



## SUBLETHAL EXPOSURE OF CATFISH *MYSTUS MONTANUS* TO DETERGENT AND THEIR INFLUENCE ON BIOCHEMICAL CONTENT OF REPRODUCTIVE ORGANS

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### ABSTRACT

*The freshwater bodies are main source of drinking water and cheap protein in the form fish. However, these water bodies are become severely polluted due to domestic, industrial and agricultural discharge and detergents. The surfactant is a main constituent of detergents, it is also used in shampoos and toilet soaps for cleaning and other purposes in large quantity. The effect of detergents namely Surf Excel and Nirma powder on biochemical content in gonads of fresh water catfish, *Mystus montanus* was evaluate. The 96-hours acute toxicity ( $LC_{50}$ ) of Surf excel and Nirma powder is 22.0mg/L and it is 23.5 mg/L respectively. The test animals exposed to  $1/3^{rd}$  and  $2/3^{rd}$  sublethal concentrations of  $LC_{50}$  values of both detergents for 96hrs. After 24, 48, 72 and 96 hours of exposure the glycogen, protein and lipid levels were estimated in Gonads. The amount of glycogen, protein and lipid contents in reproductive tissues such as ovaries and testis decreases with an increase in concentrations of both detergents and time of exposure. It may affect the process of development and maturation of gonads.*

Key words:- detergent, gonad, biochemical content

### **Introduction**

The major rivers and aquatic bodies of world are highly polluted due to the domestic, industrial and agricultural discharge and detergents. Human being for cleaning and other purposes is using a huge amount of detergent powders, flakes and various shampoos and toilet soaps. Due to surface-active property of detergents, it becomes a powerful cleaning

agent. The major component of synthetic detergents is surfactant. There is a variety of surfactants like alkyl benzene sulphonate (ABS), linear benzene sulphonate (LAS), alkyl sulphates (AS), alkyl ethoxy sulphates (AES), ethoxylates (AE), alkyl phenol ethoxylates (APE), Cetyl trimethyl Ammonium Bromide (CTAB) etc. These surfactants are also used in Washing bars, shaving creams, fabric softeners, additives for food, paint, leather and textile items; pesticides, defoliant, antiseptics, disinfectants have surfactants. The amount of these substances is very large and their toxicity is severe. These detergents form foam in the water bodies, which diminish the air-water interaction leading to lack of oxygen to the aquatic animals under the water surface. The detergents and surfactants are non-degradable pollutant accumulated in water body. Then detergents enter the food web through uptake by vegetation, planktons and fishes. Though non-target organisms are also affected and the accumulated toxicants may reach human being as they form a staple food for them. There have been few attempts to study the influence of aquatic toxicants on the fish reproduction. Fish reproduction is affected by the direct or indirect exposure to aquatic pollution [1]. It is understood that the reproduction is basic to the survival of the maximum number of young and hence the success of the fish species. Therefore, in this review, influence of aquatic pollutants on the reproductive systems is considered.

The LC<sub>50</sub> values of two detergents to *Mystus montanus* by using static bioassays. The Litchfield and Wilcoxon graphical method used to evaluate 96h LC<sub>50</sub> values. At the time of toxicity test, the fishes exposed to different concentration of detergents exhibited several behavioural changes like restlessness, rapid swimming, loses equilibrium, suffering from breathing and bleeding through gill filaments. Opercula ventilation rate as well as visual examination of dead fish indicates lethal effects of the detergent on the fish. Hemorrhage occurs at the base of body appendages (fins) and along the belly. There occurs loss of nervous control, fishes along lateral side of body. Body becomes slimy due to excess mucus secretion from epithelium of gills [2].

It has been suggests that not only concentration of toxicant causes major damages to testis but also the duration of exposure. When a dose of LAS has increased, this surfactant chemical causes cellular damage, checks the growth and maturation of sex cells and there occur a continuous decrease in Gonadosomatic index (GSI). The exposure of fishes to LAS, it results in the decrease of fertility potential in male *H. fossilis* [3]. The levels of glycogen and protein levels were decreases in Muscles and Liver in experimental fishes as an increase in concentration of detergents and duration of exposure [4]. The effect of surfactant LAS on potential reproduction on marine calanoid copepod population was observed [5]. In most

teleosts, the testes are paired and elongated organs attached to the dorsal body wall and in some they are combined into a single sac. The teleost testis, as in mammals, is composed of steroid hormone-secreting endocrine interstitial cells and sperm-producing lobular or tubular compartments. Whereas ovary in teleost is a hollow paired structure however, in some species, these are fused into one solid, single organ during their early development. The ovary consists of oogonia, oocytes and their surrounding follicle cells, supporting tissue or stroma [6].

The exposure of fishes to LAS it causes histoanatomical damage of fish testis in terms like concentration of spermatogenic cells, vacuolation of tubular cells and distortion of seminiferous cells along with inflammatory lesions. When fishes were exposed for short duration about 24hrs, the symptoms appeared even after 24hrs of exposure to LAS are frightening. The fish testes are quite vulnerable to this pollutant that is show a 29.13% decrease in GSI. After longer duration of exposures (72 and 96 hr) even with low doses, there occurs shrinkage of interstitial cells and vacuolation of tubular cells that has resulted in peculiar starry sky appearance of the testicular tissue [7]. The exposure of fishes to the carbamide for a long time causes abnormal development of lobular structure, disbanding of germinal epithelium, scattered LC with homogeneous liquified cytoplasm, prominent vacuolization and necrosis during preparatory and maturing phase [8].

