“Effects of Linear Alkyl Benzene Sulphonate on different tissues in local fresh water fish”.

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Final Report

Project undertaken by

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Introduction:

We live on a planet surrounded inside and out with water. All living organisms require water, a basic need for their survival. Although water is actually not chemically very complicated, it is the unique physical and chemical properties of water that are responsible for the existence of life on this planet. These unique properties of water provide the frame work and method for interacting with living processes.

All organisms take in water which is chemically in the form of a solution of a variety of inorganic and organic substances which also form a part of their metabolism. Pure water therefore in a general sense means water which is free of pollutants or substances unwanted by the beneficiary organisms. Water is received by water bodies from various point and non-point sources, and may contain pollutants to a lesser or greater extent. In aquatic ecosystems, not only the flora, but also the fauna, are exposed largely to all substances in their immediate environment, water. As such, water is taken by the fauna as part of nutrition as also for respiratory purposes. The presence of undesirable substances in water therefore have a direct effect on all flora and fauna within it. Fish are particularly sensitive to a wide variety of chemicals.

Linear Alkyl benzene Sulphonate (LABS) is one of the most widely used surfactants in the world, primarily in laundry detergents and cleaning products. It is a soft acid slurry and is the main raw material for synthetic detergent industries in the formulation of Washing Powder, Detergent Powder, Detergent Cake, Liquid Soap, Cleaning Powder, Scouring Bar, Oil Soaps etc. In Textile Industries it is used as mercerizing or washing agent. It is also used for increasing the surface area of distempers.

The surfactant properties of LABS resulted in large quantities of foam on its release into streams and rivers. Sewage sludge, which is widely used as a fertilizer or disposed of by land application, often contains potentially toxic compounds and elements in significant concentrations. A major component is LABS which is frequently used in industrial and household detergents.

Inhalation, ingestion or skin contact with the material may cause severe injury or death. Dilution in water may cause corrosive and/or toxic effects in addition to polluting a water body. Considering these problems, it is essential to study the toxicity of LABS to fish, mainly since aquatic organisms are the primary targets of such a pollutant, and because

major portions of population are dependent on fish as food. Hence a study was undertaken using a locally available sturdy fish species, *Clarias batrachus* .

Materials and methods:

The present work was carried out with the objective of studying the effects of LABS on fresh water fish. The presence of detergents, of which LABS is a component, is ubiquitous in water bodies. LABS of LOBA make was used for the work. The specimens used for the present work were of average length 6-8 inches and weight 20 gm. Classification of the *Clarias* is as follows:

Phylum	: Chordata
Group	: Vertebrata
Subphylum	: Gnathostomata
Series	: Pisces
Class	: Actinopterygii
Order	: Siluriformes
Family	: Clariidae
Genus	: *Clarias*
Species	: *batrachus (Linn.)*

The fish were collected from fresh water bodies and then acclimatized in aquaria using dechlorinated tap water at room temperature for about 15 days and fed with commercially available floating type fish food.

Fig. 1*: Clarias* being feed on floating fish food

Fig. 2: Measurement of length of *Clarias*

The acclimatized specimens were subjected to acute toxicity tests to determine a safe dosage limits for chronic exposure. The specimens so exposed to a safe dose limit of LABS for a longer duration were then sacrificed for harvesting the tissues.

Histopathological effects of LABS were studied on the intestine and liver tissue by standard methods of microtechnique, with paraffin embedding, section cutting and double staining by the Haematoxylin Eosin staining technique.

Acute Toxicity Tests

Acclimatized and healthy fish were kept in aquaria, using dechlorinated tap water at room temperature as dilution water. The dilution water was characterized, and the result is presented in Table 1.

LABS is highly soluble in water. A stock of the test solution was prepared using 10 ml/1000ml of dilution water and then diluted for use as per the requirement.

