	0.03	10	6	60%
4	0.04	10	8	80%
5	0.05	10	10	100%

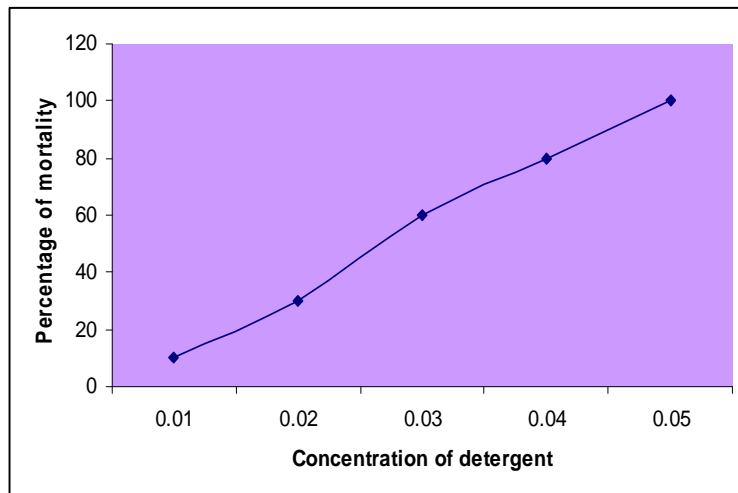
**TABLE. 2: Concentration of Protein in the muscle tissue the *Catla catla* exposed to detergents.**

Treatment	Organ	Total Protein	
		0 <sup>th</sup> day	7 <sup>th</sup> day
Control	Muscle	8.5 ± 0.40	10.25 ± 0.040
Experimental (Treated with detergents)	Muscle	8.5 ± 0.40	6.25 ± 0.10

Mean ± SD



**Fig. 1: Protein Change in the Muscle *Catla catla* against concentration of detergents.**



**Fig. 2: Percentage of Mortality rate of *Catla catla* against concentration of detergents.**

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