A decrease in testicular proteins, RNA, lipids and ascorbic acid and elicited elevation of cholesterol and phospholipids in *C. punctatus* has noticed with treatment of Dimecron and carbofuran. When the fishes were exposed to Fenitrothion and carbofuran, it causes decrease in total ovarian protein, RNA, lipids and ascorbic acid but enhanced cholesterol and lipids during different phases of ovarian cycle [9]. The treatment of phosphamidon to scale carp causes flattening of seminiferous tubules, germinal epithelium appeared degenerated and squamated and the LC were atrophied and vacuolated. However, there is no apparent change in the production of milt could detect in phosphamidon treated scale carps [10]. The reproductive process was delay in both sexes by affecting the gonadal development, as well as inhibits the maturation process of spermatocytes and oocytes, postponing ovulation, breaking and clumping of yolk material in oocytes and suppressing the synthesis of gonadal steroids via hypothalamo-hypophysial-gonadal axis [11].

## Material and Methods

During investigation, the method of collection and maintenance of fishes and experimentation recommended in the APHA were followed [12]. As per requirement, Surf excel and Nirma powder weighed accurately and dissolved in water before adding the fishes

into the aquarium. Ten moderate size fishes selected from acclimatized stock for the experiment having length ranging from 12.3cm to 14.5cm and weight ranging from 18.72gm to 23.86gms. The average mortality in each concentration was taken to determine the LC<sub>50</sub> value by plotting a graph, taking concentration on X-axis and mortality on Y-axis [13]. According to graphical plots the 50% mortality values of Surf excel and Nirma for 96 hours were 20.0 mg/litre and 23.5 mg/litre respectively. The experimental fishes were subjected to sub-lethal concentrations namely 1/3<sup>rd</sup> and 2/3<sup>rd</sup> of LC<sub>50</sub> of Surf excel and Nirma as per suggestions for different exposure periods (24, 48, 72 and 96 hours) [14]. Through experiment, the equal number of fish was maintained as a control for similar duration of exposure [15]. In the scarified fishes, the biochemical parameter such as Glycogen was estimated by Anthrone method given by Carroll [16] and protein was analysed by adopting method of Biuret reagent. Whereas Lipid by chloroform and methanol by using Potassium Chloride (KCl).

## Results and discussion

When fishes were exposed to 1/3<sup>rd</sup> and 2/3<sup>rd</sup> sublethal concentration of Surf excel and Nirma demonstrates decrease in the amount of glycogen, protein and fat contents in reproductive tissues such as ovaries and testis with an increase in period of exposure.

**Glycogen content:** - When fishes were treating with the 1/3<sup>rd</sup> and 2/3<sup>rd</sup> sublethal concentrations of Surf excel and Nirma slight decreases have noticed to both sublethal concentrations of two detergents in Ovary and Testis upto 48hrs. There was insignificant decrease has been observed with 1/3<sup>rd</sup> sublethal concentrations upto 72 hrs. However, at 2/3<sup>rd</sup> sublethal concentration there is a significant reduction occurs from 72hrs onwards.

**Protein content:** - The exposure of fishes to the sublethal concentrations of Surf excel and Nirma powder, shows insignificant decrease with 1/3<sup>rd</sup> sublethal concentrations upto 96hrs in both gonads. However, with 2/3<sup>rd</sup> sublethal concentration upto 48hrs an insignificant decrease was noticed, but thereafter there is significant decrease has observed to both sublethal concentration of both detergents.

**Lipid content:** - The Lipid content in both gonads of fish demonstrate an insignificant decrease with 1/3<sup>rd</sup> sublethal concentration of both detergents upto 96hrs. Although, with 2/3<sup>rd</sup> sublethal concentration of both detergents upto 48hrs an insignificant decrease was observed, but subsequently there is significant decrease has detected.

The amount of glycogen, protein and fat contents in reproductive tissues such as ovaries and testis decrease with an increase in concentration of both detergents and increase in time of exposure. This reduction may affect the process of development and maturation of gonads such as oogenesis and spermatogenesis. It may affect whole reproduction process will be disturbed.

Table: -1

Effects of Surf excel and Nirma powder on glycogen (--mg/gm) content in Ovary of *Mystus montanus* at various sublethal concentrations.

Exposure Time	Concentration of detergents –mg/litre.				
		Surf excel		Nirma powder	
	Control	6.67mg/lit.	13.3mg/lit.	7.833mg/lit.	15.67mg/lit.
24hrs	6.5 ± 0.229129	6.3 * ± 0.15	6.15* ± 0.15	6.3* ± 0.15	6.2* ± 0.229129
48hrs	6.6 ± 0.15	6.15 * ± 0.15	5.75 * ± 0.229129	6.2 * ± 0.31225	6.0 * ± 0.15
72hrs	6.55 ± 0.15	5.6 * ± 0.31225	5.2 ** ± 0.377492	5.8 ** ± 0.31225	5.5 ** ± 0.229129
96hrs	6.6 ± 0.216333	5.35 ** ± 0.229129	4.8 ** ± 0.15	5.45 ** ± 0.229129	4.95 ** ± 0.15

Values are expressed in ---mg/gram of wet weight of tissue,

± = Standard deviation of three observation.