Table 1: Characteristics of Dilution Water

Parameters	Values *
Temperature º C	25-27
pH	7.5-8.2
Total Alkalinity as $CaCO_3$	156-190
Total Hardness as $CaCO_3$	142-172
Ca Hardness as $CaCO_3$	80-94
Mg Hardness as $CaCO_3$	62-78
Dissolved Oxygen	6.9-7.3
Calcium as Ca	32-38
Magnesium as Mg	14-18
Sodium as Na	36-38
Potassium as K	2-4
Chloride	126

*All the values are expressed as mg/L except temperature and pH.

Appropriate controls were invariably maintained. They were exposed to dilution water as specified in Table 1 above.

The test fish were periodically exposed to fresh test solutions of various dilutions in water, once every 24 hours. The dilution water as well as test solution was replaced every 24 hours. 10 liters of water containing 10 fishes was used in each aquarium during the acute toxicity test. On an average, ten concentrations were studied, and tests were run in duplicate for each concentration.

At higher concentrations, fish showed irregular rapid movements, upside down movements, peeling of the skin, and red patches.

Fig. 3: Peeling of the epidermal tissue

Fig. 4: Haemorrhagic patches on the body

Mortality was also seen to be associated with the appearance of red patches on the caudal region. The fishes were presumed dead when they showed no reaction on prodding.

Chronic Toxicity Tests:

Experiments were carried out using a 20 litre rectangular glass aquarium. A continuous flow of 10 litres of water containing a sub lethal dose of either LABS was maintained into the aquarium. A safe sub lethal concentration of LABS was arrived at based on acute toxicity tests. This safe concentration of LABS used for the experiments was $5\mu l l^{-1}$.

The incoming solution was delivered at the bottom of the aquarium and water was allowed to spill out from an outlet at the upper level of water, thus maintaining the volume of water in the aquarium constant. Experiments were continued for a time interval of 15 and 30 days. Experimental fish showed a gradual decline in feeding. 20 numbers of fish were introduced in the aquarium for each concentration of LABS.

A parallel control was run along with every concentration under the same experimental conditions. The fishes were removed alive from the aquaria after every 15 days of exposure. They were then sacrificed and were dissected and individual tissues were preserved in Bouin's fixative for 7-8 days until further processing.

Different tissues from the test fish, viz. the Liver and Intestine were fixed in aqueous Bouin's fixative. The tissue was then thoroughly washed in running water, and dehydrated through increasing grades of alcohol: 30%, 50%, 70%, 90% and Absolute alcohol. The tissues were then cleared using Xylene and then embedded in paraffin wax (Melting point 58-60°C).

The tissue was sectioned at the thickness of 6-8 µ, using a manually operated rotary microtome (Weswox). The section ribbons were spread on glass slides using Meyer's albumin. The sections were first deparaffinised using two changes of Xylene and then hydrated for staining.

The standard double staining method for histological study using Haematoxylin and Eosin stains was followed. The tissue sections were hydrated through decreasing grades of alcohol: Absolute alcohol, 90%, 70%, 50% and 30% alcohol, and then treated with distilled water. Sections were then stained with aqueous Haematoxylin (Delafield's) stain solution of MERCK make. Tissue sections were then washed in running tap water for removal of excess stain and proper fixing of the stain. This was followed by a dehydration of the tissue sections through 30%, 50% and 70% alcohol, for staining with alcoholic (in 70% alcohol) Eosin stain.

The sections were washed after staining with Eosin in 70% alcohol to remove excess stain, and dehydrated further through 90% and absolute alcohol. The tissue sections were then cleared using Xylene, which also acts as a miscibility intermediate between alcohol and the mountant medium.

The cleared sections were mounted in DPX mountant, covered with cover glass of appropriate size, and allowed to dry. Stained sections so prepared were observed under compound microscope for histopathological effects on tissue.

Results:

Following histopathological effects were seen on the tissues viz. intestine and liver of *Clarias* after chronic exposure to either LABS for periods of 15 and 30 days.

The Intestine:

Control

The general histological structure of the intestine shows a simple mucoid epithelium, overlying a submucosa which is often richly endowed with eosinophilic granular

cells and limited by a dense muscularis mucosa and a fibroelastic layer. Control intestine of *Clarias* is shown in Fig. 5.