\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Graph-1**-Effects of Surf excel and Nirma powder on glycogen (---mg/gm) content in Ovary of *Mystus montanus* at various sublethal concentrations.

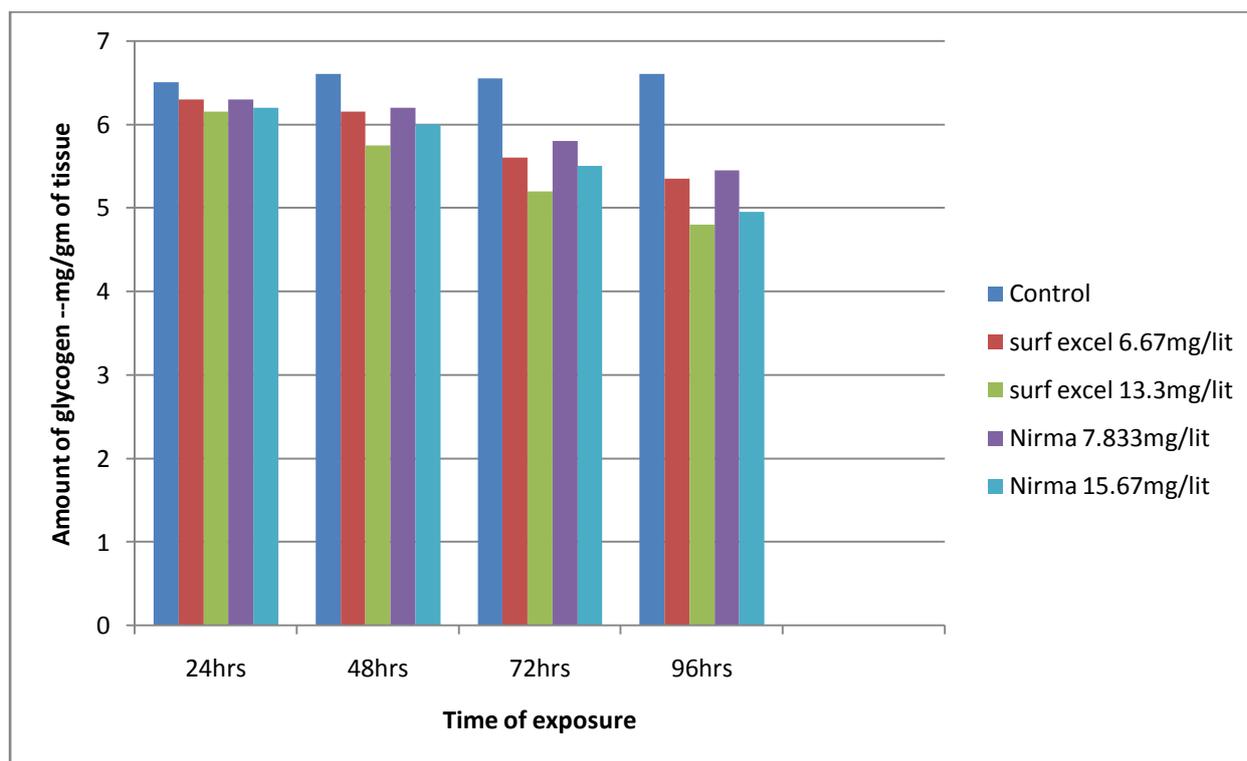


Table: -2

Effects of Surf excel and Nirma powder on glycogen (---mg/gm) content in Testis of *Mystus montanus* at various sublethal concentrations.

Exposure Time	Concentration of detergents –mg/litre.				
		Surf excel		Nirma powder	
	Control	6.67mg/lit.	13.3mg/lit.	7.833mg/lit.	15.67mg/lit.
24hrs	5.3 ± 0.229129	5.25* ± 0.15	5.1* ± 0.15	5.25 * ± 0.3	5.15* ± 0.377492
48hrs	5.4 ± 0.3	5.15 * ± 0.229129	4.85 * ± 0.229129	5.2 * ± 0.173205	5.0 * ± 0.229129
72hrs	5.45 ± 0.229129	4.8 * ± 0.259808	4.4 ** ± 0.229129	4.95 * ± 0.15	4.8 ** ± 0.3
96hrs	5.35 ± 0.229129	4.6 ** ± 0.31225	4.3 ** ± 0.229129	4.65 ** ± 0.3	4.6 ** ± 0.229129

Values are expressed in ---mg/gram of wet weight of tissue

± = Standard deviation of three observation.

\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Graph-2**-Effects of Surf excel and Nirma powder on glycogen (--mg/gm) content in Tetis of *Mystus montanus* at various sublethal concentrations.

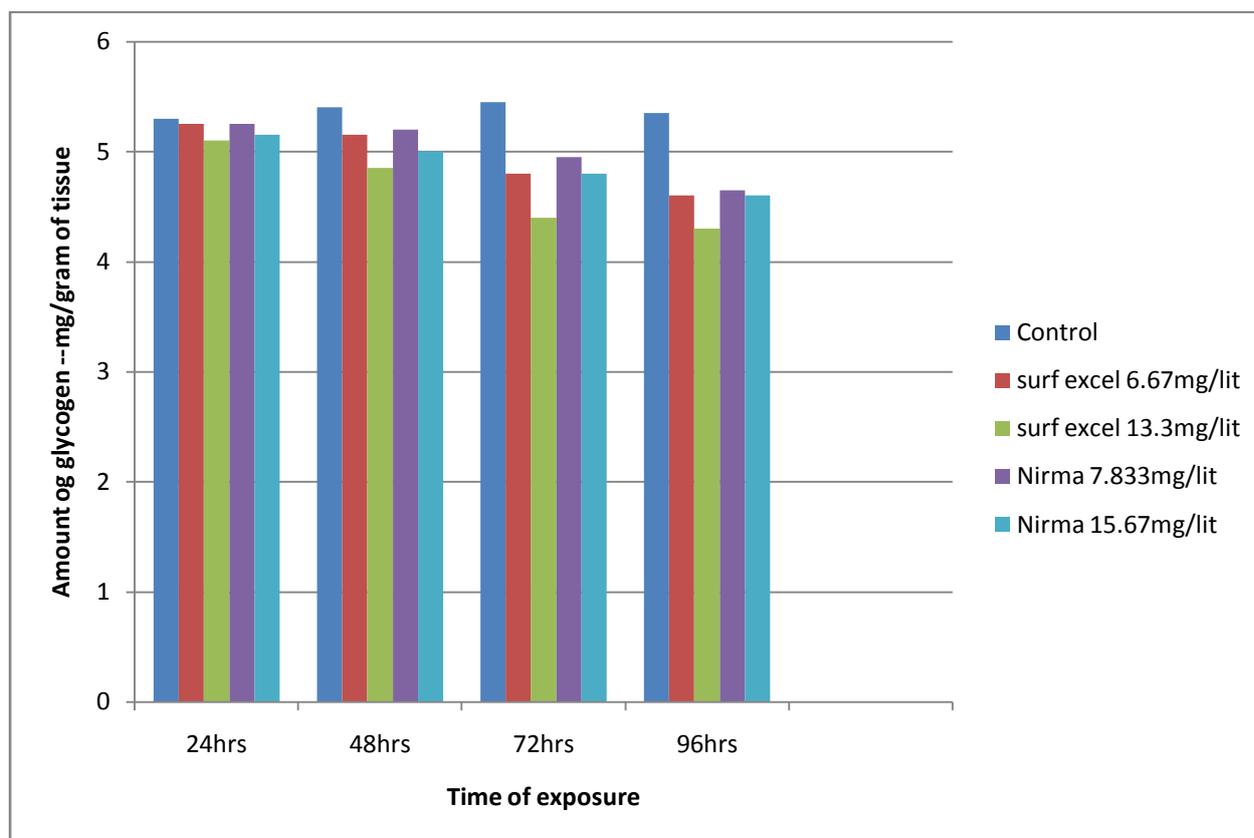


Table: -3

Effects of Surf excel and Nirma powder on Protein (--mg/gm) content in Ovary of *Mystus montanus* at various sublethal concentrations.

Exposure Time	Concentration of detergents –mg/litre.				
		Surf excel		Nirma powder	
	Control	6.67mg/lit.	13.3mg/lit.	7.833mg/lit.	15.67mg/lit.
24hrs	43.611 ±1.27297	42.222 * ±2.096867	43.11067* ± 1.734989	43.333* ± 1.667	41.38867** ± 1.734588
48hrs	44.1663 ± 1.6665	41.38867 * ± 1.73499	40.8887 * ± 1.734588	41.11067 * ± 1.734989	39.1663 ** ± 2.204478
72hrs	44.444 ± 2.097	40.944* ± 0.20486	38.88867 ** ± 1.734588	38.61067* ± 2.09733	38.16633 ** ± 1.6665
96hrs	43.61067 ± 2.096867	38.833* ± 1.667	38.27767** ± 2.09733	39.38867* ± 1.734588	38.837** ± 1.667

Values are expressed in ---mg/gram of wet weight of tissue

± = Standard deviation of three observation.

\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Graph-3**-Effects of Surf excel and Nirma powder on Protein (--mg/gm) content in Ovary of *Mystus montanus* at various sublethal concentrations.