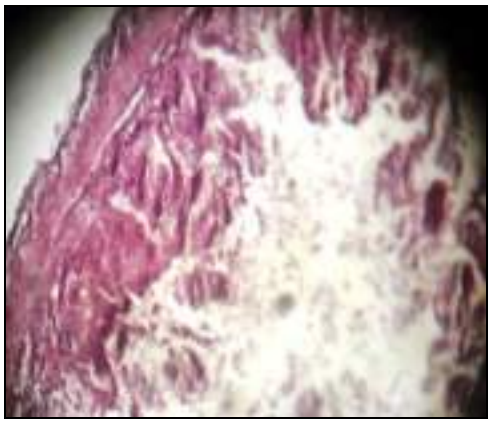

Fig. 5: T.S. Intestine of *Clarias* (Control 15 Days: Magnification: 10X)

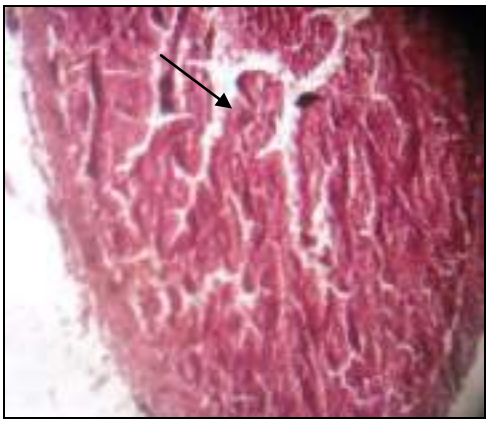

Fig. 6: T.S. Intestine of *Clarias* (Expeimental 15 Days: Magnification: 10X)

Exposure of *Clarias* to LABS showed following results. A 15 day exposure showed severe hypertrophy of villi with partial occlusion of the intestinal lumen. (Fig. 6), whereas 30 days exposure showed complete occlusion of the intestinal lumen (Fig. 8)

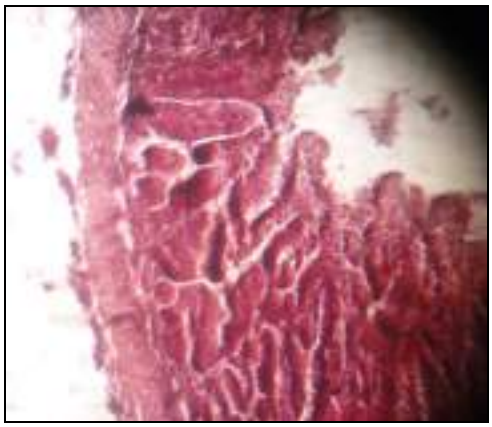

Fig. 7: T.S. Intestine of *Clarias* (Control for 30 days Magnification: 10X)

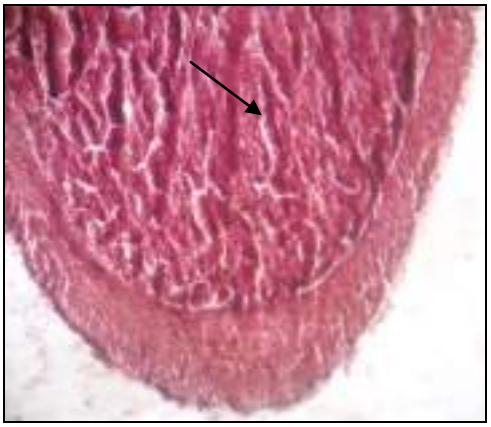

Fig. 8: T.S. Intestine of *Clarias* (Experimental for 30 days Magnification: 10X)

The Liver:

Control

The fish liver is composed of a large number of polyhedral hepatic cells containing a granular cytoplasm. The nuclei of liver cells are vesicular with large nucleoli. Numerous

bile ductules, bile capillaries and sinusoids are noticed. Sinusoids, which are irregularly distributed between the hepatocytes, are few in number, and lined by endothelial cells with prominent nuclei.