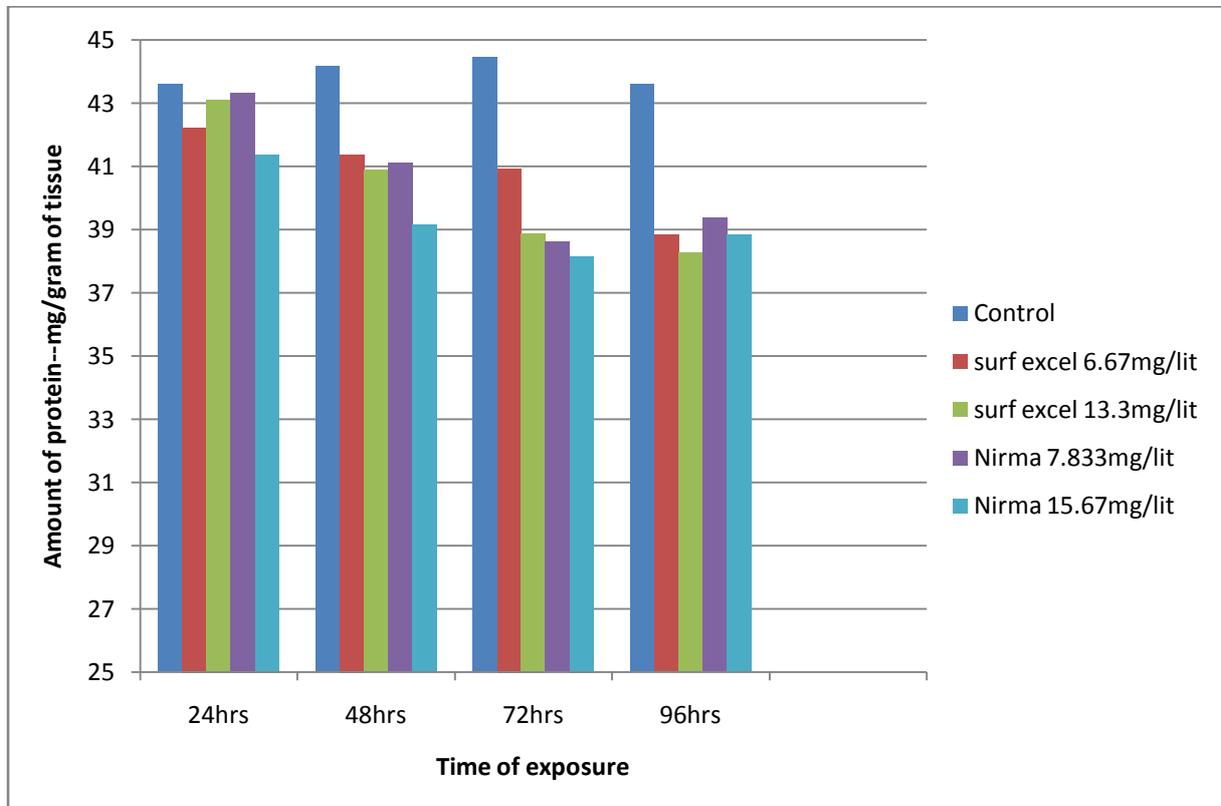


Table-4

Effects of Surf excel and Nirma powder on Protein (--mg/gm) content in Testis of *Mystus montanus* at various sublethal concentrations.

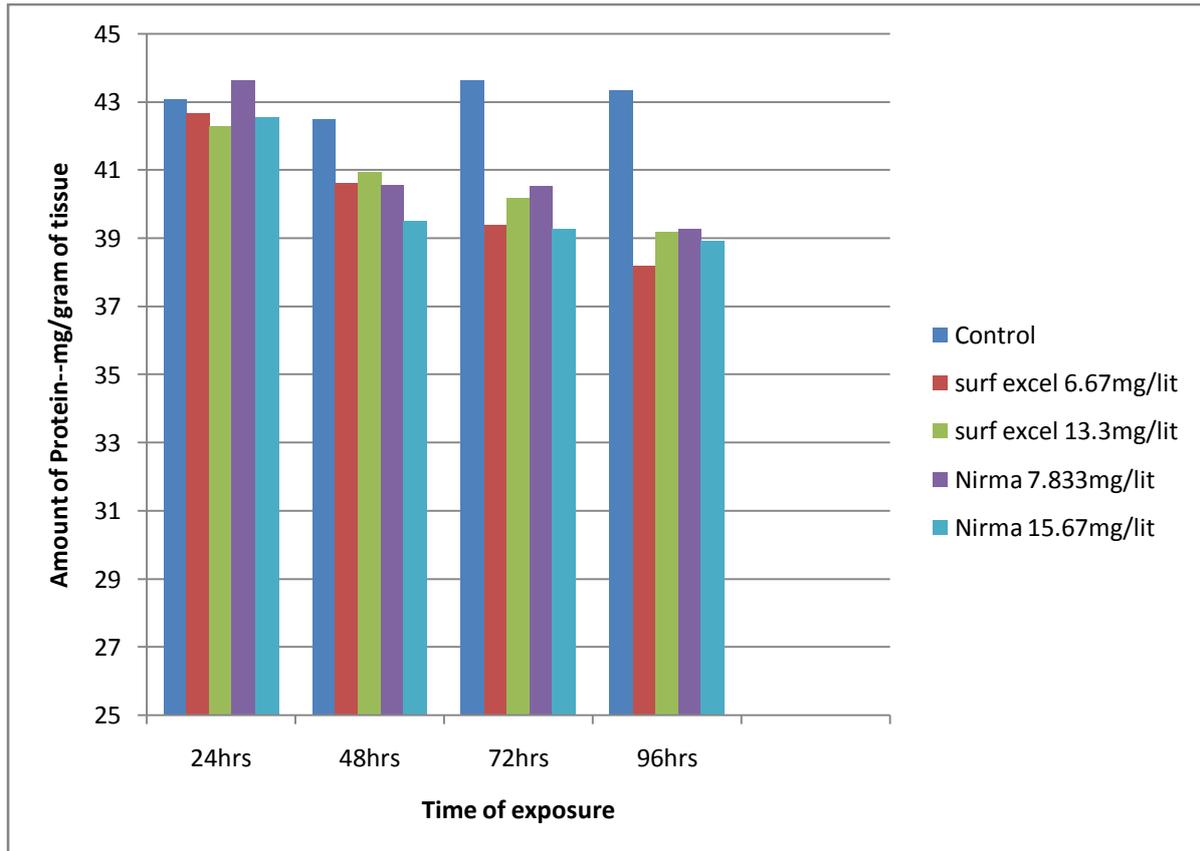
Exposure Time	Concentration of detergents –mg/litre.				
	Control	Surf excel		Nirma powder	
		6.67mg/lit.	13.3mg/lit.	7.833mg/lit.	15.67mg/lit.
24hrs	43.0553 ± 2.204478	42.6663* ± 2.204478	42.27767* ± 2.09733	43.61067* ± 1.734989	42.55533* ± 2.096867
48hrs	42.4997 ±1.7354989	40.61067 * ± 2.09733	40.944* ± 2.096867	40.5553 ± 2.096867	39.49967** ± 2.205045
72hrs	43.61067 ±1.734989	39.38867* ± 1.734588	40.16633 * ± 2.204478	40.49967* ± 1.6665	39.27767** ± 2.09733
96hrs	43.333 ± 2.204856	38.61067* ± 2.096867	39.1663** ± 2.096867	39.27767* ± 2.09733	38.8887** ± 1.734588

Values are expressed in ---mg/gram of wet weight of tissue

± = Standard deviation of three observation.

\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Graph-4**-Effects of Surf excel and Nirma powder on Protein (--mg/gm) content in Testis of *Mystus montanus* at various sublethal concentrations.



**Table-5**

Effects of Surf excel and Nirma powder on Lipid (--mg/gm) content in Ovary of *Mystus montanus* at various sublethal concentrations.

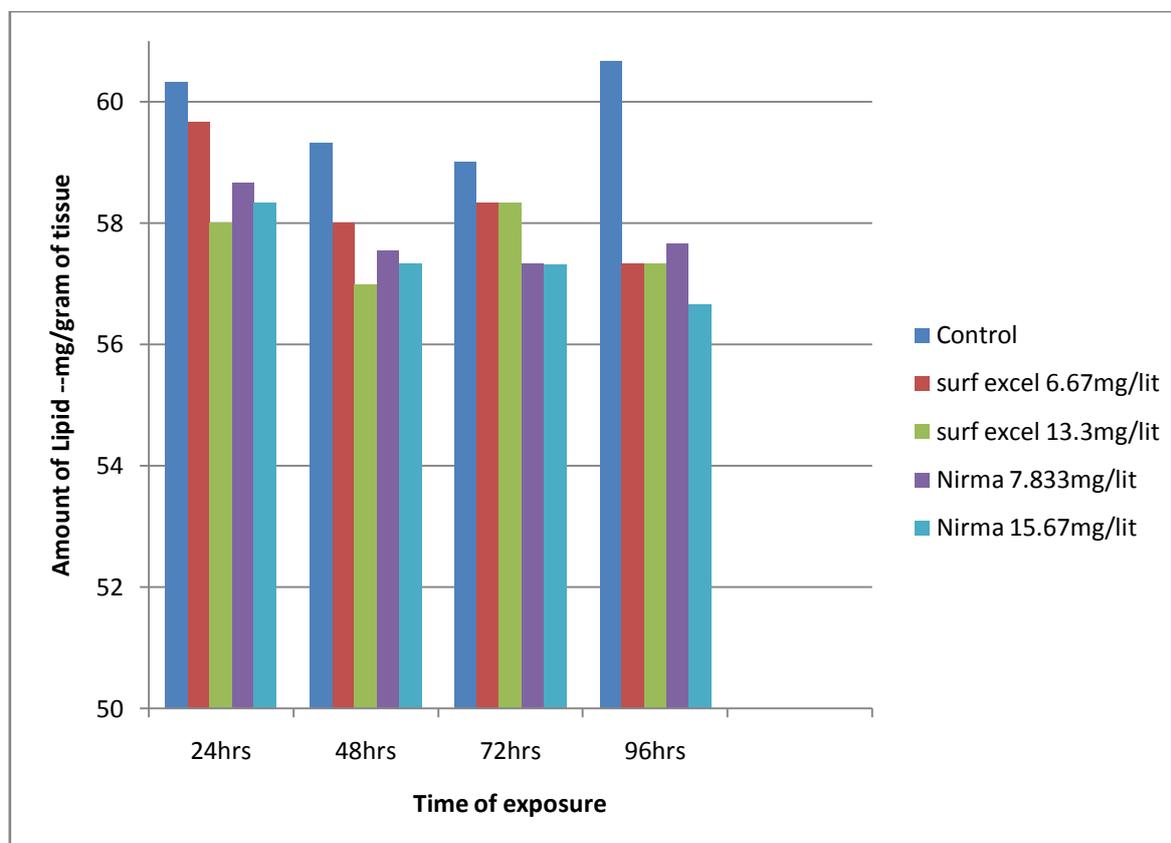
Exposure Time	Concentration of detergents –mg/litre.				
		Surf excel		Nirma powder	
	Control	6.67mg/lit.	13.3mg/lit.	7.833mg/lit.	15.67mg/lit.
24hrs	60.3333 ± 2.08166	59.6667 * ± .527525	58.0** ± 2.0	58.66667 * ± 1.527525	58.3333 * ± 2.51661
48hrs	<b>59.3333</b> ± 1.527525	58.0 ** ± 2.0	57.0 * ± 2.0	57.54203 * ± 2.0	57.3333* ± 1.527525
72hrs	<b>59.0</b> ±2.645751	58.3333* ± 2.08166	58.3333* ±1.527525	58.3333* ± 1.527525	57.326 * ± 2.0
96hrs	<b>60.6667</b> ± 2.081666	57.333* ± 2.51661	57.333* ±1.527525	57.66667* ± 2.081666	56.6667 ** ± 1.527525