Fig. 9: T.S. Liver of *Clarias* (Control 15 Days: Magnification: 10X)

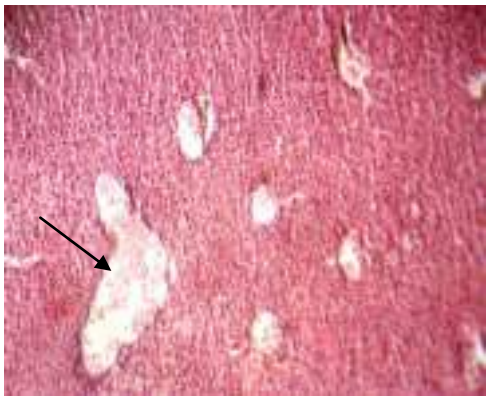

Fig. 10: T.S. Liver of *Clarias* (Experimental 15 Days: Magnification: 10X)

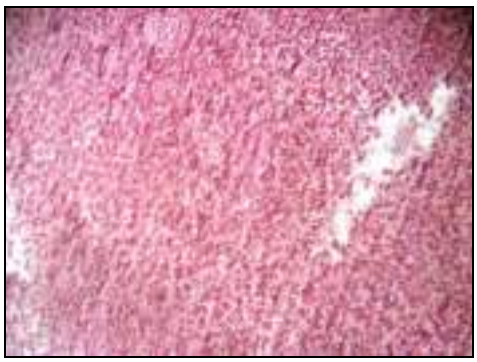

Fig. 11: T.S. Liver of *Clarias* (Control 30 Days: Magnification: 10X)

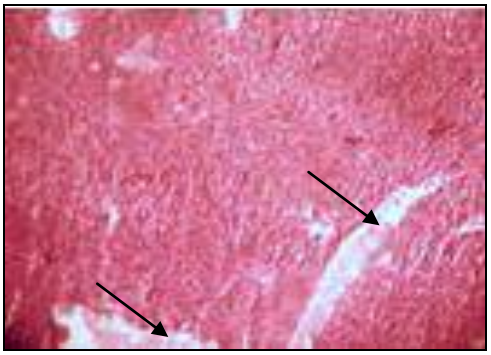

Fig. 12: T.S. Liver of *Clarias* (Experimental 30 Days: Magnification: 10X)

On exposure of *Clarias* to LABS for 15 days, there was enlargement of sinusoids (Fig. 10), showing necrosis along the endothelium.

Necrosis was observed to increase further on a longer exposure period of *Clarias* for 30 days to LABS (Fig. 12), along with an increase in the number and size of sinusoids in the liver tissue.

Summary:

In the present work, the effects LABS on tissues of the fish species, *Clarias batrachus* (Linn.) have been studied. The study encompasses histopathological effects of this compound on the tissues intestine and liver.

Damage to both intestine and liver tissue was very severe with necrosis, degeneration of connective tissue, enlargement of blood filled interstitial spaces and occlusion of lumen.

It is probable that chronic exposure resulted in hypertrophy of intestinal layers, and changes in the liver histology are a reflection of the attempts of the liver to detoxify and biotransform the ingress of LABS into the blood and body tissues.

The main objective of this study was to see the histopathological changes and destruction, if any, in the internal tissues due to chronic toxicity exposure for a period of 30 days. Samples were collected accordingly at time intervals of 15 and 30 days.

The villi in the intestine provide more surface area for absorption and enzymatic activity. Exposure to LABS on longer exposure of 15 days showed that villi get fused with each other, and thus the intestinal lumen space was reduced considerably.

This situation became more acute as the exposure period increased to 30 days, where complete occlusion of the intestinal lumen occurred.

Liver of fish exposed to LABS showed enlargement of sinusoids, with necrosis, which was significant even at 15 days exposure. These changes became finer as the exposure time increased.

Conclusion:

It can be inferred that tissue damage increases in proportion to the duration of exposure. A safe dose in case of exposure to a compound can be determined on the basis of toxicity studies, though it may differ from one species to another and between different compounds, in a given water body. Possible strategies for remediation can also be designed after studying the degree of damage due to the pollutant substance.