Values are expressed in ---mg/gram of wet weight of tissue

± = Standard deviation of three observation.

\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Graph-5**-Effects of Surf excel and Nirma powder on Lipid (--mg/gm) content in Ovary of *Mystus montanus* at various sublethal concentrations.



**Table-6**

Effects of Surf excel and Nirma powder on Lipid (--mg/gm) content in Testis of *Mystus montanus* at various sublethal concentrations.

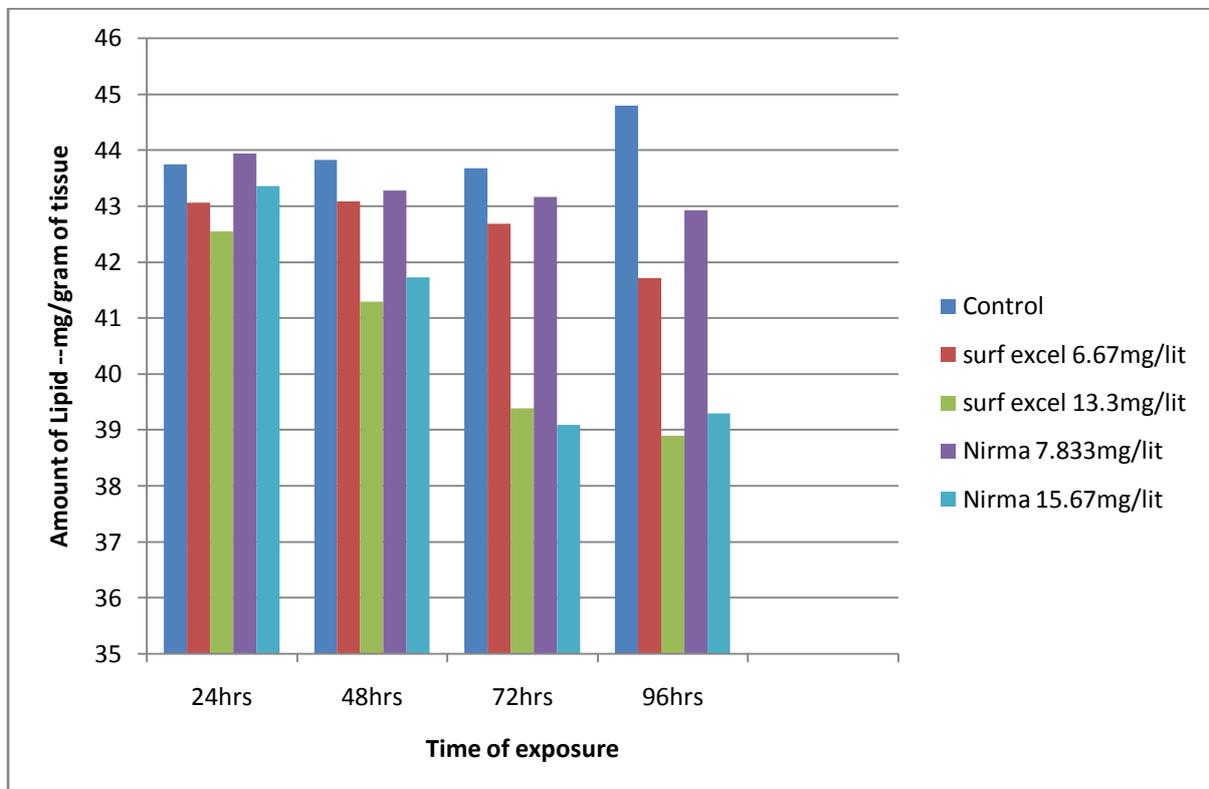
Exposure Time	Concentration of detergents –mg/litre.				
		Surf excel		Nirma powder	
	Control	6.67mg/lit.	13.3mg/lit.	7.833mg/lit.	15.67mg/lit.
24hrs	43.74603 ± 1.5892	43.0586* ± 1.20152	42.54203* ± 1.17571	43.94927* ± 1.189681	43.34998* ± 0.613626
48hrs	43.81824 ± 1.482246	43.08667* ±1.000823	41.2918 ** ±1.276868	43.27704* ± 1.002299	41.72732* ± 1.961442
72hrs	43.67716 ±1.235467	42.67996* ± 1.19643	39.39088** ± 1.497885	43.15878* ± 0.81497	39.08867** ± 0.932906
96hrs	44.78785 ± 1.224591	41.71499* ±1.739633	38.89553** ± 1.763384	42.91694* ± 0.880581	39.29393** ± 0.816048

Values are expressed in ---mg/gram of wet weight of tissue,

± = Standard deviation of three observation.

\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Graph-6**-Effects of Surf excel and Nirma powder on Lipid (--mg/gm) content in Testis of *Mystus montanus* at various sublethal concentrations.



The results and findings of present investigation show an agreement to the results and findings of many authors. Saksena and Agrawal, N. E. Chandanshive (2015), Barton et al. (2002), Maruthanayagam (1997), Saxena et al. (1986) and many other authors were made similar observations.

The surfactants are major constituents of detergents. These are poisonous to living fauna, can be easily absorbed through gill or intestine, and damage the epithelium of mouth and gill. It may result in poor consumption of food and inhibit in oxygen uptake. The fish fauna from polluted water getting difficulty to find prey and they lost to taste. This may affect the enzymatic actions and functioning of various organs resulting into poor digestion of food and interference with physiology of fish. The toxicity of detergents changes composition of biochemical contents in the organs of animals. The first indication of contamination strain is changes in biochemical contents in the body of an organism. At the time of stress, an organism requires adequate amount of energy that can supplied from reserve biochemical materials. The investigation shown that amount of protein decreased with increase in concentrations of detergents and time of exposure [17]. The exposure of fresh water prawn *Macrobrachium lamarrei* to various sublethal concentrations of detergent shows decrease in protein, lipid and carbohydrate content in the tissues like hepatopancreas, muscles and gills [18].

The reduction in ovarian total protein and alkaline phosphate occurs on exposure of *Calarius batrachus* to Mercuric chloride but there is an increased in the glycogen, acid phosphatase and cholesterol levels [19]. The phenomenon of intersex is commonly occurring in the cyprinid fish at lowland rivers in the United Kingdom. In both sex of cyprinid fish, reproductive ducts and several cyprinids female also have germ cells (oocytes) within a predominantly male testis. In female cyprinid fish exposed to effluent, there was higher rate of oocyte atresia and significantly, decrease in fecundity in effluent-exposed fish compared with female from the references sites [20]. There is a harmful effect on abnormal development (feminization) of secondary sexual characteristics, as well as on gonad development and differentiation and in male *Gambusia affinis* when exposed to treated sewage effluent [21].

The intersex is a commonly occurring process in the tilapia collected from Era-Jiin River of Taiwan due to endocrine disrupting chemicals, especially when the ratio of intersex was equal and these fishes were natural inhabitants on this river [22]. It is reported that 20% male *Puntius javanicus* were found to be feminized on July 19, 2012 collection from that

river. Some histological samples indicated the presence of testis-ova in fish gonad, which indicated the occurrence of intersex of this species [23]. The studies on effects detergents on reproduction have suggested that as the quantity of detergent is increase in water, it severely affects the reproduction in guppy. There is alteration of ovary of fish, *Poecilia reticulata* also observed. This has noticed that when fishes were exposed to detergents for long duration, causes more damages to all types of developing follicles. After 30 days of exposure to detergents, almost all the entire developing and vitellogenic follicle had deteriorated. During these experiments, it has noticed that ovary losses maturing and developing mature oocyte, while the no. of immature and atretic oocyte increases [24].

When fishes exposed to detergents for long period, causes hypertrophy and hyperplasia of the cell types and oestrous cycle of fish become irregular. In *Poecilia reticulata*, eggs formation of get affected due to detergents [25]. The investigation shows that a decrease in feeding, body growth and conversion efficiency of *Oreochromis mossambicus* reared in different concentrations of detergents [26]. The pollution may affect liver size through energy consumption, supplied by glycogen and fat stores from the liver, resulting in decreases of liver size [27]. The fecundities were low at the impacted area for carp and pumpkinseed and the trade-off for common carp are consistent with documented effects of contaminants on fecundity because the pollutants may affect different levels of fish reproduction [28]. The field studies have demonstrated that there was an alteration in gonadal weight and fecundity with exposure to aromatic hydrocarbons, PCBs, and other chlorinated compounds [29].

In the present investigation, it is noticed that the levels of glycogen, protein and lipid contents in reproductive tissues such as ovaries and testis is decrease with an increase in concentration of detergents and time of exposure. These biochemical glycogen, protein and lipid are essential for the normal development of sperm and ova. This decline level may affect the process of developmental and maturation process of gonads such as oogenesis and spermatogenesis. There will be a significant decline in the ovarian weight as well as decline in number of ova (fecundities) in the ovaries of fish. There is a possibility of delay in ovulation and insemination process and thus affecting the breeding or spawning process in fishes. Due to pollution stress, hatching process and development of embryo will affect, and there will be possibility of number of abnormalities in developed embryos. Thus, whole reproduction process will be disturbed.

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